European Journal of Pharmacology 586 (2008) 1–8

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Metalloporphyrins as a therapeutic drug class against peroxynitrite in cardiovascular diseases involving ischemic reperfusion injury

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ARTICLE INFO

Article history:
Received 5 November 2007
Received in revised form 18 January 2008
Accepted 6 February 2008
Available online 5 March 2008

Keywords:
Metalloporphyrin
Peroxynitrite
Ischemic reperfusion injury
Scavenger/decomposition catalyst
Heart
Stroke

ABSTRACT

Peroxynitrite is well-recognized as being capable of inducing damaging cellular effects and has been identified as a mediator of cell damage in numerous disease states, including cardiovascular diseases. Metalloporphyrins are a class of molecule that represents an exciting new pharmacological approach to reducing peroxynitrite levels. These compounds catalyze the conversion of the harmful peroxynitrite molecules into less toxic derivatives and can be considered the reasonable intervention to reduce the toxicity of peroxynitrite. Several compounds have been synthesized and tested with promising results. Differences in the metalloporphyrin structure affect their reactivity. Iron-based metalloporphyrins display the highest rate of peroxynitrite decomposition with the narrowest scope of side reactions, whereas manganese-based metalloporphyrins react slower and with more pronounced secondary reactions, notably functioning as superoxide dismutase mimetics. This review examines the evidence that peroxynitrite is operative in patients with cardiovascular disease focusing on metalloporphyrin peroxynitrite decomposition catalysts and the evidence for their utility in the pharmacological treatment of major cardiovascular diseases. The data suggest that modification of peroxynitrite-induced cardiovascular injury is an intriguing and useful treatment approach.

Contents

1. Introduction

Peroxynitrite, formed by the reaction of nitric-oxide (NO) and superoxide anions, is a potent oxidant molecule that has been receiving increasing attention for its capacity to react with and alter biochemical pathways and biological processes. At low concentrations, peroxynitrite may participate naturally in cell signaling (Pacher et al., 2007), however, the damaging effect of high peroxynitrite levels is of considerable concern. Peroxynitrite-induced nitration or oxidation of proteins results in the formation of *S*-nitrosothiols with thiol groups of proteins (van der Vliet et al., 1998). As thiols are highly modifiable residues, used in numerous cellular reactions and signaling pathways, peroxynitrite has the potential to disrupt many of normal processes with a resulting deregulation of many cellular functions. Peroxynitrite can also damage DNA, by inducing strand breaks through modification of the bases or the sugar–phosphate backbone (Burney et al., 1999; Yermilov et al., 1995). As a consequence of its toxic nature, peroxynitrite may play a role in the pathophysiology of cancer (Sawa et al., 2006), neurodegenerative diseases (Torreilles et al., 1999),

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doi:10.1016/j.ejphar.2008.02.078

atherosclerosis (White et al., 1994) and a number of important other cardiovascular diseases (Pacher et al., 2007).

Counteracting the damage associated with peroxynitrite may be accomplished by a number of different approaches, which include: (i) preventing the formation of peroxynitrite; (ii) scavenging or decomposing peroxynitrite; or (iii) repairing peroxynitrite-induced damage. This latter option may be exceedingly difficult due to the sheer number of events that would need to be addressed and thus likely would not be feasible. Prevention of peroxynitrite formation centers on an inhibition of nitric-oxide and superoxide formation. This is not an optimal strategy as it may preclude any beneficial effects of nitric oxide in the body and/or be extremely difficult because of the large number of diverse biochemical processes leading to superoxide ion generation. Thus scavenging or decomposing peroxynitrite appears to be a most reasonable intervention to reduce the toxicity of peroxynitrite. A class of compounds that scavenge or decompose peroxynitrite are certain metalloporphyrins, which represent an exciting new class of therapeutic agents. The purpose of this review is to examine the clinical data that peroxynitrite is formed in major cardiovascular diseases, discuss metalloporphyrins as peroxynitrite decomposition catalysts and evaluate their potential as novel therapeutic agents in the treatment of cardiovascular diseases.

2. Peroxynitrite formation in patients with cardiovascular disease

There is limited available clinical data on peroxynitrite generation in patients with cardiovascular disease. Studies in patients with acute coronary syndromes or ischemia–reperfusion have demonstrated increased production of oxidative products of nitric oxide from the heart (Akiyama et al., 1998; Hayashi et al., 2001). Importantly there is histological evidence of peroxynitrite-induced cardiac effects in the myocardium of patients with acute coronary syndromes (Marfella et al., 2004). Experimental data have consistently shown the cardiotoxicity of peroxynitrite on the myocyte element of the heart (Keira et al., 2002; Levrand et al., 2006; Rabkin et al., 2007; Rabkin and Kong, 2000). While NO has a bifunctional role in cell death (Chung et al., 2001; Tsang et al., 2004), there is a strong line of experimental evidence suggesting an adverse effect of NO in acute myocardial infarction as mice lacking nitric-oxide synthase-type 2 (iNOS) have less cardiac cell death and lesser mortality after acute myocardial infarction (Feng et al., 2001; Sam et al., 2001). These iNOS−/− mutant mice have less peroxynitrite generation and less immunohistochemical evidence of myocardial effects of peroxynitrite (Feng et al., 2001). The converse has also been established as chronic beta-adrenergic receptor stimulation induces peroxynitrite formation and aggravates myocardial ischemia/reperfusion injury by provoking inducible nitric-oxide synthase-mediated nitrate stress (Hu et al., 2006).

Atherosclerosis is one of the underlying elements of many current cardiovascular diseases because the resultant impairment in tissue blood flow, ischemia, can lead to myocardial infarction, stroke and heart failure. There is considerable information supporting a role for peroxynitrite in atherosclerosis. Atherosclerotic lesions show evidence of peroxynitrite formation specifically the presence of 3-nitrotyrosine by either histochemistry, HPLC or gas liquid chromatography (Buttery et al., 1996; Leeuwenburgh et al., 1997; Luoma et al., 1998; Morton et al., 2003; Sucu et al., 2003). Low density lipoproteins (LDL) isolated from human aortic atherosclerotic lesions have a ninety fold increase in protein bound 3-nitrotyrosine compared to the amount in circulating LDL, suggesting that peroxynitrite formed in the vascular wall promotes atherosclerotic vascular disease (Leeuwenburgh et al., 1997). Atherosclerosis may also be produced by peroxynitrite-induced changes in lipids and lipoproteins. High density lipoproteins (HDL) from patients with established coronary artery disease contain twice as much 3-nitrotyrosine as HDL from plasma of healthy subjects, indicating peroxynitrite-induced nitration of HDL (Ferretti et al., 2006; Pennathur et al., 2004) HDL is susceptible to structural modifications of both surface and core components of HDL by peroxynitrite with a resulting limitation in the antiinflammatory and cytoprotective properties of HDL that may promote atherosclerosis (Ferretti et al., 2006). Peroxynitrite may also contribute to atherogenesis through a mechanism involving endoplasmic reticulum stress (Dickhout et al., 2005).

While there is much less data on peroxynitrite in patients with stroke compared to those with cardiac disease, similar kinds of data are available. Patients with stroke have higher plasma peroxynitrite concentrations than controls suggest peroxynitrite is operative in the pathogenesis of ischemic brain injury (Nanetti et al., 2007). Human brain after ischemic stroke shows evidence of protein nitration by NO-derived peroxynitrite (Forster et al., 1999). There is more evidence for a role for peroxynitrite in heart failure. Histochemical evidence of peroxynitrite effects on the myocardium have also been found in heart of patients with septic shock (Rossi et al., 2007) and severe heart failure from idiopathic dilated cardiomyopathy (Hunt et al., 2002; Lokuta et al., 2005). Peroxynitrite is a potent inducer of tissue damage during systemic inflammatory responses and circulatory shock (Evgenov and Liaudet, 2005). Experimental data support a pathophysiologic role for peroxynitrite in heart failure as shown by the ability of peroxynitrite to inactivate myofibrillar creatine kinase, alter myocardial Ca^{2+} handling all of which can lead to a reduction in cardiac contractility (Katori et al., 2006; Mihm et al., 2001). The ability of hydralazine to improve the prognosis of some patients with heart failure has been speculated to be due to is action to inhibit peroxynitrite formation (Daiber et al., 2005).

The role of peroxynitrite in contraction and relaxation of cardiac and vascular smooth muscle is complex (Adachi et al., 2004; Katori et al., 2006). The cardiovascular impact of peroxynitrite depends on the site of its generation — intravascular or intracellular, as well as the underlying conditions (diseases) of the myocardium and vasculature (Adachi et al., 2004; Katori et al., 2006). Peroxynitrite generated within the cardiomyocyte can impair contractile function by directly altering Ca^{2+} handling (Katori et al., 2006). Sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) is *S*-glutathiolated by low concentrations of peroxynitrite with resulting increase in Ca^{2+}-uptake activity of SERCA decreasing intracellular Ca^{2+} concentration and relaxing cardiac and vascular smooth muscle (Adachi et al., 2004). The effect of peroxynitrite on vascular relaxation can be mitigated in atherosclerosis (Adachi et al., 2004). Reduced myocardial SERCA2a activity correlated with nitrotyrosine levels in idiopathic dilated cardiomyopathy suggesting that peroxynitrite-induced Ca^{2+} pump inactivation contributes to heart failure in this form of cardiomyopathy (Lokuta et al., 2005). Idiopathic dilated cardiomyopathy is associated with increased iNOS expression resulting in an increase in NO generation (Vanderheyden et al., 2004). Experimentally, consistent observations are found as iNOS-deficient mice displayed much less hypertrophy, dilation, fibrosis, and left ventricular dysfunction in response to hypertension induced by chronic transverse aortic constriction (Zhang et al., 2007b). Although there is controversy about the extent to which NO generation is harmful in heart failure (Heger et al., 2002; Mungrue et al., 2002), the amount of peroxynitrite generated in the cardiac myocyte, from NO, can be a determinant of the extent of left ventricular systolic dysfunction because of the direct effect of peroxynitrite on myocardial Ca^{2+} handling (Katori et al., 2006).

3. Nature of metalloporphyrins

Porphyrins are aromatic macrocyclic structures derived from a basic structure composed of four pyrrole rings joined by methylene bridges (Fig. 1). As a result of the extended conjugated ring system arising from the orientation of the electron orbitals, these molecules distribute charge throughout their structure, making them stable compounds (Schleyer, 2001). The International Union of Pure and Applied Chemistry's (IUPAC) Compendium of Chemical Terminology

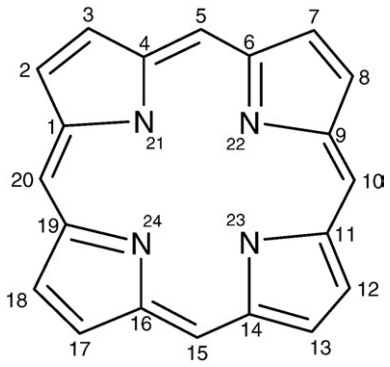

Fig. 1. Porphyrin structure and nomenclature. The basic porphyrin structure is composed of four pyrrole rings linked together with methylene bridges. Atom numbering (1–20) begins on a methylene bridge-linked carbon and proceeds through the pyrrole ring and the subsequent methylene and pyrrole carbons. Nitrogen numbering continues (21–24) beginning from the same starting pyrrole ring.

has provided a nomenclature such that the outer ring of carbon atoms are given the designations of 1–20, beginning with the top-left pyrrole group and proceeding clockwise, and the four nitrogen atoms within the ring continue the numbering from 21–24 (Fig. 1) (Moss et al., 1995). Substituents generally attach to the carbon atoms. Following standard IUPAC naming rules of listing the attachment position followed by the number and type of substituents, compounds take on names such as 5,10,15,20-tetrakis(*N*-methyl-4′-pyridyl)porphyrin, which (thankfully) have acceptable shorthand names such as TMPyP. The central hole of the porphyrin happens to be of appropriate size in which can fit one of several metal ions. The result is a highly stable metalloporphyrin, capable of binding to other molecules. Metalloporphyrins are involved in numerous biological reactions, with their specificity and reactivity depending on the metal ion center and the nature of the chemical groups attached to the outer ring (Fig. 2). Perhaps the most well-known metalloporphyrin is heme, which is a protoporphyrin IX molecule with a central iron atom. Heme acts as a prosthetic group required for the proper function of several hemoproteins, such as haemoglobin, guanylate cyclase, or cytochromes (Ponka and Ponka, 1999). This example illustrates the core importance of metalloporphyrins in regulating biological function.

4. Metalloporphyrins as peroxynitrite scavengers/decomposition catalysts

Peroxynitrite is highly reactive with sulfhydryls, where via one- or two-electron transfers, it can cause their oxidation, the formation of thiyl radicals (Quijano et al., 1997), or the formation of *S*-nitrosothiols with thiol groups of proteins (van der Vliet et al., 1998). Peroxynitrite-induced modifications can also interfere with normal phosphorylation signaling, in particular at tyrosine residues (Li et al., 1998). As thiols and tyrosines are used in numerous signaling pathways and cellular reactions, the potential for peroxynitrite to disrupt normal processes is large. Furthermore, peroxynitrite can cause lipid peroxidation (Radi et al., 1991), and DNA strand breaks and mutations through modification of the bases or the sugar–phosphate backbone (Burney et al., 1999; Yermilov et al., 1995), all of which are cytotoxic to the cell.

Whereas peroxynitrite is relatively stable, its protonated conjugate — peroxynitrous acid, with a pK_a of 6.8, is not stable and decays at a rate of 1.3 s^{-1} under normal conditions (Pryor et al., 1997). At neutral pH, the conversion of peroxynitrite to nitrate is accelerated by CO_2 (Zhang et al., 1997), through the formation of nitrosoperoxycarbonate, which is itself a highly reactive compound that can also damage cells (Uppu et al., 1996). In this sense, CO_2 should not be considered a peroxynitrite "scavenger" because it is merely converted into another toxic species (Crow, 2000).

Metalloporphyrin peroxynitrite decomposition catalysts represent a class of compounds that specifically degrade peroxynitrite with a minimal number of additional reactions, and so hold the potential to combat peroxynitrite-mediated disease (Salvemini et al., 1998). Naturally occurring metalloporphyrins, such as myeloperoxidase (Floris et al., 1993), were first observed to react with peroxynitrite, which led to the development of several synthetic compounds. Aside from varying the metal centers of these compounds, these compounds also have different functional groups attached to the porphyrin rings which affect their reactivity. Several compounds have been synthesized with differing functional groups. Anionic groups add an increased negative charge to the compound, resulting in a decreased reactivity with peroxynitrite (Crow, 1999). Conversely, cationic or neutral groups tend to have an increased affinity for peroxynitrite (Crow, 1999). Thus, optimal compounds may contain a Fe center and non-negative functional groups which would electrostatically promote peroxynitrite-binding and would introduce a minimal amount of steric hindrance such that the peroxynitrite was free to approach the metal center.

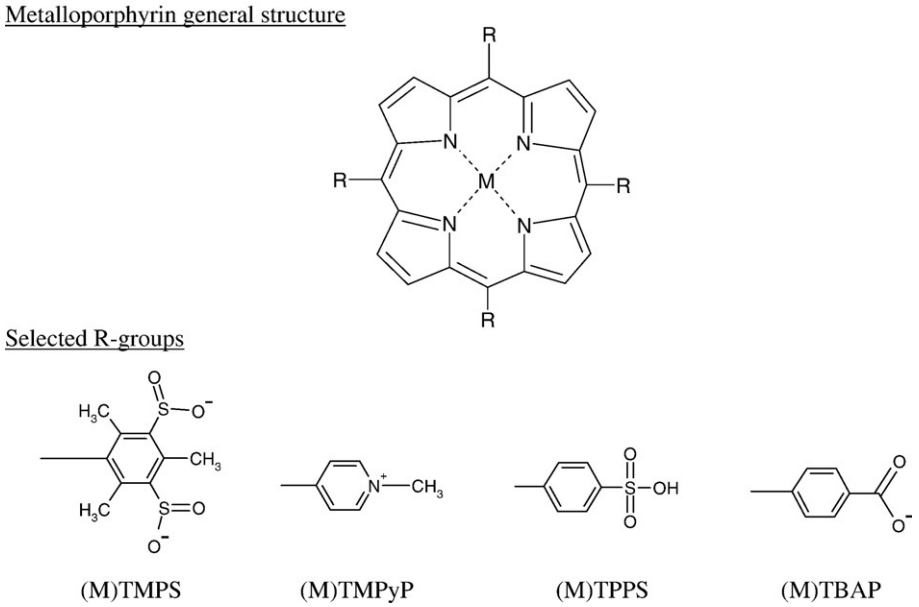

Fig. 2. Selected metalloporphyrin structures. Metalloporphyrin structure is shown, represented by the basic porphyrin structure with a bound central metal ion and covalently attached R-groups. Selected R-groups that extend from the methylene bridge carbons are shown.

5. Modes of metalloporphyrins catalyze peroxynitrite decomposition

Compounds that catalyze the decomposition of peroxynitrite have been used pharmacologically to reduce peroxynitrite levels. Examples of these catalysts include compounds such as 5,10,15,20-tetrakis(2,4,6-trimethyl-3, 3-disulfonatophenyl)porphyrinato iron (III) (FeTMPS), 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron (III) (FeTPPS), 5,10,15,20-tetrakis(*N*-methyl-4′-pyridyl)porphyrinato iron (III) (FeTM-PyP), and FP15 (FeCl tetrakis-2-(*N*-triethylene glycol monomethyl ether) pyridyl porphyrin) (Fig. 2). Studies strongly suggest that their function is dependent on the combination of both the porphyrin and the metal center, demonstrated by the activity of FeTMPS, and how $FeCl_3$ or H_2TMPS were unable to catalyze the decomposition (Stern et al., 1996).

Studies have also begun to provide insight into the molecular mechanics of peroxynitrite decomposition by these compounds (Fig. 3). Peroxynitrite initially binds to the metal center via its distal oxygen atom, following which nitrogen dioxide (NO_2) is released upon the formation of an oxo-iron(IV) species (Shimanovich and Groves, 2001). Oxidized FeTMPS reacted rapidly with both nitrite and nitrogen dioxide to regenerate the reduced form of the compound (forming respectively nitrogen dioxide or nitrate), whereas oxidized FeTMPyP reacted very slowly with these species (Shimanovich and Groves, 2001). For FeTMPS, the oxo-iron(IV) species only represented 55% of the total catalyst, with the rest regenerating the reduced iron(III) species (Shimanovich and Groves, 2001). However, for FeTMPyP, the oxo-iron(IV) species formed rapidly and remained (Lee et al., 1998). It was found that, to explain the rapid rate of peroxynitrite decomposition by FeTMPS, these oxidized species have a catalytic ability as well to convert peroxynitrite to nitrate (Lee et al., 1998; Shimanovich and Groves, 2001). Interestingly, Lee et al. (1998) observed with FeTMPyP that excess peroxynitrite slowed the rate of catalysis. They contend that the iron(III) species reacts with peroxynitrite rapidly to yield nitrite and the oxo-iron(IV) species. When stoichiometric amounts of FeTMPyP and peroxynitrite are combined, the rate of intermediate formation approximately equalled the rate of peroxynitrite decomposition. However, where excess peroxynitrite is present, it can react with the oxo-iron(IV) species to form a secondary intermediate, whose formation and decay occurs 10-fold slower and thus serves to limit the overall rate of catalysis. These observations therefore indicate that the efficiency of these compounds is dependent on the concentrations of peroxynitrite and the compound itself, as well as the presence of antioxidants that could modify the redox state of the compound, thus altering the catalytic rate.

Furthermore, kinetic studies of FeTMPS and FeTMPyP revealed that differences in the metalloporphyrin structure affect their reactivity (Shimanovich and Groves, 2001). These conclusions suggest that the electrophilicity of the pyridinium groups attached to FeTMPyP causes the cationic metal center to be more reactive, compared to the negatively charged groups on FeTMPS causing it to exert less of an electrophilic effect on the metal center and thus be less reactive. In addition, the size of the side groups attached to FeTMPS sterically hinders free access to the metal center, also contributing to its lower intrinsic reactivity. Interestingly, in vivo studies implicate FeTMPS as having a higher activity that FeTMPyP (Misko et al., 1998), which may be a result of the presence of biological antioxidants or some other interplay with cellular factors.

6. Differences in efficacy for decomposing peroxynitrite

While not all metalloporphyrin compounds have been analyzed, of the ones that have had their reaction kinetics studied, most catalyze the decomposition of peroxynitrite effectively (Table 1). The compound FeTMPyP has been observed to catalyze the breakdown the most rapidly of the iron-based metalloporphyrins, with a rate constant of $7.0\times10^5\pm0.9$ M^{-1} s^{-1} (Crow, 1999). FeTPPS and FePPIX are comparably rapid, with rate constants of $3.3\times10^5\pm0.4$ M^{-1} s^{-1} and $3.7\times10^5\pm0.2$ M^{-1} s^{-1} respectively (Crow, 1999). FeTMPS accelerates the reaction an order of magnitude slower, with a rate constant of 6.0×10^4 M^{-1} s^{-1} (Shimanovich and Groves, 2001). By comparison, the manganese-based metalloporphyrin MnTMPyP decomposes peroxynitrite even slower, at a rate of $4.8\times10^4\pm0.4$ M^{-1} s^{-1}, whereas MnTBAP did not accelerate the reaction at all (Crow, 1999). However, when provided with the antioxidant ascorbate, to regenerate the Mn(III)

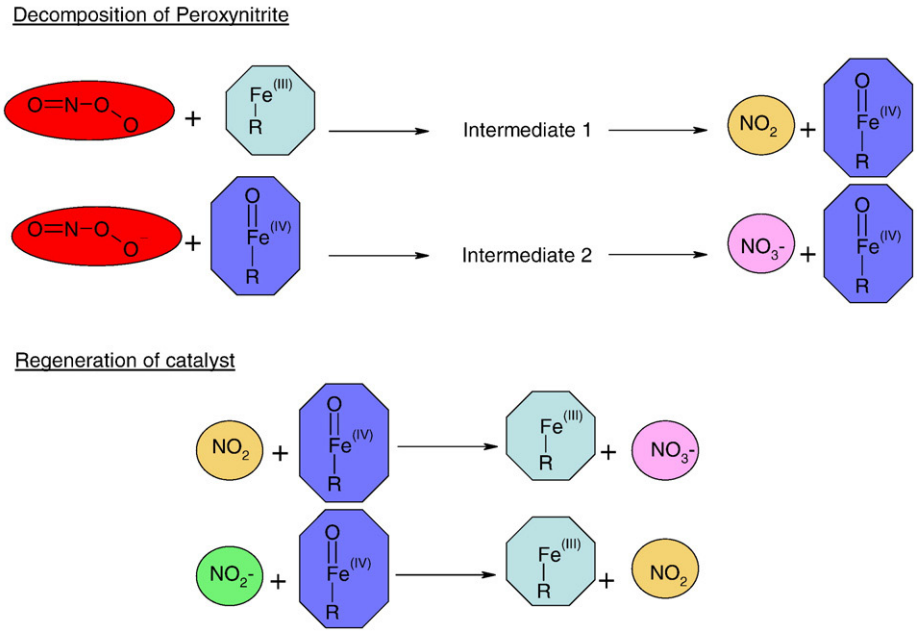

Fig. 3. Peroxynitrite decomposition mechanism. Decomposition of peroxynitrite by Fe-based catalysts is described. Peroxynitrite binds to Fe(III) or Fe(IV), with subsequent degradation. The Fe(IV) is converted back to Fe(III) through reduction by nitrogen dioxide or nitrite, with production of nitrate or nitrogen dioxide respectively. 'R' represents the porphyrin component of the catalyst. Two NO_2 can form N_2O_4, which spontaneously hydrolyzes to yield NO_2^- and NO_3^- (Ignarro et al., 1993) and is not included as it occurs independent of a metalloporphyrin.

Table 1
Metalloporphyrins as peroxynitrite decomposition catalysts

Symbol	Name	Porphyrin charge	Rate constant ($M(E-1)$ $s(E-1)$)	Reference
FePPIX	Iron protoporphyrin IX	Anionic	3.7E5±0.2	Crow (1999)
FeTMPS	5,10,15,20-Tetrakis(2,4,6-trimethyl-3,5-disulfonato)porphyrinato iron(III)	Anionic	6.0E5	Shimanovich and Groves (2001)
FeTMPyP	5,10,15,20-Tetrakis(*N*-methyl-4′-pyridyl)porphyrinato iron(III)	Cationic	7.9E5±0.9	Crow (1999)
FeTPPS	5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrinato iron(III)	Anionic	3.3E5±0.4	Crow (1999)
MnTBAP	5,10,15,20-Tetrakis(benzoic acid)porphyrinato manganese(III)	Anionic	None 3.3E3±0.4 (with antioxidant)	Crow (1999)
MnTMPyP	5,10,15,20-Tetrakis(*N*-methyl-4′-pyridyl)porphyrinato manganese(III)	Cationic	4.8E4±0.4 2.2E6±0.3 (with antioxidant)	Crow (1999), Lee et al. (1998)
FP15	FeCl tetrakis-2-(triethylene glycol monomethyl ether) pyridyl porphyrin	Neutral?	Unknown	Szabo et al. (2002)
FeTBAP	5,10,15,20-Tetrakis(benzoic acid)porphyrinato iron(III)	Anionic	Unknown	
MnPPIX	Manganese protoporphyrin IX	Anionic	Unknown	
WW85	–	Unknown	Unknown	

Selected metalloporphyrin peroxynitrite decomposition catalysts are shown. Porphyrin charge may affect the affinity for peroxynitrite, and is based on structural descriptions. Where known, rate constants are provided for catalyzed peroxynitrite decomposition in the absence or antioxidants, or in their presence where noted.

reaction center from the Mn(IV) formed after each reaction cycle, these compounds demonstrated reaction rates of $1.8\times10^{6}\pm0.3$ M^{-1} s^{-1} (Lee et al., 1998) and $3.3\times10^{3}\pm0.4$ M^{-1} s^{-1} (Crow, 1999) respectively. Given that biological antioxidants may not be readily available in some diseases, Fe-based compounds may be functionally more appropriate when considering these compounds as treatments.

7. Peroxynitrite decomposition catalysts as therapeutics

Several manganese(III)- and iron(III)-based compounds have been devised and implemented in therapeutic settings. Fe-based compounds may be more efficient when considering these compounds as therapeutic treatments. The main difference between these two types of compounds lies in their requirements for regeneration. Mn(III) or Fe(III) are the reactive centers that initially bind to and catalyze the decomposition of peroxynitrite, which involves a change of their oxidation state to Mn(IV) or Fe(IV). However, the Mn(IV) needs the presence of cellular antioxidants in order to regenerate the Mn(III) and catalyze another cycle, whereas the Fe(IV) does not and it can also catalyze the breakdown of peroxynitrite (Crow, 1999). The availability of biological antioxidants may be compromised in conditions of oxidative stress found in certain diseases (Lee et al., 1998; Salvemini et al., 1998), which in turn compromises the ability of Mn-containing metalloporphyrins to decompose peroxynitrite.

In conditions with impaired cardiac systolic or diastolic dysfunction, Fe-containing peroxynitrite decomposition catalysts appear to be promising therapeutic agents. Cytokines (interleukin-1 beta, interferon-gamma, and tumor necrosis factor-alpha) induce a marked decline in myocardial contractile function accompanied by markers of peroxynitrite formation (Ferdinandy et al., 2000). FeTPPS inhibited this decline in myocardial function, concomitant with reduction in indicators of peroxynitrite effects such as nitrotyrosine formation (Ferdinandy et al., 2000). Similarly, endotoxin-induced (cytokine-mediated) myocardial inflammation and contractile dysfunction were improved by FeTPPS (Lancel et al., 2004). This may, however, be specific for the type of cytokine, as interleukin (IL)-6-induced reductions in cardiac contractility were not immediately blocked by FeTPPS, but it may have a later action (Yu et al., 2005). Cholesterol-enriched diet-induced hyperlipidemia, in the rat, leads to an increase in cardiac peroxynitrite formation with an associated deterioration of cardiac performance, as reflected in a significant elevation in left ventricular end-diastolic pressure (LVEDP) (Onody et al., 2003). FeTPPS was able to minimize the development of this abnormality of diastolic function by normalize LVEDP in the cholesterol-fed group (Onody et al., 2003). FP15 inhibited the protein nitration associated with a reduction of diabetes, and prevented the reduction in cardiac contractility in diabetic rats (Szabo et al., 2002). FP15 significantly prevented the loss of cardiac function in aging rats as well as improving the ability of aortic rings to relax (Radovits et al., 2007). FP15 also protected the heart from the cardiotoxic effects of the antitumor drug doxorubicin, which is generally recognized as heart failure and which is associated with increased cardiac nitric-oxide and peroxynitrite generation (Pacher et al., 2003).

In shock states, FeTPPS has the potential to antagonize reductions in cardiac contractility, endothelial function and the severity of the hemodynamic consequences of endotoxin-induced shock (Table 2). Lower doses (10–100 mg/kg i.v.) of FeTPPS were beneficial in rats with endotoxin-induced shock (Cuzzocrea et al., 2000), as it inhibited, in a dose-dependent manner, *Escherichia coli* lipopolysaccharide (LPS)-induced hypotension, tissue injury, and improved mortality rate. Furthermore, when administered (100 mg/kg i.v.) 1 h before LPS, FeTPPS prevented the LPS-induced aortic contractile and endothelial dysfunction (Cuzzocrea et al., 2006). MnTBAP, 15 mg kg^{-1} i.v., before the administration of endotoxin in rats ameliorated the development of vascular hyporeactivity and endothelial dysfunction (Zingarelli et al., 1997).

In stroke, FeTPPS 3 mg/kg intravenously administered at 2 and 6 h after middle cerebral artery occlusion in the rat, but not later administration times, significantly reduced the infarct volume, edema volume and neurological deficits produced by the arterial occlusion (Thiyagarajan et al., 2004). FeTPPS treatment (1 and 3 mg kg^{-1}, i.p.) reduces neurological symptoms — hyperlocomotion, memory impairment and CA1 hippocampal neuronal damage in ischemia–reperfusion in the gerbil (Sharma et al., 2007). FeTPPS treatment also attenuated the increased malondialdehyde (MDA) levels and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) positive cells after cerebral IR injury. Following middle cerebral artery occlusion, FeTMPyP caused a significant reduction of cerebral infarction, as well as exhibiting neuroprotective effects due to attenuation of peroxynitrite-dependent PARP activation and DNA damage (Sharma

Table 2
Animal models of clinical conditions that suggest a potential beneficial role for metalloporphyrins

Condition	Agent	Reference
Heart failure	FeTPPS	Ferdinandy et al. (2000), Onody et al. (2003)
	FP15	Pacher et al. (2003), Radovits et al. (2007), Szabo et al. (2002)
Endotoxic shock	FeTPDS	Cuzzocrea et al. (2006)
	MnTBAP	Zingarelli et al. (1997)
Stroke	FeTPPS	Sharma et al. (2007), Thiyagarajan et al. (2004)
	FeTMPyP	Dhar et al. (2006), Sharma et al. (2004)
Myocardial infarction	FP15	Bianchi et al. (2002), Tao et al. (2006), Zhang et al. (2007a)
	FeTMPyP	Wang et al. (2007)

et al., 2004; Thiyagarajan et al., 2004). Studies also show that FeTMPyP, administered intravenously to gerbils 30 min prior to carotid artery occlusion-induced cerebral ischemia and reperfusion, restored locomotor activity, memory impairment, and reduced the extent of neuron loss in the hippocampal pyramidal region (Dhar et al., 2006). Decreases in penile nitric-oxide-dependent autonomic nerve and microvascular function are restored by FeTMPyP in diabetes mellitus (Nangle et al., 2004).

In myocardial infarction or myocardial ischemia/reperfusion injury (IR), porphyrin, FP15 (FeCl tetrakis-2-(*N*-triethylene glycol monomethyl ether) pyridyl porphyrin), has shown positive results in experiments. FP15 reduces myocardial infarct size, following ischemia/reperfusion (Bianchi et al., 2002) and appears to be especially effective in myocardial infarction in the aging rat (Zhang et al., 2007a). Furthermore, myocardial apoptosis is reduced by FP15, likely involving an attenuation of apoptosis signal-regulating kinase-1 and a prevention of the nitration-induced inhibition of the cellular antioxidant thioredoxin (Tao et al., 2006). FeTMPyP reduced myocardial ischemia/reperfusion-induced injury and cardiomyocyte apoptosis (Wang et al., 2007). FeTMPS reduced the extent of injury following ischemia/reperfusion in a model of splanchnic artery occlusion shock, possibly through a mechanism involving the inhibition of adhesion molecule expression and peroxynitrite-mediated tissue damage (Cuzzocrea et al., 2000). FeTMPyP provides limited protection against intestinal ischemia–reperfusion injury in neonatal rats (Stefanutti et al., 2007). WW85 is another novel metalloporphyrin. It has been the focus of a limited number of studies. However, it has been tested in cardiac transplant rejection and shown to prolong survival, improve function, and decrease rejection, all independently of leukocytes or cytokines (Pieper et al., 2005).

The issue of selectivity or specificity of metalloporphyrins as peroxynitrite scavengers has not been adequately addressed for all of the members of this class of compounds. It is a key to consider that agents such as manganese(III) tetrakis(4-benzoic acid)porphyrin (MnTBAP) are superoxide dismutase mimetics as well as peroxynitrite scavengers. MnTBAP reduces 3-nitrotyrosine formation in myocardial ischemia–reperfusion, reducing infarct size in the rat (Levrand et al., 2006) and preventing diabetic cardiomyopathy in the mouse (Cai et al., 2005). It also blocked cytokine-mediated apoptosis in cardiac myocytes (Arstall et al., 1999). Similarly, MnTMPyP also scavenges superoxide anions with results such as reduced cardiac hypertrophy (Amin et al., 2001; Xiao et al., 2002) and decreased apoptosis in a rat model of cardiac transplantation (Nilakantan et al., 2006). Agents such as these are successful, but the extent to which the benefit can be attributed to peroxynitrite scavenging, rather than to being a superoxide dismutase mimetic, needs to be determined. Metalloporphyrins are more likely to be useful in treatment of acute diseases such as ischemia–reperfusion injury in stroke and myocardial infarction than as long term therapy to prevent conditions such as atherosclerosis that may take decades to develop in man. As Fe-based compounds appear to have a more limited specificity for peroxynitrite, and considering the higher rates of reaction of these compounds in mediating its decomposition, they may be the more optimal choice of metalloporphyrin to use pharmacologically.

8. Metalloporphyrin toxicity

To the extent that peroxynitrite functions as a signaling molecule in normal physiology, it may be anticipated that peroxynitrite decomposition catalysts may prevent or limit this normal function. Thus the major concern in the potential use of these agents is that the role of peroxynitrite is not always damaging to the heart or the vasculature. Under some conditions, peroxynitrite can attenuate the extent of myocardial necrosis after ischemia/reperfusion injury perhaps through peroxynitrite-mediated preservation of coronary vascular endothelial function (Nossuli et al., 1997).

The adverse effect profile of these peroxynitrite decomposition catalysts has not been completely defined. Some data suggest that high concentrations of FeTPPS may damage the integrity of the blood-brain barrier (Levrand et al., 2006; Tan et al., 2004). Studies need to be conducted to more fully elucidate the potential toxicity of these metalloporphyrins.

9. Summary

Metalloporphyrin peroxynitrite decomposition catalysts represent a class of compounds that specifically degrade peroxynitrite with a minimal number of additional reactions. These compounds hold the potential to combat the expanding list of diseases that are being found to be mediated all or in part through peroxynitrite. Manganese-based compounds have been demonstrated to be useful in catalyzing the decomposition of peroxynitrite, yet the ability of iron-based metalloporphyrins to do so without the requisite of cellular antioxidants, as well as displaying minimal secondary reactions, makes iron-based metalloporphyrin compounds a preferential focus for further study. The experimental data show encouraging results suggesting that these metalloporphyrin merit attention as pharmacological agents in treating cardiovascular diseases associated with excess peroxynitrite generation.

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