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Effects of tempol and redox-cycling nitroxides in models of oxidative stress

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Abstract

Tempol is a redox cycling nitroxide that promotes the metabolism of many reactive oxygen species (ROS) and improves nitric oxide bioavailability. It has been studied extensively in animal models of oxidative stress. Tempol has been shown to preserve mitochondria against oxidative damage and improve tissue oxygenation. Tempol improved insulin responsiveness in models of diabetes mellitus and improved the dyslipidemia, reduced the weight gain and prevented diastolic dysfunction and heart failure in fat-fed models of the metabolic syndrome. Tempol protected many organs, including the heart and brain, from ischemia/reperfusion damage. Tempol prevented podocyte damage, glomerulosclerosis, proteinuria and progressive loss of renal function in models of salt and mineralocorticosteroid excess. It reduced brain or spinal cord damage after ischemia or trauma and exerted a spinal analgesic action. Tempol improved survival in several models of shock. It protected normal cells from radiation while maintaining radiation sensitivity of tumor cells. Its paradoxical pro-oxidant action in tumor cells accounted for a reduction in spontaneous tumor formation. Tempol was effective in some models of neurodegeneration. Thus, tempol has been effective in preventing several of the adverse consequences of oxidative stress and inflammation that underlie radiation damage and many of the diseases associated with aging. Indeed, tempol given from birth prolonged the life span of normal mice. However, presently tempol has been used only in human subjects as a topical agent to prevent radiation-induced alopecia.

Keywords

Reactive oxygen species; oxidative stress; blood vessels; kidney; heart; brain

1. Introduction

1.1. Scope of the review

The rapid increase of interest in the role of “oxidative stress” in a range of disease processes has focused attention on drugs that prevent the generation of “reactive oxygen species (ROS)” or enhance their metabolism. The response to such interventions can give insight

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into the underlying role of ROS in the pathophysiology and may point to future therapeutic targets. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that has been studied extensively in animal models of increased ROS and its effects on hypertension and endothelial function. The chemistry of tempol (Carroll et al., 2000; Bonini et al., 2002; Soule et al., 2007; Wilcox & Pearlman, 2008) and its effects on the regulation of blood pressure (BP) and endothelial function (Wilcox & Pearlman, 2008; Simonsen et al., 2009a; Simonsen et al., 2009b) have been reviewed recently. Therefore, the review will not consider these aspects of tempol, except where necessary to describe new findings. The introduction will provide a very brief overview of the major chemical reactions of tempol, but readers are refered to the published reviews for further information on this, and on the effects of tempol on blood pressure and the endothelium.

1.2. Overview of chemical reactions

Tempol can shuttle between the nitroxide radical, the reduced hydroxylamine and the oxidized oxoammonium cation form with 1 and 2 electron transfer reactions (Figure 1A). These are facilitated by “boat and chair” conformational changes that underlie the rapidity and catalytic nature of the reactions. The reaction of tempol with superoxide anion ($O_2^{\cdot -}$) to form hydrogen peroxide (H_2O_2) accounts for its “superoxide dismutase (SOD) mimetic” action. Although the reported reaction rates between tempol and $O_2^{\cdot -}$ vary widely, a recent study reported that the effectiveness of tempol in catalyzing the metabolism of cellular $O_2^{\cdot -}$ was similar to native SOD and only slightly less than the cell permeabilized pegylated form of SOD (PEG-SOD) (Figure 2). Tempol was as effective in metabolizing cellular $O_2^{\cdot -}$ as N-acetyl cystein (NAC), but unlike tempol, NAC paradoxically enhanced $O_2^{\cdot -}$ generation at lower concentrations. Tempol was significantly more effective than other frequently used “antioxidants” and was far more effective than vitamins (Figure 2). Among a group of 6-member ring nitroxides, the *in vitro* SOD-mimetic activity in metabolizing $O_2^{\cdot -}$ closely paralleled their *in vitro* actions (Patel et al., 2006), consistent with the concept that the biological efficacy of tempol depended on its facility to promote the metabolism of $O_2^{\cdot -}$.

Nevertheless, some studies have documented important additional biochemical actions of nitroxides to facilitate metabolism of a wide range of ROS and reactive nitrogen species. Indeed, a comparison of the efficacy of tempol in metabolizing cellular $O_2^{\cdot -}$ or H_2O_2 or in protecting cells from damaging effects of hydroxyl radical (·OH) demonstrated that the sensitivity of tempol was greatest for ·OH, intermediate for H_2O_2 and least for $O_2^{\cdot -}$. Tempol is effective as a catalase-like agent and in preventing the generation of ·OH from H_2O_2 in the presence of transition metals in the Fenton reaction (Soule et al., 2007; Wilcox & Pearlman, 2008). Thus, tempol is best considered a general purpose redox cycling agent rather than a specific SOD mimetic (Wilcox & Pearlman, 2008). Tempol is an ampholyte with a high capacity to permeate cell membranes, the gastrointestinal tract (GIT) or the blood-brain barrier which accounts for its effectiveness after oral administration and its central nervous system actions.

The reaction between $O_2^{\cdot -}$ and nitric oxide (NO) yields peroxynitrite ($ONOO^-$) which is highly oxidant. Carroll et al demonstrated that tempol inhibited nitration of test compounds by a large molar excess of $ONOO^-$ and was recovered intact as tempol thereafter, leading to the conclusion that tempol acted catalytically (Carroll et al., 2000). The reaction entailed oxidation of tempol by $ONOO^-$ derived radicals such as ·OH, carbonate or nitrogen dioxide radicals to the oxammonium cation which itself was reduced back to tempol while oxidizing $ONOO^-$ to oxygen (O_2) and NO. The details of these interactions were given by Carroll et al (Carroll et al., 2000) and were confirmed by Bonini et al (Bonini et al., 2002). Since peroxynitrite is implicated in many pathophysiologic conditions of oxidative stress, this is likely an important mechanism of tissue protection by tempol as, for example, in traumatic brain injury (Deng-Bryant et al., 2008; Xiong & Hall, 2009).

Saito et al demonstrated an additional reaction of tempol that did not involve the nitroxide radical (Figure 1B). They reported that ·OH interacts with the 4-position of the piperidine ring of tempol to form 4-oxo-2,2,6,6-tetramethylpiperidine-N-oxyl (tempone) (Saito et al., 2003). However, at physiologic levels of pH, this reaction accounted for only about 10% of the reduction of ·OH by tempol (Deffner & Schimmack, 1976; Saito et al., 2003) and was rapidly reversible since tempone was metabolized in cells (Kroll et al., 1999) or in mice (Kroll et al., 1999; Kroll & Borchert, 1999) to tempol over 10 minutes. High performance liquid chromatography confirmed that the reaction of tempol with ·OH yielded tempol hydroxylamine (tempol-H) and tempone (Kudo et al., 2008).

1.3. Development of knowledge concerning nitroxides

An extensive series of studies by Mitchell, Krishna, Samuni, Russo, Hahn, Cotrim and colleagues reviewed recently (Saito et al., 2003; Wilcox & Pearlman, 2008) defined the biophysical properties of nitroxides and their roles as radiation protection agents. The authors demonstrated that tempol protected cells (Mitchell et al., 1991; DeGraff et al., 1992a; Hahn et al., 1992b) and animals (Hahn et al., 1992a; DeGraff et al., 1992a; Liebmann et al., 1994; Hahn et al., 1997; Hahn et al., 1998; Hahn et al., 2000) from the effects of radiation. They reported that these effects of tempol were relatively specific for well oxygenated normal tissues, whereas the hypoxic environment of tumors promoted a rapid reduction of tempol to tempol-H that lacked radioprotective effects (Hahn et al., 1997; Cotrim et al., 2005; Cotrim et al., 2007). This led the way to the first use of tempol in human subjects where it is undergoing clinical trial as a topical agent to protect the hair and skin of patients receiving cranial radiotherapy for cancer(Metz et al., 2004).

Nishiyama and colleagues demonstrated that tempol protected the kidneys from glomerulosclerosis in aldosterone-and salt-dependent models of hypertension (Nishiyama & Abe, 2004; Nishiyama et al., 2004a; Nishiyama et al., 2004b) and protected the heart in models of cardiac hypertrophy and cardiac failure (Zhang et al., 2005a; Guo et al., 2006). They demonstrated the importance of the mitogen-activated protein kinase pathway as a target for the tissue protective effects of tempol (Zhang et al., 2004; Nishiyama & Abe, 2004; Nishiyama et al., 2004a; Nishiyama et al., 2004b; Zhang et al., 2005a). Fujita and colleagues demonstrated that a reduction of ROS with tempol in models of mineralocorticosteroid excess and salt prevented the mineralocorticosteroid receptor signalling and preserved podocyte function in the glomerulus. They demonstrated that tempol had an anti-proteinuric action and preserved cardiac diastolic relaxation and thereby prevented diastolic heart failure (Nagase et al., 2007; Matsui et al., 2008; Nagase & Fujita, 2008; Wang et al., 2008a) in models of the metabolic syndrome (Furukawa et al., 2004; Nagase et al., 2006; Ando & Fujita, 2009) and diabetes mellitus (DM) (Asaba et al., 2007).

Schnackenberg, Welch, Wilcox et al related the biological effects of acute intravenous nitroxides in hypertensive models to their *in vitro* SOD mimetic activity (Patel et al., 2006), to the generation of vascular H_2O_2 (Chen et al., 2007c), to enhancement of NO signaling (Schnackenberg et al., 1998; Welch & Wilcox, 2001), to inhibition of the sympathetic nervous system and to activation of adenosine triphosphate-dependent potassium channels (Chen et al., 2007b). The effects of more prolonged oral tempol administration were not accompanied by increased vascular H_2O_2 (Chen et al., 2007c) but were related to correction of oxidative stress (Schnackenberg & Wilcox, 1999) and salt sensitivity (Welch et al., 2005b). They reported that tempol improved the efficiency with which the hypertensive rat kidney used oxygen for tubular sodium transport and thereby improved the oxygen tension in the renal cortex and medulla (Welch et al., 2003; Welch et al., 2005a). They showed further that tempol restored the endothelium dependent relaxation/nitric oxide (EDRF/NO) responses and diminished the endothelium dependent contraction factor (EDCF) responses in the renal afferent arterioles and mesenteric resistance vessels in models of oxidative stress

and restored NO signalling between renal tubular epithelial cells in the macula densa and the renal afferent arteriole (Wilcox & Welch, 2000; Welch et al., 2000; Schnackenberg et al., 2000; Schnackenberg & Wilcox, 2001; Welch & Wilcox, 2001; Wang et al., 2003a; Wang et al., 2004; Wang et al., 2006). This restoration by tempol of NO signalling within the juxtaglomerular apparatus may maintain renal blood flow and protect against salt-dependent increases in BP.

In an extensive set of studies of the protective roles of tempol, Thiemermann et al reported that pretreatment with tempol prevented ischemia/reperfusion damage in the kidney (Chatterjee et al., 2000; Patel et al., 2002; Thiemermann, 2003), heart (McDonald et al., 1999; McCormick et al., 2006), liver (Sepodes et al., 2004) and brain (Cuzzocrea et al., 2000c), prevented experimental colitis (Cuzzocrea et al., 2000b), arthritis (Cuzzocrea et al., 2000d) and pleurisy (Cuzzocrea et al., 2000a) and provided protection against shock from hemorrhage (Mota-Filipe et al., 1999), gram-positive (Zacharowski et al., 2000) or gram-negative (Leach et al., 1998) bacteria and multiple organ failure (Cuzzocrea et al., 2001).

Gariboldi, Monti and colleagues reported that, in contrast to its effects to reduce ROS in normal cells (Mitchell et al., 2003; Erker et al., 2005), tempol administered to malignant cells, or rodent models of cancer, induced oxidative stress, p21 expression, and cell death (Gariboldi et al., 2000; Ravizza et al., 2004b). In these models of malignancy, tempol impaired mitochondrial function (Monti et al., 2001), induced apoptosis (Monti et al., 2001) and exerted anti-proliferative effects (Gariboldi et al., 1998; Gariboldi et al., 2003). Tempol had synergistic anti-tumor activity with some chemotherapeutic agents, including doxorubicin and temozolomide (Ravizza et al., 2004b; Gariboldi et al., 2006) which they related to the interruption by tempol of the multidrug resistance pathway (Ravizza et al., 2004b).

1.4. Tempol doses and toxicity

Cellular actions of tempol, for example, in preventing $O_{2}^{\cdot -}$ generation in vascular smooth muscle cells (VSMCs) stimulated by angiotensin II (Ang II), are characterized by dose-dependent effects with high efficacy but limited potency. Thus, although tempol was as effective as native SOD in preventing $O_{2}^{\cdot -}$ generation by Ang II in VSMCs, its ED_{50} was 10-fold higher (Luo et al., 2009). Fully effective concentrations of tempol *ex vivo* to restore relaxation responses to acetylcholine in isolated microvessels from rodent models of oxidative stress, were usually 10^{-4}M (Wang et al., 2003a; Wang et al., 2004) or even 10^{-3}M (Schnackenberg & Wilcox, 2001). Likewise, 10^{-4} to 10^{-3}M tempol was used to increase Ang II-stimulated NO activity in rat isolated, perfused vasa recta capillaries (Zhang et al., 2005b). The *in vivo* antihypertensive response to acute intravenous bolus doses of tempol showed dose dependency. There was a threshold dose of 17 μmol · kg^{-1} and a maximal dose of about 130 μmol · kg^{-1} (Patel et al., 2006; Chen et al., 2007b). The antihypertensive response to tempol infused subcutaneously from an osmotic minipump required 200 nmol · kg^{-1} · min^{-1} in the spontaneously hypertensive rat (SHR) (Welch et al., 2005b). A similar rate of infusion was effective in preventing hypertension and parameters of ROS in Ang II-infused mice (Kawada et al., 2002). Tempol has been widely effective when added to the drinking water of rats or mice in concentrations between 1 and 6 mmol · l^{-1} without any evidence of dose-dependency within this range (Wilcox & Pearlman, 2008). Thus, oral doses of 1-2 mmol · l^{-1} of drinking water are generally used. Care is needed to protect tempol from light, and to replace the water daily, since tempol solutions discolor and may lose effectiveness. The lower limits of the effective oral dose of tempol have not been established and 1 mmol · l^{-1} of oral tempol may be above that required. Nevertheless, assuming that rats drink about 25-30 ml of fluid daily, 1 mmol · l^{-1} of tempol translates into an intake of about 7 mg per day or about 1.5 to 2 gram of oral tempol for an 80 kg human subject. Tempol has not yet been administrated to human subjects. However, these

considerations suggest that it may not be very potent when given alone. The finding that tempol is effective when added to the drinking water of rats or mice has reduced the need for intraperitoneal (ip) dosing. Tempol, given at 87 μmol · kg^{-1} as a once daily ip dose has been effective in reducing the BP in hypertensive rats (Adeagbo et al., 2003; Awe et al., 2003).

Doses of tempol will be given in this review for key studies or in circumstances where they fall outside these generally accepted ranges.

Tempol has been well-tolerated in many animal studies. The toxic doses of tempol are approximately 1 to 2 mmol · kg^{-1} whether given ip (Hahn et al., 1998) or iv (Matsumoto et al., 2004). Thus, as discussed previously (Wilcox & Pearlman, 2008), the effective doses of tempol are well below those that cause toxic manifestations.

2. Mitochondrial function and oxygenation

Mitochondrial ROS are produced largely as a byproduct of O_2 metabolism by the respiratory chain, in particular by complexes I and III in the inner mitochondrial membrane and by monoamine oxidase in the outer membrane (Chance et al., 1979; Poderoso et al., 1996; Cadenas & Davies, 2000). Mitochondria are also targets for ROS. Tempol prevented Ang II from stimulating mitochondrial ROS (Kimura et al., 2005a). The oral administration of tempol (300 mg · kg^{-1} · d^{-1}) to rats prevented the effect of tumor necrosis factor alpha (TNFα) to increase $O_2^{\cdot -}$ production by the mitochondrial complex 1 subunit in cardiac cells whereas apocynin, which inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, was not effective thereby demonstrating the ability of tempol to inhibit mitochondrial-derived ROS (Mariappan et al., 2009). Rats transgenic for the renin-2 (ren-2) gene had hepatic mitochondrial damage and dysfunction that were prevented by tempol (Wei et al., 2009b). In contrast, tempol was relatively ineffective in preserving mitochondrial function in the injured spinal cord where mitochondrial Ca^{++} overload, rather than excessive mitochondrial ROS, was the major factor contributing to dysfunction (Patel et al., 2009).

Mitochondria have a low partial pressure of oxygen (PO_2) and a high rate of production of ROS. This accounts for their being a major site for reduction of tempol to the hydroxylamine (Iannone et al., 1990). Efforts have been made to further enhance the mitochondrial accumulation of tempol to provide a targeted protection of mitochondria against oxidative damage. Mitochondria maintain a substantial electrical potential of about -180 mV across their inner membrane. This membrane also recognizes a peptide signal sequence contained in the cyclopeptide antibiotic, gramicidin S that directs proteins to the mitochondrial matrix. Both of these characteristics have been employed to partition drugs into mitochondria (Murphy, 1997). An early studied failed to show that mitochondrial targeted nitroxide compounds were more effective as mitochondrial antioxidants, perhaps because of considerable cellular and mitochondrial accumulation of native tempol itself (Dessolin et al., 2002). However, the highly cationic SOD mimetic Mn (III) meso-tetrakis (N-ethylpyridium-2-yl) porphyrin was concentrated in mitochondria isolated from the heart of mice after systemic injection (Spasojevic et al., 2007). Moreover, complexing of vitamin E (Smith et al., 1999) or tempol (Murphy, 1997; Trnka et al., 2008) with tetraphenylphosphonium yielded highly cationic compounds whose mitochondrial uptake were enhanced several hundred fold. The tempol conjugate, termed mito-tempo, was a mitochondrial anti-oxidant (Trnka et al., 2008) but was found to be no more effective than tempol itself in inhibiting depolarization of the inner mitochondrial membrane during apoptosis induced by oxidative stress (Dessolin et al., 2002) and was significantly less effective than tempol in preventing $O_2^{\cdot -}$ accumulation in mitochondrial membranes, perhaps because the ionic form was excluded from lipid compartments (Kruglov et al.,

2008). Nevertheless, complexing of spin traps such as tempol with ubiquinone enhanced their mitochondrial uptake and improved cellular protection against H_2O_2-induced apoptosis (Kelso et al., 2001), prevented oxidative activation of mitochondrial uncoupling proteins (Murphy et al., 2003), prevented the development of tolerance to nitroglycerin (Esplugues et al., 2006), and moderated ischemia-reperfusion damage in rat hearts (Esplugues et al., 2006). Nitroxides conjugated with fragments of gramicidin S also were concentrated in mitochondria where they retained their ability to reduce $O_2^{\cdot -}$ and combat cellular apoptosis (Wipf et al., 2005; Jiang et al., 2007).

Thus, oral tempol alone in several studies achieved fully effective concentrations in mitochondria where it exerted antioxidant and protective actions. Strategies to increase mitochondrial accumulation of nitroxides by linking them to moieties that enhance uptake across the mitochondrial membrane have not enhanced their efficacy consistently.

NO is rapidly bioinactivated in the tissues by $O_2^{\cdot -}$ (Gryglewski et al., 1986). NO inhibits tissue O_2 usage in part by completing with O_2 in the mitochandria (Boveris et al., 2000). Thus, blockade of nitric oxide synthase (NOS) (Laycock et al., 1998; Wolin et al., 1999) or induction of oxidative stress (Palm et al., 2003; Welch et al., 2005a) both enhanced O_2 usage by the rat heart or kidney. Conscious dogs with oxidative stress due to hyperhomocysteinemia had an increased cardiac oxygen usage that was corrected by tempol (Suematsu et al., 2007). Likewise, the Mn-SOD (+/-) mouse model of mitochondrial oxidative stress had increased whole body O_2 usage and decreased work capacity (Kinugawa et al., 2005b) that were improved by seven days of tempol administration (Kinugawa et al., 2005b). The NOS-1 knockout mouse had an increased agonist-induced myocardial O_2 consumption that was attributed to a defective mitochondrial NO generation and was corrected by tempol (Kinugawa et al., 2005a). Hearts from elderly Fisher 344 rats had a blunted reduction in O_2 usage with NO that was related to excessive ROS since tempol or apocynin corrected the response (Adler et al., 2003). Infusion of tempol corrected the increased cardiac (Kinugawa et al., 2003) or renal (Welch et al., 2005a) O_2 usage caused by an Ang II infusion. A rat kidney made ischemic by clipping its artery had a reduced PO_2 that was corrected by an infusion of tempol (Welch et al., 2003).

There is normally a close linear relationship between the renal oxygen usage (Q_{O2}) and the chemical work for the tubular transport of sodium (T_{Na}) (Welch et al., 2001; Welch & Wilcox, 2001). The slope of the line relating Q_{O2} to T_{Na} defines the efficiency with which the kidney uses O_2 for Na^+ transport. This slope was increased by agents that increased ROS or Ang II or decreased NO, and was decreased by tempol or angiotensin receptor blockers (ARB) (Figure 3A). There were corresponding changes in renal cortical or medullary PO_2. Thus, Ang II infusion reduced the PO_2 in the renal cortex and medulla but this was prevented in rats given tempol (Figure 3B). The administration of tempol to diabetic rats moderated the renal hypoxia yet paradoxically increased the renal expression of hypoxia inducible factors and heme oxygenases (Rosenberger et al., 2008). An improvement in the oxygenation of the heart, kidneys and skeletal muscles may contribute to the apparently beneficial effects of tempol in some models of heart and kidney failure, and in the protection of organs from ischemia-reperfusion or hypoxia-reoxygenation injury. These are described later.

3. Metabolic effects of tempol

3.1. Insulin resistance, metabolic syndrome and diabetes mellitus (DM)

DM type 1, whose hallmark is insulinopenia, often originates from destruction of the pancreatic islet cells. Rats given streptozotocin (STZ) to damage the pancreatic islet cells are a well characterized model of DM type 1. DM type 2, which often develops later in life, is a

condition of insufficient insulin to meet the body's needs because of obesity, insulin resistance and prolonged hyperglycemia. The metabolic syndrome is a clinical complex that includes central obesity, hypertriglyceridemia, hyperuricemia, hypertension, reduced levels of high density lipoprotein cholesterol with insulin resistance as a prime component (Hall et al., 1993). The presence of the metabolic syndrome increased the cardiovascular risk at any level of BP and was a precursor of DM type 2 (Grundy et al., 2005). Tempol has several mechanisms of action that mitigate the effects of reduced insulin release or insulin resistance in animal models of these conditions (Banday et al., 2005).

Tempol prevented the ROS generation and the dysfunction of pancreatic islet cells in a model of free fatty acid-induced insulin resistance (Oprescu et al., 2007) and enhanced insulin secretion from rat cultured pancreatic islet cells subject to oxidative stress by high ambient glucose concentrations. Tempol also enhanced insulin releases *in vivo* in glucose-infused rats (Tang et al., 2007). Thus tempol promoted insulin release from the pancreas.

Tempol can enhance insulin-stimulated glucose uptake. Thus tempol increased the membrane abundance of the glucose transporter-1 and enhanced glucose uptake into endothelial cells (ECs) and VSMCs (Alpert et al., 2004). An infusion of tempol prevented the severe insulin resistance and the tyrosine phosphorylation and inactivation of the insulin receptor in the skeletal muscles and adipocytes of rats fed a high salt diet and infused with Ang II to increase ROS (Ogihara et al., 2002). Thus tempol can both enhance the expression of the glucose transporter and enhance the cellular actions of insulin.

Tempol corrected insulin resistance, and the associated renal damage, in several rat models of the metabolic syndrome. Ten weeks of oral tempol given to obese Zucker rats fed a high fat diet reduced the elevated plasma levels of glucose and corrected the increased plasma levels of insulin (Rafikova et al., 2008; Ebenezer et al., 2009). This was associated with normalization of BP, dyslipidemia, C-reactive protein and renal ROS (Ebenezer et al., 2009). However, the oral administration of tempol (1 mmol $\cdot$ l^{-1} of drinking water) for 4 weeks to the SHR/cps model of the metabolic syndrome did not change insulin sensitivity (Matsui et al., 2008). Oral tempol improved the hyperinsulinemia and/or the insulin responsiveness of rats fed a high fructose diet (Onuma & Nakanishi, 2004) or rats transgenic for the ren-2 gene with excessive Ang II and ROS generation (Blendea et al., 2005; Wei et al., 2008) or Dahl salt sensitive (DSS) rats (Zhou et al., 2009), or elderly, obese Fisher 244 rats (Asghar & Lokhandwala, 2006). Crossing of the SHR with a leptin receptor mutant/obese rat and feeding a high salt diet created a rat model of the metabolic syndrome in which adipocytes secreted an adipokine that released aldosterone from the adrenal glands (Nagase et al., 2006). This model had glomerular podocyte damage, proteinuria and enhanced renal expression of the aldosterone-dependent signaling factor serum glucocorticoid kinase-1 (Sgk-1) (Nagase & Fujita, 2008) Tempol reduced the nuclear expression of the mineralocorticosteroid receptor (MR) in the kidneys and its downstream signalling pathways and reduced renal damage, despite little change in BP (Nagase et al., 2007).

Hyperuricemia frequently accompanies the metabolic syndrome and may contribute to oxidative damage (Feig et al., 2008). Induction of hyperuricemia in the rat led to hypertension, renal oxidative stress and damage (Sanchez-Lozada et al., 2008). Tempol prevented the hypertension and the activation of neutrophil oxidase (Nox)-4-dependent NADPH oxidase, reduced the renal Ang II levels and the afferent arteriopathy and maintained the glomerular capillary pressure in this model (Sanchez-Lozada et al., 2008). Thus, tempol has been quite effective in preventing or improving some of the metabolic and signalling defects in rat models of the metabolic syndrome including the lipid profile and insulin sensitivity which may contribute to its preservation of organ function in these models.

ROS are implicated in many of the complication of DM (Brownlee, 2005; Simmons, 2006) including impaired microvascular function. Diabetic patients, or animal models, have profound vascular endothelial dysfunction (Schnackenberg & Wilcox, 2001), vascular proliferation and neoangiogenesis and accelerated atherosclerosis and thrombosis that predispose to ischemia and infarction of organs (Veglio et al., 1993; Brownlee, 2005; Simmons, 2006). The conducted vasomotor dilator or constrictor responses of cremasteric microvascular arterioles *in vivo* were impaired in STZ induced diabetic mice but were corrected by a combined administration of tempol and the protein kinase C beta II inhibitor, LY-341,684 (Rai et al., 2008). Tempol improved vasodilation to hypoxia without changing vasoconstriction to hyperoxia in cerebral vessels from obese Zucker rats (Phillips et al., 2005) and restored nNOS-dependent vasodilation of cerebral arterioles from a rat model of DM type 1 (Arrick et al., 2007). DSS rats had oxidative stress and insulin resistance accompanied by impaired insulin-stimulated endothelium-dependent relaxation of the aorta and a reduced activation of vascular NOS by insulin that were improved by an ARB or by oral tempol (Zhou et al., 2009). Blood vessel from the leptin deficient (db) mouse model of DM type 2 (Gao et al., 2007) or the obese Zucker rat (Phillips et al., 2005) had impaired flow-induced vascular dilations that were corrected by tempol (Bouvet et al., 2007). The addition of tempol to the bath of aortas from apolipoprotein E-deficient mice improved EDRF/NO responses (Kitayama et al., 2007) and blunted nuclear factor kappa B (NFκB)-dependent activation of TNF-α that activated NADPH oxidase to generate ROS (Kitayama et al., 2007). Rats treated with streptozocin to cause DM had systemic and vascular oxidative stress and profound defects in EDRF/NO that were corrected by tempol (Nassar et al., 2002). Perfused afferent arterioles isolated from the kidneys of rabbits with DM type 1 had impaired EDRF/NO responses and developed an acetylcholine-induced EDCF response both of which were improved by addition of tempol to the bath (Schnackenberg & Wilcox, 2001). Thus tempol restored vasodilation and endothelial function in several models of diabetes (Phillips et al., 2005).

The mechanism of endothelial dysfunction in models of DM has been studied quite extensively. ECs exposed to high glucose concentrations had a reduced S-nitrosylation of endothelial (e) NOS (Wadham et al., 2007) and an uncoupled eNOS which generated ROS both of which were prevented by tempol administration (Brodsky et al., 2004; Chander et al., 2004). The uncoupling of eNOS was ascribed to oxidation of tetrahydrobiopterin to dihydrobiopterin which is an ineffective co-factor for NOS-dependent NO generation. ECs exposed to high glucose also had a reduced expression of guanosine triphosphate cylohydrolase-1 that is required for biopterin synthesis (Brodsky et al., 2004; Chander et al., 2004) that was corrected by tempol (Xu et al., 2007). Thus tempol improved NO production in ECs from models of DM by correcting the uncoupling of eNOS and restoring its synthesis of NO rather than ROS.

Tempol prevented or moderated some of the impared vascular regeneration, remodeling and angiogenesis in models of DM type 2. Oral tempol (1 mmol $\cdot l^{-1}$) given for four weeks to the fructose-fed rat model of type 2 DM prevented the accelerated neointimal proliferation and VSMC apoptosis in injured carotid arteries (Jagadeesha et al., 2005). The obese Zucker rat model of type 2 DM had reduced skeletal muscle vasodilation and vascular rarefaction that were improved by four weeks of oral tempol (Frisbee, 2005). Eight weeks of oral tempol administration to the db/db mouse model of type 2 DM reduced ROS and plasma glucose and reduced the vascular matrix expression and remodeling (San Martin et al., 2007).

Some 40% of patients with type 1 or 2 DM develop nephropathy after a latent period of about 15 years. Tempol blocked the effect of glucose on rat glomerular mesangial cells to generate vascular endothelial growth factor that has been implicated in diabetic nephropathy (Xia et al., 2007). Whereas rat models of DM developed proteinuria, mesangial expansion

and eventually glomerulosclerosis, the black 6 stain of mice have been more resistant, and no rodent strains develop the unique renal manifestations of nodular glomerulosclerosis. Therefore, the relevance of these models of diabetic nephropathy has been questioned (Hsueh et al., 2007). Nevertheless, NOS blockade in rats with insulinopenic DM caused hypertension and a fall in glomerular filtration rate (GFR) that were prevented by tempol. The authors suggested that tempol substituted for impaired NO generation to stabilize the BP and the GFR of diabetic rats (Brands et al., 2004). Cu/Zn SOD knockout mice developed more severe nephropathy and reduction in GFR during STZ-induced DM that were reduced by oral tempol (450 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ for 5 weeks) (DeRubertis et al., 2007). Tempol corrected several metabolic changes in rats with streptozotocin-induced DM including renal cortical $O_2^{\cdot -}$ and nitrotyrosine accumulation (Chen et al., 2006), increased renal $p47^{phox}$ expression in cell membranes, decreased SOD and catalase activities and mesangial matrix accumulation and transforming growth factor beta (TGFβ), fibronectin and periodic acid Schiff staining in the glomerulus (Asaba et al., 2007). Despite this, tempol did not prevent the modest increase in proteinuria in these diabetic rats which the authors attributed to a failure of tempol to reduce myeloperoxidase activity leading to hypochloride accumulation in the diabetic kidneys (Asaba et al., 2007). This was surprising since these authors had reported that apocynin, which also blocks superoxide generation, was effective in preventing proteinuria in this model (Asaba et al., 2005). The obese, hypertensive Zucker rat is a model of the metabolic syndrome and type II DM with enhanced expression of ROS in the kidneys and blood vessels. Nine weeks of tempol administration to these rats improved insulin sensitivity and reduced renal inflammation, proliferation and fibrosis but failed to reduce the proteinuria (Rafikova et al., 2008). Since tempol increased the renal activity of SOD but not catalase, and failed to prevent renal protein oxidation in this model, the authors speculated that ongoing H_2O_2 generation may have limited the protective effect of tempol (Rafikova et al., 2008). Prostaglandins generated in renal vessels and glomeruli in response to protein kinase C (PKC)-dependent signalling have been implicated in both the early hyperfiltration and the late renal functional impairment in rat model of DM type 1 diabetes (Craven et al., 1992; DeRubertis & Craven, 1993; DeRubertis & Craven, 1994). The increased PG release and cyclooxygenase (COX)-2 expression in the kidneys of rats with STZ-induced DM were prevented by one month of tempol (Li et al., 2005) but tempol did not interrupt the renal COX-1 pathway (Li et al., 2005). Thus tempol has been effective in reversing the increased ROS generation, reduced GFR, inflammation and fibrosis in the renal cortex in models of DM and in correcting COX-2 signalling but has not been effective in preventing the proteinuria that is a hallmark of diabetic nephropathy.

Diabetes is often accompanied by renal salt retention, salt-sensitivity of BP, neuropathy and cardiomyopathy. Some evidence suggests that tempol might be effective in countering these complications. Activation of dopamine type 1 receptors in the renal proximal tubule during volume expansion contributes to decreased sodium reabsorption and protects against the salt sensitivity that is common in patients with diabetes or the metabolic syndrome. Rat models of type I (Marwaha & Lokhandwala, 2006) and type 2 DM (Banday et al., 2005; Banday et al., 2007) had defective dopamine type 1 receptor-like -G protein coupling in the kidneys that were restored by oral tempol. Rats fed a high fat diet developed renal oxidative stress and increased renal expression and activity of the bumetanide-sensitive Na-K-2Cl cotransporter which were corrected by oral tempol (1 $mmol \cdot l^{-1}$) (Riazi et al., 2009). Diabetic neuropathy occurs in conjunction with microvascular disease. Epineural arterioles from diabetic rats had impaired acetylcholine-induced relaxations that were improved by tempol, α-lipoic acid or arginine (Coppey et al., 2003). Rats with STZ-induced DM had excessive oxidative stress and apoptotic cells in the heart that were prevented by one week of oral tempol or an ARB, but not by a beta-1 adrenoceptor blocker (Fiordaliso et al., 2007). Tempol restored NO bioactivity in the heart of obese, insulin-resistant mice (Bender et al., 2007).

Collectively, these data suggest that tempol can prevent endothelial dysfunction and mitigate some of the vascular, renal, cardiac, neural and mitochondrial defects in models of DM.

3.2. Leptin, paraoxonase-1 (PON-1), adiponectin and food intake

Leptin reduces food intake. However, when infused into rats, leptin increased lipid peroxidation products and decreased the excretion of NO metabolites (Beltowski et al., 2005a; Beltowski et al., 2005b) and increased the BP. These effects were prevented by tempol (Wojcicka et al., 2008) or apocynin (Beltowski et al., 2005c). Tempol also prevented the effects of leptin infusion to increase the ouabain sensitive (Beltowski et al., 2005a) and insensitive (Beltowski et al., 2007) Na^+/K^+ ATPase activities in the kidneys. However tempol did not prevent a reduced food intake with leptin (Beltowski et al., 2005b). Thus, tempol prevented the increased ROS, reduced NO and the increased renal energy use with leptin while preserving its effects on food intake. Moreover, prolonged administration of tempol to normal mice reduced their plasma levels of leptin and increased the tissue levels of mitochondrial uncoupling protein-2 which reduces mitochondrial ROS generation (Mitchell et al., 2003). Thus, tempol reduced leptin release and altered the response to leptin in the rat.

PON-1 is a circulating protein that has been associated with reduced rates of coronary events and atherosclerosis (Mackness et al., 2003). Tempol blocked the effect of leptin infusion to reduce PON-1 activity in the kidneys and aorta (Beltowski et al., 2005b). Thus, increased leptin generation in the metabolic syndrome may contribute to oxidative stress and a reduction in PON-1, high-density lipoproteins and NO generation that can be corrected by tempol.

Adiponectin is an adipokine that counters inflammation, atherosclerosis and insulin resistance (Ouchi et al., 2000; Ouchi et al., 2001). Circulating levels of adiponectin were reduced in patients or animal models of obesity, diabetes or acute coronary syndromes (Kumada et al., 2003). However, subsequent studies reported that plasma levels of adiponectin were increased in patients with stable congestive heart failure (CHF) (George et al., 2006) in proportion to the severity of the CHF (Nakamura et al., 2006). In patients with CHF, serum adiponectin was positively correlated with high density lipoprotein cholesterol and negatively with plasma triglycerides (von Eynatten et al., 2006). The authors concluded that adiponectin may mediate part of its proposed anti-atherosclerotic properties by influencing high density lipoprotein cholesterol concentrations and might be a therapeutic target for atherogenic dyslipidemia. Oral tempol (2 mmol $\cdot l^{-1}$) or tetrahydrobiopterin given to rats prevented the effects of Ang II infusion to reduce the plasma levels and adipose tissue expression of adiponectin (Hattori et al., 2005). However, until a clearer picture emerges about the benefits or adverse effects of adiponectin, it is hard to predict whether a suppression of adiponectin by tempol would be of benefit or harm.

The oral administration of tempol (1 mmol $\cdot l^{-1}$ of drinking water for ten weeks) to obese/Zucker rats fed a high fat diet reduced the abdominal fat and the weight gain, without changing the food intake (Ebenezer et al., 2009). This suggests that tempol may modify fat metabolism to limit the abdominal obesity that occurs with excessive caloric intake and which contributes to insulin resistance. Mice fed tempol from birth had a reduced weight gain, but appeared fully healthy (Mitchell et al., 2003; Schubert et al., 2004).

These intriguing results suggest that tempol may protect against obesity and the development of the metabolic syndrome.

3.3. Dyslipidemia

The db/db mouse which harbors a mutation in the gene expressing $p47^{phox}$ (Huang et al., 2000) and in the leptin receptor is a model of the metabolic syndrome. When fed a regular diet over 12 weeks, they develop obesity, hyperglycemia despite hyperinsulinemia, and a 50% increase in total cholesterol and a four-fold increase in triglycerides (San Martin et al., 2007). Their aortas have increased NADPH oxidase activity and increased expression of bone morphogenic protein-4 and osteopontin which are cytokines that have been implicated in matrix remodeling. Eight weeks of oral tempol (2 mmol $\cdot l^{-1}$) corrected the increased aortic superoxide levels and the hypercholesterolemia, and reduced the elevated plasma triglycerides and aortic expression of the cytokines (San Martin et al., 2007). The obese Zucker rat fed a high fat diet developed pronounced dyslipidemia with a four-fold increase in plasma triglycerides, a doubling of plasma very low-density lipoprotein cholesterol and total cholesterol and a 40% reduction in the protective high-density lipoprotein cholesterol. All of these changes were prevented by ten weeks of oral tempol (1 mmol $\cdot l^{-1}$) (Ebenezer et al., 2009). Tempol also prevented the excessive weight gain during high fat feeding, reduced the blood glucose and normalized the blood insulin and creatinine levels (Figure 4). Thus, tempol prevented many of the adverse effects of a high fat intake in rodents with a genetically susceptible state that adversely effected lipid and carbohydrate metabolism and renal function. The striking increase in high-density lipoprotein cholesterol produced by tempol in this model is quite unusual and would predict strong protection against cardiovascular disease. However, oral tempol (1 mmol $\cdot l^{-1}$), given to the SHR with leptin deficiency and fed a high salt diet which is another rodent model of the metabolic syndrome, did not reduce serum triglycerides, but did reduce serum fatty acids (Matsui et al., 2008). Hypercholesterolemia induced by cholesterol feeding of rabbits (Pfister, 2006) or genetic deletion of apolipoprotein E in mice (Goodwill et al., 2008) caused endothelial dysfunction and enhanced vascular contractility attributed to signaling via the thromboxane prostanoid receptor. Incubation of aortas from mice with genetic deletion of apolipoprotein E with tempol improved endothelial function and corrected the excessive vascular generation of thromboxane A_2 (Goodwill et al., 2008).

4. Antifibrotic effects

Parenchymal fibrosis in the heart or kidneys is considered an irreversible and adverse outcome that contributes to cardiomyopathy and chronic kidney disease (CKD). Several cytokines, notably TGFβ and TNFα, are activated by ROS and could mediate fibrotic responses or epidermal to mesenchymal cellular transformation.

Rats infused with Ang II had activated NADPH oxidase, increased expression of TGFβ, type 1 collagen, tissue inhibitor of matrix metalloprotease (TIMP)-1 and -2, and myofibroblasts and increased collagen volume in the kidneys or heart all of which were abrogated by oral tempol or apocynin (both at 120 mg $\cdot kg^{-1} \cdot day^{-1}$) (Zhao et al., 2008a). However, tempol was not effective in preventing the cardiac fibrosis and collagen accumulation in a mouse model of pressure overload following aortic constriction (Moens et al., 2008). Thus, tempol may prevent fibrosis in conditions such as prolonged Ang II infusion that induces ROS generation.

The effects of tempol on ROS generation in fibroblasts have been well studied. Generation of $O_2^{\cdot -}$ by xanthine + xanthine oxidase in the culture medium of lung fibroblasts increased intracellular $O_2^{\cdot -}$, TGFβ and collagen. These effects were related to $O_2^{\cdot -}$ rather than to H_2O_2 and were prevented by tempol (Qi et al., 2009). Stimulation of cardiac fibroblasts with Ang II, or blockade of SOD with diethyldithiocarbonate, increased intracellular ROS and stimulated collagen 1 and fibronectin synthesis. These were prevented by coincubation with tempol, PEG-SOD or EUK-8 which is another SOD mimetic drug (Lijnen et al., 2008).

Similarly, oral tempol reduced the expression of TNFα and NFκB in the soleus muscle of ren-2 rats that are transgenic for the renin gene (Wei et al., 2008). Treatment of cultured fibroblasts with TNFα increased intracellular Ca^{++} concentration and ROS which were prevented by tempol (Mariappan et al., 2007). Moreover, feeding obese Zucker rats a high fat diet doubled renal cortical expression of TNFα. These effects were prevented by additional tempol (1 mmol $\cdot$ l^{-1}) to the drinking water for 10 weeks (Ebenezer et al., 2009). Thus tempol may interrupt a feed-forward cycle whereby ROS activate cytokineses such as TNFα that further enhances ROS generation in fibroblasts that promote matrix accumulation and thereby lead to further fibrosis.

5. Major organ effects of tempol

5.1. Kidneys

Ischemia and reperfusion, or anoxia and reoxygenation, are potent stimuli to ROS generation from xanthine oxidase and contribute to organ injury (Berry & Hare, 2004; Hare, 2004). Ischemia-reperfusion of the kidneys depleted renal Cu/Zn SOD and glutathione-S-transferase, thereby reducing intracellular antioxidant defenses (Davies et al., 1995). Pretreatment with tempol (55 to 550 μmol $\cdot$ kg^{-1} iv) diminished the renal injury and the renal dysfunction of rats following 45 minutes of renal ischemia and reperfusion (Chatterjee et al., 2000; Fujii et al., 2005) and reduced the excessive spillover of norepinephrine and endothelin-1 into the renal vein (Fujii et al., 2005). Tempone also protected the rat kidney against ischemia-reperfusion damage and reduced renal markers of oxidative and nitrosative stress but did not reduce the BP in this model (Patel et al., 2002). Rats with the two kidney, one clip (2K,1C) model of Goldblatt hypertension have an ischemic, clipped kidney with a downregulated expression of renal Cu/Zn SOD (Son et al., 2008). Tempol reduced the renal histologic damage, TNFα expression (Son et al., 2008) and hypoxia, and improved the renal blood flow (Palm et al., 2008). Thus, tempol may correct problems arising from a reduction in renal SOD expression that accompanies acute or prolonged renal ischemia.

Renal dysfunction and proteinuria can be initiated by damage to glomerular podocytes (Pavenstadt et al., 2003). Ten weeks of oral tempol (1 mmol $\cdot$ l^{-1}) given to fat-fed obese Zucker rats (Ebenezer et al., 2009) prevented glomerular podocyte damage and foot process effacement, desmin expression, glomerular hypertrophy, increased blood urea nitrogen and serum creatinine concentrations and moderated the increased albumin excretion. These effects were confirmed in one other study in a similar model (Nagase & Fujita, 2008) but in another tempol moderated the renal inflammatory, fibrotic and sclerotic changes and the insulin resistance without affecting the proteinuria (Rafikova et al., 2008). During states of NO deficiency, tempol may correct a local imbalance within the podocyte environment between NO and ROS since it prevented the increase in albumin permeably of isolated glomeruli incubated with the endogenous NOS inhibitor, asymmetric dimethylarginine (Sharma et al., 2009). Additionally, tempol protected kidneys from damage in a wide range of hypertensive models associated with increased ROS (Wilcox & Pearlman, 2008).

Loss of more than half of the functional renal mass in adults evoked compensatory changes of hypertrophy and glomerular hyperfiltration which initially mitigated the loss of renal function but, over time, led to glomerular hypertension, proteinuria, glomerular sclerosis and a progressive decline in renal function (Hostetter et al., 1981; Kotchen et al., 2000). TGF-β has been implicated in the renal fibrosis developing in this and other models of CKD (Kelly et al., 1999; Dahly et al., 2002). A rat model of reduced renal mass (RRM) produced by unilateral nephrectomy and infarction of two thirds of the remaining kidney developed a rise in BP by day 3 that was prevented by tempol (1.5 mmol $\cdot$ kg^{-1} $\cdot$ d^{-1} sc). Tempol, unlike vitamin E, reduced renal insufficiency and proteinuria (Tain et al., 2007) and preserved EDRF/NO responses (Hasdan et al., 2002). This is relevant since cardiovascular disease,

associated with endothelial dysfunction, is the primary cause of death in patients with CKD (Modlinger et al., 2004). Eighteen weeks after 5/6 nephrectomy, homozygous acatalasemic mutant mice developed accelerated tubulointerstitial fibrosis and lipid peroxidation that were not prevented by oral administration of tempol, implying that tempol did not prevent excessive H_2O_2 generation in this model (Kobayashi et al., 2005). Mice subjected to surgical 5/6 nephrectomy developed oxidative stress, hypertension, azotemia, glomerular sclerosis and tubulointerstitial damage all of which were prevented by oral tempol (3 mmol $\cdot l^{-1}$) (Hobo et al., 2009). This effect was ascribed to a reduced expression of midkine which is a circulating cytokine produced in damaged kidneys that activated the angiotensin converting enzyme and thereby increased angiotensin-induced generation of ROS (Hobo et al., 2009). Rats with 5/6 nephrectomy assessed non-invasively with telemetric recording had only a modest increase in BP unless they were fed a high salt intake which led to substantial hypertension and worsening proteinuria, azotemia, systemic oxidative stress, renal glomerulosclerosis and tubulointerstitial damage. These deleterious effects of salt were all reduced by 12 weeks of oral tempol (1 mmol $\cdot l^{-1}$) (Li et al., 2008). In contrast, a similar schedule of tempol administration to rats with RRM but without a high salt diet was ineffective (Quiroz et al., 2009). Thus, tempol has been quite effective in delaying the onset of CKD and vascular endothelial dysfunction and in preventing salt-sensitivity and salt-induced renal damage in rodent models of CKD.

Tempol protected the kidneys and/or maintained the renal hemodynamics (Wang et al., 2008b) of rats with shock due to endotoxin, hemorrhage or gram positive bacteremia (Leach et al., 1998; Mota-Filipe et al., 1999; Zacharowski et al., 2000; Kentner et al., 2002; Thiemermann, 2003).

Rat mesangial cells incubated with homocysteine developed oxidative stress, cellular proliferation and expression of TIMP-1. All of these effects were blocked by tempol (Yang & Zou, 2003). Increased mechanical strain imposed on isolated mesangial cells stimulated the phosphorylation of extracellular signal-regulated kinases (ERK) and the $p47^{phox}$ component of NADPH oxidase. Remarkably, these effects were blocked in the absence of added Ang II by olmesartan which is an inverse agonist ARB, and also by tempol (Yatabe et al., 2009). The authors concluded that mesangial strain stimulated angiotensin type 1 receptors (AT1-Rs) to activate ERK and NADPH oxidase. Thus, tempol prevented glomerular mesangial cells from developing oxidative stress in response to a variety of biochemical and mechanical stressors.

Tempol protected renal tubular epithelial cells against the cytotoxic effects of the redox-cycling quinolone paraquat (Samai et al., 2007) or H_2O_2 (Chatterjee et al., 2000; Asghar & Lokhandwala, 2004). However, tempol given to rats before and twice daily after vancomycin, did not prevent tubular necrosis whereas chelation of iron with 2,3-dihydroxybenzoic acid was very effective (Naghibi et al., 2007). Nevertheless, daily intraperitoneal doses of tempol reduced rat renal tubular damage following gentamicin-induced acute tubular necrosis (Karatas et al., 2004). Thus, tempol protected against some, but not all, nephrotoxins.

Tempol has been tested in a number of MR-dependent models. Beswick et al reported that the administration of tempol for 4 weeks to rats with deoxycorticosterone acetate-salt hypertension decreased renal inflammatory cell infiltration and NFK-B expression (Beswick et al., 2001). Nishiyama et al reported that a six week administration of oral tempol (3 mmol $\cdot l^{-1}$) to rats with hypertension from aldosterone infusion and a high salt diet reduced the proteinuria, glomerular cell proliferation and mesangial matrix accumulation (Nishiyama et al., 2004a) and reduced the glomerulosclerosis (Nishiyama & Abe, 2004). These protective effects were equivalent to those produced by blockade of the MR with eplerenone and were

associated with decreased renal cortical NADPH oxidase expression, ROS generation, and mitogen-activated protein kinase activity. Oral tempol (6 mmol $\cdot l^{-1}$) given to rats infused with aldosterone and a high salt diet, protected the glomerular podocytes from damage and upregulation of Sgk-1. Tempol also prevented aldosterone-induced Sgk-1 expression in cultured podocytes *ex vivo* (Shibata et al., 2007). Thus the protective effects of tempol on the kidneys and glomeruli of salt-dependent models of renal damage may entail interruption of the signal cascade that follows MR activation.

Tempol was effective in preventing renal damage in many salt-dependent models of hypertension. Manning et al reported that tempol infused iv over 3 weeks into DSS rats fed a high salt diet decreased protein excretion and glomerulosclerosis and maintained the ratio of kidney: body weight despite no changes in renal hemodynamics (Meng et al., 2003). Hisaki et al confirmed that tempol given orally over 5 weeks to this model prevented the adverse changes in the kidney of arteriosclerosis, matrix accumulation and expansion of the interstitium (Hisaki et al., 2005). Oral tempol (3 mmol $\cdot l^{-1}$) given to DSS rats fed salt reduced the proteinuria, glomerular sclerotic and proliferative changes, collagen deposition (Nishiyama et al., 2004b), the fall in GFR and the kidney damage whereas an equally antihypertensive treatment with hydralazine was less protective (Hoagland et al., 2003). An important new finding was that pharmacologic blockade of 20-hydroxyeicosatetraenoic acid blunted the antihypertensive and nephroprotective effects of tempol (Hoagland et al., 2003). The authors concluded that an increase in 20-hydroxyeicosatetraenoic acid may underlie these protective effects of tempol. These results suggest that tempol can exert renoprotective effects in salt-sensitive hypertension relatively independent of changes in BP.

Although the oral administration of tempol to rats with STZ-induced insulinopenic DM reduced parameters of ROS and BP, it failed to modify the modest structural changes in the kidneys or the albuminuria (Asaba et al., 2007) whereas apocynin was effective (Onozato et al., 2008). On the other hand, tempol increased the renal expression of EC-SOD, decreased NADPH oxidase activity, limited renal structural damage and prevented renal collagen IV expression and proteinuria in an apparently similar rat model of DM, but using younger rats (Peixoto et al., 2009). Thus, the efficacy of tempol for renal protection in models of DM type 1 is incomplete.

The oral administration of tempol reduced oxidative stress and albuminuria in rats with acute glomerular immune injury due to the administration of an anti-glomerular basement membrane antibody (Duann et al., 2006). This was considered a direct effect of tempol on the glomerulus since it moderated the enhanced albumin permeability of isolated glomeruli with oxidative stress.

Tempol was equally effective as an ARB in reducing the BP and lipid peroxidation products of rats with 2K,1C hypertension, but, unlike the ARB, it maintained the GFR in the clipped kidney. Tempol was effective in preventing vascular remodeling in the aorta in this model (Figure 5).

Thus, tempol has been generally renoprotective in models of ischemia-reperfusion injury and shock and in several chronic models of the metabolic syndrome or mineralocorticosteroid or salt-induced renal damage but has not been consistently effective in models of type 1 DM.

5.2. Heart

Tempol has been effective in preventing cardiac ROS generation and several of the adverse consequences. ROS have complicated effects on cardiac cells. Generally, ROS promote hypertrophic and contractile responses by increasing intracellular Ca^{++} and signaling via

inositol-3-phosphate and promote interstitial fibrosis (Suzuki & Ford, 1999). Indeed, tempol blocked ROS and hypertrophic responses to atrial natriuretic peptide in rat neonatal cardiac myocytes (Laskowski et al., 2006). Uninephrectomized apolipoprotein e (-/-) mice had enhanced ROS and developed an aortic atherosclerotic plaque burden with a reduced capillary length density and higher interstitial volume in the heart associated with increased aortic expression of nitrotyrosine, TGFβ, vascular endothelial growth factor and collagen. These effects were reduced or prevented in mice given tempol or an angiotensin converting enzyme inhibitor for 12 weeks (Piecha et al., 2008). Lipopolysaccharide or TNFα released during septicemia enhanced ROS in cardiomyocytes, reduced cardiac contractility, impared the efficiency with which the cells used oxygen, reduced cardiomyocyte ATP levels and caused cardiac mitochondrial dysfunction by opening the mitochondrial membrane pore (Mariappan et al., 2007; Mariappan et al., 2009). These effects were prevented by oral tempol (300 $\mu mol \cdot kg^{-1}$) but not apocynin (Mariappan et al., 2007; Mariappan et al., 2009), suggesting that tempol interrupted primarily mitochondrial ROS generation in this model.

Tempol blocked the cardiac fibrosis, myofibroblast proliferation and cardiac collagen accumulation produced by an infusion of Ang II (Zhao et al., 2008b). The administration of tempol and vitamin C to rats after NOS blockade moderated the cardiac hypertrophy and the adrenomedullin signalling without reducing the hypertension (Bell et al., 2007). These studies established the efficacy of tempol in preventing cardiac ROS, fibrosis, and apoptosis in several rodent models associated with lipopolysaccharide, cytokines, reduced NO or prolonged Ang II.

In other models, ROS promoted apoptosis and decreased cardiac contractility. Thus, tempol reduced ROS production and apoptosis in cardiac cells exposed to high glucose concentrations (Fiordaliso et al., 2007), blocked apoptotic responses of cardiomyocytes to aldosterone signaling via a non-genomic pathway (Hayashi et al., 2008) and inhibited the Ca^{++} transient within cardiac myosites stimulated by pressure-flow stress (Belmonte & Morad, 2008).

These different effects of tempol may relate to preservation of NO which has complicated effects on cardiomyocytes because of spatial confinement and differential effects of NO generation by specific NOS isoforms (Barouch et al., 2002). For example, Gonzales et al (Gonzalez et al., 2008) studied the acute effect of tempol on the ionotropic state of the rat isolated heart. The addition of 100 μM tempol did not affect contractility but tempol did prevent the increased contractility caused by a modest concentration of a NO donor (Paolocci et al., 2000; Espey et al., 2002; Schrammel et al., 2003; Koshiishi et al., 2007) yet did not prevent the increased contractility with higher concentrations of NO (Gonzalez et al., 2008).

Tempol has been effective in protecting the heart against injury and dysfunction following ischemia and reperfusion or myocardial infarction. Tempol prevented post-ischemic activation of NADPH oxidase and xanthine oxidase in the guinea pig (Duda et al., 2007) or rat heart or coronary vessels (Zhang et al., 2006). Tempol given to rats or rabbits after coronary ligation and reperfusion reduced the infarct size of the myocardium by up to 60% when given during the reperfusion period (McDonald et al., 1999; Zacharowski et al., 2007), protected the heart against oxidative damage due to ischemia-reperfusion injury (Zeltcer et al., 1997; McDonald et al., 1999; Zeltcer et al., 2002; Li et al., 2002; Hoffman et al., 2003; Kutala et al., 2006; McCormick et al., 2006), and prevented reperfusion arrhythmias in one study (Guo et al., 2005) but did not change, or increased, the frequency of ventricular arrhythmias in another (Neckar et al., 2008). Long term administration of oral tempol (2 $mmol \cdot l^{-1}$) for six weeks after coronary artery ligation prevented the signs of cardiac dysfunction and failure manifest as an increase in left ventricular end diastolic pressure and

volume, reduced ejection fracton and enhanced renal sympathetic nerve activity and plasma norepinephrine (Shi et al., 2009).

A note of caution derives from the report of Kimura et al in the rat (Kimura et al., 2005b) that a 30 minute pressor infusion of Ang II prior to acute coronary occlusion diminished the infarct size. This pharmacologic preconditioning depended on the generation of ROS and was prevented by tempol.

Diastolic heart failure which accounts for almost one half of the cases of chronic congestive cardiac failure is a complication of hypertensive or ischemic cardiac disease and remodeling that leads to impaired diastolic relaxation. Presently, the therapy for diastolic heart failure is unsatisfactory. Tempol has been effective in preventing or reversing cardiac hypertrophy and diastolic dysfunction in several rat models of salt sensitive hypertension. Tempol (3 mmol $\cdot l^{-1}$) added to the drinking water of DSS rats fed a high salt diet for 10 weeks reduced the BP moderately, but normalized the left ventricular hypertrophy and cardiac relaxation and reduced the cardiac expression of brain natriuretic peptide, TGFβ, connective tissue growth factor, collagen types I and III, the $p22^{phox}$ and neutrophil oxidase (Nox)-2 components of NADPH oxidase and mitochondrial uncoupling protein 2 (Guo et al., 2006). Thus, inhibition of cardiac ROS by tempol prevented the cardiac fibrosis, remodeling and defective relaxation that underlie diastolic heart failure. Indeed, four weeks of oral tempol (1 mmol $\cdot l^{-1}$) or eplerenone to SHR given a high salt diet, which is a model of the metabolic syndrome, moderated the diastolic dysfunction when assessed directly by echocardiography and cardiac catheterization, and prevented the cardiac perivascular fibrosis and upregulation of the mineralocorticosteroid signaling pathways (Matsui et al., 2008) (Figure 6). Likewise, oral tempol or eplerenone given for six weeks to rats infused with Ang II and fed a high salt diet prevented the diastolic dysfunction and cardiac oxidative stress in this model also (Wang et al., 2008a). Oral tempol given to DSS rats fed a high salt diet prevented the development of left ventricular dilation and cardiac failure (Hasegawa et al., 2006) and, when given to rats infused with aldosterone and fed a high salt diet, reduced the collagen accumulation in the kidney and the fibrosis in the heart and aorta substantially (Iglarz et al., 2004). Thus tempol can prevent the development of left ventricular hypertrophy, cardiac inflammation and fibrosis and diastolic dysfunction and cardiac failure in salt sensitive rodent models by actions that may entail interruption of mineralocorticosteroid receptor signaling pathways.

High sugar diets increase mortality and left ventricular dysfunction in models of pressure overload. Tempol given to fructose-fed mice with pressure overload prevented the cardiac ROS accumulation, hypertrophy and impaired left ventricular ejection fraction whereas these effects were not seen in mice fed a low sugar chow (Chess et al., 2008). Another study of fructose-fed mice with pressure overload reported that ROS were generated in the heart from uncoupled NOS (Moens et al., 2008). Tetrahydrobiopterin recoupled NOS and diminished the late development of cardiac fibrosis and hypertrophy whereas oral tempol did not recouple NOS was not effective in preventing the adverse cardiac changes. Thus, in this model, the main problem appears to be ROS generated from an uncoupled NOS and preventing this was more effective than a general antioxidant strategy with tempol.

The administration of tempol to several rat models of hypertension not associated with salt loading has not shown benefit in preventing cardiac hypertrophy or dysfunction. The administration of tempol (3 mmol $\cdot l^{-1}$) for 2 weeks to rats infused with aldosterone but maintained on a normal salt diet prevented hypertension but failed to prevent the myocardial changes, in contrast to treatment with another antioxidant NAC (Yoshida et al., 2005). Tempol infused into rats with the beta-adrenergic agonist, isoproterenol prevented the increased cardiac collagen accumulation but not the cardiac hypertrophy (Zhang et al.,

2005a). Likewise, oral tempol given to rats infused with thyroxine for 6 weeks did not prevent the cardiac hypertrophy, despite a substantial reduction in the MAP and indices of oxidative stress (Moreno et al., 2005). It is not clear presently why the beneficial cardiac effects of tempol in models of hypertension appear specific to those associated with high salt intake but this may relate to interruption of MR signaling pathways that are activated in these salt-sensitive models (Nagase & Fujita, 2008).

As reviewed under “3.1. Metabolic effects of tempol – Insulin resistance, metabolic syndrome and diabetes mellitus”, tempol protected the heart from some of the adverse effects of diabetes type 1 or 2. For example, an infusion of tempol (550 μmol · kg^{-1}) into hyperglycemic dogs normalized their coronary endothelial dysfunction and coronary wall oscillatory shear stress (Gross et al., 2003). Tempol given to SHR made diabetic with STZ reduced the cardiac indices of ROS and the cardiac apoptosis (Fiordaliso et al., 2007).

Repeated exposure of rat isolated aortic segments to nitrates led to tolerance as shown by diminishing relaxation that was corrected by tempol. This was ascribed to a reduction in excessive H_2O_2 accumulation, since the beneficial effects of tempol were prevented by catalase (Ghatta et al., 2007). Pretreatment of rat aortic rings with tempol, vitamin C, uric acid, or the PKC inhibitor, chelerythrine all prevented the development of tolerance to the vasodilating actions of nitroglycerin (bou-Mohamed et al., 2004). Thus, the prevention of nitrate tolerance by tempol likely involves a prevention of H_2O_2-induced activation of PKC signalling.

Many investigators have studied the effects of tempol on central sympathetic stimulation, since excitation of the SNS during heart failure predisposes to bad outcomes. A rabbit model of chronic heart failure from paced ventricular tachycardia had enhanced renal sympathetic nerve activity which was reduced by intracerebroventricular (icv) administration of an ARB, an NADPH oxidase inhibitor, apocynin or tempol (Gao et al., 2004). The addition of tempol or apocynin to Ang II-stimulated cultured neuronal cells reversed the increased expression of the AT1-R, and NADPH oxidase activity (Liu et al., 2008). The rostral ventrolateral medulla (RVLM) and paraventricular nucleus (PVN) are brain stem centres that regulate the sympathetic neural discharge. The 2K,1C Goldblatt model of Ang II excess had increased expression of the AT1-R and NADPH oxidase subunits in these nuclei (Oliveira-Sales et al., 2009). Injection of tempol (1 to 5 mmol · l^{-1}) into the RVLM of these rats reduced their BP and renal sympathetic nerve discharge (Oliveira-Sales et al., 2009). Moreover, prolonged Ang II infusion into rats increased the expression of NFκB and AT1-R in the PVN, and increased the BP, plasma cytokines, renal sympathetic nerve activity and plasma norepinephrine (Kang et al., 2009). The infusion of tempol (80 μg · h^{-1}) into the PVN reduced these parameters which was blocked by the NFκB antagonist pyrrolidine dithiocarbamate. The authors concluded that tempol prevented Ang II-induced superoxide formation in these brain stem centers that activated NFκB-induced sympathoexcitation (Kang et al., 2009). Indeed, intravenous tempol (120 μmol · kg^{-1}) given to normal or hypertensive rats reduced their BP, heart rate and renal sympathetic nerve activity concomitant with a significant reduction in the spontaneous discharge of neurons in the PVN and RVLM (Wei et al., 2009a). These effects of tempol were independ of NO since they occurred after nitric oxide synthase blockade and were ascribed to prevention of the formation of ·OH by tempol since they were prevented by systemic administration of ·OH scavenger, dimethyl sulfoxide (Wei et al., 2009a). Rats with heart failure due to coronary artery ligation had increased expression in the hypothalamic PVN of the AT1-R, pro-inflammatory cytokines, NADPH oxidase components and NFκB, and increased plasma levels of norepinephrine. Icv infusion of the ARB, losartan or of tempol (80 μg · h^{-1}) corrected these parameters and decreased plasma levels of Ang II and left ventricular end diastolic pressure (Kang et al., 2008). The authors concluded that $O_2^{\cdot -}$ stimulates NFκB in

the PVN to sustain the neurohumoral activation that worsened heart failure. Similarly, an icv infusion of tempol for seven days into rabbits with congestive cardiac failure due to chronic ventricular tachycardia decreased the expression of AT1-Rs in the RVLM (Liu et al., 2008). Six weeks of oral tempol (2 mmol $\cdot$ l^{-1}) given to rats with a prior myocardial infarction caused by coronary artery ligation normalized renal sympathetic nerve discharge, plasma norepinephrine, left ventricular end diastolic pressure and contraction (Shi et al., 2009). Administration of tempol and apocynin to rabbits with chronic heart failure corrected the increased expression in the RVLM of the AT1-R (Shi et al., 2009) and the cytosolic phosphorylation of c-JUN N-terminal kinases protein and the activator protein-1 DNA binding activity (Liu et al., 2008).

Tempol also diminishes reflex activation of the SNS. The cardiac sympathetic afferent reflex which was activated by epicardial bradykinin or Ang II was enhanced in rats with chronic heart failure and led to enhanced renal sympathetic nerve activity (Ding et al., 2009). This was prevented by microinjection of tempol into the PVN (Han et al., 2007). Rabbits with chronic heart failure had increased peripheral chemoreceptor sensitivity that was corrected by local application of tempol to the carotid body chemoreceptor nerve (Li et al., 2007). These studies have established that ROS generated in brain stem cardiovascular nuclei can lead to sustained angiotensin-dependent activation of the SNS. Tempol, whether given systemically or into these nuclei, can prevent the activation of the SNS which might contribute to beneficial effects in models of heart failure where ongoing SNS drive in the heart limits cardiac function and in the kidney where the sympathic drive may contribute to azotemia and renal NaCl and fluid retention. The inhibition of the SNS by tempol during heart failure can involve reduced afferent input and reduced reflex activation as well as reduced brain-stem SNS drive.

Tempol has also been effective in reducing some manifestation of right heart failure. Tempol (500 μmol $\cdot$ kg^{-1} $\cdot$ $24h^{-1}$) normalized the right ventricular systolic pressure and reduced the right ventricular hypertrophy in a rat model of hypoxic right heart failure and pulmonary hypertension (Elmedal et al., 2004) and prevented hypoxic vasoconstriction of rat pulmonary arteries (Knock et al., 2009). Administration of tempol to neonatal rats subjected to hypoxia from birth limited pulmonary oxidative stress and attenuateal right ventricular remodeling. However, tempol slowed cellular differentiation in the distal air spaces of these hypoxic neonatal rats (Jankov et al., 2008). Tempol administration to transgenic Ren2 rats limited the increased pulmonary artery pressure and the hypertrophy of the pulmonary artery and right ventricle (DeMarco et al., 2008). Adrenomedullin is a vasodilator that activates antioxidant pathways whose expression in pulmonary vessels is upregulated by hypoxia (Matsui et al., 2004). Adrenomedullin +/- mice subjected to 3 weeks of hypoxia had enhanced pulmonary artery remodeling and ROS, both of which were normalized by the administration of tempol (Matsui et al., 2004). Tempol prevented the hypoxic pulmonary vasoconstriction, independent of NO, in isolated rat lungs (Hodyc et al., 2007). Thus, tempol may improve manifestations of right heart failure due to prolonged hypoxia, but experience in neonatal animals suggest that it might have adverse effects in reducing pulmonary cellular differentiation.

Tempol, hydralazine and PEG-SOD added to aortic valve interstitial cell cultures stimulated by TGF-β all suppressed calcifying nodule formation (Kennedy et al., 2009). The authors suggested that anti-oxidants may protect against the development of the valvular calcification that occurs in the elderly.

5.3. Blood vessels

Angiogenesis can preserve tissue perfusion following vascular damage, ischemia and hypoxia. ROS have complex effects on angiogenesis. Since ROS are required for some

angiogenic responses, tempol might be anticipated to inhibit angiogenesis. Indeed both tempol and SOD inhibited angiogenesis in chick chorioallantoic membranes. This was attributed to an effect of tempol to reduce H_2O_2-dependent inducible NOS expression in this model (Polytarchou & Papadimitriou, 2004). Treatment of human umbilical vein endothelial cells with tempol, SOD, 4-(2-aminoethyl)-benzenesulfonyl fluoride to block NADPH oxidase, catalase or L-nitro arginine methylester all inhibited cell migration, proliferation and endothelial NOS activity (Polytarchou & Papadimitriou, 2005). These studies have established an anti-angiogenic action of tempol, likely related to a reduction in H_2O_2-induced NO generation in tissues or cell models.

On the other hand, ROS generated in response to hyperglycemia (Zhang et al., 2008b) accelerated the senescence of stem and progenitor cells and inhibited angiogenesis (Dernbach et al., 2004). Tempol largely prevented the inhibition of proliferation of progenitor cells by high glucose concentrations (Zhang et al., 2008b). Other studies have documented a pro-angiogenic action of tempol that may relate to a decrease in $O_2^{\cdot -}$ and thereby to increased NO bioactivity. Ischemia of the hind-limb of mice increased oxidative stress, collateral vessel formation, capillary density and tissue blood flow and decreased NO bioactivity, apoptosis and the expression of extracellular SOD (Kim et al., 2007). Since these effects of ischemia were diminished in EC-SOD (-/-) mice, but were rescued by infusion of tempol, they were related to a reduction in vascular $O_2^{\cdot -}$ (Kim et al., 2007). An impaired collateral blood vessel growth after ileal artery occlusion in the SHR model of oxidative stress was restored by oral tempol (1 and 5 mmol $\cdot l^{-1}$) or apocynin (NADPH oxidase inhibitor) but not by an angiotensin converting enzyme inhibitor or an ARB (Miller et al., 2007). Tempol promoted the healing response of capillary ECs after a scrape wound (Braunhut et al., 1996). Two weeks of treatment of salt-loaded stroke-prone spontaneously hypertensive rats (SHRsp) with an ARB (candesartan 1 mg $\cdot kg^{-1} \cdot day^{-1}$) or tempol (5 mg $\cdot kg^{-1} \cdot day^{-1}$) but not a diuretic (trichlormethiazide 1.6 mg $\cdot kg^{-1} \cdot day^{-1}$) reduced markers of ROS and markedly increased circulating endothelial progenitor cells (Yu et al., 2008).

These conflicting effects with tempol on angiogenesis are not presently resolved. It may be that an inhibitory effect of tempol on angiogenesis is mediated by reduced tissue levels of H_2O_2 in some circumstances but a stimulant effect of tempol on angiogenesis is mediated by reduced tissue levels of $O_2^{\cdot -}$ and enhanced NO in others.

Vascular injury, diabetes, hyperperfusion or hypertension cause vascular remodeling that enhances contractility and resistance to flow (Folkow, 1978; Schiffrin, 2004) and contributes to accelerated neointimal growth and atherosclerosis (Tong et al., 2008). Vascular remodeling predicted poor cardiovascular outcomes in hypertensive patients and therefore is a target for therapeutic intervention (Schiffrin, 2001). Tempol diminished the enhanced VSMC medial migration and vascular remodeling in the injured arteries (Jagadeesha et al., 2009). Ligation of branches of the mesenteric artery caused high flow-induced vascular remodeling in surviving arteries that was diminished in obese Zucker diabetic rats but restored by three weeks of oral tempol (Belin de Chantemele et al., 2009). However, the administration of hydralazine or tempol for two weeks prevented the flow-induced remodeling of the mesenteric arteries of old rats (Dumont et al., 2008). The administration of tempol or apocynin to the adrenomedullin +/-mouse blocked the exaggerated generation of ROS and the intimal hyperplasia after femoral artery damage (Kawai et al., 2004). Oral tempol (1 mmol $\cdot l^{-1}$) for six weeks given to salt-fed, SHRsp reduced the media:lumen ratio of small mesenteric resistance vessels (Park et al., 2002a). Oral administration of tempol (18 mg/kg/day) for 8 weeks to rats with 2K,1C Goldblatt hypertension corrected the increased media:lumen ratio of the aorta (vascular remodeling) whereas apocynin was ineffective despite a similar reduction in hypertension (Castro et al., 2009). Tempol corrected the effects of high medium glucose to increase migration of cultured VSMCs by preventing the

oxidation and inactivation of the sarcoplasmic/endoplasmic reticulum calcium adenosine triphosphatase that mediates the inhibitory effects of NO on cell migration (Tong et al., 2008). Thus, tempol has proved effective in preventing or reversing the vascular remodeling accompanying vascular repair or prolonged hyperglycemia or hypertension in several models.

Some of the apparently beneficial effects of tempol on blood vessel remodelling have been ascribed its reduction of vascular matrix metalloproteases (MMPs). MMPs are zinc-containing endopeptidases that promote the degradation of excessive extracellular matrix components and thereby prime VSMCs for migration and proliferation and prime monocytes for invasion (Newby, 2006). The expression and activity of vascular MMPs are increased by ROS (Grote et al., 2003; Valentin et al., 2005). Indeed, the oral administration of tempol (18 $mg \cdot kg^{-1} \cdot d^{-1}$ for 8 weeks) prevented the excessive systemic and vascular ROS generation, aortic expression and activity of MMP-2 and the vascular remodeling in a rat model of 2K, 1C Goldblatt hypertension (Castro et al., 2009). The oral administration of tempol and apocynin together (120 $mg \cdot kg^{-1} \cdot d^{-1}$ of each for four weeks) to rats infused with Ang II attenuated the cardiac expression of NADPH oxidase, MMP-1 and -2, TGF-β and collagen-1, and reduced the cardiac fibrosis (Zhao et al., 2008b). Tempol prevented the increased aortic expression of MMP-3 of rats developing an aneurysm due to perfusion with elastase (Sinha et al., 2007) and reduced the aortic expression of MMP-3 and collagen in ovariectomized rats (Lam et al., 2009). Thus, a reduction in vascular MMPs may contribute to the effects of tempol to reduce vascular and cardiac remodeling and inflammation (Chow et al., 2007).

5.4. Brain, spinal cord, peripheral nerve, eye and ear

Tempol has been effective in protecting neuronal cells against ischemic and toxic challenges in several models. Tempol reduced oxidative damage in cerebral synaptosomes from gerbils subjected to hyperoxic brain damage (Howard et al., 1996). Cerebral edema, which exacerbated hypoxic brain damage, was reduced by tempol in a brain slice model (MacGregor et al., 2003).

Ischemia-reperfusion injury of the brain produced by ligation and release of a cerebral artery, induced oxidative stress and increased cell $[Ca^{++}]$ that contributed to the neuronal cell death (Cuzzocrea et al., 2000c; Schild & Reiser, 2005) and defective vascular regulation (Sun et al., 2008). Tempol diminished the cerebral oxidative stress that followed ischemia-reperfusion or hypoxia-reoxygenation injury to the brain and preserved neuronal viability (Rak et al., 2000; Cuzzocrea et al., 2000c; Leker et al., 2002; Behringer et al., 2002; Hu et al., 2003; Kato et al., 2003; Mehta et al., 2004; Schild & Reiser, 2005). Tempol infused one hour after middle cerebral arterial occlusion in SHR improved subsequent motor performance and cognitive function and reduced the cerebral infarct size (Leker et al., 2002). Tempol given intravenously to dogs prior to induced cardiac arrest provided substantial protection against the subsequent impairment of brain function (Behringer et al., 2002). Tempol was as effective as blockade of N-methyl-D-aspartate (NMDA) receptors with dexanabinol in neuroprotection after middle cerebral artery occlusion in the rat. This suggests that the neural protection provided by tempol entailed preservation of bioactive NO and/or a reduction in peroxinitrate. Indeed, tempol blocked the enhanced NMDA-induced neurotoxicity in rat cultured cortical brain cells following induction of inducible NOS, suggesting that $ONOO^-$ was the damaging species (Hewett et al., 1994; Teichner et al., 2003).

A reduction in BP after an acute ischemic stroke can worsen the neurologic deficit because the blood flow to the ischemic brain tissue at the margin of the infarcted region is no longer autoregulated. Therefore, the benefits of tempol to protect the brain from ischemia-

reperfusion injury might be limited by the associated hypotension. The nitroxide 3-carbamoyl-PROXYL given at the time of reperfusion caused dose-dependent cerebral protection without reducing the BP (Hu et al., 2003). As with other protective treatments, the time at which tempol was given was critical. Thus, iv tempol given at the time of reperfusion reduced lipid peroxidation in the brain and reduced the infract size, but if given 15 minutes after reperfusion, it was not effective (Kato et al., 2003).

Tempol protected cultured neuronal cells against hypoxia-reoxygenation damage (Tabakman et al., 2002; Yamada et al., 2003; Lang-Rollin et al., 2003) and prevented phosphorylation of ERK-1 and -2 in ischemic brain tissue (Wakade et al., 2008). Tanycytes cultured from the hypothalamus supported axonal regeneration but did not survive grafting to the brain because of sensitivity to ROS which was prevented by tempol and vitamin C (Prieto & Alonso, 1999). This suggests possible roles for tempol in protecting hypoxic neuronal cells and aiding regeneration in the brain.

ARBs may have a special protective role against stroke in patients with hypertension (Dahlöf et al., 2002). Tempol also had neuroprotective effects in a model of hypertension in the SHRsp infused with Ang II where tempol prevented cerebral neuronal cell loss and preserved the blood-brain barrier (Kim-Mitsuyama et al., 2005).

Tempol protected the brain or spinal cord from oxidative damage in several models of traumatic injury (Zhang et al., 1998; Trembovler et al., 1999; Hillard et al., 2004; Hillard et al., 2007). Tempol (300 mg $\cdot$ kg^{-1}) given to rats at the time of traumatic injury to the spinal cord (Xiong & Hall, 2009) or to the cerebral cortex (Deng-Bryant et al., 2008) reduced protein nitration and mitochondrial respiratory dysfunction and reduced calpain-mediated protein degradation. These effects were attributed to prevention of mitochondrial oxidative damage in the injured spinal cord (Patel et al., 2009) but were confined to a therapeutic window of thirty to sixty minutes after cord damage (Patel et al., 2009; Xiong & Hall, 2009). However, repeated doses of tempol after cerebral injury improved recovery of motor function (Merenda et al., 2008; Deng-Bryant et al., 2008). Tempol also was protective in transient focal cerebral ischemia (Rak et al., 2000) and prevented constriction of rat epineurial arterioles during exposure to high levels of oxygen (Sakai et al., 2007). A subdural hematoma induced cerebral oxidative stress and cerebral infarction that were reduced in rats given tempol (55 μmol $\cdot$ kg^{-1} iv) (Kwon et al., 2003).

Tempol protected the eye against retinal apoptosis, loss of retinal neurones and $ONOO^-$ accumulation after injection of NMDA (el-Remessy et al., 2003) and protected retinal blood vessels of rats from adverse effects of infused Ang II or from DM (Chen et al., 2007a). Oxidative damage of retinal pigment epithelial cells may underlie macula dengeneration (Zhou et al., 2008). The reduced form of tempol, tempol-H was more effective than Trolox or α-tocopherol in protecting these cells from photooxidation (Zhou et al., 2008). The tempol derivative 1-hydroxy-4-cyclopropanecarbonyloxy-2,2,6,6-tetramethylpiperidine hydrochloride given to albino rats protected their eyes from photodamage (Tanito et al., 2007). Tempol prevented the development of autoimmune uveitis in a rat model (Zamir et al., 1999) and protected the lens from cataract caused by H_2O_2 (Reddan et al., 1999; Zigler, Jr. et al., 2003; Akiyama et al., 2009) or radiation (Sasaki et al., 1998). Tempol protected retinal ganglion cells from the toxic effects of hypoxia or from TNFα (Tezel & Yang, 2004). Thus, a number of studies have demonstrated protective effects of tempol on retinal blood vessels, the retinal pigment cells and the lens in rodent models of ocular damage.

Guinea pigs subjected to excessive noise developed frequency-dependent auditory threshold shifts that were mitigated by oral tempol (3 mmol $\cdot$ l^{-1}) (Minami et al., 2007). Tempol prevented acoustic damage to the cochlear in the mouse (Murashita et al., 2006).

5.5. Gastrointestinal tract (GIT) and liver

Tempol prevented some of the damaging effect of nonsteroidal anti-inflammatory drugs on the GIT (Rachmilewitz et al., 1994; Davies & Jamali, 1997) and the development of gastric ulceration (Rachmilewitz et al., 1994; Samuni et al., 1999; Jia et al., 2007) and capsaicin- and ethanol-(Karmeli et al., 1995) or iodoacetamide-induced gastric damage (Karmeli et al., 1996). Tempol prevented ischemia-reperfusion damage to the small intestine (Udassin et al., 1998; Victorino et al., 2008) and was protective in a rat model of colitis (Cuzzocrea et al., 2000b; Park et al., 2002b; Bhattacharyya et al., 2009) or pancreatitis (Sledzinski et al., 1995) but failed to protect against granulomatous lesions in the small intestine in a rat model of Crohn's disease (Rachmilewitz et al., 1997).

Tempol protected the liver from ethanol-induced damage and the formation of megamitochondria (Matsuhashi et al., 1998). Tempol conjugated to amino acids protected the liver from ischemia-reperfusion injury (Bi et al., 2008). Tempol prevented hepatocyte toxicity from hypoxia, cyanide or antimycin (Niknahad et al., 1995) and reduced hepatic reperfusion damage (Blonder et al., 2000; Sepodes et al., 2004). Tempol reduced hepatic ROS, steatosis, fibrosis and hepatic mitochondrial damage in rats transgenic for the ren-2 gene. Thus, tempol was effective in models of both alcoholic and non-alcoholic liver disease (Wei et al., 2009b).

Encephalophy and raised intracranial pressure can complicate hepatic failure. The addition of ammonia to cultured brain astrocytes caused oxidation, nitration and activation of the Na/K/2Cl type 1 transporter that led to neuronal cell swelling which was prevented by tempol and other antioxidants (Jayakumar et al., 2008).

5.6. Lung

The administration of tempol to a model of lung transplant prevented pulmonary edema during warm ischemia and improved pulmonary oxygenation (Hodyc et al., 2008). Tempol protected cultured pulmonary epithelial cells from the damaging effects of ozone (Castagna et al., 2009) and prevented the generation of TGF-beta 1 and collagen by lung fibroblasts stimulated with $O_2^{\cdot -}$ (Qi et al., 2009).

5.7. Skeletal muscle

Superoxide in skeletal muscle reduced tetanic force development whereas incubation of muscle with tempol enhanced the maximal force development significantly (Edwards et al., 2007). Intravenous injections of tempol (52 mg $\cdot$ kg^{-1}) or vitamin C (76 mg $\cdot$ kg^{-1}) before electrical stimulation of rat spinotrapezius muscle increased muscle O_2 tension and usage yet reduced maximum tone generation (Herspring et al., 2008). Thus, it is unclear what effects tempol may have on exercise ability. As recently reviewed in detail (Bonetto et al., 2009), there is increasing evidence that oxidative stress initiates muscle atrophy in aging, cancer, diabetes, neurological disease and sepsis. However, there is no experience with the use of tempol in these conditions.

6. Additional actions of tempol

6.1. Protection from radiation and ultraviolet light

Cellular irradiation evoked a sustained increase in ROS over 8 days (Gao et al., 2008). The ensuing cellular damage likely entailed $O_2^{\cdot -}$ since overexpression of Cu/Zn-SOD (SOD-1) conferred radioresistance (Gao et al., 2008).

Mitchell et al demonstrated that tempol protected cells (Mitchell et al., 1991; DeGraff et al., 1992a) and mice (Hahn et al., 1992a; Liebmann et al., 1994) from the lethal effects of

radiation without apparent toxicity. Tempol and stem cell factor acted synergistically to protect mice from lethal doses of radiation (Liebmann et al., 1994). These effect of tempol were related to a reduction in tissue $O_2^{\cdot -}$ since the nitroxide was radioprotective whereas the hydroxylamine was not (Mitchell et al., 1991). Tempol protected against whole body radiation with a dose-modifying factors of 1 to 4 (Hahn et al., 1992a). The important finding that tempol protected oxygenated (Mitchell et al., 1991; Krishna & Samuni, 1993) but not hypoxic cells (Millar et al., 1985) from radiation-induced cell death provided insight into how nitroxides may provide radioprotection of normal cells without preventing radiosensitivity of hypoxic tumor cells (Millar et al., 1977; Millar et al., 1978; Millar et al., 1983). Thus, Hahn et al reported that pretreatment of mice with ip tempol (1.6 mmol $\cdot$ kg^{-1}) protected the bone marrow, but not an implanted tumor, against radiation. Studies with electron paramagnetic resonance or magnet resonance imaging (MRI) led to the conclusion that this was due to a rapid bio-reduction of tempol to its non-radioprotective hydroxylamine analogue in the tumor (Hahn et al., 1997; Cotrim et al., 2007). Tempol and mouth shielding completely protected mouse salivary glands from damage during head and neck irradiation (Vitolo et al., 2004). Cotrim et al showed that the decay of the tempol nitroxide signal studied by MRI within a salivary gland tumor in mice was faster than in normal tissues (Cotrim et al., 2007) which could account for the finding that tempol given before radiation protected the normal salivary glands from radiation damage without modifying the anti-tumor effect of the radiation on a tumor implanted in the leg (Cotrim et al., 2005; Cotrim et al., 2007). This differential effect of tempol was attributed to a 2-fold faster reduction of tempol in the tumor cells to the hydroxylamine, compared to the submandibular gland (Cotrim et al., 2007). Recently, Mitchell et al have proposed the use of MRI after nitroxide injection to detect the ideal time for radiotherapy when the normal tissues, but not the tumor, are protected (Hyodo et al., 2008).

However, other studies have shown that nitroxide hydroxylamines also can provide radiation protection under certain conditions. Both nitroxides, and their corresponding hydroxylamines, protected cells from DNA fragmentation by oxidants generated by the Cu^{++} -catalyzed Huber-Weiss reaction. However, under conditions that prevented the reconversion of the hydroxylamine to the nitroxide, only nitroxide radicals protected against radiation damage (Mitchell et al., 1991; Xavier et al., 2002). Normally, tempol-hydroxylamine can be oxidized *in vivo* to tempol within 10 minutes when it becomes effective in radiation protection (Mitchell et al., 1991; Sasaki et al., 1998; Hahn et al., 2000; Matsumoto et al., 2004). Thus, radiation protection by tempol-hydroxylamine in these conditions likely follows from its re-conversion to the active tempol nitroxide. However, intraocular injection of tempol, but not tempol-hydroxylamine, lessened radiation-induced DNA strand breaks in the rabbit eye (Sasaki et al., 1998). Thus not all environments are effective in oxidizing tempol-hydroxylamine to the radioprotective nitroxide radical.

The structural requirements for radiation protection by nitroxides showed that six-member piperidine nitroxides, such as tempol were effective for radiation protection and their hydroxylamines were efficiently reoxygenated to the nitroxide radical *in vivo* (Matsumoto et al., 2004). Other water-soluble nitroxides, such as 3-carbamoyl-PROXYL, provided similar *in vivo* radioprotection as tempol but did not lower the BP (Hahn et al., 1998). Among a family of nitroxides, those that were positively charged [e.g. tempamine and 3-aminomethyl-PROXYL] provided the best binding to DNA and the best radioprotection. The amphilite tempol was intermediate and negatively charged nitroxides were least effective (Hahn et al., 1992b). Radioprotective effects of nitroxides required cell permeation. Thus, tempol, but not the cell impermeable nitroxide 4-trimethyl-ammonium-2,2,6,6-tetramethylpiperidine-1-oxyl iodide, protected human lymphoblastoid cells against radiation-induced cell death (Samuni et al., 2004a) and protected human peripheral blood lymphocytes against radiation-induced chromosomal aberrations (Johnstone et al., 1995).

Radiation protection was quite specific for nitroxide radicals in one study since N-acetyl cystein, which also is a cell-permeable antioxidant, was not effective (Samuni et al., 2004a). These studies have established that radioprotection by nitroxides requires that they be capable of redox cycling, be positively charged or neutral and be cell membrane permeable.

Several studies have examined the basis for radiation protection by tempol. This entailed a reduction of transition metals, SOD-like activity, scavenging of oxy- and carbon-based free radicals (Hahn et al., 1994), a diminished formation of ·OH (Polytarchou et al., 2006), and a reduction in ROS generated within mitochondria (Miura et al., 2000) which could account for the greater efficacy of the mitochondrial targeted hemigramicidin-S-conjugated tempo (Jiang et al., 2008). Tempol protected lipids (Samuni et al., 1997) and acyl chains (Samuni & Barenholz, 1997) from radiation-induced degradation, albumin from carbonyl formation (Damiani et al., 2000) and cells from DNA strand breaks (Damiani et al., 1999). Thus, nitroxides protect cells and their components from a wide range of damaging effect of radiation by redox cycling from the nitroxide to the hydroxylamine species.

Organ damage after irradiation is compounded by endothelial dysfunction, disruption of the microcirculation and ischemia (Jordan et al., 1978; Law, 1981; Hopewell et al., 1986; Kantak et al., 1993; Hatoum et al., 2006). This may be an additional protective site of tempol since it can promote the healing of damaged capillary ECs (Braunhut et al., 1996).

A topical formulation of tempol which prevented radiation-induced alopecia in guinea-pigs (Cuscela et al., 1996) is undergoing a phase lb clinical study to evaluate its safety and side effect profile for protection against radiation-induced skin damage and alopecia in patients receiving head irradiation for brain metastases (Metz et al., 2004). Tempol was well tolerated and effective in eight of nine patients in retaining hair after irradiation (Metz et al., 2004).

These extensive experimental studies have established that tempol confers strong protection against the damaging effects of radiation on normal cells and tissues while generally preserving the damaging effects of radiation on malignant cells.

ROS are implicated in photodamage and aging of the skin (De et al., 1978; Pence & Naylor, 1990; Bissett et al., 1992; Miyachi, 1995). Tempol was effective in protecting cells (Weaver & Chon, 1966), skin (Bernstein et al., 2001; Yuen et al., 2002) and dermal fibroblasts (Yan et al., 2005) from UV light damage or photoaging. A tempol derivative was more effective than common ingredients of sunscreen formulations in protecting against UV damage (Damiani et al., 2002). Tempol penetrated well into skin where it was effective in protecting from damage caused by x-irradiation or UV light (Bernstein et al., 2001).

6.2. Anti-tumor and cancer-preventative actions and interactions with cancer chemotherapeutic agents

ROS participate in both cellular apoptosis and proliferation according to ROS species, concentration and cell type (Schubert et al., 2004). Thus, H_2O_2 induced VSMC proliferation at low micromolar concentrations but apoptosis at higher concentrations (Stone & Yang, 2006). Tempol can prevent the effect of ROS to activate serine/threonine-specific protein kinase-signalling pathways and induce apoptosis in several studies of normal cells (Fiordaliso et al., 2007; Song et al., 2007; Wakade et al., 2008). Yet ROS in the vasculature mediate the proliferative and remodeling responses of VSMCs to Ang II, platelet derived growth factor, thrombin and inflammatory stimuli (Lambeth, 2007).

Tumors and tumor cells produced excess ROS, often from NADPH oxidase (Szatrowski & Nathan, 1991; Burdon, 1995; Burdon, 1996; Droge, 2002; Chamulitrat et al., 2003;

Lambeth, 2007). Malignant tumor cells proliferated despite increased ROS production in most (Toyokuni et al., 1995; Kondo et al., 1999; Martin et al., 2007) but not all assay systems (Ko et al., 2007). Therefore, a reduction in tumor ROS by tempol might not be anticipated to have anti-tumor actions. However, the situation is complicated since preservation of bioactive NO by tempol can cause apoptosis, depending on the concentration and underlying state of proliferation (Muhl et al., 1996; Shami et al., 1998; Hajri et al., 1998), whereas ROS can cause DNA damage and thereby become mutagenic or cytotoxic at higher concentrations (Schubert et al., 2004).

Several studies have demonstrated that tempol reduced tumor growth or incidence. Important insights derived from studies by Gariboldi, Monti and colleagues of apparently paradoxical oxidant-like effects of tempol in neoplastic cells that underlied its antiproliferative and antitumor actions (Gariboldi et al., 1998; Gariboldi et al., 2000; Gariboldi et al., 2003; Gariboldi et al., 2006). Thus, whereas tempol diminished apoptosis in normal lymphocytes (Kobayashi & Schmid-Schonbein, 2006), it induced apoptosis in a human promyelocytic leukemic cell line (Gariboldi et al., 2000; Monti et al., 2001). Nitroxides caused oxidative stress, cell death (Gariboldi et al., 2000), decreased mitochondrial membrane potential and increased mitochandrial ROS generation in neoplastic human promyelocytic leukemic cells (Gariboldi et al., 2000; Monti et al., 2001). The cytotoxic effect of tempol on tumor cells was related to glutathione depletion and hypoxia (Metodiewa et al., 2000; Samuni et al., 2004b) via reactions of tempol with glutathione (Glebska et al., 2003) or with heme (Yin et al., 2004). Tempol elicited distinct signalling mechanisms in human breast cancer cells culminating in apoptotic cell death (Suy et al., 1998).

Further insight came from studies of mice deficient in the ataxia-telangiectasia gene with a high incidence of lymphoid tumors and neurodegeneration (Reliene et al., 2008). Although tempol prevented these effects and doubled their lifespan (Schubert et al., 2004; Reliene & Schiestl, 2007; Reliene et al., 2008), this remarkable effect was dissociated from its antioxidant actions. Thus tumor formation was unaffected when the tissue levels of ROS were enhanced by deletion of the genes for SOD-1 or -2 or reduced by α-tocopherol (Erker et al., 2006). Rather, the ability of nitroxides to inhibit tumor growth was ascribed to an *increase* in tumor cell ROS (Gariboldi et al., 1998; Gariboldi et al., 2000; Monti et al., 2001) which explained the observation that the anti-oxidant drug NAC blocked the anti-tumor effect of tempol in breast cancer cells (Gariboldi et al., 2006).

The observation that the incidence of cancer was lower in those consuming a diet rich in naturally occurring antioxidants and that ROS were increased in cancerous tissues suggested that antioxidants might prevent tumor formation (Schubert et al., 2004). Indeed, normal mice fed tempol from birth had a 4-fold reduction in spontaneous tumorgenesis (Mitchell et al., 2003). Tempol reduced glioma formation in rats (Gariboldi et al., 2003) and delayed the development of tumors (Gariboldi et al., 2003; Zhang et al., 2008a), and prolonged the lifespan, of cancer-prone mice harboring a p53 gene deletion (Erker et al., 2005). These differential effect of tempol on tumor cells suggested that tempol could be a novel agent for cancer prevention or chemotherapy (May et al., 1998; Schor et al., 2004).

A note of caution comes from some studies which have demonstrated potential tumorgenic effects of tempol. Tempol caused post-transcriptional activation of the urokinase receptor (Lejeune et al., 2006) which is associated with progression of human prostate cancer (Rabbani & Xing, 1998). Rats subjected to severe exercise showed DNA strand breaks that were enhanced by oral tempol (200 $\mu mol \cdot kg^{-1} \cdot d^{-1}$) (Wierzba et al., 2006).

Tempol interacts with many chemotherapeutic agents. Tempol potentiated the cell death and/or apoptosis, and upregulated p22 protein expression (Ravizza et al., 2004b) in Jurkat tumor cells (Sugimoto et al., 2002), human Burkitt lymphoma cells (Shacter et al., 2000) and colon cancer cells (Ravizza et al., 2004b). Tempol reduced ROS and diminished toxicity of the chemotherapeutic agents doxorubicin, cisplatin, AraC or VP-16 (Czepas et al., 2008). Tempol reduced the lethality of 6-mercaptopurine and enhanced its anti-tumor effect (Konovalova et al., 1996a; Konovalova et al., 1996b). Tempol reduced glutathione levels and synergetically enhanced the cytotoxic effects of the methylating agent, temozolomide in human glioblastoma cells (Ravizza et al., 2004a).

A limitation of chemotherapy with anthracycline agents such as doxorubicin has been the development of multidrug resistance (Longley & Johnston, 2005). Tempol synergized with doxorubicin in causing cytotoxicity of human breast adenocarcinoma cells by inhibiting the multidrug resistance pathway and thereby enhanced the cellular uptake of doxorubicin (Gariboldi et al., 2006).

An enhancement of chemotherapeutic efficacy by tempol has not been a universal finding. Apoptosis induced by the chemotherapeutic agents VP-16 and cisplatin in human B leukemic cells (Senturker et al., 2002) or by antimycin A in Hela cancer cells was unaffected by tempol (Han et al., 2008b). Tirapazamine becomes an active anti-tumor agent after reduction to nitrogen oxide anion radical which was prevented by tempol (Khan & O'Brien, 1995). The cytotoxicity of mitomycin C, or its quinolone redox-cycling analogues, were inhibited by tempol (Samuni et al., 2002).

Tempol protected normal cells from the cytotoxic effect of some chemotherapeutic agents. It prevented the cardiotoxicity of adriamycin (DeGraff et al., 1994; Monti et al., 1996), the hepatotoxicity of antimycin (Niknahad et al., 1995), the cytotoxicity of the semiquinone redox cycling agents, such as streptonigrin (DeGraff et al., 1994), the neurotoxicity of 6-hydroxydopamine (Purpura et al., 1996; Weinberg et al., 2004) and the spinal cord toxicity of etanidazole (Palayoor et al., 1994).

Thus, tempol has selective, but not universal, effects that can both enhance chemotherapeutic efficacy of anti-cancer agents against tumor cells while protecting some normal cells from these adverse effects.

6.3. Toxicity from drugs, metals, pollutants, and food additives

Paraquat is a redox-cycling quinolone used as a broad-spectrum herbicide which, if ingested, causes widespread oxidative damage and multisystem failure. Tempol protected cultured kidney cells from toxic effects of paraquat or related compounds (Zhang et al., 1994; Samuni et al., 2002; Samai et al., 2007). The addition of tempol to the bath of isolated, perfused renal afferent arterioles prevented the graded vasoconstriction produced by paraquat (Schnackenberg et al., 2000). Thus tempol might be effective against paraquat toxicity.

Tempol can prevent the effects of free iron to catalyze the production of ·OH from H_2O_2 by the Fenton reaction (Goralska et al., 2000). However, tempol has complicated effects on iron metabolism that can affect the outcome. Thus, high concentrations of tempol added to lens epithelial cells reduced the incorporation of iron into ferritin and increased the pool of free iron (Goralska et al., 2000) whereas lower concentrations of tempol protected against iron release during hypoxia (Borisenko et al., 2000). Tempol given to mice with deletion of the gene for the iron regulatory protein-2 restored iron homeostasis in the brain and attenuated the associated progressive neuromuscular dysfunction (Ghosh et al., 2008). The profound

endothelial dysfunction that occurs the day after injection of iron gluconate into rats was prevented by co-administration of tempol or NAC, but not by ascorbate (Nouri et al., 2007).

Tempol prevented cuprous ions from forming a superoxide generating complex with glutathione (Speisky et al., 2009). Tempol protected human lymphocytes from toxic effects of chromium and cadmium salts (Lewinska et al., 2008), protected arterial endothelium from the dysfunction caused by nanomolar concentrations of mercury chloride (Wiggers et al., 2008) and protected cultured juxtaglomerular (Han et al., 2008c) and lung cells (Han et al., 2008a) from apotosis by arsenic trioxide.

The aortas from Apo E gene deleted mice exposed to gasoline engine exhaust had enhanced endothelin-1 expression and upregulation of membrane metalloprotease-2 and -9 and TIMP-2 which were corrected by oral tempol (41 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}) (Lund et al., 2009a).

Carrageenans are sulphated polysaccharides which are used widely as food additives. They can cause inflammatory colitis in animals that was prevented by tempol (Bhattacharyya et al., 2008). Tempol prevented hepatotoxicity from H_2O_2 generated by high concentrations of hydralazine (Tafazoli & O'Brien, 2008), the anti-malarial compound amodiaquine (Tafazoli & O'Brien, 2009) or tetramethylphenylenediamine (Pourahmad et al., 2008). Tempol prevented the genomic damage caused by dopamine (Stopper et al., 2009) or by ROS (DeGraff et al., 1992b).

6.4. Anti-neurodegenerative action

Studies in animal models, and limited clinical information, suggest that some neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis might be associated with increases in ROS (Moosmann & Behl, 2002). Thus, patients with Parkinson's disease have a high frequency of single nucleotide polymorphisms in the gene for Mn-SOD (Shimoda-Matsubayashi et al., 1996; Grasbon-Frodl et al., 1999). Patients with the inherited form of amyotrophic lateral sclerosis have changes in the gene for SOD-1 that lead to its oligomerization and to an increase in ROS formation (Valentine & Hart, 2003). These observation suggest a therapeutic potential for tempol in these conditions (Grasbon-Frodl et al., 1999). Indeed, tempol given to mice diminished the severity of the Parkinsonian syndrome that followed intrastratal administration of 6-hydroxydopamine (Liang et al., 2005) and protected against neurotoxicity and striatal dopamine depletion after malonate challenge in a model of neurodegenerative disease (Matthews et al., 1999). Incubation of neuronal cells with dopamine increased the formation of tempol-hydroxylamine implying that tempol had reacted with ·OH generated in the cells by dopamine (Kudo et al., 2008).

The accelerated age-dependent cognitive decline of mice transgenic for the human renin gene was prevented by tempol (Inaba et al., 2009). Tempol prevented the progressive neurodegenerative disorder of mice with a knockout for the iron regulatory protein-2 (Ghosh et al., 2008). Tempol, and the peroxisome proliferator-activator receptor gamma agonist, pioglitazone fully preserved cerebrovascular function in mice transgenic for the amyloid precursor protein as a model of Alzheimer's disease (Nicolakakis et al., 2008).

6.5. Effects on inflammation and pain

Many studies have shown anti-inflammatory effects of tempol. For example, tempol blocked the release of cytokines from cultured ECs challenged with macrophage chemotactic protein-1 or interleukin-6 (Volk et al., 2000), reduced the pleural exudation and the polymorphonuclear cell migration in a rat carrageenan-induced model of pleuracy (Kurihara et al., 2002), reduced the inflammation in a rodent model of periodontitis (Di et al., 2005)

and delayed the manifestations of collagen-induced arthritis in the rat (Cuzzocrea et al., 2000d).

Generation of ROS in the central nervous system can induce pain. Thus, the intrathecal injection of antimycin A or rotenone into the spinal cord of mice to induce mitochondrial ROS generation evoked long-lasting hyperalgesia that was prevented by intrathecal tempol (Kim et al., 2008). Intrathecal tempol prevented thermal and mechanical hypersensitivity in a rat model of neuropathic pain (Tanabe et al., 2009). Tempol and NAC diminished chronic neuropathic pain in models of post-ischemic (Coderre et al., 2004) or chronic injury-induced pain (Tal, 1996) and reduced the hyperalgesia and normalized the evoked responses in the spinal cord of rats given intradermal capsaicin (Lee et al., 2007). Tempol prevented the central component of pain sensitization after injection of capsaicin into the foot (Schwartz et al., 2008). These data suggest potent analgesic effects of tempol mediated by reduction of ROS in the spinal cord.

6.6. Effects on infection

Superoxide is required for killing bacteria within phagolysosomes of activated leukocytes. Therefore, inhibition of $O_2^{\cdot -}$ generation with tempol might limit the host defense against bacterial infection. This concept was tested in pigs injected with live Pseudomonas aeruginosa bacteria which led to hypotension, a progressive deterioration in mucosal microcirculation and renal and hemostatic dysfunction. However, tempol given with iv fluid infusion 12 hours after the induction of sepsis reduced all of these parameters. It is remarkable that in this and other models of sepsis, tempol prevented a catastrophic fall in BP in contrast to its usual effect to lower BP (Matejovic et al., 2007). These studies suggest that tempol does not impair the ability to respond to an acute and severe systemic infection with aerobic bacteria.

The pathogenicity of malaria parasites involves the generation of ·OH following release of iron within erythrocytes (Schwartz et al., 1999). Tempol inhibited parasite growth in the erythrocytes (Schwartz et al., 1999) and protected erythrocytes from oxidative hemolysis (Wu et al., 1997; Li et al., 2006) likely by limiting ·OH formation by iron-catalyzed metabolism of H_2O_2. Erythrocytes from mice infected with the malaria parasite Plasmodium berghei reduced nitroxides at an accelerated rate (imply a hypoxic or reducing environment) that was corrected by chloroquine (Deslauriers et al., 1987).

On the other hand, two studies have detected effects of tempol that might promote infection. Tempol enhanced the growth of the anaerobic bacteria Campylobacter species. These bacteria normally cannot grow in media with an O_2 content above 15% but tolerated high O_2 contents in the presence of tempol (Hodge & Krieg, 1994). Oral tempol given to mice for four months after injection with *Leishmania amazonensis* inhibited inducible NOS expression and NO derived oxidants and increased the parasite burden (Linares et al., 2008). Thus, tempol might increase the pathogenicity of anaerobic bacteria and reduce host resistance to certain parasitic infections.

6.7. Shock and multiorgan failure

Tempol has been effective in ameliorating the effects of septic shock in several experimental settings. Tempol diminished the myocardial depression (Peng et al., 2005), preserved the renal blood flow and the GFR (Wang et al., 2003b; Wang et al., 2008b), ameliorated the renal and liver injury and preserved organ blood flow and metabolism (Leach et al., 1998; Zacharowski et al., 2000) in rodent or porcine (Matejovic et al., 2007) models of septic or endotoxic shock. Rats treated with lipopolysaccharide (LPS) developed shock and multiorgan failure accompanied by a rapid decline in the expression of Cu/Zn-SOD in the

kidney (Leach et al., 1998). Tempol (550 μmol · kg^{-1}), given before endotoxemia, attenuated organ dysfunction (Leach et al., 1998) and restored vascular NO generation in mesenteric vessels without perturbing their reactivity to NE, suggesting that tempol should preserve sympathetic vascular responses (Hernanz et al., 2004). Microinjections of tempol or the inducible NOS inhibitor, S-methylisothiourea into the rostroventral medulla of rats treated with LPS preserved brain stem mitochondrial function and cell viability and diminished the cardiovascular depression (Chan et al., 2005). Prolonged ischemia and reperfusion of the intestine led to local mucosal damage (Berber et al., 2009) and to pulmonary capillary dysfunction and leak ("shock lung") that were prevented by infusion of polynitroxylated albumin and tempol (Zhang et al., 2000). Tempol alone had a similar effect and reduced the mortality (Teke et al., 2008). Tempol, or a metalloporphyrin antioxidant, administered to mice after an injection of LPS, restored the renal expression of EC-SOD and the renal hemodynamics (Wang et al., 2003b). Since the beneficial effects of tempol were prevented by blockade of inducible NOS, the protection by tempol was ascribed to preservation of NO generated from inducible NOS and inhibition of peroxynitrate formation (Wang et al., 2003b). Tempol given to rats with multiorgan failure due to sepsis improved the hemodynamics and survival, and reduced the polymorphonuclear cell accumulation in the lungs and liver (Liaw et al., 2005). Tempol, NAC or blockade of NADPH oxidase all prevented TNFα generation by LPS in cardiomyocytes (Peng et al., 2005) that has been implicated in sepsis-induced myocardial depression (Pogrebniak et al., 1991).

Tempol given with fluid resuscitation has been effective in alleviating many of the adverse consequences of hemorrhagic shock. Tempol improved the survival of rats following hemorrhage (Kentner et al., 2002) and diminished the associated hemodynamic instability, the multi-organ dysfunction (Mota-Filipe et al., 1999) and the hepatic damage (Paxian et al., 2002; Paxian et al., 2003). Tempol (170 μmol · kg^{-1} · h^{-1} iv) given to rats during reperfusion four hours after hypotensive hemorrhage prevented the delayed circulatory failure (Mota-Filipe et al., 1999) but when given without adequate volume resuscitation, tempol enhanced the mortality (Kentner et al., 2007).

Tempol also has been effective in mitigating the consequences of severe circulatory failure. Tempol protected the brain following cardiac arrest (Behringer et al., 2002) and ameliorated the multiorgan failure, DNA damage and impaired mitochondrial respiration in rats after zymosan (Cuzzocrea et al., 2001). Tempol given to swine one hour after chest injury reduced their requirements for fluid infusion and reduced pulmonary inflammation, but did not modify mortality (Maxwell et al., 2000). Hind limb ischemia and reperfusion in the rat led to multiorgan dysfunction with edema, inflammation and the release of lactate dehydrogenase and creatine phosphokinase from unaffected muscle which were ameliorated by tempol given at the time of reperfusion (Arieli et al., 2008).

Thus, tempol can diminish organ damage in models of septic, hemorrhagic or ischemic shock, and multiorgan failure. Further work is needed to determine whether tempol is effective in the more challenging setting of alleviating the consequences of established shock, and whether tempol is the best nitroxide for use in these conditions.

6.8. Fertility and teratogenicity

Tempol improved the effectiveness of bull semen for artificial insemination (Foote et al., 2002) and, when added to ram semen, improved its motility and fertility (Mara et al., 2005). The addition of tempol to goat semen did not perturb pregnancy rates or the frequency of kidding (Mara et al., 2007).

Tempol (1 mmol · l^{-1}) added to control medium did not disrupt embryogenesis and prevented the effects of hyperglycemic culture media to cause malformed embryos (Ryu et

al., 2007). The authors concluded that tempol might provide protection against diabetic teratogenicity (Ryu et al., 2007). The pregnant BPH/5 mouse developed proteinuria, hypotension, excessive placenta ROS and impaired fetal growth and survival which was considered a model of human preeclampsia. These effects were prevented by tempol (Hoffmann et al., 2008).

6.9. Aging and other protective actions

Tempol has been effective in preventing or reversing some of the effects of aging. Tempol or apocynin restored the capacity of NO to inhibit cardiac O_2 usage (Adler et al., 2003) and restored EDRF/NO responses of cerebral arterioles isolated from aged Fisher-344 rats (Mayhan et al., 2008) or aged SHR (Payne et al., 2003). The restoration of EDRF/NO responses by tempol in blood vessels from aged rodents (Lesniewski et al., 2009; Durrant et al., 2009; Modrick et al., 2009; Lund et al., 2009b) was attributed to substitution for the decreased expression of vascular EC-SOD during aging (Lund et al., 2009b). Tempol prevented the hyperphosphorylation and inactivation of the dopamine D1 receptor and restored the natriuretic effect of dopamine in aged rats (Beheray et al., 2000; Fardoun et al., 2006).

The addition of tempol to the culture medium of Drosophila melanogaster increased their life span (Izmaylov & Obukhova, 1996). Finally, mice fed tempol from birth appeared fully healthy and lived 40% longer (Mitchell et al., 2003).

7. Conclusions

Redox-cycling nitroxides effectively reduced cellular, tissue and whole animal ROS in a wide range of animal studies and models complicated by oxidative stress. During long term administration, tempol had multiple actions to diminish oxidative and nitrosative stress. For example, tempol catalased the dismutation of $O_2^{\cdot -}$, facilitated catalase-like metabolism of H_2O_2 and prevented Fenton signalling and ·OH formation. These primary effects were accompanied by reduced lipid peroxidation, protein carbonyl formation, tyrosine nitration and DNA damage, and enhanced bioactivity of NO in blood vessels and organs.

Tempol reduced renal, cardiac, cerebral and gastrointestinal damage in animal models of ischemia-reperfusion injury. Tempol prevented vascular remodeling and preserved mitochondrial function in blood vessels from hypertensive models.

Tempol has metabolic actions related to improved mitochondrial function, cell membrane signalling and NO activity. Tempol reversed insulin resistance in models of oxidative stress by improving insulin release and glucose uptake, restoring leptin and adiponectin responses and reducing food intake. It ameliorated the elevated plasma lipid levels while increasing the protective high density lipoprotein cholesterol in models of the metabolic syndrome. It was effective in moderating renal and vascular complications of diabetes in rodent models.

Tempol has exerted other protective effects against toxic, environmental, pharmacologic or radiation-induced challenges. It protected normal tissues from radiation damage yet paradoxically maintained the tumoricidal actions of radiation. This was related to the low PO_2 and high $O_2^{\cdot -}$ in tumors that reduced the nitroxide tempol to the hydroxylamine that did not confer radiation protection. Whereas tempol enhanced proliferation in normal cells, it reduced tumor incidence and had apoptotic and cytotoxic actions on tumor cells which synergized with chemotherapeutic agents in some cancer models. These effects have been related to differential cell signalling by tempol in neoplastic and normal cell lines.

Tempol has been remarkably effective in improving the function and survival of animals after hemorrhagic or septic shock. It prevented toxic reactions to iron and drugs such as paraquat, prevented nitrate tolerance and has some promise for reducing neurodegenerative diseases and the aging process. Indeed, tempol, or related nitroxides, could have a role in preventing many of the devastating consequences of aging on the cardiovascular, renal and cerebrovascular systems. However, tempol presently has only been used in human subjects as a topical agent to prevent radiation-induced alopecia.

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Reference List

Adeagbo AS, Joshua IG, Falkner C, Matheson PJ. Tempol, an antioxidant, restores endothelium-derived hyperpolarizing factor-mediated vasodilation during hypertension. Eur J Pharmacol. 2003; 481:91–100. [PubMed: 14637180]

Adler A, Messina E, Sherman B, Wang Z, Huang H, Linke A, et al. NAD(P)H oxidase-generated superoxide anion accounts for reduced control of myocardial O_2 consumption by NO in old Fischer 344 rats. Am J Physiol. 2003; 285:H1015–1022.

Akiyama N, Umeda IO, Sogo S, Nishigori H, Tsujimoto M, Natori S. 5-S-GAD, a novel radical scavenging compound, prevents lens opacity development. Free Radic Biol Med. 2009; 46:511–519. [PubMed: 19084592]

Alpert E, Altman H, Totary H, Gruzman A, Barnea D, Barash V, et al. 4-Hydroxy tempol-induced impairment of mitochondrial function and augmentation of glucose transport in vascular endothelial and smooth muscle cells. Biochem Pharmacol. 2004; 67:1985–1995. [PubMed: 15130774]

Ando K, Fujita T. Metabolic syndrome and oxidative stress. Free Radic Biol Med. 2009; 47:213–218. [PubMed: 19409982]

Arieli D, Nahmany G, Casap N, Ad-El D, Samuni Y. The effect of a nitroxide antioxidant on ischemia-reperfusion injury in the rat in vivo hind limb model. Free Radic Res. 2008; 42:114–123. [PubMed: 18297604]

Arrick DM, Sharpe GM, Sun H, Mayhan WG. nNOS-dependent reactivity of cerebral arterioles in Type 1 diabetes. Brain Res. 2007; 1184:365–371. [PubMed: 17991456]

Asaba K, Tojo A, Onozato ML, Goto A, Fujita T. Double-edged action of SOD mimetic in diabetic nephropathy. J Cardiovasc Pharmacol. 2007; 49:13–19. [PubMed: 17261958]

Asaba K, Tojo A, Onozato ML, Goto A, Quinn MT, Fujita T, et al. Effects of NADPH oxidase inhibitor in diabetic nephropathy. Kidney Int. 2005; 67:1890–1898. [PubMed: 15840036]

Asghar M, Lokhandwala MF. Antioxidant supplementation normalizes elevated protein kinase C activity in the proximal tubules of old rats. Exp Biol Med (Maywood). 2004; 229:270–275. [PubMed: 14988520]

Asghar M, Lokhandwala MF. Antioxidant tempol lowers age-related increases in insulin resistance in Fischer 344 rats. Clin Exp Hypertens. 2006; 28:533–541. [PubMed: 16820349]

Awe SO, Tsakadze NL, D'Souza SE, Adeagbo AS. Tert-butyl hydroperoxide-mediated vascular responses in DOCA-salt hypertensive rats. Vascul Pharmacol. 2003; 40:51–57. [PubMed: 12646410]

Banday AA, Fazili FR, Marwaha A, Lokhandwala MF. Mitogen-activated protein kinase upregulation reduces renal D1 receptor affinity and G-protein coupling in obese rats. Kidney Int. 2007; 71:397–406. [PubMed: 17191082]

Banday AA, Marwaha A, Tallam LS, Lokhandwala MF. Tempol reduces oxidative stress, improves insulin sensitivity, decreases renal dopamine D1 receptor hyperphosphorylation, and restores D1

receptor-G-protein coupling and function in obese Zucker rats. Diabetes. 2005; 54:2219–2226. [PubMed: 15983225]

Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature. 2002; 416:337–339. [PubMed: 11907582]

Beheray S, Kansra V, Hussain T, Lokhandwala MF. Diminished natriuretic response to dopamine in old rats is due to an impaired D1-like receptor-signaling pathway. Kidney Int. 2000; 58:712–720. [PubMed: 10916094]

Behringer W, Safar P, Kentner R, Wu X, Kagan VE, Radovsky A, et al. Antioxidant tempol enhances hypothermic cerebral preservation during prolonged cardiac arrest in dogs. J Cereb Blood Flow Metab. 2002; 22:105–117. [PubMed: 11807400]

Belin de Chantemele EJ, Vessieres E, Guihot AL, Toutain B, Maquignau M, Loufrani L, et al. Type 2 diabetes severely impairs structural and functional adaptation of rat resistance arteries to chronic changes in blood flow. Cardiovasc Res. 2009; 81:788–796. [PubMed: 19050009]

Bell D, Zhao Y, McCoy FP, Devine AB, McDermott BJ. Differential effects of an anti-oxidant intervention on cardiomyocyte expression of adrenomedullin and intermedin and their receptor components in chronic nitric oxide deficiency. Cell Physiol Biochem. 2007; 20:269–282. [PubMed: 17762156]

Belmonte S, Morad M. 'Pressure-flow'-triggered intracellular Ca2+ transients in rat cardiac myocytes: possible mechanisms and role of mitochondria. J Physiol. 2008; 586:1379–1397. [PubMed: 18187469]

Beltowski J, Borkowska E, Wojcicka G, Marciniak A. Regulation of renal ouabain-resistant Na^+, K^+ -ATPase by leptin, nitric oxide, reactive oxygen species, and cyclic nucleotides: implications for obesity-associated hypertension. Clin Exp Hypertens. 2007; 29:189–207. [PubMed: 17497345]

Beltowski J, Jamroz-Wisniewska A, Borkowska E, Nazar J, Marciniak A. Antioxidant treatment normalizes renal Na^+,K^+ -ATPase activity in leptin-treated rats. Pharmacol Rep. 2005a; 57:219–228. [PubMed: 15886421]

Beltowski J, Jamroz-Wisniewska A, Borkowska E, Wojcicka G. Differential effect of antioxidant treatment on plasma and tissue paraoxonase activity in hyperleptinemic rats. Pharmacol Res. 2005b; 51:523–532. [PubMed: 15829432]

Beltowski J, Wojcicka G, Jamroz-Wisniewska A, Borkowska E, Marciniak A. Antioxidant treatment normalizes nitric oxide production, renal sodium handling and blood pressure in experimental hyperleptinemia. Life Sci. 2005c; 77:1855–1868. [PubMed: 15913657]

Bender SB, Herrick EK, Lott ND, Klabunde RE. Diet-induced obesity and diabetes reduce coronary responses to nitric oxide due to reduced bioavailability in isolated mouse hearts. Diabetes Obes Metab. 2007; 9:688–696. [PubMed: 17697061]

Berber I, Aydin C, Cevahir N, Yenisey C, Gumrukcu G, Kocbil G, et al. Tempol reduces bacterial translocation after ischemia/reperfusion injury in a rat model of superior mesenteric artery occlusion. Surg Today. 2009; 39:407–413. [PubMed: 19408078]

Bernstein EF, Kong SK, Brown DB, Kwak BC, Takeuchi T, Gasparro FP, et al. The nitroxide Tempol affords protection against ultraviolet radiation in a transgenic murine fibroblast culture model of cutaneous photoaging. Exp Dermatol. 2001; 10:55–61. [PubMed: 11168580]

Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: Molecular mechanisms and pathophysiological implications. J Physiol. 2004; 555:589–606. [PubMed: 14694147]

Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD, Webb RC. Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. Hypertens. 2001; 37:781–786.

Bhattacharyya S, Dudeja PK, Tobacman JK. Carrageenan-induced NFkappaB activation depends on distinct pathways mediated by reactive oxygen species and Hsp27 or by Bcl10. Biochim Biophys Acta. 2008; 1780:973–982. [PubMed: 18452717]

Bhattacharyya S, Dudeja PK, Tobacman JK. ROS, Hsp27, and IKKbeta mediate dextran sodium sulfate (DSS) activation of IkappaBa, NFkappaB, and IL-8. Inflamm Bowel Dis. 2009; 15:673–683. [PubMed: 19085995]

Bi W, Cai J, Xue P, Zhang Y, Liu S, Gao X, et al. Protective effect of nitronyl nitroxide-amino acid conjugates on liver ischemia-reperfusion induced injury in rats. Bioorg Med Chem Lett. 2008; 18:1788–1794. [PubMed: 18328700]

Bissett DL, Chatterjee R, Hannon DP. Protective effect of a topically applied anti-oxidant plus an anti-inflammatory agent against ultraviolet radiation-induced chronic skin damage in the hairless mouse. J Soc Cosmet Chem. 1992; 43:85–92.

Blendea MC, Jacobs D, Stump CS, McFarlane SI, Ogrin C, Bahtyiar G, et al. Abrogation of oxidative stress improves insulin sensitivity in the Ren-2 rat model of tissue angiotensin II overexpression. Am J Physiol Endocrinol Metab. 2005; 288:E353–E359. [PubMed: 15494608]

Blonder JM, McCalden TA, Hsia CJ, Billings RE. Polynitroxyl albumin plus tempol attenuates liver injury and inflammation after hepatic ischemia and reperfusion. Life Sci. 2000; 67:3231–3239. [PubMed: 11191630]

Bonetto A, Penna F, Muscaritoli M, Minero VG, Fanelli FR, Baccino FM, et al. Are antioxidants useful for treating skeletal muscle atrophy? Free Radic Biol Med. 2009; 47:906–916. [PubMed: 19591922]

Bonini MG, Mason RP, Augusto O. The mechanism by which 4-hydroxy-2,2,6,6-tetramethylpiperidene-1-oxyl (tempol) diverts peroxynitrite decomposition from nitrating to nitrosating species. Chem Res Toxicol. 2002; 15:506–511. [PubMed: 11952336]

Borisenko GG, Kagan VE, Hsia CJ, Schor NF. Interaction between 6-hydroxydopamine and transferrin: “Let my iron go”. Biochem. 2000; 39:3392–3400. [PubMed: 10727233]

bou-Mohamed G, Johnson JA, Jin L, el-Remessy AB, Do K, Kaesemeyer WH, et al. Roles of superoxide, peroxynitrite, and protein kinase C in the development of tolerance to nitroglycerin. J Pharmacol Exp Ther. 2004; 308:289–299. [PubMed: 14563789]

Bouvet C, de Chantemele EB, Guihot AL, Vessieres E, Bocquet A, Dumont O, et al. Flow-induced remodeling in resistance arteries from obese Zucker rats is associated with endothelial dysfunction. Hypertens. 2007; 50:248–254.

Boveris A, Costa LE, Cadenas E, Poderoso JJ. Regulation of mitochondrial respiration by adenosine diphosphate, oxygen, and nitric oxide. Meth Enzymol. 2000; 301:188–198. [PubMed: 9919567]

Brands MW, Bell TD, Gibson B. Nitric oxide may prevent hypertension early in diabetes by counteracting renal actions of superoxide. Hypertens. 2004; 43:57–63.

Braunhut SJ, Medeiros D, Lai L, Bump EA. Tempol prevents impairment of the endothelial cell wound healing response caused by ionising radiation. Br J Cancer Suppl. 1996; 27:S157–S160. [PubMed: 8763870]

Brodsky SV, Gealekman O, Chen J, Zhang F, Togashi N, Crabtree M, et al. Prevention and reversal of premature endothelial cell senescence and vasculopathy in obesity-induced diabetes by ebselen. Cir Res. 2004; 94:377–384.

Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005; 54:1615–1625. [PubMed: 15919781]

Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med. 1995; 18:775–794. [PubMed: 7750801]

Burdon RH. Control of cell proliferation by reactive oxygen species. Biochem Soc Trans. 1996; 24:1028–1032. [PubMed: 8968506]

Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000; 29:222–230. [PubMed: 11035250]

Carroll RT, Galatsis P, Borosky S, Kopec KK, Kumar V, Althaus JS, et al. 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol) inhibits peroxynitrite-mediated phenol nitration. Chem Res Toxicol. 2000; 13:294–300. [PubMed: 10775330]

Castagna R, Davis PA, Vasu VT, Soucek K, Cross CE, Greci L, et al. Nitroxide radical TEMPO reduces ozone-induced chemokine IL-8 production in lung epithelial cells. Toxicol In Vitro. 2009; 23:365–370. [PubMed: 19162161]

Castro MM, Rizzi E, Rodrigues GJ, Ceron CS, Bendhack LM, Gerlach RF, et al. Antioxidant treatment reduces matrix metalloproteinase-2-induced vascular changes in renovascular hypertension. Free Radic Biol Med. 2009; 46:1298–1307. [PubMed: 19248829]

Chamulitrat W, Schmidt R, Tomakidi P, Stremmel W, Chunglok W, Kawahara T, et al. Association of gp91phox homolog Nox1 with anchorage-independent growth and MAP kinase-activation of transformed human keratinocytes. Oncogene. 2003; 22:6045–6053. [PubMed: 12955083]

Chan SH, Wu KL, Wang LL, Chan JY. Nitric oxide- and superoxide-dependent mitochondrial signaling in endotoxin-induced apoptosis in the rostral ventrolateral medulla of rats. Free Radic Biol Med. 2005; 39:603–618. [PubMed: 16085179]

Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979; 59:527–605. [PubMed: 37532]

Chander PN, Gealekman O, Brodsky SV, Elitok S, Tojo A, Crabtree M, et al. Nephropathy in Zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. J Am Soc Nephrol. 2004; 15:2391–2403. [PubMed: 15339988]

Chatterjee PK, Cuzzocrea S, Brown PA, Zacharowski K, Stewart KN, Mota-Filipe H, et al. Tempol, a membrane-permeable radical scavenger, reduces oxidant stress-mediated renal dysfunction and injury in the rat. Kidney Int. 2000; 58:658–673. [PubMed: 10916089]

Chen P, Guo AM, Edwards PA, Trick G, Scicli AG. Role of NADPH oxidase in Ang II in diabetes-induced retinal leukostasis. Am J Physiol Regul Integr Comp Physiol. 2007a; 293:R1619–R1629. [PubMed: 17652361]

Chen X, Patel K, Connors S, Mendonca M, Welch WJ, Wilcox CS. Acute antihypertensive action of tempol in the spontaneously hypertensive rat. Am J Physiol. 2007b; 293:H3246–H3253.

Chen Y, Pearlman A, Luo Z, Wilcox CS. Hydrogen peroxide mediates a transient vasorelaxation with tempol during oxidative stress. Am J Physiol. 2007c; 293:H2085–H2092.

Chen YJ, Li J, Quilley J. Effect of inhibition of nitric oxide synthase on renal cyclooxygenase in the diabetic rat. Eur J Pharmacol. 2006; 541:80–86. [PubMed: 16753143]

Chess DJ, Xu W, Khairallah R, O'Shea KM, Kop WJ, Azimzadeh AM, et al. The Antioxidant Tempol Attenuates Pressure Overload-Induced Cardiac Hypertrophy and Contractile Dysfunction in Mice Fed a High-Fructose Diet. Am J Physiol Heart Circ Physiol. 2008; 295:H2223–H2230. [PubMed: 18931030]

Chow AK, Cena J, Schulz R. Acute actions and novel targets of matrix metalloproteinases in the heart and vasculature. Br J Pharmacol. 2007; 152:189–205. [PubMed: 17592511]

Coderre TJ, Xanthos DN, Francis L, Bennett GJ. Chronic post-ischemia pain (CPIP): a novel animal model of complex regional pain syndrome-type I (CRPS-I; reflex sympathetic dystrophy) produced by prolonged hindpaw ischemia and reperfusion in the rat. Pain. 2004; 112:94–105. [PubMed: 15494189]

Coppey LJ, Gellett JS, Davidson EP, Yorek MA. Preventing superoxide formation in epineurial arterioles of the sciatic nerve from diabetic rats restores endothelium-dependent vasodilation. Free Radic Res. 2003; 37:33–40. [PubMed: 12653215]

Cotrim AP, Hyodo F, Matsumoto K, Sowers AL, Cook JA, Baum BJ, et al. Differential radiation protection of salivary glands versus tumor by Tempol with accompanying tissue assessment of Tempol by magnetic resonance imaging. Clin Cancer Res. 2007; 13:4928–4933. [PubMed: 17699873]

Cotrim AP, Sowers AL, Lodde BM, Vitolo JM, Kingman A, Russo A, et al. Kinetics of tempol for prevention of xerostomia following head and neck irradiation in a mouse model. Clin Cancer Res. 2005; 11:7564–7568. [PubMed: 16243832]

Craven PA, Melhem MF, DeRubertis FR. Thromboxane in the pathogenesis of glomerular injury in diabetes. Kidney Int. 1992; 42:937–946. [PubMed: 1453584]

Cuscela D, Coffin D, Lupton GP, Cook JA, Krishna MC, Bonner RF, et al. Protection from radiation-induced alopecia with topical application of nitroxides: Fractionated studies. Cancer J Sci Am. 1996; 2:273–278. [PubMed: 9166544]

Cuzzocrea S, McDonald MC, Filipe HM, Costantino G, Mazzon E, Santagati S, et al. Effects of tempol, a membrane-permeable radical scavenger, in a rodent model of carrageenan-induced pleurisy. Eur J Pharmacol. 2000a; 390:209–222. [PubMed: 10708726]

Cuzzocrea S, McDonald MC, Mazzon E, Dugo L, Lepore V, Fonti MT, et al. Tempol, a membrane-permeable radical scavenger, reduces dinitrobenzene sulfonic acid-induced colitis. Eur J Pharmacol. 2000b; 406:127–137. [PubMed: 11011044]

Cuzzocrea S, McDonald MC, Mazzon E, Filipe HM, Centorrino T, Lepore V, et al. Beneficial effects of tempol, a membrane-permeable radical scavenger, on the multiple organ failure induced by zymosan in the rat. Crit Care Med. 2001; 29:102–111. [PubMed: 11176168]

Cuzzocrea S, McDonald MC, Mazzon E, Siriwardena D, Costantino G, Fulia F, et al. Effects of tempol, a membrane-permeable radical scavenger, in a gerbil model of brain injury. Brain Res. 2000c; 875:96–106. [PubMed: 10967303]

Cuzzocrea S, McDonald MC, Mota-Filipe H, Mazzon E, Costantino G, Britti D, et al. Beneficial effects of tempol, a membrane-permeable radical scavenger, in a rodent model of collagen-induced arthritis. Arthritis Rheum. 2000d; 43:320–328. [PubMed: 10693871]

Czepas J, Koceva-Chyla A, Gwozdzinski K, Jozwiak Z. Different effectiveness of piperidine nitroxides against oxidative stress induced by doxorubicin and hydrogen peroxide. Cell Biol Toxicol. 2008; 24:101–112. [PubMed: 17610030]

Dahlöf B, Devereux RB, Kjeldsen SE, Beevers G, de Faire U, Fyhrquist F, et al. Cardiovascular morbidity and mortality in the losartan intervention for endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet. 2002; 359:995–1010. [PubMed: 11937178]

Dahly AJ, Hoagland KM, Flasch AK, Jha S, Ledbetter SR, Roman RJ. Antihypertensive effects of chronic anti-TGF-beta antibody therapy in Dahl S rats. Am J Physiol Regul Integr Comp Physiol. 2002; 283:R757–R767. [PubMed: 12185011]

Damiani E, Carloni P, Biondi C, Greci L. Increased oxidative modification of albumin when illuminated in vitro in the presence of a common sunscreen ingredient: protection by nitroxide radicals. Free Radic Biol Med. 2000; 28:193–201. [PubMed: 11281286]

Damiani E, Castagna R, Greci L. The effects of derivatives of the nitroxide tempol on UVA-mediated in vitro lipid and protein oxidation. Free Radic Biol Med. 2002; 33:128–136. [PubMed: 12086690]

Damiani E, Greci L, Parsons R, Knowland J. Nitroxide radicals protect DNA from damage when illuminated in vitro in the presence of dibenzoylmethane and a common sunscreen ingredient. Free Rad Biol Med. 1999; 26:809–816. [PubMed: 10232823]

Davies NM, Jamali F. Pharmacological protection of NSAID-induced intestinal permeability in the rat: effect of tempo and metronidazole as potential free radical scavengers. Hum Exp Toxicol. 1997; 16:345–349. [PubMed: 9257157]

Davies SJ, Reichardt-Pascal SY, Vaughan D, Russell GI. Differential effect of ischaemia-reperfusion injury on anti-oxidant enzyme activity in the rat kidney. Exp Nephrol. 1995; 3:348–354. [PubMed: 8528679]

De RG, Chan JT, Black HS, Rudolph AH, Knox JM. Systemic protection by antioxidants against UVL-induced erythema. J Invest Dermatol. 1978; 70:123–125. [PubMed: 632615]

Deffner U, Schimmack W. Letter: Radiation effects on aqueous solutions of the nitroxyl free radical TMPN (2,2,6,6-tetramethyl-4-piperidinol-N-oxyl). Int J Radiat Biol Relat Stud Phys Chem Med. 1976; 29:71–75. [PubMed: 178616]

DeGraff W, Hahn SM, Mitchell JB, Krishna MC. Free radical modes of cytotoxicity of adriamycin and streptonigrin. Biochem Pharmacol. 1994; 48:1427–1435. [PubMed: 7945443]

DeGraff WG, Krishna MC, Kaufman D, Mitchell JB. Nitroxide-mediated protection against X-ray- and neocarzinostatin-induced DNA damage. Free Radic Biol Med. 1992a; 13:479–487. [PubMed: 1459474]

DeGraff WG, Krishna MC, Russo A, Mitchell JB. Antimutagenicity of a low molecular weight superoxide dismutase mimic against oxidative mutagens. Environ Mol Mutagen. 1992b; 19:21–26. [PubMed: 1310080]

DeMarco VG, Habibi J, Whaley-Connell AT, Schneider RI, Heller RL, Bosanquet JP, et al. Oxidative stress contributes to pulmonary hypertension in the transgenic (mRen2)27 rat. Am J Physiol Heart Circ Physiol. 2008; 294:H2659–H2668. [PubMed: 18424632]

Deng-Bryant Y, Singh IN, Carrico KM, Hall ED. Neuroprotective effects of tempol, a catalytic scavenger of peroxynitrite-derived free radicals, in a mouse traumatic brain injury model. J Cereb Blood Flow Metab. 2008; 28:1114–1126. [PubMed: 18319733]

Dernbach E, Urbich C, Brandes RP, Hofmann WK, Zeiher AM, Dimmeler S. Antioxidative stress-associated genes in circulating progenitor cells: evidence for enhanced resistance against oxidative stress. Blood. 2004; 104:3591–3597. [PubMed: 15161665]

DeRubertis FR, Craven PA. Eicosanoids in the pathogenesis of the functional and structural alterations of the kidney in diabetes. Am J Kidney Dis. 1993; 22:727–735. [PubMed: 8238021]

DeRubertis FR, Craven PA. Activation of protein kinase C in glomerular cells in diabetes. Mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. Diabetes. 1994; 43:1–8. [PubMed: 8262306]

DeRubertis FR, Craven PA, Melhem MF. Acceleration of diabetic renal injury in the superoxide dismutase knockout mouse: effects of tempol. Metabolism. 2007; 56:1256–1264. [PubMed: 17697870]

Deslauriers R, Butler K, Smith IC. Oxidant stress in malaria as probed by stable nitroxide radicals in erythrocytes infected with Plasmodium berghei. The effects of primaquine and chloroquine. Biochim Biophys Acta. 1987; 931:267–275. [PubMed: 3315005]

Dessolin J, Schuler M, Quinart A, De GF, Ghosez L, Ichas F. Selective targeting of synthetic antioxidants to mitochondria: towards a mitochondrial medicine for neurodegenerative diseases? Eur J Pharmacol. 2002; 447:155–161. [PubMed: 12151007]

Di PR, Mazzon E, Zito D, Maiere D, Britti D, Genovese T, et al. Effects of Tempol, a membrane-permeable radical scavenger, in a rodent model periodontitis. J Clin Periodontol. 2005; 32:1062–1068. [PubMed: 16174269]

Ding Y, Li YL, Zimmerman MC, Davisson RL, Schultz HD. Role of CuZn superoxide dismutase on carotid body function in heart failure rabbits. Cardiovasc Res. 2009; 81:678–685. [PubMed: 19091790]

Droge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002; 82:47–95. [PubMed: 11773609]

Duann P, Datta PK, Pan C, Blumberg JB, Sharma M, Lianos EA. Superoxide dismutase mimetic preserves the glomerular capillary permeability barrier to protein. J Pharmacol Exp Ther. 2006; 316:1249–1254. [PubMed: 16303918]

Duda M, Konior A, Klemenska E, Beresewicz A. Preconditioning protects endothelium by preventing ET-1-induced activation of NADPH oxidase and xanthine oxidase in post-ischemic heart. J Mol Cell Cardiol. 2007; 42:400–410. [PubMed: 17156794]

Dumont O, Pinaud F, Guihot AL, Baufreton C, Loufrani L, Henrion D. Alteration in flow (shear stress)-induced remodelling in rat resistance arteries with aging: improvement by a treatment with hydralazine. Cardiovasc Res. 2008; 77:600–608. [PubMed: 18006444]

Durrant JR, Seals DR, Connell ML, Russell MJ, Lawson BR, Folian BJ, et al. Voluntary wheel running restores endothelial function in conduit arteries of old mice: Direct evidence for reduced oxidative stress, increased superoxide dismutase activity and down-regulation of NADPH oxidase. J Physiol. 2009; 587:3271–3285. [PubMed: 19417091]

Ebenezer PJ, Mariappan N, Elks CM, Haque M, Francis J. Diet-induced Renal Changes in Zucker Rats Are Ameliorated by the Superoxide Dismutase Mimetic TEMPOL. Obesity (Silver Spring). 2009; 17:1994–2002. [PubMed: 19424163]

Edwards JN, Macdonald WA, van der PC, Stephenson DG. O2(*-) production at 37 degrees C plays a critical role in depressing tetanic force of isolated rat and mouse skeletal muscle. Am J Physiol Cell Physiol. 2007; 293:C650–C660. [PubMed: 17459949]

el-Remessy AB, Khalil IE, Matragoon S, bou-Mohamed G, Tsai NJ, Roon P, et al. Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. Am J Pathol. 2003; 163:1997–2008. [PubMed: 14578199]

Elmedal B, de Dam MY, Mulvany MJ, Simonsen U. The superoxide dismutase mimetic, tempol, blunts right ventricular hypertrophy in chronic hypoxic rats. Br J Pharmacol. 2004; 141:105–113. [PubMed: 14656807]

Erker L, Schubert R, Elchuri S, Huang TT, Tarin D, Mueller K, et al. Effect of the reduction of superoxide dismutase 1 and 2 or treatment with alpha-tocopherol on tumorigenesis in Atm-deficient mice. Free Radic Biol Med. 2006; 41:590–600. [PubMed: 16863992]

Erker L, Schubert R, Yakushiji H, Barlow C, Larson D, Mitchell JB, et al. Cancer chemoprevention by the antioxidant tempol acts partially via the p53 tumor suppressor. Hum Mol Genet. 2005; 14:1699–1708. [PubMed: 15888486]

Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP, et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann N Y Acad Sci. 2002; 962:195–206. [PubMed: 12076975]

Esplugues JV, Rocha M, Nunez C, Bosca I, Ibiza S, Herance JR, et al. Complex I dysfunction and tolerance to nitroglycerin: an approach based on mitochondrial-targeted antioxidants. Circ Res. 2006; 99:1067–1075. [PubMed: 17053193]

Fardoun RZ, Asghar M, Lokhandwala M. Role of oxidative stress in defective renal dopamine D1 receptor-G protein coupling and function in old Fischer 344 rats. Am J Physiol Renal Physiol. 2006; 291:F945–F951. [PubMed: 16757732]

Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. N Engl J Med. 2008; 359:1811–1821. [PubMed: 18946066]

Fiordaliso F, De AN, Bai A, Cuccovillo I, Salio M, Maria SD, et al. Effect of beta-adrenergic and renin-angiotensin system blockade on myocyte apoptosis and oxidative stress in diabetic hypertensive rats. Life Sci. 2007; 81:951–959. [PubMed: 17825849]

Folkow B. Cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension. Clin Sci Mol Med. 1978; 55:3s–22s.

Foote RH, Brockett CC, Kaproth MT. Motility and fertility of bull sperm in whole milk extender containing antioxidants. Anim Reprod Sci. 2002; 71:13–23. [PubMed: 11988368]

Frisbee JC. Reduced nitric oxide bioavailability contributes to skeletal muscle microvessel rarefaction in the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol. 2005; 289:R307–R316. [PubMed: 15802560]

Fujii T, Takaoka M, Ohkita M, Matsumura Y. Tempol protects against ischemic acute renal failure by inhibiting renal noradrenaline overflow and endothelin-1 overproduction. Biol Pharm Bull. 2005; 28:641–645. [PubMed: 15802802]

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004; 114:1752–1761. [PubMed: 15599400]

Gao L, Wang W, Li YL, Schultz HD, Liu D, Cornish KG, et al. Superoxide mediates sympathoexcitation in heart failure: roles of angiotensin II and NAD(P)H oxidase. Circ Res. 2004; 95:937–944. [PubMed: 15459075]

Gao X, Belmadani S, Picchi A, Xu X, Potter BJ, Tewari-Singh N, et al. Tumor necrosis factor-alpha induces endothelial dysfunction in Lepr(db) mice. Circ. 2007; 115:245–254.

Gao Z, Sarsour EH, Kalen AL, Li L, Kumar MG, Goswami PC. Late ROS accumulation and radiosensitivity in SOD1-overexpressing human glioma cells. Free Radic Biol Med. 2008; 45:1501–1509. [PubMed: 18790046]

Gariboldi M, Rimoldi V, Supino R, Favini E, Monti E. The nitroxide tempol induces oxidative stress, $p21^{WAF1/CIP1}$, and cell death in HL60 cells. Free Rad Biol Med. 2000; 29:633–641. [PubMed: 11033415]

Gariboldi MB, Lucchi S, Caserini C, Supino R, Oliva C, Monti E. Antiproliferative effect of the piperidine nitroxide TEMPOL on neoplastic and nonneoplastic mammalian cell lines. Free Radic Biol Med. 1998; 24:913–923. [PubMed: 9607601]

Gariboldi MB, Ravizza R, Petterino C, Castagnaro M, Finocchiaro G, Monti E. Study of in vitro and in vivo effects of the piperidine nitroxide Tempol--a potential new therapeutic agent for gliomas. Eur J Cancer. 2003; 39:829–837. [PubMed: 12651210]

Gariboldi MB, Terni F, Ravizza R, Meschini S, Marra M, Condello M, et al. The nitroxide Tempol modulates anthracycline resistance in breast cancer cells. Free Radic Biol Med. 2006; 40:1409–1418. [PubMed: 16631531]

George J, Patal S, Wexler D, Sharabi Y, Peleg E, Kamari Y, et al. Circulating adiponectin concentrations in patients with congestive heart failure. Heart. 2006; 92:1420–1424. [PubMed: 16621874]

Ghatta S, Hemmer RB, Uppala S, O'rourke ST. Role of endogenous hydrogen peroxide in the development of nitrate tolerance. Vascul Pharmacol. 2007; 46:247–252. [PubMed: 17157562]

Ghosh MC, Tong WH, Zhang D, Ollivierre-Wilson H, Singh A, Krishna MC, et al. Tempol-mediated activation of latent iron regulatory protein activity prevents symptoms of neurodegenerative disease in IRP2 knockout mice. Proc Natl Acad Sci U S A. 2008; 105:12028–12033. [PubMed: 18685102]

Glebska J, Skolimowski J, Kudzin Z, Gwozdzinski K, Grzelak A, Bartosz G. Pro-oxidative activity of nitroxides in their reactions with glutathione. Free Radic Biol Med. 2003; 35:310–316. [PubMed: 12885593]

Gonzalez DR, Fernandez IC, Ordenes PP, Treuer AV, Eller G, Boric MP. Differential role of S-nitrosylation and the NO-cGMP-PKG pathway in cardiac contractility. Nitric Oxide. 2008; 18:157–167. [PubMed: 18023373]

Goodwill AG, Stapleton PA, James ME, D'audiffret AC, Frisbee JC. Increased Arachidonic Acid-Induced Thromboxane Generation Impairs Skeletal Muscle Arteriolar Dilation with Genetic Dyslipidemia. Microcirculation. 2008; 15:621–631. [PubMed: 18720229]

Goralska M, Holley B, McGahan MC. The effects of Tempol on ferritin synthesis and Fe metabolism in lens epithelial cells. Biochim Biophys Acta. 2000; 1497:51–60. [PubMed: 10838158]

Grasbon-Frodl EM, Kosel S, Riess O, Muller U, Mehraein P, Graeber MB. Analysis of mitochondrial targeting sequence and coding region polymorphisms of the manganese superoxide dismutase gene in German Parkinson disease patients. Biochem Biophys Res Commun. 1999; 255:749–752. [PubMed: 10049782]

Gross ER, LaDisa JF Jr, Weihrauch D, Olson LE, Kress TT, Hettrick DA, et al. Reactive oxygen species modulate coronary wall shear stress and endothelial function during hyperglycemia. Am J Physiol Heart Circ Physiol. 2003; 284:H1552–H1559. [PubMed: 12543642]

Grote K, Flach I, Luchtefeld M, Akin E, Holland SM, Drexler H, et al. Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species. Circ Res. 2003; 92:e80–e86. [PubMed: 12750313]

Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circ. 2005; 112:2735–2752.

Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in breakdown of endothelium-derived vascular relaxing factor. Nature. 1986; 320:454–456. [PubMed: 3007998]

Guo P, Nishiyama A, Rahman M, Nagai Y, Noma T, Namba T, et al. Contribution of reactive oxygen species to the pathogenesis of left ventricular failure in Dahl salt-sensitive hypertensive rats: effects of angiotensin II blockade. J Hypertens. 2006; 24:1097–1104. [PubMed: 16685210]

Guo R, Gao XY, Wang W, Wang HJ, Zhang F, Zhang Y, et al. Tempol reduces reperfusion-induced arrhythmias in anaesthetized rats. Pharmacol Res. 2005; 52:192–198. [PubMed: 15967386]

Hahn SM, DeLuca AM, Coffin D, Krishna CM, Mitchell JB. In vivo radioprotection and effects on blood pressure of the stable free radical nitroxides. Int J Radiat Oncol Biol Phys. 1998; 42:839–842. [PubMed: 9845107]

Hahn SM, Krishna CM, Samuni A, DeGraff W, Cuscela DO, Johnstone P, et al. Potential use of nitroxides in radiation oncology. Cancer Res. 1994; 54:2006s–2010s. [PubMed: 8137329]

Hahn SM, Krishna MC, DeLuca AM, Coffin D, Mitchell JB. Evaluation of the hydroxylamine Tempol-H as an in vivo radioprotector. Free Radic Biol Med. 2000; 28:953–958. [PubMed: 10802227]

Hahn SM, Sullivan FJ, DeLuca AM, Krishna CM, Wersto N, Venzon D, et al. Evaluation of tempol radioprotection in a murine tumor model. Free Radic Biol Med. 1997; 22:1211–1216. [PubMed: 9098095]

Hahn SM, Tochner Z, Krishna CM, Glass J, Wilson L, Samuni A, et al. Tempol, a stable free radical, is a novel murine radiation protector. Cancer Research. 1992a; 52:1750–1753. [PubMed: 1551104]

Hahn SM, Wilson L, Krishna CM, Liebmann J, DeGraff W, Gamson J, et al. Identification of nitroxide radioprotectors. Radiat Res. 1992b; 132:87–93. [PubMed: 1410280]

Hajri A, Metzger E, Vallat F, Coffy S, Flatter E, Evrard S, et al. Role of nitric oxide in pancreatic tumour growth: in vivo and in vitro studies. Br J Cancer. 1998; 78:841–849. [PubMed: 9764573]

Hall JE, Brands MW, Dixon WN, Smith MJ Jr. Obesity-induced hypertension: Renal function and systemic hemodynamics. Hypertens. 1993; 22:292–299.

Han Y, Shi Z, Zhang F, Yu Y, Zhong MK, Gao XY, et al. Reactive oxygen species in the paraventricular nucleus mediate the cardiac sympathetic afferent reflex in chronic heart failure rats. Eur J Heart Fail. 2007; 9:967–973. [PubMed: 17719272]

Han YH, Kim SH, Kim SZ, Park WH. Apoptosis in arsenic trioxide-treated Calu-6 lung cells is correlated with the depletion of GSH levels rather than the changes of ROS levels. J Cell Biochem. 2008a; 104:862–878. [PubMed: 18393359]

Han YH, Kim SH, Kim SZ, Park WH. Intracellular GSH levels rather than ROS levels are tightly related to AMA-induced HeLa cell death. Chem Biol Interact. 2008b; 171:67–78. [PubMed: 17935707]

Han YH, Kim SZ, Kim SH, Park WH. Intracellular GSH level is a factor in As4.1 juxtaglomerular cell death by arsenic trioxide. J Cell Biochem. 2008c; 104:995–1009. [PubMed: 18247324]

Hare JM. Nitroso-redox balance in the cardiovascular system. N Eng J Med. 2004; 351:2112–2114.

Hasdan G, Benchetrit S, Rashid G, Green J, Bernheim J, Rathaus M. Endothelial dysfunction and hypertension in 5/6 nephrectomized rats are mediated by vascular superoxide. Kidney Int. 2002; 61:586–590. [PubMed: 11849400]

Hasegawa H, Takano H, Kohro T, Ueda K, Niitsuma Y, Aburatani H, et al. Amelioration of hypertensive heart failure by amlodipine may occur via antioxidative effects. Hypertens Res. 2006; 29:719–729. [PubMed: 17249528]

Hatoum OA, Otterson MF, Kopelman D, Miura H, Sukhotnik I, Larsen BT, et al. Radiation induces endothelial dysfunction in murine intestinal arterioles via enhanced production of reactive oxygen species. Arterioscler Thromb Vasc Biol. 2006; 26:287–294. [PubMed: 16322529]

Hattori Y, Akimoto K, Gross SS, Hattori S, Kasai K. Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. Diabetologia. 2005; 46:1066–1074. [PubMed: 15864528]

Hayashi H, Kobara M, Abe M, Tanaka N, Gouda E, Toba H, et al. Aldosterone nongenomically produces NADPH oxidase-dependent reactive oxygen species and induces myocyte apoptosis. Hypertens Res. 2008; 31:363–375. [PubMed: 18360057]

Hernanz R, Alonso MJ, Zibrandtsen H, Alvarez Y, Salaices M, Simonsen U. Measurements of nitric oxide concentration and hyporeactivity in rat superior mesenteric artery exposed to endotoxin. Cardiovasc Res. 2004; 62:202–211. [PubMed: 15023567]

Herspring KF, Ferreira LF, Copp SW, Snyder BS, Poole DC, Musch TI. Effects of antioxidants on contracting spinotrapezius muscle microvascular oxygenation and blood flow in aged rats. J Appl Physiol. 2008; 105:1889–1896. [PubMed: 18845782]

Hewett SJ, Csernansky CA, Choi DW. Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS. Neuron. 1994; 13:487–494. [PubMed: 7520256]

Hillard VH, Peng H, Das K, Murali R, Moorthy CR, Etlinger JD, et al. Inhibition of x-irradiation-enhanced locomotor recovery after spinal cord injury by hyperbaric oxygen or the antioxidant nitroxide tempol. J Neurosurg Spine. 2007; 6:337–343. [PubMed: 17436923]

Hillard VH, Peng H, Zhang Y, Das K, Murali R, Etlinger JD, et al. Tempol, a nitroxide antioxidant, improves locomotor and histological outcomes after spinal cord contusion in rats. J Neurotrauma. 2004; 21:1405–1414. [PubMed: 15672631]

Hisaki R, Fujita H, Saito F, Kushiro T. Tempol attenuates the development of hypertensive renal injury in Dahl salt-sensitive rats. Am J Hypertens. 2005; 18:707–713. [PubMed: 15882555]

Hoagland KM, Maier KG, Roman RJ. Contributions of 20-HETE to the antihypertensive effects of Tempol in Dahl salt-sensitive rats. Hypertens. 2003; 41:697–702.

Hobo A, Yuzawa Y, Kosugi T, Kato N, Asai N, Sato W, et al. The growth factor midkine regulates the renin-angiotensin system in mice. J Clin Invest. 2009; 119:1616–1625. [PubMed: 19451697]

Hodge JP, Krieg NR. Oxygen tolerance estimates in Campylobacter species depend on the testing medium. J Appl Bacteriol. 1994; 77:666–673. [PubMed: 7822225]

Hodyc D, Hnilickova O, Hampl V, Herget J. Pre-arrest administration of the cell-permeable free radical scavenger tempol reduces warm ischemic damage of lung function in non-heart-beating donors. J Heart Lung Transplant. 2008; 27:890–897. [PubMed: 18656803]

Hodyc D, Snorek M, Brtnicky T, Herget J. Superoxide dismutase mimetic tempol inhibits hypoxic pulmonary vasoconstriction in rats independently of nitric oxide production. Exp Physiol. 2007; 92:945–951. [PubMed: 17496002]

Hoffman A, Goldstein S, Samuni A, Borman JB, Schwalb H. Effect of nitric oxide and nitroxide SOD-mimic on the recovery of isolated rat heart following ischemia and reperfusion. Biochem Pharmacol. 2003; 66:1279–1286. [PubMed: 14505807]

Hoffmann DS, Weydert CJ, Lazartigues E, Kutschke WJ, Kienzle MF, Leach JE, et al. Chronic tempol prevents hypertension, proteinuria, and poor feto-placental outcomes in BPH/5 mouse model of preeclampsia. Hypertens. 2008; 51:1058–1065.

Hopewell JW, Campling D, Calvo W, Reinhold HS, Wilkinson JH, Yeung TK. Vascular irradiation damage: its cellular basis and likely consequences. Br J Cancer Suppl. 1986; 7:181–191. [PubMed: 3521701]

Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol. 1981; 241:F85–F93. [PubMed: 7246778]

Howard BJ, Yatin S, Hensley K, Allen KL, Kelly JP, Carney J, et al. Prevention of hyperoxia-induced alterations in synaptosomal membrane-associated proteins by N-tert-butyl-alpha-phenylnitrone and 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (Tempol). J Neurochem. 1996; 67:2045–2050. [PubMed: 8863512]

Hsueh W, Abel ED, Breslow JL, Maeda N, Davis RC, Fisher EA, et al. Recipes for creating animal models of diabetic cardiovascular disease. Circ Res. 2007; 100:1415–1427. [PubMed: 17525381]

Hu G, Lyeth BG, Zhao X, Mitchell JB, Watson JC. Neuroprotection by the stable nitroxide 3-carbamoyl-proxyl during reperfusion in a rat model of transient focal ischemia. J Neurosurg. 2003; 98:393–396. [PubMed: 12593628]

Huang CK, Zhan L, Hannigan MO, Ai Y, Leto TL. P47(phox)-deficient NADPH oxidase defect in neutrophils of diabetic mouse strains, C57BL/6J-m db/db and db/+ J Leukoc Biol. 2000; 67:210–215. [PubMed: 10670582]

Hyodo F, Soule BP, Matsumoto K, Matusmoto S, Cook JA, Hyodo E, et al. Assessment of tissue redox status using metabolic responsive contrast agents and magnetic resonance imaging. J Pharm Pharmacol. 2008; 60:1049–1060. [PubMed: 18644197]

Iannone A, Tomasi A, Vannini V, Swartz HM. Metabolism of nitroxide spin labels in subcellular fraction of rat liver. I. Reduction by microsomes. Biochim Biophys Acta. 1990; 1034:285–289. [PubMed: 2114173]

Iglarz M, Touyz RM, Viel EC, Amiri F, Schiffrin EL. Involvement of oxidative stress in the profibrotic action of aldosterone: interaction with the renin-angiotensin system. Am J Hypertens. 2004; 17:597–603. [PubMed: 15243979]

Inaba S, Iwai M, Furuno M, Tomono Y, Kanno H, Senba I, et al. Continuous activation of renin-angiotensin system impairs cognitive function in renin/angiotensinogen transgenic mice. Hypertens. 2009; 53:356–362.

Izmaylov DM, Obukhova LK. Geroprotector efficiency depends on viability of control population: life span investigation in D. melanogaster. Mech Ageing Dev. 1996; 91:155–164. [PubMed: 9055239]

Jagadeesha DK, Lindley TE, Deleon J, Sharma RV, Miller F, Bhalla RC. Tempol therapy attenuates medial smooth muscle cell apoptosis and neointima formation after balloon catheter injury in carotid artery of diabetic rats. Am J Physiol Heart Circ Physiol. 2005; 289:H1047–H1053. [PubMed: 15833798]

Jagadeesha DK, Miller FJ Jr, Bhalla RC. Inhibition of Apoptotic Signaling and Neointimal Hyperplasia by Tempol and Nitric Oxide Synthase following Vascular Injury. J Vasc Res. 2009; 46:109–118. [PubMed: 18714161]

Jankov RP, Kantores C, Pan J, Belik J. Contribution of xanthine oxidase-derived superoxide to chronic hypoxic pulmonary hypertension in neonatal rats. Am J Physiol Lung Cell Mol Physiol. 2008; 294:L233–L245. [PubMed: 18083771]

Jayakumar AR, Liu M, Moriyama M, Ramakrishnan R, Forbush B III, Reddy PV, et al. Na-K-Cl cotransporter-1 in the mechanism of ammonia-induced astrocyte swelling. J Biol Chem. 2008; 283:33874–33882. [PubMed: 18849345]

Jia YT, Wei W, Ma B, Xu Y, Liu WJ, Wang Y, et al. Activation of p38 MAPK by reactive oxygen species is essential in a rat model of stress-induced gastric mucosal injury. J Immunol. 2007; 179:7808–7819. [PubMed: 18025227]

Jiang J, Belikova NA, Hoye AT, Zhao Q, Epperly MW, Greenberger JS, et al. A mitochondria-targeted nitroxide/hemigramicidin S conjugate protects mouse embryonic cells against gamma irradiation. Int J Radiat Oncol Biol Phys. 2008; 70:816–825. [PubMed: 18262096]

Jiang J, Kurnikov I, Belikova NA, Xiao J, Zhao Q, Amoscato AA, et al. Structural requirements for optimized delivery, inhibition of oxidative stress and anti-apoptotic activity of targeted nitroxides. J Pharmacol Exp Ther. 2007; 320:1050–1060. [PubMed: 17179468]

Johnstone PA, DeGraff WG, Mitchell JB. Protection from radiation-induced chromosomal aberrations by the nitroxide Tempol. Cancer. 1995; 75:2323–2327. [PubMed: 7712443]

Jordan SW, Key CR, Gomez LS, Agnew J, Barton SL. Late effects of radiation on the mouse kidney. Exp Mol Pathol. 1978; 29:115–129. [PubMed: 680088]

Kang YM, Ma Y, Elks C, Zheng JP, Yang ZM, Francis J. Cross-talk between cytokines and renin-angiotensin in hypothalamic paraventricular nucleus in heart failure: role of nuclear factor-kappaB. Cardiovasc Res. 2008; 79:671–678. [PubMed: 18469338]

Kang YM, Ma Y, Zheng JP, Elks C, Sriramula S, Yang ZM, et al. Brain nuclear factor-kappa B activation contributes to neurohumoral excitation in angiotensin II-induced hypertension. Cardiovasc Res. 2009; 82:503–512. [PubMed: 19246475]

Kantak SS, Diglio CA, Onoda JM. Low dose radiation-induced endothelial cell retraction. Int J Radiat Biol. 1993; 64:319–328. [PubMed: 8105010]

Karatas Y, Secilmis MA, Karayaylali I, Doran F, Buyukafsar K, Singirik E, et al. Effect of tempol (4-hydroxy tempo) on gentamicin-induced nephrotoxicity in rats. Fundam Clin Pharmacol. 2004; 18:79–83. [PubMed: 14748758]

Karmeli F, Eliakim R, Okon E, Rachmilewitz D. Ketotifen and nitroxides decrease capsaicin-augmented ethanol-induced gastric damage in rats. Dig Dis Sci. 1995; 40:1140–1146. [PubMed: 7729277]

Karmeli F, Okon E, Rachmilewitz D. Sulphydryl blocker induced gastric damage is ameliorated by scavenging of free radicals. Gut. 1996; 38:826–831. [PubMed: 8984018]

Kato N, Yanaka K, Hyodo K, Homma K, Nagase S, Nose T. Stable nitroxide Tempol ameliorates brain injury by inhibiting lipid peroxidation in a rat model of transient focal cerebral ischemia. Brain Res. 2003; 979:188–193. [PubMed: 12850585]

Kawada N, Imai E, Karber A, Welch WJ, Wilcox CS. A mouse model of angiotensin II slow pressor response: role of oxidative stress. J Am Soc Nephrol. 2002; 13:2860–2868. [PubMed: 12444204]

Kawai J, Ando K, Tojo A, Shimosawa T, Takahashi K, Onozato M, et al. Endogenous adrenomedullin protects against vascular respnse to injury in mice. Circ. 2004; 109:1147–1153.

Kelly FJ, Anderson S, Thompson MM, Oyama TT, Kennefick TM, Corless CL, et al. Acute and chronic renal effects of recombinant human TGF-beta2 in the rat. J Am Soc Nephrol. 1999; 10:1264–1273. [PubMed: 10361864]

Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, et al. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem. 2001; 276:4588–4596. [PubMed: 11092892]

Kennedy JA, Hua X, Mishra K, Murphy GA, Rosenkranz AC, Horowitz JD. Inhibition of calcifying nodule formation in cultured porcine aortic valve cells by nitric oxide donors. Eur J Pharmacol. 2009; 602:28–35. [PubMed: 19056377]

Kentner R, Safar P, Behringer W, Wu X, Henchir J, Ma L, et al. Small volume resuscitation with tempol is detrimental during uncontrolled hemorrhagic shock in rats. Resuscitation. 2007; 72:295–305. [PubMed: 17112648]

Kentner R, Safar P, Behringer W, Wu X, Kagan VE, Tyurina YY, et al. Early antioxidant therapy with Tempol during hemorrhagic shock increases survival in rats. J Trauma. 2002; 53:968–977. [PubMed: 12435951]

Khan S, O'Brien PJ. Molecular mechanisms of tirapazamine (SR 4233, Win 59075)-induced hepatocyte toxicity under low oxygen concentrations. Br J Cancer. 1995; 71:780–785. [PubMed: 7710944]

Kim HW, Lin A, Guldberg RE, Ushio-Fukai M, Fukai T. Essential role of extracellular SOD in reparative neovascularization induced by hindlimb ischemia. Circ Res. 2007; 101:409–419. [PubMed: 17601801]

Kim HY, Chung JM, Chung K. Increased production of mitochondrial superoxide in the spinal cord induces pain behaviors in mice: The effect of mitochondrial electron transport complex inhibitors. Neurosci Lett. 2008; 447:87–91. [PubMed: 18832013]

Kim-Mitsuyama S, Yamamoto E, Tanaka T, Zhan Y, Izumi Y, Izumiya Y, et al. Critical role of angiotensin II in excess salt-induced brain oxidative stress of stroke-prone spontaneously hypertensive rats. Stroke. 2005; 36:1083–1088. [PubMed: 15817892]

Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, et al. Mitochondria-derived reactive oxygen species and vascular MAP kinases: comparison of angiotensin II and diazoxide. Hypertens. 2005a; 45:438–444.

Kimura S, Zhang GX, Nishiyama A, Shokoji T, Yao L, Fan YY, et al. Role of NAD(P)H oxidase- and mitochondria-derived reactive oxygen species in cardioprotection of ischemic reperfusion injury by angiotensin II. Hypertens. 2005b; 45:860–866.

Kinugawa S, Huang H, Wang Z, Kaminski PM, Wolin MS, Hintze TH. A defect of neuronal nitric oxide synthase increases xanthine oxidase-derived superoxide anion and attenuates the control of myocardial oxygen consumption by nitric oxide derived from endothelial nitric oxide synthase. Circ Res. 2005a; 96:355–362. [PubMed: 15637297]

Kinugawa S, Post H, Kaminski PM, Zhang X, Xu Z, Huang H, et al. Coronary microvascular endothelial stunning after acute pressure overload in the conscious dog is caused by oxidant processes. Circ. 2003; 108:2940.

Kinugawa S, Wang Z, Kaminski PM, Wolin MS, Edwards JG, Kaley G, et al. Limited exercise capacity in heterozygous manganese superoxide dismutase gene-knockout mice: roles of superoxide anion and nitric oxide. Circ. 2005b; 111:1480–1486.

Kitayama J, Faraci FM, Lentz SR, Heistad DD. Cerebral vascular dysfunction during hypercholesterolemia. Stroke. 2007; 38:2136–2141. [PubMed: 17525390]

Knock GA, Snetkov VA, Shaifta Y, Connolly M, Drndarski S, Noah A, et al. Superoxide constricts rat pulmonary arteries via Rho-kinase-mediated Ca(2+) sensitization. Free Radic Biol Med. 2009; 46:633–642. [PubMed: 19103285]

Ko CH, Shen SC, Yang LY, Lin CW, Chen YC. Gossypol reduction of tumor growth through ROS-dependent mitochondria pathway in human colorectal carcinoma cells. Int J Cancer. 2007; 121:1670–1679. [PubMed: 17597109]

Kobayashi M, Sugiyama H, Wang DH, Toda N, Maeshima Y, Yamasaki Y, et al. Catalase deficiency renders remnant kidneys more susceptible to oxidant tissue injury and renal fibrosis in mice. Kidney Int. 2005; 68:1018–1031. [PubMed: 16105032]

Kobayashi N, Schmid-Schonbein GW. Intrinsic disturbance of cellular redox balance enhances blood lymphocyte apoptosis in the spontaneously hypertensive rat. Free Radic Biol Med. 2006; 41:484–492. [PubMed: 16843829]

Kondo S, Toyokuni S, Iwasa Y, Tanaka T, Onodera H, Hiai H, et al. Persistent oxidative stress in human colorectal carcinoma, but not in adenoma. Free Radic Biol Med. 1999; 27:401–410. [PubMed: 10468215]

Konovalova NP, D'iachkovskaia RF, Volkova LM, Varfolomeev VN. Nitroxyl radical Tempol as a modulator of toxic and antineoplastic effect of 6-mercaptopurine. Vopr Onkol. 1996a; 42:57–63. [PubMed: 8801743]

Konovalova NP, Diatchkovskaya RF, Volkova LM, Varfolomeev VN. Modulatory effect of tempol on toxicity and antitumor activity of 6-mercaptopurine and on P450 cytochrome level. Neoplasma. 1996b; 43:341–346. [PubMed: 8996555]

Koshiishi I, Takajo T, Tsuchida K. Regulation of S-thiolation and S-nitrosylation in the thiol/nitric oxide system by radical scavengers. Nitric Oxide. 2007; 16:356–361. [PubMed: 17293136]

Kotchen TA, Piering AW, Cowley AW, Grim CE, Gaudet D, Hamet P, et al. Glomerular hyperfiltration in hypertensive African Americans. Hypertens. 2000; 35:822–826.

Krishna MC, Samuni A. The effect of oxygen at physiological levels on the detection of free radical intermediates by electron paramagnetic resonance. Free Radic Res Commun. 1993; 18:239–247. [PubMed: 8396553]

Kroll C, Borchert HH. Metabolism of the stable nitroxyl radical 4-oxo-2,2,6, 6-tetramethylpiperidine-N-oxyl (TEMPONE). Eur J Pharm Sci. 1999; 8:5–9. [PubMed: 10072473]

Kroll C, Langner A, Borcic S. Nitroxide metabolism in the human keratinocyte cell line HaCaT. Free Rad Biol Med. 1999; 26:850–857. [PubMed: 10232828]

Kruglov AG, Subbotina KB, Saris NE. Redox-cycling compounds can cause the permeabilization of mitochondrial membranes by mechanisms other than ROS production. Free Radic Biol Med. 2008; 44:646–656. [PubMed: 18053818]

Kudo W, Yamato M, Yamada K, Kinoshita Y, Shiba T, Watanabe T, et al. Formation of TEMPOL-hydroxylamine during reaction between TEMPOL and hydroxyl radical: HPLC/ECD study. Free Radic Res. 2008; 42:505–512. [PubMed: 18484414]

Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol. 2003; 23:85–89. [PubMed: 12524229]

Kurihara N, Yanagisawa H, Sato M, Tien CK, Wada O. Increased renal vascular resistance in zinc-deficient rats: role of nitric oxide and superoxide. Clin Exp Pharmacol Physiol. 2002; 29:1096–1104. [PubMed: 12390298]

Kutala VK, Khan M, Mandal R, Potaraju V, Colantuono G, Kumbala D, et al. Prevention of postischemic myocardial reperfusion injury by the combined treatment of NCX-4016 and Tempol. J Cardiovasc Pharmacol. 2006; 48:79–87. [PubMed: 17031260]

Kwon TH, Chao DL, Malloy K, Sun D, Alessandri B, Bullock MR. Tempol, a novel stable nitroxide, reduces brain damage and free radical production, after acute subdural hematoma in the rat. J Neurotrauma. 2003; 20:337–345. [PubMed: 12866813]

Lam KK, Cheng PY, Hsiao G, Chen SY, Shen HH, Yen MH, et al. Estrogen deficiency-induced alterations of vascular MMP-2, MT1-MMP, and TIMP-2 in ovariectomized rats. Am J Hypertens. 2009; 22:27–34. [PubMed: 19023275]

Lambeth JD. Nox enzymes, ROS, and chronic disease: An example of antagonistic pleiotropy. Free Radic Biol Med. 2007; 43:332–347. [PubMed: 17602948]

Lang-Rollin IC, Rideout HJ, Noticewala M, Stefanis L. Mechanisms of caspase-independent neuronal death: energy depletion and free radical generation. J Neurosci. 2003; 23:11015–11025. [PubMed: 14657158]

Laskowski A, Woodman OL, Cao AH, Drummond GR, Marshall T, Kaye DM, et al. Antioxidant actions contribute to the antihypertrophic effects of atrial natriuretic peptide in neonatal rat cardiomyocytes. Cardiovasc Res. 2006; 72:112–123. [PubMed: 16890211]

Law MP. Radiation-induced vascular injury and its relation to late effects in normal tissues. Adv Radiat Biol. 1981; 9:37–73.

Laycock SK, Vogel T, Forfia PR, Tuzman J, Xu X, Ochoa M, et al. Role of nitric oxide in the control of renal oxygen consumption and the regulation of chemical work in the kidney. Circ Res. 1998; 82:1263–1271. [PubMed: 9648722]

Leach M, Frank S, Olbrich A, Pfeilschifter J, Thiemermann C. Decline in the expression of copper/zinc superoxide dismutase in the kidney of rats with endotoxic shock: effects of the superoxide anion radical scavenger, tempol, on organ injury. Br J Pharmacol. 1998; 125:817–825. [PubMed: 9831920]

Lee I, Kim HK, Kim JH, Chung K, Chung JM. The role of reactive oxygen species in capsaicin-induced mechanical hyperalgesia and in the activities of dorsal horn neurons. Pain. 2007; 133:9–17. [PubMed: 17379413]

Lejeune D, Hasanuzzaman M, Pitcock A, Francis J, Sehgal I. The superoxide scavenger TEMPOL induces urokinase receptor (uPAR) expression in human prostate cancer cells. Mol Cancer. 2006; 5:21. [PubMed: 16756681]

Leker RR, Teichner A, Lavie G, Shohami E, Lamensdorf I, Ovadia H. The nitroxide antioxidant tempol is cerebroprotective against focal cerebral ischemia in spontaneously hypertensive rats. Exp Neurol. 2002; 176:355–363. [PubMed: 12359177]

Lesniewski LA, Connell ML, Durrant JR, Folian BJ, Anderson MC, Donato AJ, et al. B6D2F1 Mice are a suitable model of oxidative stress-mediated impaired endothelium-dependent dilation with aging. J Gerontol A Biol Sci Med Sci. 2009; 64:9–20. [PubMed: 19211548]

Lewinska A, Wnuk M, Slota E, Bartosz G. The nitroxide antioxidant Tempol affects metal-induced cyto- and genotoxicity in human lymphocytes in vitro. Mutat Res. 2008; 649:7–14. [PubMed: 17950026]

Li H, Ma L, Hsia CJ, Zweier JL, Kuppusamy P. Polynitroxyl-albumin (PNA) enhances myocardial infarction therapeutic effect of tempol in rat hearts subjected to regional ischemia-reperfusion. Free Radic Biol Med. 2002; 32:712–719. [PubMed: 11937297]

Li J, Chen YJ, Quilley J. Effect of tempol on renal cyclooxygenase expression and activity in experimental diabetes in the rat. J Pharmacol Exp Ther. 2005; 314:818–824. [PubMed: 15879008]

Li P, Wang D, Onozato M, Welch WJ, Wilcox CS. Tempol attenuates the development of salt-induced hypertension in rats with chronic renal failure. J Am Soc Nephrol Abstract. 2008; 19:376A.

Li WG, Zhang XY, Wu YJ, Gao MT, Zheng RL. The relationship between structure and antioxidative activity of piperidine nitroxides. J Pharm Pharmacol. 2006; 58:941–949. [PubMed: 16805954]

Li YL, Gao L, Zucker IH, Schultz HD. NADPH oxidase-derived superoxide anion mediates angiotensin II-enhanced carotid body chemoreceptor sensitivity in heart failure rabbits. Cardiovasc Res. 2007; 75:546–554. [PubMed: 17499230]

Liang Q, Smith AD, Pan S, Tyurin VA, Kagan VE, Hastings TG, et al. Neuroprotective effects of TEMPOL in central and peripheral nervous system models of Parkinson's disease. Biochem Pharmacol. 2005; 70:1371–1381. [PubMed: 16144694]

Liaw WJ, Chen TH, Lai ZZ, Chen SJ, Chen A, Tzao C, et al. Effects of a membrane-permeable radical scavenger, Tempol, on intraperitoneal sepsis-induced organ injury in rats. Shock. 2005; 23:88–96. [PubMed: 15614137]

Liebmann J, DeLuca AM, Epstein A, Steinberg SM, Morstyn G, Mitchell JB. Protection from lethal irradiation by the combination of stem cell factor and tempol. Radiat Res. 1994; 137:400–404. [PubMed: 7511822]

Lijnen P, Petrov V, van PJ, Fagard R. Inhibition of superoxide dismutase induces collagen production in cardiac fibroblasts. Am J Hypertens. 2008; 21:1129–1136. [PubMed: 18756259]

Linares E, Giorgio S, Augusto O. Inhibition of in vivo leishmanicidal mechanisms by tempol: Nitric oxide down-regulation and oxidant scavenging. Free Radic Biol Med. 2008; 44:1668–1676. [PubMed: 18313408]

Liu D, Gao L, Roy SK, Cornish KG, Zucker IH. Role of oxidant stress on AT1 receptor expression in neurons of rabbits with heart failure and in cultured neurons. Circ Res. 2008; 103:186–193. [PubMed: 18566341]

Longley DB, Johnston PG. Molecular mechanisms of drug resistance. J Pathol. 2005; 205:275–292. [PubMed: 15641020]

Lund AK, Lucero J, Lucas S, Madden MC, McDonald JD, Seagrave JC, et al. Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways. Arterioscler Thromb Vasc Biol. 2009a; 29:511–517. [PubMed: 19150882]

Lund DD, Chu Y, Miller JD, Heistad DD. Protective Effect of Extracellular Superoxide Dismutase on Endothelial Function During Aging. Am J Physiol Heart Circ Physiol. 2009b; 296:H1920–H1925. [PubMed: 19376805]

Luo Z, Chen Y, Chen S, Welch WJ, Andresen BT, Jose PA, et al. Comparison of inhibitors of superoxide generation in vascular smooth muscle cells. Br J Pharmacol. 2009; 157:935–943. [PubMed: 19466991]

MacGregor DG, Avshalumov MV, Rice ME. Brain edema induced by in vitro ischemia: causal factors and neuroprotection. J Neurochem. 2003; 85:1402–1411. [PubMed: 12787060]

Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, et al. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circ. 2003; 107:2775–2779.

Mara L, Accardo C, Pilichi S, Dattena M, Chessa F, Chessa B, et al. Benefits of TEMPOL on ram semen motility and in vitro fertility: a preliminary study. Theriogenology. 2005; 63:2243–2253. [PubMed: 15826687]

Mara L, Dattena M, Pilichi S, Sanna D, Branca A, Cappai P. Effect of different diluents on goat semen fertility. Anim Reprod Sci. 2007; 102:152–157. [PubMed: 17383124]

Mariappan N, Elks CM, Fink B, Francis J. TNF-induced mitochondrial damage: a link between mitochondrial complex I activity and left ventricular dysfunction. Free Radic Biol Med. 2009; 46:462–470. [PubMed: 19041937]

Mariappan N, Soorappan RN, Haque M, Sriramula S, Francis J. TNF-{alpha}-induced mitochondrial oxidative stress and cardiac dysfunction: restoration by superoxide dismutase mimetic Tempol. Am J Physiol Heart Circ Physiol. 2007; 293:H2726–H2737. [PubMed: 17675574]

Martin V, Herrera F, Garcia-Santos G, Antolin I, Rodriguez-Blanco J, Rodriguez C. Signaling pathways involved in antioxidant control of glioma cell proliferation. Free Radic Biol Med. 2007; 42:1715–1722. [PubMed: 17462539]

Marwaha A, Lokhandwala MF. Tempol reduces oxidative stress and restores renal dopamine D1-like receptor- G protein coupling and function in hyperglycemic rats. Am J Physiol Renal Physiol. 2006; 291:F58–F66. [PubMed: 16478977]

Matejovic M, Krouzecky A, Rokyta R Jr, Radej J, Kralova H, Treska V, et al. Effects of combining inducible nitric oxide synthase inhibitor and radical scavenger during porcine bacteremia. Shock. 2007; 27:61–68. [PubMed: 17172982]

Matsuhashi T, Karbowski M, Liu X, Usukura J, Wozniak M, Wakabayashi T. Complete suppresion of ethanol-induced formation of megamitochondria by 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-OH-TEMPO). Free Radic Biol Med. 1998; 24:139–147. [PubMed: 9436623]

Matsui H, Ando K, Kawarazaki H, Nagae A, Fujita M, Shimosawa T, et al. Salt excess causes left ventricular diastolic dysfunction in rats with metabolic disorder. Hypertens. 2008; 52:287–294.

Matsui H, Shimosawa T, Itakura K, Guanqun X, Ando K, Fujita T. Adrenomedullin can protect against pulmonary vascular remodeling induced by hypoxia. Circ. 2004; 109:2246–2251.

Matsumoto K, Krishna MC, Mitchell JB. Novel pharmacokinetic measurement using electron paramagnetic resonance spectroscopy and simulation of in vivo decay of various nitroxyl spin probes in mouse blood. J Pharmacol Exp Ther. 2004; 310:1076–1083. [PubMed: 15105413]

Matthews RT, Klivenyi P, Mueller G, Yang L, Wermer M, Thomas CE, et al. Novel free radical spin traps protect against malonate and MPTP neurotoxicity. Exp Neurol. 1999; 157:120–126. [PubMed: 10222114]

Maxwell RA, Gibson JB, Fabian TC, Proctor KG. Effects of a novel antioxidant during resuscitation from severe blunt chest trauma. Shock. 2000; 14:646–651. [PubMed: 11131916]

May JM, Qu ZC, Mendiratta S. Protection and recycling of alpha-tocopherol in human erythrocytes by intracellular ascorbic acid. Arch Biochem Biophys. 1998; 349:281–289. [PubMed: 9448716]

Mayhan WG, Arrick DM, Sharpe GM, Sun H. Age-related alterations in reactivity of cerebral arterioles: role of oxidative stress. Microcirculation. 2008; 15:225–236. [PubMed: 18386218]

McCormick J, Barry SP, Sivarajah A, Stefanutti G, Townsend PA, Lawrence KM, et al. Free radical scavenging inhibits STAT phosphorylation following in vivo ischemia/reperfusion injury. Faseb J. 2006; 20:2115–2117. [PubMed: 16935931]

McDonald MC, Zacharowski K, Bowes J, Cuzzocrea S, Thiemermann C. Tempol reduces infarct size in rodent models of regional myocardial ischemia and reperfusion. Free Rad Biol Med. 1999; 27:493–503. [PubMed: 10490268]

Mehta SH, Webb RC, Ergul A, Tawfik A, Dorrance AM. Neuroprotection by tempol in a model of iron-induced oxidative stress in acute ischemic stroke. Am J Physiol Regul Integr Comp Physiol. 2004; 286:R283–R288. [PubMed: 14592931]

Meng S, Cason GW, Gannon AW, Racusen LC, Manning RD Jr. Oxidative stress in Dahl salt-sensitive hypertension. Hypertens. 2003; 41:1346–1352.

Merenda A, Gugliotta M, Holloway R, Levasseur JE, Alessandri B, Sun D, et al. Validation of brain extracellular glycerol as an indicator of cellular membrane damage due to free radical activity after traumatic brain injury. J Neurotrauma. 2008; 25:527–537. [PubMed: 18363507]

Metodiewa D, Skolimowski J, Kochman A, Koceva-Chyla A. The paradoxical apoptotic effects of novel nitroxide antioxidants on Yoshida sarcoma cells in vivo: a commentary. Anticancer Res. 2000; 20:2593–2599. [PubMed: 10953331]

Metz JM, Smith D, Mick R, Lustig R, Mitchell J, Cherakuri M, et al. A phase I study of topical Tempol for the prevention of alopecia induced by whole brain radiotherapy. Clin Cancer Res. 2004; 10:6411–6417. [PubMed: 15475427]

Millar BC, Fielden EM, Smithen CE. Polyfunctional radiosensitizers III. Effect of the biradical (Ro-03-303) in combination with other radiosensitizers on the survival of hypoxic V-79 cells. Radiat Res. 1977; 69:489–499. [PubMed: 847098]

Millar BC, Fielden EM, Smithen CE. Polyfunctional radiosensitizers IV. The effect of contact time and temperature on sensitization of hypoxic Chinese hamster cells in vitro by bifunctional nitroxyl compounds. Br J Cancer Suppl. 1978; 3:73–79. [PubMed: 277261]

Millar BC, Jenkins TC, Fielden EM, Jinks S. Polyfunctional Radiosensitizers. VI. Dexamethasone inhibits shoulder modification by uncharged nitroxyl biradicals in mammalian cells irradiated in vitro. Radiat Res. 1983; 96:160–172. [PubMed: 6622648]

Millar BC, Jenkins TC, Smithen CE, Jinks S. Polyfunctional radiosensitizers. VII. Radiosensitization by conformationally-restricted isomers of a nitroxyl biradical in vitro. Radiat Res. 1985; 101:111–122. [PubMed: 3969438]

Miller SJ, Norton LE, Murphy MP, Dalsing MC, Unthank JL. The role of the renin-angiotensin system and oxidative stress in spontaneously hypertensive rat mesenteric collateral growth impairment. Am J Physiol Heart Circ Physiol. 2007; 292:H2523–H2531. [PubMed: 17277018]

Minami SB, Yamashita D, Ogawa K, Schacht J, Miller JM. Creatine and tempol attenuate noise-induced hearing loss. Brain Res. 2007; 1148:83–89. [PubMed: 17359945]

Mitchell JB, De Graff W, Kaufman D, Krishna MC, Samuni A, Finkelstein E, et al. Inhibition of oxygen-dependent radiation-induced damage by the nitroxide superoxide dismutase mimetic, tempol. Arch Biochem Biophys. 1991; 289:62–70. [PubMed: 1654848]

Mitchell JB, Xavier S, DeLuca AM, Sowers AL, Cook JA, Krishna MC, et al. A low molecular weight antioxidant decreases weight and lowers tumor incidence. Free Rad Biol Med. 2003; 34:93–102. [PubMed: 12498984]

Miura Y, Anzai K, Ueda J, Ozawa T. Novel approach to in vivo screening for radioprotective activity in whole mice: in vivo electron spin resonance study probing the redox reaction of nitroxyl. J Radiat Res (Tokyo). 2000; 41:103–111. [PubMed: 11037578]

Miyachi Y. Photoaging from an oxidative standpoint. J Dermatol Sci. 1995; 9:79–86. [PubMed: 7772578]

Modlinger PS, Wilcox CS, Aslam S. Nitric oxide, oxidative stress and progression of chronic renal failure. Semin Nephrol. 2004; 24:354–365. [PubMed: 15252775]

Modrick ML, Didion SP, Sigmund CD, Faraci FM. Role of Oxidative Stress and AT1 Receptors in Cerebral Vascular Dysfunction with Aging. Am J Physiol Heart Circ Physiol. 2009; 296:H1914–H1919. [PubMed: 19395552]

Moens AL, Takimoto E, Tocchetti CG, Chakir K, Bedja D, Cormaci G, et al. Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy. Circ. 2008; 117:2626–2636.

Monti E, Cova D, Guido E, Morelli R, Oliva C. Protective effect of the nitroxide tempol against the cardiotoxicity of adriamycin. Free Radic Biol Med. 1996; 21:463–470. [PubMed: 8886796]

Monti E, Supino R, Colleoni M, Costa B, Ravizza R, Gariboldi MB. Nitroxide TEMPOL impairs mitochondrial function and induces apoptosis in HL60 cells. J Cell Biochem. 2001; 82:271–276. [PubMed: 11527152]

Moosmann B, Behl C. Antioxidants as treatment for neurodegenerative disorders. Expert Opin Investig Drugs. 2002; 11:1407–1435.

Moreno JM, Rodriguez Gomez I, Wangensteen R, Osuna A, Bueno P, Vargas F. Cardiac and renal antioxidant enzymes and effects of tempol in hyperthyroid rats. Am J Physiol. 2005; 289:E776–E783.

Mota-Filipe H, McDonald MC, Cuzzocrea S, Thiemermann C. A membrane-permeable radical scavenger reduces the organ injury in hemorrhagic shock. Shock. 1999; 12:255–261. [PubMed: 10509626]

Muhl H, Sandau K, Brune B, Briner VA, Pfeilschifter J. Nitric oxide donors induce apoptosis in glomerular mesangial cells, epithelial cells and endothelial cells. Eur J Pharmacol. 1996; 317:137–149. [PubMed: 8982730]

Murashita H, Tabuchi K, Hoshino T, Tsuji S, Hara A. The effects of tempol, 3-aminobenzamide and nitric oxide synthase inhibitors on acoustic injury of the mouse cochlea. Hear Res. 2006; 214:1–6. [PubMed: 16516419]

Murphy MP. Selective targeting of bioactive compounds to mitochondria. Trends Biotechnol. 1997; 15:326–330. [PubMed: 9263481]

Murphy MP, Echtay KS, Blaikie FH, sin-Cayuela J, Cocheme HM, Green K, et al. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. J Biol Chem. 2003; 278:48534–48545. [PubMed: 12972420]

Nagase M, Fujita T. Aldosterone and glomerular podocyte injury. Clin Exp Nephrol. 2008; 12:233–242. [PubMed: 18317876]

Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxical activation of the mineralocorticoid receptor: role of oxidative stress. Hypertens. 2007; 50:877–883.

Nagase M, Yoshida S, Shibata S, Nagase T, Gotoda T, Ando K, et al. Enhanced aldosterone signaling in the early nephropathy of rats with metabolic syndrome: possible contribution of fat-derived factors. J Am Soc Nephrol. 2006; 17:3438–3446. [PubMed: 17082236]

Naghibi B, Ghafghazi T, Hajhashemi V, Talebi A, Taheri D. The effect of 2,3-dihydroxybenzoic acid and tempol in prevention of vancomycin-induced nephrotoxicity in rats. Toxicology. 2007; 232:192–199. [PubMed: 17296255]

Nakamura T, Funayama H, Kubo N, Yasu T, Kawakami M, Saito M, et al. Association of hyperadiponectinemia with severity of ventricular dysfunction in congestive heart failure. Circ J. 2006; 70:1557–1562. [PubMed: 17127799]

Nassar T, Kadery B, Lotan C, Da'as N, Kleinman Y, Haj-Yehia A. Effects of the superoxide dismutase-mimetic compound tempol on endothelial dysfunction in streptozotocin-induced diabetic rats. Eur J Pharmacol. 2002; 436:111–118. [PubMed: 11834254]

Neckar J, Ostadal B, Kolar F. Acute but not chronic tempol treatment increases ischemic and reperfusion ventricular arrhythmias in open-chest rats. Physiol Res. 2008; 57:653–656. [PubMed: 18783306]

Newby AC. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. Cardiovasc Res. 2006; 69:614–624. [PubMed: 16266693]

Nicolakakis N, Aboulkassim T, Ongali B, Lecrux C, Fernandes P, Rosa-Neto P, et al. Complete rescue of cerebrovascular function in aged Alzheimer's disease transgenic mice by antioxidants and pioglitazone, a peroxisome proliferator-activated receptor gamma agonist. J Neurosci. 2008; 28:9287–9296. [PubMed: 18784309]

Niknahad H, Khan S, O'Brien PJ. Hepatocyte injury resulting from the inhibition of mitochondrial respiration at low oxygen concentrations involves reductive stress and oxygen activation. Chem Biol Interact. 1995; 98:27–44. [PubMed: 7586049]

Nishiyama A, Abe Y. Aldosterone and renal injury. Nippon Yakurigaku Zasshi. 2004; 124:101–109. [PubMed: 15277728]

Nishiyama A, Yao L, Nagai Y, Miyata K, Yoshizumi M, Kagami S, et al. Possible contributions of reactive oxygen species and mitogen-activated protein kinase to renal injury in aldosterone/salt-induced hypertensive rats. Hypertens. 2004a; 24:841–848.

Nishiyama A, Yoshizumi M, Hitomi H, Kagami S, Kondo S, Miyatake A, et al. The SOD mimetic tempol ameliorates glomerular injury and reduces mitogen-activated protein kinase activity in Dahl salt-sensitive rats. J Am Soc Nephrol. 2004b; 15:306–315. [PubMed: 14747377]

Nouri P, Chen Y, Wilcox CS. Tempol prevents the attenuation of endothelium-dependent relaxation response to acetylcholine caused by intravenous injection of iron dextran. J Am Soc Nephrol Abstract. 2007; 18:638A.

Ogihara T, Asano T, Ando K, Chiba Y, Sakoda H, Anai M, et al. Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. Hypertens. 2002; 40:872–879.

Oliveira-Sales EB, Nishi EE, Carillo BA, Boim MA, Dolnikoff MS, Bergamaschi CT, et al. Oxidative stress in the sympathetic premotor neurons contributes to sympathetic activation in renovascular hypertension. Am J Hypertens. 2009; 22:484–492. [PubMed: 19229193]

Onozato ML, Tojo A, Leiper J, Fujita T, Palm F, Wilcox CS. Expression of DDAH and PRMT isoforms in the diabetic rat kidney; effects of angiotensin II receptor blockers. Diabetes. 2008; 57:172–180. [PubMed: 17909098]

Onuma S, Nakanishi K. Superoxide dismustase mimetic tempol decreases blood pressure by increasing renal medullary blood flow in hyperinsulinemic-hypertensive rats. Metabolism. 2004; 53:1305–1308. [PubMed: 15375786]

Oprescu AI, Bikopoulos G, Naassan A, Allister E, Tang C, Park E, et al. Free fatty acid-induced reduction in glucose stimulated insulin secretion evidence for a role of oxidative stress in vitro and in vivo. Diabetes. 2007; 56:2927–2937. [PubMed: 17717282]

Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circ. 2001; 103:1057–1063.

Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circ. 2000; 102:1296–1301.

Palayoor ST, Bump EA, Malaker K, Langley RE, Saroff DM, Delfs JR, et al. Modification of the aerobic cytotoxicity of etanidazole. Int J Radiat Oncol Biol Phys. 1994; 29:289–293. [PubMed: 8195021]

Palm F, Cederberg J, Hansell P, Liss P, Carlsson PO. Reactive oxygen species cause diabetes-induced decrease in renal oxygen tension. Diabetologia. 2003; 46:1153–1160. [PubMed: 12879251]

Palm F, Connors S, Mendonca M, Welch WJ, Wilcox CS. Angiotensin II type 2 receptors and nitric oxide sustain oxygenation in the clipped kidney of early Goldblatt hypertensive rats. Hypertens. 2008; 51:345–351.

Paolocci N, Ekelund UE, Isoda T, Ozaki M, Vandegaer K, Georgakopoulos D, et al. cGMP-independent inotropic effects of nitric oxide and peroxynitrite donors: potential role for nitrosylation. Am J Physiol Heart Circ Physiol. 2000; 279:H1982–H1988. [PubMed: 11009488]

Park JB, Touyz RM, Chen X, Schiffrin EL. Chronic treatment with a superoxide dismutase mimetic prevents vascular remodeling and progression of hypertension in salt-loaded stroke-prone spontaneously hypertensive rats. Am J Hypertens. 2002a; 15:78–84. [PubMed: 11824865]

Park JH, Ma L, Oshima T, Carter P, Coe L, Ma JW, et al. Polynitroxylated starch/TPL attenuates cachexia and increased epithelial permeability associated with TNBS colitis. Inflammation. 2002b; 26:1–11. [PubMed: 11936750]

Patel K, Chen Y, Dennehy K, Blau J, Mendonca M, Tarpey M, et al. Acute antihypertensive action of nitroxides in the spontaneously hypertensive rat. Am J Physiol Regul Integr Comp Physiol. 2006; 290:R37–R43. [PubMed: 16179488]

Patel NS, Chatterjee PK, Chatterjee BE, Cuzzocrea S, Serraino I, Brown PA, et al. TEMPONE reduces renal dysfunction and injury mediated by oxidative stress of the rat kidney. Free Radic Biol Med. 2002; 33:1575–1589. [PubMed: 12446215]

Patel SP, Sullivan PG, Pandya JD, Rabchevsky AG. Differential effects of the mitochondrial uncoupling agent, 2,4-dinitrophenol, or the nitroxide antioxidant, Tempol, on synaptic or nonsynaptic mitochondria after spinal cord injury. J Neurosci Res. 2009; 87:130–140. [PubMed: 18709657]

Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. Physiol Rev. 2003; 83:253–307. [PubMed: 12506131]

Paxian M, Bauer I, Kaplan D, Bauer M, Rensing H. Hepatic redox regulation of transcription factors activator protein-1 and nuclear factor-kappaB after hemorrhagic shock in vivo. Antioxid Redox Signal. 2002; 4:711–720. [PubMed: 12470498]

Paxian M, Bauer I, Rensing H, Jaeschke H, Mautes AE, Kolb SA, et al. Recovery of hepatocellular ATP and “pericentral apoptosis” after hemorrhage and resuscitation. FASEB J. 2003; 17:993–1002. [PubMed: 12773482]

Payne JA, Reckelhoff JF, Khalil RA. Role of oxidative stress in age-related reduction of NO-cGMP-mediated vascular relaxation in SHR. Am J Physiol Regul Integr Comp Physiol. 2003; 285:R542–R551. [PubMed: 12816742]

Peixoto EB, Pessoa BS, Biswas SK, Lopes de Faria JB. Antioxidant SOD Mimetic Prevents NADPH Oxidase-Induced Oxidative Stress and Renal Damage in the Early Stage of Experimental Diabetes and Hypertension. Am J Nephrol. 2009; 29:309–318. [PubMed: 18849601]

Pence BC, Naylor MF. Effects of single-dose ultraviolet radiation on skin superoxide dismutase, catalase, and xanthine oxidase in hairless mice. J Invest Dermatol. 1990; 95:213–216. [PubMed: 2380580]

Peng T, Lu X, Feng Q. Pivotal role of gp91phox-containing NADH oxidase in lipopolysaccharide-induced tumor necrosis factor-alpha expression and myocardial depression. Circ. 2005; 111:1637–1644.

Pfister SL. Aortic thromboxane receptor deficiency alters vascular reactivity in cholesterol-fed rabbits. Atherosclerosis. 2006; 189:358–363. [PubMed: 16515789]

Phillips SA, Sylvester FA, Frisbee JC. Oxidant stress and constrictor reactivity impair cerebral artery dilation in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol. 2005; 288:R522–R530. [PubMed: 15514104]

Piecha G, Koleganova N, Gross ML, Geldyyev A, Ritz E. Oxidative stress after uninephrectomy alters heart morphology in the apolipoprotein E -/- mouse. J Hypertens. 2008; 26:2220–2229. [PubMed: 18854764]

Poderoso JJ, Carreras MC, Lisdero C, Riobo N, Schopfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. Arch Biochem Biophys. 1996; 328:85–92. [PubMed: 8638942]

Pogrebniak H, Matthews W, Mitchell J, Russo A, Samuni A, Pass H. Spin trap protection from tumor necrosis factor cytotoxicity. J Surg Res. 1991; 50:469–474. [PubMed: 2038186]

Polytarchou C, Kardamakis D, Katsoris P, Papadimitriou E. Antioxidants modify the effect of X rays on blood vessels. Anticancer Res. 2006; 26:3043–3047. [PubMed: 16886632]

Polytarchou C, Papadimitriou E. Antioxidants inhibit angiogenesis in vivo through down-regulation of nitric oxide synthase expression and activity. Free Radic Res. 2004; 38:501–508. [PubMed: 15293558]

Polytarchou C, Papadimitriou E. Antioxidants inhibit human endothelial cell functions through down-regulation of endothelial nitric oxide synthase activity. Eur J Pharmacol. 2005; 510:31–38. [PubMed: 15740722]

Pourahmad J, O'Brien PJ, Chan K, Shakouri A. Tetramethylphenylenediamine-induced hepatocyte cytotoxicity caused by lysosomal labilisation and redox cycling with oxygen activation. Chem Biol Interact. 2008; 172:39–47. [PubMed: 18221936]

Prieto M, Alonso G. Differential sensitivity of cultured tanycytes and astrocytes to hydrogen peroxide toxicity. Exp Neurol. 1999; 155:118–127. [PubMed: 9918711]

Purpura P, Westman L, Will P, Eidelman A, Kagan VE, Osipov AN, et al. Adjunctive treatment of murine neuroblastoma with 6-hydroxydopamine and tempol. Cancer Res. 1996; 56:2336–2342. [PubMed: 8625308]

Qi S, den Hartog GJ, Bast A. Superoxide radicals increase transforming growth factor-beta1 and collagen release from human lung fibroblasts via cellular influx through chloride channels. Toxicol Appl Pharmacol. 2009; 237:111–118. [PubMed: 19268487]

Quiroz Y, Ferrebuz A, Vaziri ND, Rodriguez-Iturbe B. Effect of chronic antioxidant therapy with superoxide dismutase-mimetic drug, tempol, on progression of renal disease in rats with renal mass reduction. Nephron Exp Nephrol. 2009; 112:e31–e42. [PubMed: 19342872]

Rabbani SA, Xing RH. Role of urokinase (uPA) and its receptor (uPAR) in invasion and metastasis of hormone-dependent malignancies. Int J Oncol. 1998; 12:911–920. [PubMed: 9499455]

Rachmilewitz D, Karmeli F, Okon E, Samuni A. A novel antiulcerogenic stable radical prevents gastric mucosal lesions in rats. Gut. 1994; 35:1181–1188. [PubMed: 7959222]

Rachmilewitz D, Okon E, Karmeli F. Sulphydryl blocker induced small intestinal inflammation in rats:a new model mimicking Crohn's disease. Gut. 1997; 41:358–365. [PubMed: 9378392]

Rafikova O, Salah EM, Tofovic SP. Renal and metabolic effects of tempol in obese ZSF1 rats--distinct role for superoxide and hydrogen peroxide in diabetic renal injury. Metabolism. 2008; 57:1434–1444. [PubMed: 18803950]

Rai A, Riemann M, Gustafsson F, Holstein-Rathlou NH, Torp-Pedersen C. Streptozotocin-induced diabetes decreases conducted vasoconstrictor response in mouse cremaster arterioles. Horm Metab Res. 2008; 40:651–654. [PubMed: 18792878]

Rak R, Chao DL, Pluta RM, Mitchell JB, Oldfield EH, Watson JC. Neuroprotection by the stable nitroxide Tempol during reperfusion in a rat model of transient focal ischemia. J Neurosurg. 2000; 92:646–651. [PubMed: 10761655]

Ravizza R, Cereda E, Monti E, Gariboldi MB. The piperidine nitroxide Tempol potentiates the cytotoxic effects of temozolomide in human glioblastoma cells. Int J Oncol. 2004a; 25:1817–1822. [PubMed: 15547722]

Ravizza R, Gariboldi MB, Passarelli L, Monti E. Role of the p53/p21 system in the response of human colon carcinoma cells to Doxorubicin. BMC Cancer. 2004b; 4:92. [PubMed: 15601469]

Reddan JR, Giblin FJ, Kadry R, Leverenz VR, Pena JT, Dziedzic DC. Protection from oxidative insult in glutathione depleted lens epithelial cells. Exp Eye Res. 1999; 68:117–127. [PubMed: 9986749]

Reliene R, Fleming SM, Chesselet MF, Schiestl RH. Effects of antioxidants on cancer prevention and neuromotor performance in Atm deficient mice. Food Chem Toxicol. 2008; 46:1371–1377. [PubMed: 18037553]

Reliene R, Schiestl RH. Antioxidants suppress lymphoma and increase longevity in atm-deficient mice. J Nutr. 2007; 137:229S–232S. [PubMed: 17182831]

Riazi S, Tiwari S, Sharma N, Rash A, Ecelbarger CM. Abundance of the Na-K-2Cl cotransporter NKCC2 is increased by high-fat feeding in Fischer 344 X Brown Norway (F1) rats. Am J Physiol Renal Physiol. 2009; 296:F762–F770. [PubMed: 19193725]

Rosenberger C, Khamaisi M, Abassi Z, Shilo V, Weksler-Zangen S, Goldfarb M, et al. Adaptation to hypoxia in the diabetic rat kidney. Kidney Int. 2008; 73:34–42. [PubMed: 17914354]

Ryu S, Kohen R, Samuni A, Ornoy A. Nitroxide radicals protect cultured rat embryos and yolk sacs from diabetic-induced damage. Birth Defects Res A Clin Mol Teratol. 2007; 79:604–611. [PubMed: 17626261]

Saito K, Takeshita K, Ueda J, Ozawa T. Two reaction sites of a spin label, TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), with hydroxyl radical. J Pharm Sci. 2003; 92:275–280. [PubMed: 12532377]

Sakai N, Mizuno R, Ono N, Kato H, Ohhashi T. High oxygen tension constricts epineurial arterioles of the rat sciatic nerve via reactive oxygen species. Am J Physiol Heart Circ Physiol. 2007; 293:H1498–H1507. [PubMed: 17513489]

Samai M, Sharpe MA, Gard PR, Chatterjee PK. Comparison of the effects of the superoxide dismutase mimetics EUK-134 and tempol on paraquat-induced nephrotoxicity. Free Radic Biol Med. 2007; 43:528–534. [PubMed: 17640563]

Samuni A, Karmeli F, Moshen M, Rachmilewitz D. Mechanisms underlying gastric antiulcerative activity of nitroxides in rats. Free Radic Res. 1999; 30:133–140. [PubMed: 10193581]

Samuni AM, Barenholz Y. Stable nitroxide radicals protect lipid acyl chains from radiation damage. Free Radic Biol Med. 1997; 22:1165–1174. [PubMed: 9098090]

Samuni AM, Barenholz Y, Crommelin DJ, Zuidam NJ. Gamma-irradiation damage to liposomes differing in composition and their protection by nitroxides. Free Radic Biol Med. 1997; 23:972–979. [PubMed: 9358239]

Samuni AM, DeGraff W, Cook JA, Krishna MC, Russo A, Mitchell JB. The effects of antioxidants on radiation-induced apoptosis pathways in TK6 cells. Free Radic Biol Med. 2004a; 37:1648–1655. [PubMed: 15477016]

Samuni AM, DeGraff W, Krishna MC, Mitchell JB. Nitroxides as antioxidants: Tempol protects against EO9 cytotoxicity. Molecular and Cellular Biochemistry. 2002; 234/235:327–333. [PubMed: 12162451]

Samuni Y, Gamson J, Samuni A, Yamada K, Russo A, Krishna MC, et al. Factors influencing nitroxide reduction and cytotoxicity in vitro. Antioxid Redox Signal. 2004b; 6:587–595. [PubMed: 15130285]

San Martin A, Du P, Dikalova A, Lassegue B, Aleman M, Gongora MC, et al. Reactive oxygen species-selective regulation of aortic inflammatory gene expression in type 2 diabetes. Am J Physiol Heart Circ Physiol. 2007; 292:H2073–H2082. [PubMed: 17237245]

Sanchez-Lozada LG, Soto V, Tapia E, vila-Casado C, Sautin YY, Nakagawa T, et al. Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia. Am J Physiol Renal Physiol. 2008; 295:F1134–F1141. [PubMed: 18701632]

Sasaki H, Lin LR, Yokoyama T, Sevilla MD, Reddy VN, Giblin FJ. TEMPOL protects against lens DNA strand breaks and cataract in the x-rayed rabbit. Invest Ophthalmol Vis Sci. 1998; 39:544–552. [PubMed: 9501865]

Schiffrin EL. Effects of antihypertensive drugs on vascular remodeling: do they predict outcome in response to antihypertensive therapy? Curr Opin Neph Hypertens. 2001; 10:617–624.

Schiffrin EL. Remodeling of resistance arteries in essential hypertension and effects of antihypertensive treatment. Am J Hypertens. 2004; 17:1192–1200. [PubMed: 15607629]

Schild L, Reiser G. Oxidative stress is involved in the permeabilization of the inner membrane of brain mitochondria exposed to hypoxia/reoxygenation and low micromolar Ca2+ FEBS J. 2005; 272:3593–3601. [PubMed: 16008559]

Schnackenberg C, Wilcox CS. Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Isoprostaglandin F_{2a}. Hypertens. 1999; 33:424–428.

Schnackenberg CG, Welch WJ, Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. Hypertens. 1998; 32:59–64.

Schnackenberg CG, Welch WJ, Wilcox CS. TP receptor-mediated vasoconstriction in microperfused afferent arterioles: role O_2^- and NO. Am J Physiol. 2000; 48:F302–F308.

Schnackenberg CG, Wilcox CS. The SOD mimetic tempol restores vasodilation in afferent arterioles of experimental diabetes. Kidney Int. 2001; 59:1859–1864. [PubMed: 11318957]

Schor NF, Kagan VE, Liang Y, Yan C, Tyurina Y, Tyurin V, et al. Exploiting oxidative stress and signaling in chemotherapy of resistant neoplasms. Biochemistry (Mosc). 2004; 69:38–44. [PubMed: 14972016]

Schrammel A, Gorren AC, Schmidt K, Pfeiffer S, Mayer B. S-nitrosation of glutathione by nitric oxide, peroxynitrite, and $\cdot NO/O_2\cdot^-$ Free Radic Biol Med. 2003; 34:1078–1088. [PubMed: 12684093]

Schubert R, Erker L, Barlow C, Yakushiji H, Larson D, Russo A, et al. Cancer chemoprevention by the antioxidant tempol in Atm-deficient mice. Hum Mol Genet. 2004; 13:1793–1802. [PubMed: 15213104]

Schwartz E, Samuni A, Friedman I, Hempelmann E, Golenser J. The role of superoxide dismutation in malaria parasites. Inflammation. 1999; 23:361–370. [PubMed: 10443798]

Schwartz ES, Lee I, Chung K, Chung JM. Oxidative stress in the spinal cord is an important contributor in capsaicin-induced mechanical secondary hyperalgesia in mice. Pain. 2008; 138:514–524. [PubMed: 18375065]

Senturker S, Tschirret-Guth R, Morrow J, Levine R, Shacter E. Induction of apoptosis by chemotherapeutic drugs without generation of reactive oxygen species. Arch Biochem Biophys. 2002; 397:262–272. [PubMed: 11795881]

Sepodes B, Maio R, Pinto R, Marques C, Mendes-do-Vale J, McDonald MC, et al. Tempol, an intracelullar free radical scavenger, reduces liver injury in hepatic ischemia-reperfusion in the rat. Transplant Proc. 2004; 36:849–853. [PubMed: 15194291]

Shacter E, Williams JA, Hinson RM, Senturker S, Lee YJ. Oxidative stress interferes with cancer chemotherapy: inhibition of lymphoma cell apoptosis and phagocytosis. Blood. 2000; 96:307–313. [PubMed: 10891466]

Shami PJ, Sauls DL, Weinberg JB. Schedule and concentration-dependent induction of apoptosis in leukemia cells by nitric oxide. Leukemia. 1998; 12:1461–1466. [PubMed: 9737697]

Sharma M, Zou Z, Miura H, Papapetropoulos A, McCarthy ET, Sharma R, et al. ADMA Injures Glomerular Filtration Barrier: Role of Nitric Oxide and Superoxide. Am J Physiol Renal Physiol. 2009

Shi Z, Chen AD, Xu Y, Chen Q, Gao XY, Wang W, et al. Long-term administration of tempol attenuates postinfarct ventricular dysfunction and sympathetic activity in rats. Pflugers Arch. 2009; 458:247–257. [PubMed: 19132383]

Shibata S, Nagase M, Yoshida S, Kawachi H, Fujita T. Podocyte as the target for aldosterone: roles of oxidative stress and Sgk1. Hypertens. 2007; 49:355–364.

Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun. 1996; 226:561–565. [PubMed: 8806673]

Simmons RA. Developmental origins of diabetes: the role of oxidative stress. Free Radic Biol Med. 2006; 40:917–922. [PubMed: 16540386]

Simonsen U, Christensen FH, Buus NH. The effect of tempol on endothelium-dependent vasodilatation and blood pressure. Pharmacol Ther. 2009a; 122:109–124. [PubMed: 19268689]

Simonsen U, Rodriguez-Rodriguez R, Dalsgaard T, Buus NH, Stankevicius E. Novel approaches to improving endothelium-dependent nitric oxide-mediated vasodilatation. Pharmacol Rep. 2009b; 61:105–115. [PubMed: 19307698]

Sinha I, Pearce CG, Cho BS, Hannawa KK, Roelofs KJ, Stanley JC, et al. Differential regulation of the superoxide dismutase family in experimental aortic aneurysms and rat aortic explants. J Surg Res. 2007; 138:156–162. [PubMed: 17196988]

Sledzinski Z, Wozniak M, Antosiewicz J, Lezoche E, Familiari M, Bertoli E, et al. Protective effect of 4-hydroxy-TEMPO, a low molecular weight superoxide dismutase mimic, on free radical toxicity in experimental pancreatitis. Int J Pancreatol. 1995; 18:153–160. [PubMed: 8530831]

Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. Eur J Biochem. 1999; 263:709–716. [PubMed: 10469134]

Son D, Kojima I, Inagi R, Matsumoto M, Fujita T, Nangaku M. Chronic hypoxia aggravates renal injury via suppression of Cu/Zn-SOD: a proteomic analysis. Am J Physiol Renal Physiol. 2008; 294:F62–F72. [PubMed: 17959751]

Song P, Wu Y, Xu J, Xie Z, Dong Y, Zhang M, et al. Reactive nitrogen species induced by hyperglycemia suppresses Akt signaling and triggers apoptosis by upregulating phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) in an LKB1-dependent manner. Circ. 2007; 116:1585–1595.

Soule BP, Hyodo F, Matsumoto K, Simone NL, Cook JA, Krishna MC, et al. The chemistry and biology of nitroxide compounds. Free Radic Biol Med. 2007; 42:1632–1650. [PubMed: 17462532]

Spasojevic I, Chen Y, Noel TJ, Yu Y, Cole MP, Zhang L, et al. Mn porphyrin-based superoxide dismutase (SOD) mimic, Mn(III)TE-2-PyP(5+), targets mouse heart mitochondria. Free Radic Biol Med. 2007; 42:1193–1200. [PubMed: 17382200]

Speisky H, Gomez M, Burgos-Bravo F, Lopez-Alarcon C, Jullian C, Olea-Azar C, et al. Generation of superoxide radicals by copper-glutathione complexes: redox-consequences associated with their interaction with reduced glutathione. Bioorg Med Chem. 2009; 17:1803–1810. [PubMed: 19230679]

Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. Antioxid Redox Signal. 2006; 8:243–270. [PubMed: 16677071]

Stopper H, Schupp N, Fazeli G, Dietel B, Queisser N, Walitza S, et al. Genotoxicity of the neurotransmitter dopamine in vitro. Toxicol In Vitro. 2009; 23:640–646. [PubMed: 19285549]

Suematsu N, Ojaimi C, Kinugawa S, Wang Z, Xu X, Koller A, et al. Hyperhomocysteinemia alters cardiac substrate metabolism by impairing nitric oxide bioavailability through oxidative stress. Circ. 2007; 115:255–262.

Sugimoto K, Tamayose K, Sasaki M, Hayashi K, Oshimi K. Low-dose doxorubicin-induced necrosis in Jurkat cells and its acceleration and conversion to apoptosis by antioxidants. Br J Haematol. 2002; 118:229–238. [PubMed: 12100152]

Sun H, Zhao H, Sharpe GM, Arrick DM, Mayhan WG. Influence of chronic alcohol consumption on inward rectifier potassium channels in cerebral arterioles. Microvasc Res. 2008; 75:367–372. [PubMed: 18191159]

Suy S, Mitchell JB, ehleiter D, Haimovitz-Friedman A, Kasid U. Nitroxides tempol and tempo induce divergent signal transduction pathways in MDA-MB 231 breast cancer cells. J Biol Chem. 1998; 273:17871–17878. [PubMed: 9651392]

Suzuki YJ, Ford GD. Redox regulation of signal transduction in cardiac and smooth muscle. J Mol Cell Cardiol. 1999; 31:345–353. [PubMed: 10093047]

Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 1991; 51:794–798. [PubMed: 1846317]

Tabakman R, Lazarovici P, Kohen R. Neuroprotective effects of carnosine and homocarnosine on pheochromocytoma PC12 cells exposed to ischemia. J Neurosci Res. 2002; 68:463–469. [PubMed: 11992473]

Tafazoli S, O'Brien PJ. Accelerated cytotoxic mechanism screening of hydralazine using an in vitro hepatocyte inflammatory cell peroxidase model. Chem Res Toxicol. 2008; 21:904–910. [PubMed: 18393451]

Tafazoli S, O'Brien PJ. Amodiaquine-induced oxidative stress in a hepatocyte inflammation model. Toxicology. 2009; 256:101–109. [PubMed: 19059302]

Tain YL, Freshour G, Dikalova A, Griendling KK, Baylis C. Vitamin E reduces glomerulosclerosis, restores renal neuronal NOS, and suppresses oxidative stress in the 5/6 nephrectomized rat. Am J Physiol Renal Physiol. 2007; 292:F1404–F1410. [PubMed: 17200156]

Tal M. A novel antioxidant alleviates heat hyperalgesia in rats with an experimental painful peripheral neuropathy. NeuroReport. 1996; 7:1382–1384. [PubMed: 8856680]

Tanabe M, Nagatani Y, Saitoh K, Takasu K, Ono H. Pharmacological assessments of nitric oxide synthase isoforms and downstream diversity of NO signaling in the maintenance of thermal and mechanical hypersensitivity after peripheral nerve injury in mice. Neuropharmac. 2009; 56:702–708.

Tang C, Han P, Oprescu AI, Lee SC, Gyulkhandanyan AV, Chan GN, et al. Evidence for a role of superoxide generation in glucose induced {beta}-cell dysfunction in vivo. Diabetes. 2007; 56:2722–2731. [PubMed: 17682092]

Tanito M, Li F, Elliott MH, Dittmar M, Anderson RE. Protective effect of TEMPOL derivatives against light-induced retinal damage in rats. Invest Ophthalmol Vis Sci. 2007; 48:1900–1905. [PubMed: 17389526]

Teichner A, Ovadia H, Lavie G, Leker RR. Combination of dexanabinol and tempol in focal cerebral ischemia: is there a ceiling effect? Exp Neurol. 2003; 182:353–360. [PubMed: 12895446]

Teke Z, Kabay B, Ozden A, Yenisey C, Bir F, Demirkan NC, et al. Effects of tempol, a membrane-permeable radical scavenger, on local and remote organ injuries caused by intestinal ischemia/reperfusion in rats. J Surg Res. 2008; 149:259–271. [PubMed: 18533188]

Tezel G, Yang X. Caspase-independent component of retinal ganglion cell death, in vitro. Invest Ophthalmol Vis Sci. 2004; 45:4049–4059. [PubMed: 15505055]

Thiemermann C. Membrane-permeable radical scavengers (tempol) for shock, ischemia-reperfusion injury, and inflammation. Crit Care Med. 2003; 31:S76–S84. [PubMed: 12544980]

Tong X, Ying J, Pimentel DR, Trucillo M, Adachi T, Cohen RA. High glucose oxidizes SERCA cysteine-674 and prevents inhibition by nitric oxide of smooth muscle cell migration. J Mol Cell Cardiol. 2008; 44:361–369. [PubMed: 18164028]

Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett. 1995; 358:1–3. [PubMed: 7821417]

Trembovler V, Beit-Yannai E, Younis F, Gallily R, Horowitz M, Shohami E. Antioxidants attenuate acute toxicity of tumor necrosis factor-alpha induced by brain injury in rat. J Interferon Cytokine Res. 1999; 19:791–795. [PubMed: 10454350]

Trnka J, Blaikie FH, Smith RA, Murphy MP. A mitochondria-targeted nitroxide is reduced to its hydroxylamine by ubiquinol in mitochondria. Free Radic Biol Med. 2008; 44:1406–1419. [PubMed: 18206669]

Udassin R, Haskel Y, Samuni A. Nitroxide radical attenuates ischaemia/reperfusion injury to the rat small intestine. Gut. 1998; 42:623–627. [PubMed: 9659154]

Valentin F, Bueb JL, Kieffer P, Tschirhart E, Atkinson J. Oxidative stress activates MMP-2 in cultured human coronary smooth muscle cells. Fundam Clin Pharmacol. 2005; 19:661–667. [PubMed: 16313278]

Valentine JS, Hart PJ. Misfolded CuZnSOD and amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A. 2003; 100:3617–3622. [PubMed: 12655070]

Veglio F, Bertello P, Pinna G, Mulatero P, Rossi A, Gurioli L, et al. Plasma endothelin in essential hypertension and diabetes mellitus. J Hum Hypertens. 1993; 7:321–325. [PubMed: 8410922]

Victorino GP, Ramirez RM, Chong TJ, Curran B, Sadjadi J. Ischemia-reperfusion injury in rats affects hydraulic conductivity in two phases that are temporally and mechanistically separate. Am J Physiol Heart Circ Physiol. 2008; 295:H2164–H2171. [PubMed: 18790838]

Vitolo JM, Cotrim AP, Sowers AL, Russo A, Wellner RB, Pillemer SR, et al. The stable nitroxide tempol facilitates salivary gland protection during head and neck irradiation in a mouse model. Clin Cancer Res. 2004; 10:1807–1812. [PubMed: 15014035]

Volk T, Hensel M, Schuster H, Kox WJ. Secretion of MCP-1 and IL-6 by cytokine stimulated production of reactive oxygen species in endothelial cells. Mol Cell Biochem. 2000; 206:105–112. [PubMed: 10839200]

von Eynatten M, Hamann A, Twardella D, Nawroth PP, Brenner H, Rothenbacher D. Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. Clin Chem. 2006; 52:853–859. [PubMed: 16556684]

Wadham C, Parker A, Wang L, Xia P. High glucose attenuates protein S-nitrosylation in endothelial cells: role of oxidative stress. Diabetes. 2007; 56:2715–2721. [PubMed: 17704302]

Wakade C, Khan MM, De Sevilla LM, Zhang QG, Mahesh VB, Brann DW. Tamoxifen neuroprotection in cerebral ischemia involves attenuation of kinase activation and superoxide production and potentiation of mitochondrial superoxide dismutase. Endocrinol. 2008; 149:367–379.

Wang D, Chabrashvili T, Wilcox CS. Enhanced contractility of renal afferent arterioles from angiotensin-infused rabbits: roles of oxidative stress, thromboxane-prostanoid receptors and endothelium. Circ Res. 2004; 94:1436–1442. [PubMed: 15117817]

Wang D, Chen Y, Chabrashvili T, Aslam S, Borrego L, Umans J, et al. Role of oxidative stress in endothelial dysfunction and enhanced responses to Ang II of afferent arterioles from rabbits infused with Ang II. J Am Soc Nephrol. 2003a; 14:2783–2789. [PubMed: 14569088]

Wang D, Jose P, Wilcox CS. Beta1 receptors protect the renal afferent arteriole of angiotensin-infused rabbits from norepinephrine-induced oxidative stress. J Am Soc Nephrol. 2006; 17:3347–3354. [PubMed: 17108317]

Wang H, Shimosawa T, Matsui H, Kaneko T, Ogura S, Uetake Y, et al. Paradoxical mineralocorticoid receptor activation and left ventricular diastolic dysfunction under high oxidative stress conditions. J Hypertens. 2008a; 26:1453–1462. [PubMed: 18551023]

Wang W, Jittikanont S, Falk SA, Li P, Feng L, Gengaro PE, et al. Interaction among nitric oxide, reactive oxygen species, and antioxidants during endotoxemia-related acute renal failure. Am J Physiol Renal Physiol. 2003b; 284:F532–F537. [PubMed: 12556364]

Wang W, Zolty E, Falk S, Summer S, Zhou Z, Gengaro P, et al. Endotoxemia-related acute kidney injury in transgenic mice with endothelial overexpression of GTP cyclohydrolase-1. Am J Physiol Renal Physiol. 2008b; 294:F571–F576. [PubMed: 18171994]

Weaver EC, Chon HP. Spin label studies in chlamydomonas. Science. 1966; 153:301–303. [PubMed: 17780000]

Wei SG, Zhang ZH, Yu Y, Felder RB. Systemically administered tempol reduces neuronal activity in paraventricular nucleus of hypothalamus and rostral ventrolateral medulla in rats. J Hypertens. 2009a; 27:543–550. [PubMed: 19330914]

Wei Y, Clark SE, Thyfault JP, Uptergrove GM, Li W, Whaley-Connell AT, et al. Oxidative stress-mediated mitochondrial dysfunction contributes to angiotensin II-induced nonalcoholic fatty liver disease in transgenic Ren2 rats. Am J Pathol. 2009b; 174:1329–1337. [PubMed: 19246643]

Wei Y, Sowers JR, Clark SE, Li W, Ferrario CM, Stump CS. Angiotensin II-induced skeletal muscle insulin resistance is mediated by NF-kappaB activation via NADPH oxidase. Am J Physiol Endocrinol Metab. 2008; 294:E345–E351. [PubMed: 18073321]

Weinberg A, Nylander KD, Yan C, Ma L, Hsia CJ, Tyurin VA, et al. Prevention of catecholaminergic oxidative toxicity by 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl and its recycling complex with polynitroxylated albumin, TEMPOL/PNA. Brain Res. 2004; 1012:13–21. [PubMed: 15158156]

Welch WJ, Baumgärtl H, Lübbers D, Wilcox CS. Nephron pO_2 and renal oxygen usage in the hypertensive rat kidney. Kidney Int. 2001; 59:230–237. [PubMed: 11135075]

Welch WJ, Blau J, Xie H, Chabrashvili T, Wilcox CS. Angiotensin-induced defects in renal oxygenation: role of oxidative stress. Am J Physiol. 2005a; 288:H22–H28.

Welch WJ, Mendonca M, Aslam S, Wilcox CS. Roles of oxidative stress and AT_1 receptors in renal hemodynamics and oxygenation in the post-clipped 2K,1C kidney. Hypertens. 2003; 41:692–696.

Welch WJ, Mendonca M, Blau J, Karber A, Dennehy K, Lao Y, et al. Antihypertensive response to prolonged tempol in the spontaneously hypertensive rat. Kidney Int. 2005b; 68:179–187. [PubMed: 15954907]

Welch WJ, Tojo A, Wilcox CS. Roles of NO and oxygen radicals in tubuloglomerular feedback in SHR. Am J Physiol Renal Physiol. 2000; 278:F769–F776. [PubMed: 10807588]

Welch WJ, Wilcox CS. AT_1 receptor antagonist combats oxidative stress and restores nitric oxide signaling in the SHR. Kidney Int. 2001; 59:1257–1263. [PubMed: 11260386]

Wierzba TH, Olek RA, Fedeli D, Falcioni G. Lymphocyte DNA damage in rats challenged with a single bout of strenuous exercise. J Physiol Pharmacol. 2006; 57 Suppl 10:115–131. [PubMed: 17242495]

Wiggers GA, Stefanon I, Padilha AS, Pecanha FM, Vassallo DV, Oliveira EM. Low nanomolar concentration of mercury chloride increases vascular reactivity to phenylephrine and local angiotensin production in rats. Comp Biochem Physiol C Toxicol Pharmacol. 2008; 147:252–260. [PubMed: 18093879]

Wilcox CS, Pearlman A. Chemistry and antihypertensive effects of tempol and other nitroxides. Pharm Rev. 2008; 60:418–469. [PubMed: 19112152]

Wilcox CS, Welch WJ. Interaction between nitric oxide and oxygen radicals in regulation of tubuloglomerular feedback. Acta Physiol Scand. 2000; 168:119–124. [PubMed: 10691789]

Wipf P, Xiao J, Jiang J, Belikova NA, Tyurin VA, Fink MP, et al. Mitochondrial targeting of selective electron scavengers: synthesis and biological analysis of hemigramicidin-TEMPO conjugates. J Am Chem Soc. 2005; 127:12460–12461. [PubMed: 16144372]

Wojcicka G, Jamroz-Wisniewska A, Widomska S, Ksiazek M, Beltowski J. Role of extracellular signal-regulated kinases (ERK) in leptin-induced hypertension. Life Sci. 2008; 82:402–412. [PubMed: 18206959]

Wolin MS, Xie YW, Hintze TH. Nitric oxide as a regulator of tissue oxygen consumption. Curr Opin Nephrol Hypertens. 1999; 8:97–103. [PubMed: 9914866]

Wu YJ, Li WG, Zhang ZM, Tian X. Antioxidative activity of 4-oxy- and 4-hydroxy-nitroxides in tissues and erythrocytes from rats. Zhongguo Yao Li Xue Bao. 1997; 18:150–154. [PubMed: 10072969]

Xavier S, Yamada K, Samuni AM, Samuni A, DeGraff W, Krishna MC, et al. Differential protection by nitroxides and hydroxylamines to radiation-induced and metal ion-catalyzed oxidative damage. Biochim Biophys Acta. 2002; 1573:109–120. [PubMed: 12399020]

Xia L, Wang H, Munk S, Frecker H, Goldberg HJ, Fantus IG, et al. Reactive oxygen species, PKC-beta1, and PKC-zeta mediate high-glucose-induced vascular endothelial growth factor expression in mesangial cells. Am J Physiol Endocrinol Metab. 2007; 293:E1280–E1288. [PubMed: 17711990]

Xiong Y, Hall ED. Pharmacological evidence for a role of peroxynitrite in the pathophysiology of spinal cord injury. Exp Neurol. 2009; 216:105–114. [PubMed: 19111721]

Xu J, Wu Y, Song P, Zhang M, Wang S, Zou MH. Proteasome-dependent degradation of guanosine 5′-triphosphate cyclohydrolase I causes tetrahydrobiopterin deficiency in diabetes mellitus. Circ. 2007; 116:944–953.

Yamada J, Yoshimura S, Yamakawa H, Sawada M, Nakagawa M, Hara S, et al. Cell permeable ROS scavengers, Tiron and Tempol, rescue PC12 cell death caused by pyrogallol or hypoxia/reoxygenation. Neurosci Res. 2003; 45:1–8. [PubMed: 12507718]

Yan SX, Hong XY, Hu Y, Liao KH. Tempol, one of nitroxides, is a novel ultraviolet-A1 radiation protector for human dermal fibroblasts. J Dermatol Sci. 2005; 37:137–143. [PubMed: 15734282]

Yang ZZ, Zou AP. Homocysteine enhances TIMP-1 expression and cell proliferation associated with NADH oxidase in rat mesangial cells. Kidney Int. 2003; 63:1012–1020. [PubMed: 12631082]

Yatabe J, Sanada H, Yatabe MS, Hashimoto S, Yoneda M, Felder RA, et al. Angiotensin II type 1 receptor blocker attenuates the activation of ERK and NADPH oxidase by mechanical strain in mesangial cells in the absence of angiotensin II. Am J Physiol Renal Physiol. 2009; 296:F1052–F1060. [PubMed: 19261744]

Yin W, Mitra K, Stearns RA, Baillie TA, Kumar S. Conversion of the 2,2,6,6-tetramethylpiperidine moiety to a 2,2-dimethylpyrrolidine by cytochrome P450: evidence for a mechanism involving nitroxide radicals and heme iron. Biochem. 2004; 43:5455–5466. [PubMed: 15122911]

Yoshida K, Kim-Mitsuyama S, Wake R, Izumiya Y, Izumi Y, Yukimura T, et al. Excess aldosterone under normal salt diet induces cardiac hypertrophy and infiltration via oxidative stress. Hypertens Res. 2005; 28:447–455. [PubMed: 16156509]

Yu Y, Fukuda N, Yao EH, Matsumoto T, Kobayashi N, Suzuki R, et al. Effects of an ARB on endothelial progenitor cell function and cardiovascular oxidation in hypertension. Am J Hypertens. 2008; 21:72–77. [PubMed: 18091747]

Yuen KS, Nearn MR, Halliday GM. Nitric oxide-mediated depletion of Langerhans cells from the epidermis may be involved in UVA radiation-induced immunosuppression. Nitric Oxide. 2002; 6:313–318. [PubMed: 12009849]

Zacharowski K, Olbrich A, Cuzzocrea S, Foster SJ, Thiemermann C. Membrane-permeable radical scavenger, tempol, reduces multiple organ injury in a rodent model of gram-positive shock. Crit Care Med. 2000; 28:1953–1961. [PubMed: 10890647]

Zacharowski K, Zacharowski PA, Friedl P, Mastan P, Koch A, Boehm O, et al. The effects of the fibrin-derived peptide Bbeta(15-42) in acute and chronic rodent models of myocardial ischemia-reperfusion. Shock. 2007; 27:631–637. [PubMed: 17505302]

Zamir E, Zhang R, Samuni A, Kogan M, Pe'er J. Nitroxide stable radical suppresses autoimmune uveitis in rats. Free Radic Biol Med. 1999; 27:7–15. [PubMed: 10443914]

Zeltcer G, Berenshtein E, Kitrossky N, Chevion M, Samuni A. Time window of nitroxide effect on myocardial ischemic-reperfusion injury potentiated by iron. Free Radic Biol Med. 2002; 32:912–919. [PubMed: 11978493]

Zeltcer G, Berenshtein E, Samuni A, Chevion M. Nitroxide radicals prevent metal-aggravated reperfusion injury in isolated rat heart. Free Radic Res. 1997; 27:627–635. [PubMed: 9455698]

Zhang C, Xu X, Potter BJ, Wang W, Kuo L, Michael L, et al. TNF-alpha contributes to endothelial dysfunction in ischemia/reperfusion injury. Arterioscler Thromb Vasc Biol. 2006; 26:475–480. [PubMed: 16385082]

Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Abe Y. ROS during the acute phase of Ang II hypertension participates in cardiovascular MAPK activation but not vasconstriction. Hypertens. 2004; 43:117–124.

Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Yao L, et al. Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. Cardiovasc Res. 2005a; 65:230–238. [PubMed: 15621051]

Zhang QS, Eaton L, Snyder ER, Houghtaling S, Mitchell JB, Finegold M, et al. Tempol protects against oxidative damage and delays epithelial tumor onset in Fanconi anemia mice. Cancer Res. 2008a; 68:1601–1608. [PubMed: 18316625]

Zhang R, Hirsch O, Mohsen M, Samuni A. Effects of nitroxide stable radicals on juglone cytotoxicity. Arch Biochem Biophys. 1994; 312:385–391. [PubMed: 8037450]

Zhang R, Shohami E, Beit-Yannai E, Bass R, Trembovler V, Samuni A. Mechanism of brain protection by nitroxide radicals in experimental model of closed-head injury. Free Radic Biol Med. 1998; 24:332–340. [PubMed: 9433909]

Zhang S, Li H, Ma L, Trimble CE, Kuppusamy P, Hsia CJ, et al. Polynitroxyl-albumin (PNA) plus tempol attenuate lung capillary leak elicited by prolonged intestinal ischemia and reperfusion(1). Free Radic Biol Med. 2000; 29:42–50. [PubMed: 10962204]

Zhang W, Wang XH, Chen SF, Zhang GP, Lu N, Hu RM, et al. Biphasic response of endothelial progenitor cell proliferation induced by high glucose and its relationship with reactive oxygen species. J Endocrinol. 2008b; 197:463–470. [PubMed: 18492812]

Zhang Z, Rhinehart K, Solis G, Pittner J, Kwon W, Welch WJ, et al. Chronic Ang II infusion increases NO generation by rat descending vasa recta. Am J Physiol. 2005b; 288:H29–H36.

Zhao W, Chen SS, Chen Y, Ahokas RA, Sun Y. Kidney fibrosis in hypertensive rats: role of oxidative stress. Am J Nephrol. 2008a; 28:548–554. [PubMed: 18239381]

Zhao W, Zhao T, Chen Y, Ahokas RA, Sun Y. Oxidative stress mediates cardiac fibrosis by enhancing transforming growth factor-beta1 in hypertensive rats. Mol Cell Biochem. 2008b; 317:43–50. [PubMed: 18581202]

Zhou J, Jang YP, Chang S, Sparrow JR. OT-674 suppresses photooxidative processes initiated by an RPE lipofuscin fluorophore. Photochem Photobiol. 2008; 84:75–80. [PubMed: 18173705]

Zhou MS, Schulman IH, Raij L. Role of angiotensin II and oxidative stress in vascular insulin resistance linked to hypertension. Am J Physiol Heart Circ Physiol. 2009; 296:H833–H839. [PubMed: 19151253]

Zigler JS Jr, Qin C, Kamiya T, Krishna MC, Cheng Q, Tumminia S, et al. Tempol-H inhibits opacification of lenses in organ culture. Free Radic Biol Med. 2003; 35:1194–1202. [PubMed: 14607518]

Abbreviations

Ang II	angiotensin II
ARB	angiotensin receptor blockers
AT1-R	angiotensin type 1 receptor
BP	blood pressure
CHF	congestive heart failure
CKD	chronic kidney disease
COX	cyclooxygenase
db	leptin deficient
DM	diabetes mellitus
DSS rats	Dahl salt sensitive rats
ECs	endothelial cells
EDCF	endothelium dependent contraction factor
EDRF/NO	endothelium dependent relaxation/nitric oxide

eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinases
GIT	gastrointestinal tract
GFR	glomerular filtration rate
H_2O_2	hydrogen peroxide
icv	intracerebroventricular
ip	intraperitoneal
2K	1C, two kidney, one clip
LPS	lipopolysaccharide
MMPs	matrix metalloproteases
MR	mineralocorticosteroid receptor
MRI	magnet resonance imaging
NAC	N-acetyl cystein
NADPH	nicotinamide adenine dinucleotide phosphate
NFκB	nuclear factor kappa B
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
Nox	neutrophil oxidase
O_2	oxygen
$O_2^{\cdot -}$	superoxide anion
·OH	hydroxyl radical
$ONOO^-$	peroxynitrite
PEG-SOD	pegylated form of superoxide dismutase
PKC	protein kinase C
PO_2	partial pressure of oxygen
PON-1	Paraoxonase-1
PVN	paraventricular nucleus
Q_{O2}	renal oxygen usage
ren-2	renin-2
ROS	reactive oxygen species
RRM	reduced renal mass
RVLM	rostral ventrolateral medulla
Sgk-1	serum glucocorticoid kinase-1
SHR	spontaneously hypertensive rats
SHRsp	stroke-prone spontaneously hypertensive rats

SOD	superoxide dismutase
SOD-1	Cu/Zn-SOD
STZ	streptozotocin
tempol	4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl
tempol-H	tempol hydroxylamine
tempone	4-oxo-2,2,6,6-tetramethylpiperidine-N-oxyl
TGFβ	transforming growth factor beta
TIMP	tissue inhibitor of matrix metalloprotease
T_{Na}	tubular transport of sodium
TNFα	tumor necrosis factor alpha
VSMCs	vascular smooth muscle cells

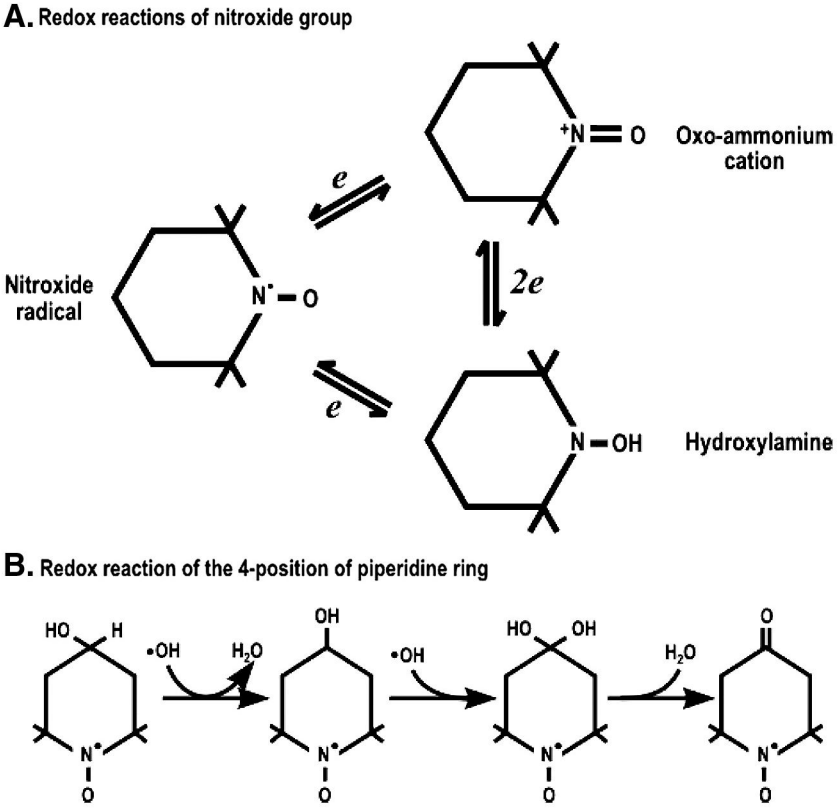

Figure 1.

Redox cycling reactions of tempol involving the nitroxide radical (Panel A) or the 4-position of the piperidine ring (Panel B).

Panel A: Reprinted from Soule, B. P., Hyodo, F., Matsumoto, K., Simone, N. L., Cook, J. A., Krishna, M. C., et al. (2007). The chemistry and biology of nitroxide compounds. *Free Radic Biol Med 42*, 1632-1650. Copyright 2007 Elsevier Limited. Used with permission.

Panel B: Reprinted from Saito, K., Takeshita, K., Ueda, J., & Ozawa, T. (2003). Two reaction sites of a spin label, TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), with hydroxyl radical. *J Pharm Sci 92*, 275-280. Copyright John Wiley & Sons Inc. Used with permission.

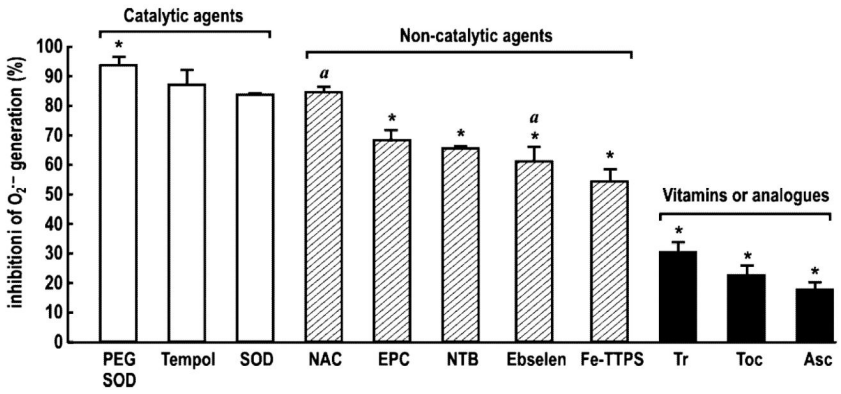

Figure 2.

Mean ± SEM values for percentage inhibition of cellular superoxide formation in cultured preglomerular vascular smooth muscle cells stimulated with angiotensin II and detected by low concentration lucigenin chemiluminence by maximum effective concentrations (10^{-3}M or 10^{-4}M) of drugs. PEG, pegolated; SOD, Cu/Zn superoxide dismutase; tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; NAC, N-acetyl-L-cysteine; EPC, (-)epicatechin; NTB, nitroblue tetrozolium; Fe-TTPS, 5,10,15,20-tetrakis (4 sulphonatophenyl) porphyronate iron; Tr, trolox; Toc, α-tocopherol; Asc, ascorbate. Compared to SOD: *, $p<0.0125$; *a*, generation of $O_2^{\cdot -}$ increased at lower drug concentration.

Data drawn from Luo, Z., Chen, Y., Chen, S., Welch, W. J., Andresen, B. T., Jose, P. A., et al. (2009). Comparison of inhibitors of superoxide generation in vascular smooth muscle cells. *Br J Pharmacol 157*, 935-943.

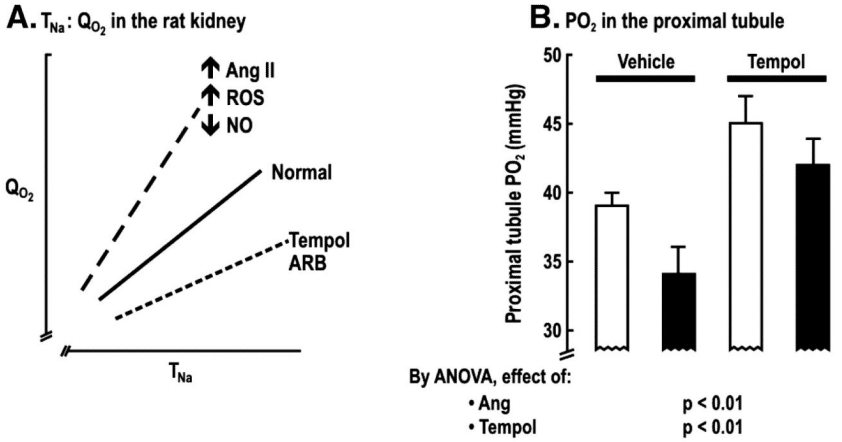

Figure 3.

Panel A depicts the relationship between renal oxygen usage (Q_{O2}) and tubular sodium transport (T_{Na}) in normal rats. It shows the effects of increases in an angiotensin II, reactive oxygen species or reduction in nitric oxide to increase the slope of the line (and decrease the efficacy of renal O_2 usage for chemical work) or tempol or an angiotensin receptor blocker given to these models to reduce the slope of the line. Panel B shows mean ± SEM values for PO_2 measured with an ultramico, coaxial electrode placed within the renal tubule by micropuncture. Rats received vehicle (open boxes) or angiotensin II at 200 ng · kg^{-1} · min by osmotic minipump (closed boxes) given with vehicle or tempol at 200 nmol · kg^{-1} · min sc for two weeks.

Panel A, data drawn from:

Welch, W. J., Blau, J., Xie, H., Chabrashvili, T., & Wilcox, C. S. (2005). Angiotensin-induced defects in renal oxygenation: role of oxidative stress. *Am J Physiol 288*, H22-H28.

Welch, W. J., Mendonca, M., Aslam, S., & Wilcox, C. S. (2003). Roles of oxidative stress and AT_1 receptors in renal hemodynamics and oxygenation in the post-clipped 2K,1C kidney. *Hypertens 41*, 692-696.

Welch, W. J., Baumgärtl, H., Lübbers, D., & Wilcox, C. S. (2003). Renal oxygenation defects in the spontaneously hypertensive rat: role of AT_1 receptors. *Kidney Int 63*, 202-208.

Welch, W. J., Baumgärtl, H., Lübbers, D., & Wilcox, C. S. (2001). Nephron PO_2 and renal oxygen usage in the hypertensive rat kidney. *Kidney Int 59*, 230-237.

Panel B, data drawn from:

Welch, W. J., Blau, J., Xie, H., Chabrashvili, T., & Wilcox, C. S. (2005). Angiotensin-induced defects in renal oxygenation: role of oxidative stress. *Am J Physiol 288*, H22-H28.

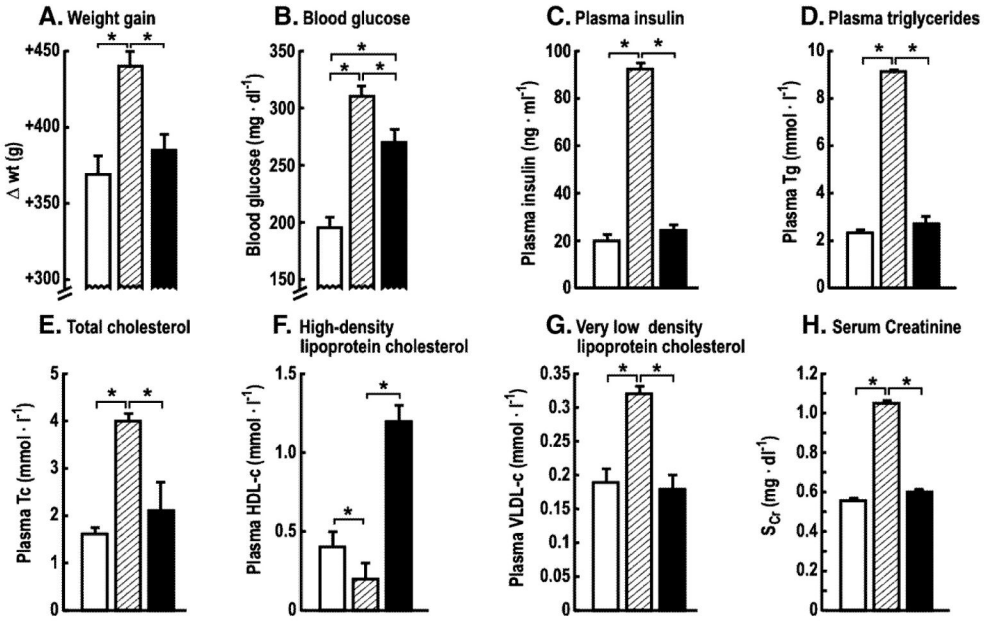

Figure 4.

Mean ± SEM values from obese Zucker rats fed for 10 weeks a reduced fat diet (14%; open boxes), a high fat diet (35%, cross-hatched boxes) as a model of the human metabolic syndrome, or a high fat diet and given tempol (1 mmol · l^{-1}) in the drinking water (closed boxes). In addition to the effects shown, tempol lowered the mean arterial pressure and renal NADPH oxidase activity and corrected albuminuria but did not modify food intake.
Data drawn from Ebenezer, P. J., Mariappan, N., Elks, C. M., Haque, M., & Francis, J. (2009). Diet-Induced Renal Changes in Zucker Rats Are Ameliorated by the Superoxide Dismutase Mimetic TEMPOL. *Obesity (Silver Spring) 17*, 1994-2002.

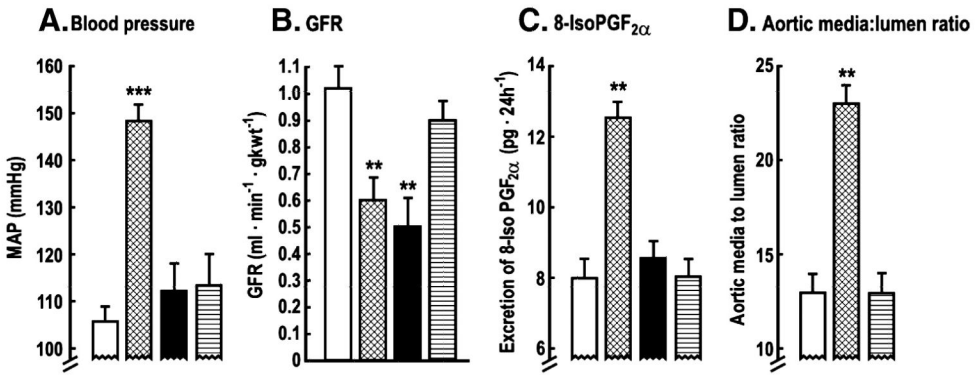

Figure 5.

Mean ± SEM values from rat studies of the two kidney, one clip (2K,1C) model of Goldblatt hypertension. Rats were studied after 3 weeks (Panel A-C) or 10 weeks (Panel D) of sham operation (open boxes) or 2K,1C hypertension and given a vehicle (grey boxes), the angiotensin receptor blocker candesartan (10 mg · kg^{-1} · day^{-1} × 14 days; solid boxes), tempol (200 nmol · kg^{-1} · min^{-1} sc × 14 days; cross-hatched boxes). Compared to 2K,1C + vehicle: *, $p<0.05$; **, $p<0.01$.

Panel A to C, data drawn from Welch, W. J., Mendonca, M., Aslam, S., & Wilcox, C. S. (2003). Roles of oxidative stress and AT_1 receptors in renal hemodynamics and oxygenation in the post-clipped 2K,1C kidney. *Hypertens 41*, 692-696.

Panel D, data drawn from Castro, M. M., Rizzi, E., Rodrigues, G. J., Ceron, C. S., Bendhack, L. M., Gerlach, R. F., et al. (2009). Antioxidant treatment reduces matrix metalloproteinase-2-induced vascular changes in renovascular hypertension. *Free Radic Biol Med 46*, 1298-1307.

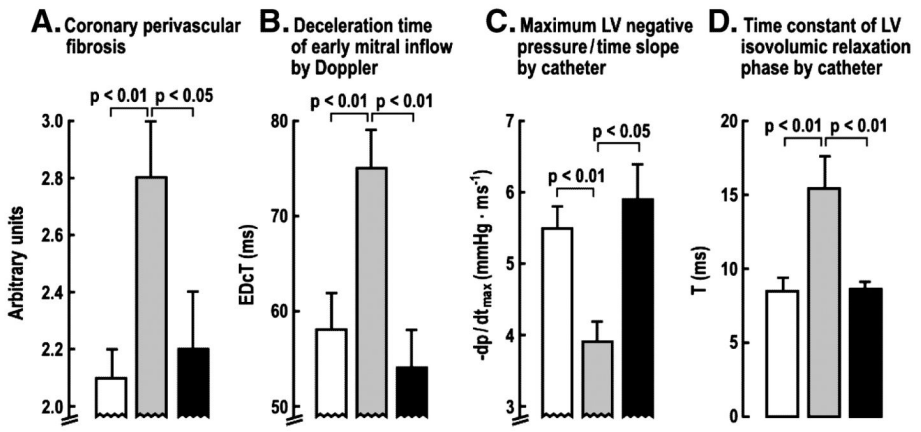

Figure 6.

Mean ± SEM values in salt-loaded spontaneously hypertensive rats with leptin receptor deficiency as a model of the metabolic syndrome. Rats were given normal salt diet (open bars), high salt diet (8% salt: grey bars) or high salt diet and oral tempol (1 mmol $\cdot$ l^{-1}) for four weeks (solid bars). There were no significant differences for the variables shown between the normal salt and the high salt plus tempol groups.

Data drawn from Matsui, H., Ando, K., Kawarazaki, H., Nagae, A., Fujita, M., Shimosawa, T., et al. (2008). Salt excess causes left ventricular diastolic dysfunction in rats with metabolic disorder. *Hypertens 52*, 287-294.