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Review

Myeloperoxidase Interactions with Nitric Oxide: A Review of Mechanistic Pathways

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Abstract: The phagocytic enzyme myeloperoxidase (MPO) plays an essential role in the inflammatory response by catalyzing formation of reactive species involved in microbial killing by generating hypochlorous acid (HOCl) from H_2O_2 and physiological (≥100 mM) Cl^- concentrations.

However, increased MPO activity has been linked to a number of pathologies with compelling evidence in initiation and progression of inflammatory events. For example, leukocyte and serum MPO levels are elevated in patients with coronary artery disease and thus may be used as a marker for cardiovascular events. MPO-derived oxidants have been linked with neurodegenerative disorders, carcinogenesis, lung disease and respiratory damage, rheumatoid arthritis, kidney damage and atherosclerosis, respectively. Recent data showed the link between increase levels of MPO and development of diabetes, implicating the enzyme as a catalyst for oxidative reactions in the vascular wall. One of the important molecules directly modulated by MPO is nitric oxide, whose bioavailability plays the central role in the development of different pathophysiologies. Thus, we reviewed and analyzed available data and proposed the comprehensive reaction pathways connecting inflammatory action of MPO and bioavailability of NO resulting in a major disturbance of normal psychological functions.

INTRODUCTION

Myeloperoxidase (MPO), hemoprotein present in neutrophils and monocytes, has an essential role in immune surveillance and host defense mechanisms. Upon phagocyte activation in peripheral tissues and fluids, MPO is secreted into both the extracellular milieu and the phagolysosome where it uses hydrogen peroxide (H_2O_2) generated during a respiratory burst by activated NADPH as co-substrate. (Hurst, 1991) A kinetic model that describes the classic myeloperoxidase cycle is presented in Figure 1.

The ground state (secreted) form of the enzyme, MPO-Fe(III), reacts in a rapid and reversible manner with H_2O_2 to form Compound I, a two e^- oxidized intermediate with Fe(IV)=O center and a resonance-stabilized porphyrin π cation radical with reduction potential of ~ -1.1 Volts (Hurst, 1991). Compound I has the ability to oxidize chloride ion normally presented in biological systems (100mM) via a single two e^- oxidation forming hypochlorous acid (HOCl) (Harrison, and Schultz, 1976).

HOCl has a potent macrobicidal and viricidal activity by playing the key role in protective and inflammatory responses in the host defense reaction (Albrich, McCarthy, and Hurst, 1981). In addition, Compound I may oxidize multiple available substrates through two sequential one e^- steps forming Compound II and MPO-Fe(III), (ground state) respectively. Some of biologically available substrates include nitrite ion, tyrosine, ascorbate, urate, estrogens, serotonin, melatonin, catecholamine, polyphenols, nitric oxide and many more. The conversion of Compound II to MPO-Fe(III) is the rate limiting step of the catalytic cycle of peroxidases. Enhancement in peroxidase catalysis due to reduction of MPO-Compound II has been noted with a series of physiological reductants like superoxide ($O_2^{\bullet -}$), melatonin, tryptophan, nitric oxide (NO), and ascorbic acid (Kettle and Candaeis, 2000), (Allegra, Furtmuller, and Regelsberger *et al.*, 2001), (Kettle, and Winterbourn,1988), (Bolscher, and Wever, 1984), (Kettle,

and Winterbourn, 19970), (Abu-Soud, and Hazen, 2000). Besides beneficial protective role that MPO has in host defense system, a number of pathophysiological conditions implicated MPO system as a sources of direct and indirect tissue injury (Daugherty *et al*., 1991), (Leeuwenburgh et al 1997).

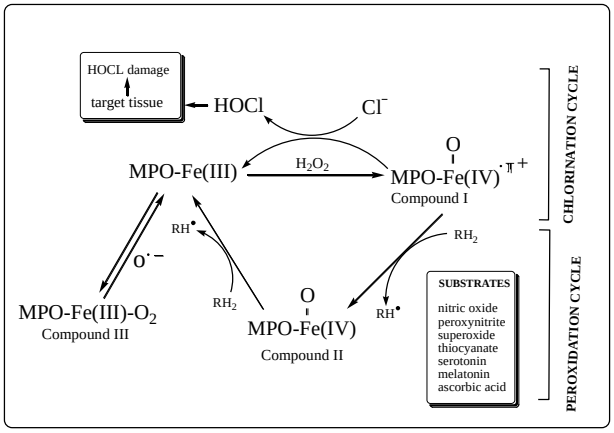

Figure 1. Classic MPO catalytic system.

A major product of MPO halogenations catalytic cycle, HOCl, has been involved in number oxidative reactions, modifying a range of biological targets such as lipids, DNA, lipoproteins and proteins (Daugherty *et al*., 1991, Leeuwenburgh *et al.*, 1997, Hazell *et al.,* 1996).The end products of these reactions have been implicated in an initiation and development of many different diseases. In a Table 1, target molecules and end product of HOCl related to specific disease are shown.

HOCl induced target molecules oxidation and end products in relation to different diseases

Oxidizing species	Target Molecule	End product	Disease
$HOCl/Cl_2$	proteins	3-chlorotyrosins	cardiovascular disease
$HOCl/Cl_2$	DNA	5-chlorouracil	carcinogenesis
$HOCl/Cl_2$	unsaturatted faty acids, cholesterol	chlorohydrines	atherosleros is
$HOCl/Cl_2$	LDL	oxidisedLDL	atherosclerosis
HOCl	NO bioavailabilty, Larginine	impared NO activity	endothelial dysfunction
HOCl/NO	proteins, lipids	chlorinated products	renal injury
HOCl	chondroitin sulfate, hyaluronic acid	degradation products	rheumatoid arthritis
HOCl	beta amyloid protein	increased ROS, structural changes	Alzheimer's disease
HOCl/MPO	mechanistic action	MPO oxidant generation NO bioavailbilty	diabetes

In addition to HOCl, MPO can generate variety of reactive oxidant species and consequently multiple distinct protein and lipid oxidation products, which have been identified in tissues associated with atherosclerosis and other inflammatory conditions (Tang, *et al*., 2006), (Daugherty *et al*., 1994), Hazell *et al.,1*997), (Leeuwenburgh,, Hardy, and Hazen, S 1997). MPO has been recently reported to be useful for identifying inflammation in the walls of coronary arteries, which in turn may indicate a risk for heart disease or heart attack (Shishehbor and Hazen, 2004). Increased levels of MPO have been detected in diabetes mellitus type 2 (Zhang, Yang, and Jennings, 2004) in joints synovial fluid of patients with developed rheumatoid arthritis(Sampaio , Fernandes, and da Silva 2012), in amyloid plaques isolated form Alzheimer type pathology tissues (Green, Mendez, and Jacob, 2004) and in kidney disease (Malle, Buch, Grone, 2003). Clearly, MPO system and its interactions with a a number of different available substrates and free radicals plays significant role in a number of pathophysiologies. Available data done *in vitro and in vivo* is extensive, but comprehensive molecular mechanism linking the reactions, inhibitory pathways, and substrate and enzymes activity under defined conditions has not been presented yet. Thus defining exact molecular mechanism of MPO activity in relationship with the surrounding system is of great importance especially when referring to a particular disease o molecular level. Thus, we analyzed available reaction pathways, our previous extended data describing the enzyme activity and kinetic of specific reactions and proposed mechanism. Apparently MPO activity is the main link between different pathways, showing the significant of ongoing inflammation in a number of pathophysiologies.

MYELOPEROXIDASE AND NITRIC OXIDE

Biological free radicals such as nitric oxide and superoxide are directly involved in MPO catalytic mechanism by a number of different pathways, thus modulating MPO activity and contributing to the detrimental effect of MPO system in the biological milieu at the sites of enzyme expression. Nitric oxide (NO), signaling molecule, plays essential bioregulatory roles in a wide range of processes, including vasodilatation, cell proliferation, nerve transmission, tumor surveillance, antimicrobial defense, and regulation of inflammatory responses (Ignarro, 1990), (Moncada, Palmer, and Higgs,1991) ,(Schmidt, and Walter, 1994), (Vincent, 1994). It is generated by a family of enzymes know as nitric oxide synthases (NOSs) which utilize molecular oxygen, NADPH, and tetrahydrobiopterin (H_4B) to convert L-arginine to NO and citrulline (Griffith, and Stuehr, 1995). Although there are three forms of NOS, only one is responsible for NO synthesis during inflammation, the cytokine inducible NOS (iNOS)(Weinberg, et al., 1999, Fang, 1997).Once expressed, it carries out continuous NO synthesis. In biological systems, enhancement of NO production by iNOS display beneficiary or harmful roles, depending on the rate of NO formation and diffusion, availability of factors that stimulate its overproduction, and local chemistry of NO degradation (Liu, et al., 1998, Gryglweski, Plamer, and Moncada, 1986).

Inducible nitric oxide synthase (iNOS) is associated with most diseases involving overproduction of NO (MacMicking, Xie, and Nathan, 1997, Nathan, 1997). Endothelial NOS (eNOS), whose expression is restricted to the vascular endothelium, is normally dormant and can generate NO upon Ca^{2+} calmoduling binding (Stuehr 1997). However, in diabetic pathology, eNOS is found to be source of superoxide radical that is directly related to the modulation of NO concentrations by forming another reactive species peroxynitrite (Gryglweski, Plamer, and Moncada, 1986). Role of MPO system on regulation of NO production has been detected in a number of *in vivo* and *in vitro* studies (Abu-Soud, Rousseau, and Stuehr, 1996, Wang *et al*., 1994). Interplay between MPO and NO can proceed via several molecular pathways. In all cases, both iNOS and eNOS can be affected structurally or kinetically, resulting in disturbed NO chemistry.

One of the possible pathways is called uncoupling of eNOS that occurs when either tetrahydrobiopterin of L-arginine concentrations are lowered or molecules are modified. As a result, eNOS switches to production of superoxide radical resulting in decreased NO generation Recent studies showed that in addition to substrate disturbance, MPO produced HOCl can induce uncoupling of eNOS by enzyme monomerisation and switch it to superoxide production ((Berka, *et al.,* 2004),(Figure 2).

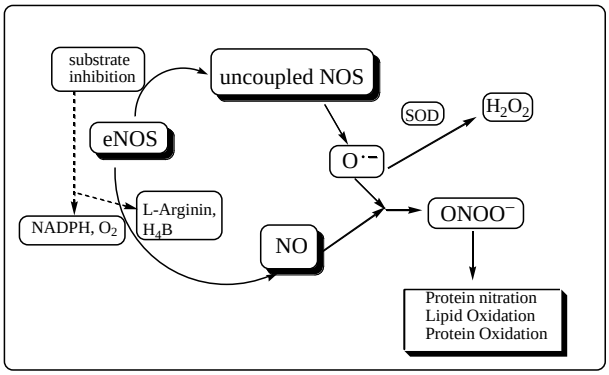

Figure 2. Uncoupling of NOS by HOCl as a source of superoxide radical.

Involvement of eNOS in superoxide radical production, and modulation of NO production caused by indirect action of MPO system is an additional pathway .directly linked to the control of inflammatory response. One of the most biologically significant molecular functions of NO is its ability to bind reversibly to many hemoproteins and nonhemoproteins thus acting either as activator or inhibitor of enzyme catalytic activity. For example, the activation of soluble guanylate cyclase takes place through the interaction with NO in vascular smooth muscle, by coordination to a heme associated with the enzyme, and resulting increase in cGMP-dependant responses induce smooth muscle relaxation associated with control of blood flow through vessels, and prevention of platelet aggregation and adherence. Several studies indicate NO activation of inter- or intracellular guanylate cyclase may play a role in many important physiologic processes, including regulation of blood pressure and coronary blood flow (Stone and Marletta, 1996, Ignarro, Wood, and Wolin, 1984, Ignarro, 1990).

Nitric oxide and NO releasing compounds have been implicated in inhibition of cytochrome P450 via the formation of an iron-nitrosyl complex that prevents access of O_2 to the catalytic site of the enzyme. Nitric oxide also mediates inhibition of mitochondrial cytochrome *c* oxidase and deenergizes mitochondria at low NO and O_2 concentrations. In the absence of L-Arg and H_4B, NO binds to the NOS heme iron at a near diffusion rate, and generates a five coordinate Fe(II)-NO complex that inhibits the catalytic activity of the enzyme (Galijasevic *et al.,* 2003) Structurally, Fe (II) is coordinated to four porphyrin nitrogens and one NO molecule, while the sixth coordinate site opposite to NO is free. Studies on MPO and its interactions with NO have demonstrated that NO modulates the catalytic activity of MPO by distinct mechanisms. Nitric oxide accelerates both the formation and decay of compound II, the rate-limiting step in the classic peroxidase cycle At higher levels of NO, reversible inhibition of MPO occurs through the formation of MPO-Fe(III)-NO complex Thus, NO serves as both a ligand and a substrate for MPO, and the overall effect of NO on the catalytic activity depends on the affinity of MPO for NO vs. H_2O_2 and their concentrations. It has been showed that NO bind to both ferric and ferrous forms of MPO, generating stable low-spin six-coordinate nitrosyl complexes. The rate of NO binding to ferrous MPO is slowed considerably with respect to ferric form, indicating that heme reduction limits the affinity of NO for the heme iron. This behavior is not typical for hemoproteins and has suggested that reduction of MPO-Fe(III) induces unusual structural, i.e., collapse or narrowing and/or electronic alterations in the heme pocket(Abu-Soud *et al.*,2002) as seen in Figure 3.

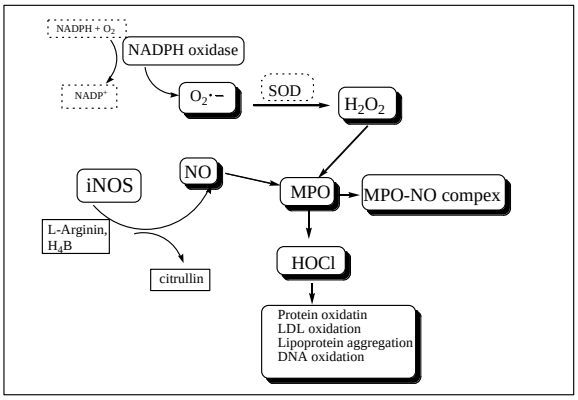

Figure 3. Interaction between MPO and NOS produced NO.

Previous studies have also indicated that heme reductions of MPO have different effects on the heme iron environment and suggest that conformational and/or electronic changes associated with heme reduction differentially affect the affinity of the heme group of mammalian heme peroxidases for diatomic ligands.

Another mechanism of interactions between MPO and iNOS suggest that the MPO system consumed NO released by iNOS during steady-state catalysis, thereby preventing the NO-induced inhibition attributed to the formation of the iNOS–nitrosyl complex. Thus, removal of NO from the iNOS milieu by the MPO system during steady-state catalysis causes a significant increase in iNOS catalytic activity, production of citrulline, and presumably production of NO. Myeloperoxidase, acting as a sink for NO efficiently activates iNOS preventing shutdown of the NO production system (Galijasevic *et al.,* 2003). However, MPO produced HOCl can react with L-arginine producing chlorinated product that can act as NOS inhibitors ultimately causing endothelial dysfunction. Number of studies confirmed that HOCL induced methylation of L-arginine inhibits NO production and contributes to the pathogenesis of inflammatory cardiovascular disease. Another reactive species, peroxynitrite $ONOO^-$, is a potent oxidant and an effective nitrating agent, promotes nitration of tyrosine residues, depletes lipid-soluble antioxidants, and initiates lipid peroxidation (van der Vliet, 1996, Lymar, , Jiang, and Hurst, 1996), Radi, 1991, Graham, 1993, Hazen, 2004). Wherever enhanced rates of NO and $O_2^{\bullet}$ production occur, peroxynitrite ($ONOO^-$) is formed in a reaction with near diffusion-controlled rate (6.7×10^9 M^{-1} s^{-1}), lowering the NO concentration achieved during the NOS reaction. The rate of $ONOO^-$ production exceeds the rate of $O_2^{\bullet-}$ removal by copper-zinc superoxide dismutase (SOD).Thus, any increase in NO production can result in $ONOO^-$ production promoting inhibition of hemoproteins such as globins, peroxidases, cytochromes P450, NOS, and

COX-2. (Herold, and Fago, 2005, Floris *et al*., 2003, Mehl *et al*., 1999, Rosen *et al*., 2002 Maréchal *et al*., 2007, Deeb *et al*., 2006).

In each case, the catalytic site of these enzymes was shown to interact with peroxynitrite and to accelerate its decomposition. This potent oxidant and its conjugate acid, peroxynitrous acid (ONOOH), are capable of promoting both protein nitration and initiation of lipid peroxidation, processes known to occur during tissue injury associated with inflammation in vivo. (Radi, 1991). Our studies demonstrated that MPO exposure to $ONOO^-$ is associated with heme depletion and protein nitration resulting with a loss of enzyme activity (Galijasevic *et al.*, 2007). Collectively, these studies showed that interplay between MPO and NO plays a major role at the sites of inflammation. By influencing MPO compound II formation, duration and decay, NO affects overall rate of peroxidation of substrates and the ability of MPO to execute one versus two electron oxidation reactions. Biologically, NO-MPO interactions as such have a broad implication on initiation and progression of local inflammatory and cardiovascular events in vivo. Molecular mechanism of MPO protective and inflammatory activity in relationship with NO is presented in Figure 4.

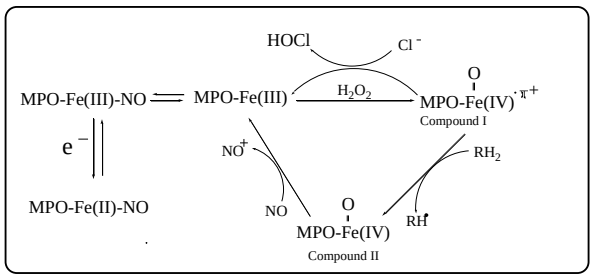

Figure 4. Catalytic cycle of MPO in the presence of nitric oxide.

MOLECULAR MECHANISM OF MPO AND NO AT THE SITES OF INFLAMMATION

Role of leukocyte derived MPO system in a destruction of microorganisms during phagocytosis is well known. Number of studies has shown that MPO system, besides its essential role in immune surveillance and host defense mechanism, has ability to promote tissue injury through a number of oxidative reactions resulting in an initiation and development of different diseases. Increased levels of MPO in walls of coronary arteries were directly related to the risk of heart disease or heart attack. (Shishehbor and Hazen 2004).MPO derived species can oxidize LDL which is considered a major event in the progression and eventual development of arthrosclerosis (Nicholls and Hazen, 2004). Another important consequence of MPO activity is a consumption of NO and induction of endothelial dysfunction. Endothelial dysfunction is defined as a loss of ability to promote vasodilatation by a shift in production and release of several vasoactive molecules. It has been linked directly to a number of diseases such as cardiovascular disease, hypertension, coronary artery disease, chronic heart failure, diabetes (Cohen, 1993, Busee, and Fleming, 1996, Harrison, 1997, Rees, Palmer and Moncada, 1989). On molecular level, a major culprit in a development of ED is decreased NO bioavailability, that is a result of impaired NO production and/or increased inactivation of NO by reactive oxygen species (Gryglewski, Palmer and Moncada, 1986). Additionally, our previous research showed involvement of MPO system by functioning as a catalytic sink for NO at the sites of inflammation thereby affecting the NO availability. Increased concentrations of MPO and reduced bioavailability of NO have been detected in models of type 2 diabetes, what is expectable considering that some of hallmarks of this disease are endothelial dysfunction, increased risk of cardiovascular diseases and elevated oxidative stress (elevated production of ROS and RNS),(Lanngenstroer and Piper, 1992, Graier, Pusch, and Wascher, 1999, Huie and Padmaja, 1993). Elevated activity and biomarkers of MPO mediated molecular damage such as 3-chlorotyrosine, protein carbonyls, 3-nitrotyrosine, lipid oxidation products and oxidized DNA have been detected in Alzheimer's disease (Green *et al*., 2004), while elevated concentrations of MPO and NO detected of synovial fluid of rheumatoid arthritis patients (Sampaio *et al*., 2012). In a **Table 1** MPO derived species, mechanisms of action and target molecules related to a different disease are presented.

Apparently, activity of MPO derived HOCl and modulation of NO bioavailability is major factor of detrimental MPO activity in a number of pathologies. Thus, the interplay between MPO products, nitric oxide , superoxide and target molecules results in a damaged molecules that can be considered as a biomarkers of atherosclerosis, diabetes, ED and continuous inflammation.

Inducible NOS is considered to be nonconstitutive, activated only after the exposure to cytokines at the sites of inflammation, while endothelial NOS is mainly associated with endothelium cell membranes. However, both type of enzyme generates NO from the amino acid L-arginine in the presence of O_2 and NADPH using cofactor tetrahydrobiopterin, H_4B. MPO and inducible NOS are both co-localized and secreted from the primary granules of activated leukocytes (Galijasevic *et al.*, 2003) hence, MPO typically performs its functions in environments where NO is formed. One mechanistic pathway of NO modulation involves inactivation of eNOS by MPO generated HOCl, inducing uncoupling of the eNOS dimere. Generally, uncoupling of NOS occurs when cofactor or substrate are lacking, resulting of production of superoxide by NOS. Dissociation of homomeric NOS can be induced by HOCl oxidation of the Zn-thiolate active center of the enzyme. Another pathway is reaction of HOCl with L-arginine giving chlorinated products that can act as NOS inhibitors, again inhibiting NO synthesis that leads to vasoconstriction manifested as an elevated blood pressure. The link between hyperglycemia and ED has been supported by a number of studies showing reduced bioavailability of NO in ED induced by MPO system (Cohen, 1993, Langenstroer and Piper, 1992). Considering all the possible reaction pathways of MPO system with NO it is clear that contribution of these interactions to ED is significant (Table 2).

Table 2. Modulation of nitric oxide bioavailabiliy.

Pathways for modulation of nitric oxide bioavailability by MPO
1. Formation of MPO-Fe(III) complex 2. Substrate for Compound I and II 3. HOCl induced L-Arginine chlrination 4. Uncoupling of iNOS by HOCl- shutdown of NO production

In inflammatory condition, superoxide radical is produced in neutrophiles during respiratory burst by NDPH oxidase. Superoxide dismutase converts it to hydrogen peroxide that is used by MPO in a production of HOCl. In addition, nonphagocyte NDPH oxidase produces superoxide radical but only a fraction of amount produced by phagocyte NDPH oxidase, and functions through intracellular signaling (Figure 5).

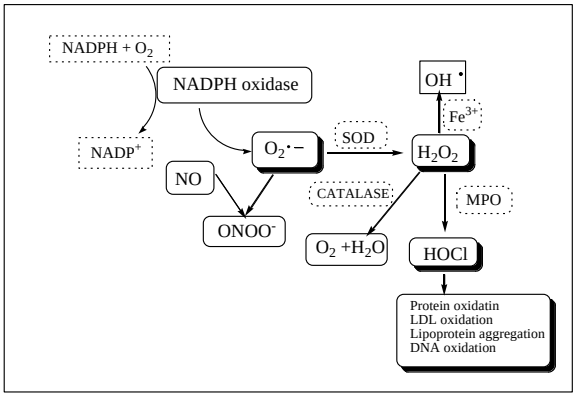

Figure 5. Fate of superoxide radical in inflammatory milieu.

However, high glucose stimulates production of ROS whose main sources is vascular NAD(P)H oxidase that can be either consume as a substrate by MPO or converted to H_2O_2 and again used by MPO leading to inflammation and diabetic vascular disease (Zhang, Yang, and Jennings, 2004). Apparently, after oxidative burst that is initial event in any inflammatory condition is stopped, stimulated MPO stays bound for vascular wall and can consume high glucose stimulated H_2O_2. Additional sources of superoxide is uncoupled NOS. The role of SOD inhibition under pathological conditions is not known yet and data are somewhat conflicting. If superoxide is not consumed directly or indirectly by MPO system, can react with available NO forming $ONOO^-$ highly toxic and reactive molecule causing tissue damage by oxidizing a number of biological molecules. Any of these pathways for superoxide consumption that are highly dependable on MPO, NOS and SOD will lead to development of pathophysiological condition either as HOCl induced tissue damage or ONOO- action in addition to ED that depends on NO bioavailability.

Considering all the available data and our previous research, we proposed the general mechanistic pathway coupling activity of MPO and NOS and their production of free radicals that can contribute directly to the initiation and development of several diseases (Figure 6).

Additional studies, but with the focus on specific concentration depended reactions done *in vivo* are necessary to further deduct the controlling conditions. As a result, the development of specific inhibitory compounds could lead to inactivation of enzymes or reaction pathways causing disturbance of normal physiological processes.

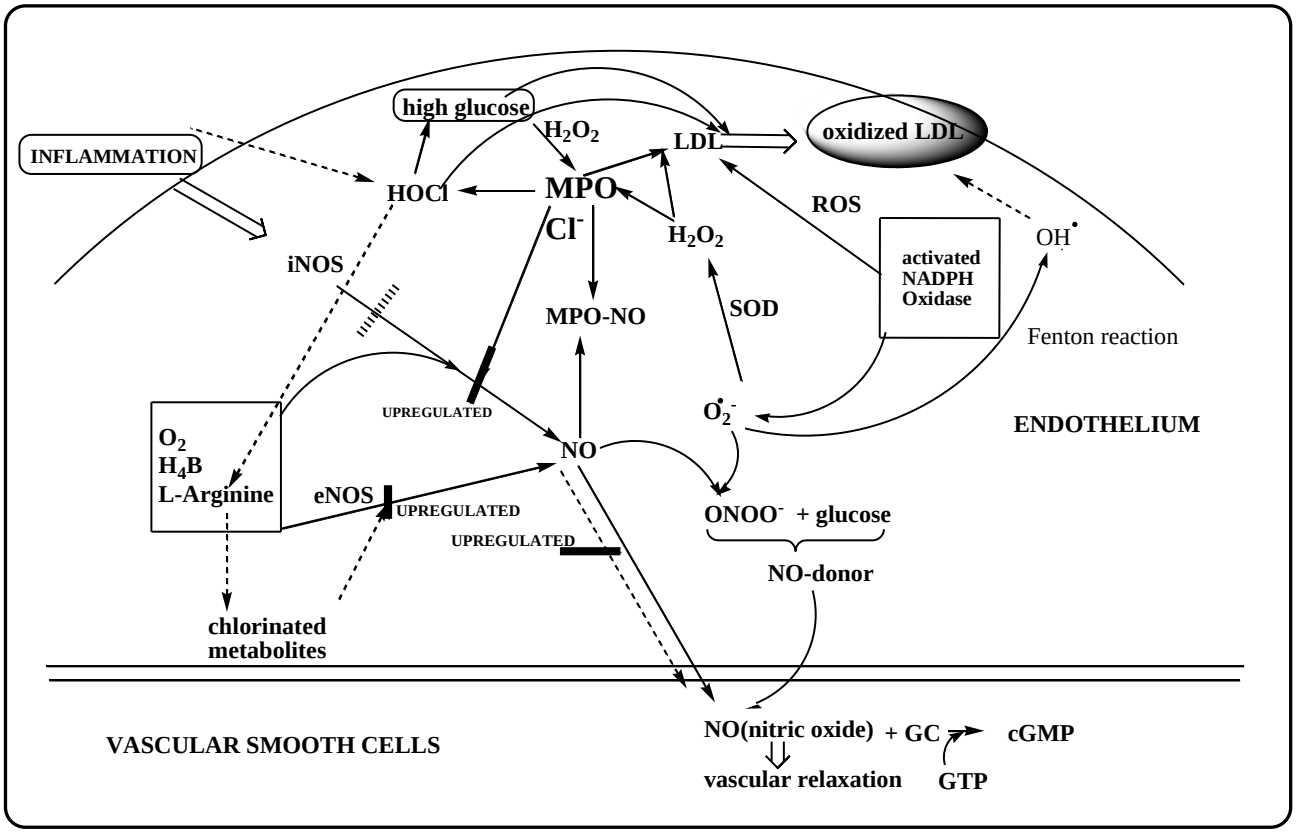

Figure 6. Comprehensive mechanism of MPO system controlled interactions with selected free radical resulting in a detrimental effect in endothelium.

REFERENCES

Abu-Soud, H.M., Hazen S.L. (2000). Nitric oxide modulates the catalytic activity of myeloperoxidase. *Journal of Biological Chemistry*, **275**, 5425-30.

Abu-Soud, H.M., Khassawneh, M.Y., Sohn, J.T., Murray, P., Haxhiu, M.A., Hazen, S.L. (2001). Peroxidases inhibit nitric oxide (NO) dependent bronchodilation: development of a model describing NO-peroxidase interactions. *Biochemistry*, **40**, 11866-75.

Abu-Sound, H. M., Ichimori, K, Presta, A, Stuehr, D.J. (2000), Electron transfer, Oxygen Binding, and nitric oxide feedback inhibition in endothenial nitric-oxide synthase. *Journal of Biological Chemistry 275***,** 17349-17357.

Abu-Sound, H.M., Rousseau, D.L., Stuehr, D.J (1996). Nitric Oxide binding to the heme of neuronal nitric-oxide synthase links its activity to changes in oxygen tension. *Journal of Biological Chemistry*, **271**, 32515-32158.

Albrich, J. M., McCarthy C. A., Hurst, J. K. (1981). Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. *Proceedings of National Academia of Sciences USA*, **78**, 210-214.

Allegra, M., Furtmuller, P.G., Regelsberger, G., Turco-Liveri, M.L., Tesoriere, L., Perretti, M., Livrea, M.A., Obinger, C. (2001). Mechanism of reaction of melatonin with human myeloperoxidase. *Biochemistry and Biophysics Research Communication*, **282**, 380-386.

Beckman, J.S, Beckman, T.W., Chen, J, Marshall P.A., Freeman, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proceedings of National Academia of Sciences USA*, **87**, 1620-1624.

Bolscher, B. G., Wever, R. A. (1984). The nitrosyl compounds of ferrous animal haloperoxidases. *Biochim. Biophys. Acta*, **788**, 1-10.

Busse, R, Fleming, I. (1996). Endothelial dysfunction in atherosclerosis. *Journal Vascular Research*, **33**, 181-194.

Cohen, R.A. (1993).Dysfunction of vascular endothelium in diabetes mellitus. *Circulation* **87**, V67-v76.

Daugherty, A., Dunn, J. L., Rateri, D., L., and Heinecke, J. W. (1994). Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *Journal of Clinical Investigation*, **94**, 437-444.

Deeb, R. S., Hao, G., Gross, S. S., Lainé, M., Qiu, J. H., Resnick, B., Barbar, E. J., Hajjar, D. P., Upmacis, R. K. (2006). Heme catalyzes tyrosine 385 nitration and inactivation of prostaglandin H2 synthase-1 by peroxynitrite. *Journal of Lipid Research*, 47, 898–911.

Fang, F.C. (1997). Perspective series: host/pathogen interactions. Mechanism of nitric oxide-related antimicrobial activity. *Journal of Clinical Investigation*, **99,** 2818-2825.

Floris, R., Piersma, S. R,; Yang, G., Jones, P., Wever, R. (2003). Interaction of myeloperoxidase with peroxynitrite: a comparison with lactoperoxidase, horseradish peroxidase and catalase. *European Journal of Biochemistry*, **215**, 767-775.

Galijasevic**,** S., Saed, G. M, Diamond, M.P., Abu-Soud. H.M. (2003). Myeloperoxidase up-regulates the catalytic activity of inducible nitric oxide synthase by preventing the nitric oxide feedback inhibition. *Proceedings of National Academia of Sciences of U S A.* **100,** 14766-14771.

Graham, A, Hogg, N., Kalyanaraman, B., O'Leary, V, Darley-Usmar, V., Moncada, S. (1993). Peroxynitrite modification of low-density lipoprotein leads to recognition by the macrophage scavenger receptor. *FEBS Letters* **330**,181-185.

Graier, W.F., Pusch, K., Waschert, T. (1999). Increased superoxide formation in endothelial cells during hyperglycemia. *Diabetes Research and Clinical Practice*, **45,** 153-160.

Green, P.S., Mendez, A.J., Jacob, J.S., Crowley, J.R., Growdon, W., Hyman, BT., Heinecke, J.W., (2004). Neuronal expression of myeloperoxidase is increased in Alzheimer's disease. *Journal of Neurochemistry*, **90,** 724-733.

Griffith, O. W. & Stuehr, D. J. (1995). Nitric oxide synthase properties and catalytic mechanism. *Annual Review of* Physiology, **57**, 707–736.

Gryglewski, R.J., Palmer, R.M., Moncada, S.(1986). Superoxide anion is involved in the breakdown of endothelium – derived vascular relaxing factor. *Nature*, **320**, 453-456.

Hazen S.L., Zhang, R., Shen, Z., Wu, W., Podrez E.A., MacPherson, J.C., Schmitt, D., Mitra, S.N, Mukhopadhyay, C., Chen. Y., Cohen, P.A., Hoff, H.F., Abu-Soud, H.M. (1999). Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation. *In vivo*. *Circculation Research*, **85**, 950-958.

Hazell, L. J., Arnold, L., Flowers, D., Waeg, G., Malle, E., Stocker, R. (1996). Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *Journal of Clinical Investigation*, **97**, 1535-1544.

Hazen, S. L., Gaut, J. P., Hus, F. F., Crowley, J. R., d'Avignon, A. Heinecke, J. W. (1997). p-Hydroxyphenylacetaldehyde, the major product of L-tyrosine oxidation by the myeloperoxidase-H_2O_2-chloride system of phagocytes, covalently modifies epsilon-amino groups of protein lysine residues. *Journal of Biological Chemistry*, **272**, 16990-16998.

Harrison, D.G. (1997).Cellular and molecular mechanisms of endothelial cell dysfunction. *Journal of Clinical Investigation*, **100**, 2153-2157.

Harrison, J. E., Schultz J. (1976). Studies on the chlorinating activity of myeloperoxidase. *Journal of Biological Chemistry*, **251**, 1371-1374.

Hurst, J. K. (1991). Myleperoxidase-active site structure and catalytic mechanisms. In: Everse J.; Everse K., Grisham, M. B., eds. Peroxidases in Chemistry

and Biology. 1st edn. Boca Raton: *CRC Press*, 37-62.

Huie, R.E. Padmaja, S. (1993). The reaction of NO with superoxide. *Free Radicals Research Communication*, **18**, 195-199.

Herold, S., Fago, A.(2005). Reactions of peroxynitritewith globin proteins and their possible physiological role. Comp. Biochem. Physiol. Mol. Integr. Physiol. **142**,124–129.

Ignarro, L. J. (1990). Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annual Review of Pharmacology and* Toxicology, **30**, 535-560.

Ignarro, L.J., Wood, K.S., Wolin, M.S. (1984). Regulation of purified soluble guanylate cyclase by porphyrins and metalloporphyrins: a unifying concept. *Advanced Cyclic Nucleotide Protein Phosphorylation Research*, **17**, 267-7.

Ignarro, L.J. (1990). Haem-dependent activation of guanylate cyclase and cyclic GMP formation by endogenous nitric oxide: a unique transduction mechanism for transcellular signaling. *Pharmacol Toxicol.* **67**:1-7.

Kettle, A.J., Candaeis, L.P. (2000). Oxidation of tryptophan by redox intermediates of myeloperoxidase and inhibition of hypochlorous acid production. *Redox Report*, **5**,179-84.

Kettle, A. J., Winterbourn, C. C. (1988). Superoxide modulates the activity of myeloperoxidase and optimizes the production of hypochlorous acid. *Biochemistry Journal*, **252**, 529-536.

Kettle, A. J., Winterbourn, C. C. (1997). Myeloperoxidase: a key regulator of neutrophil oxidant production. *Redox Report*, **3**, 3-15.

Langenstroer, P., Piper, G.M. (1992). Regulation of spontaneous EDRF release in diabetic rat aorta by oxygen free radicals. *American Journal of Physiology*, **263**, H257-262.

Liu, X., Miller, M.J., Joshu, M.S., Sadowska-Krowica, H., Clark, D., A., Lmacaster, J.R., (1998). Diffucuion limited reactions of free nitric oxide witrh erytrocites. *Journal of Biological Chemistry***, 273,** 18709-16713.

Leeuwenburgh, C., Hardy, M. M., Hazen, S. L., Wagner, P., Oh-ishi, S., Steinbrecher, U. P., Heinecke, J.,W. (1997). Reaction nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *Journal of Biological Chemistry*, **272**, 1433-1436.

Leeuwenburgh, C., Rasmussen, J. E., Hsu, F. F., Mueller, D.M., Pennathur, S., Heinecke, J.,W. (1997). Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *Journal of Biological Chemistry*, **272, 3520**-3526.

Lymar, S.V., Jiang, Q, Hurst J.K. (1996). Mechanism of carbon dioxide-catalyzed oxidation of tyrosine by peroxynitrite. *Biochemistry*. **35**, 7855-7861.

Malle, E., Buch, T., Grone, HJ., (2003). MPO in kidney disease. *Kidney International*, **64**, 1956 -1967.

MacMicking, J., Xie, Q.W., Nathan, C. (1997). Nitric oxide and macrophage function. *Annual Reviews in Immunology*, **15**, 323-350.

Maréchal, A., Mattioli, T. A., Stuehr, D. J., Santolini, J. (2007). Activation of peroxynitrite by inducible nitric-oxide synthase: a direct source of nitrative stress. *Journal of Biological Chemistry*, **282**, 14101–14112.

Mc Veigh, G.E, Brennan, G.M, Johnston, G.D, (1992). Impaired andothelium- dependent and independent vasodilatation in patients with type 2 diabetes mellitus. *Diabetologia* **35**:771-776.

Mehl, M., Daiber, A., Herold, S., Shoun, H., Ullrich, V. (1999). Peroxynitrite reaction with heme proteins. *Nitric Oxide* **3**,142–152.

Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacology Review*, **43,**109–142.

Nicholls, S.J, Hazen, S.L. (2004). The role of myeloperoxidase in the pathogenesis of coronary artery disease. *Japanese Journal of Infectious Diseases*, **57**, S21-2.

Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics*, **288**, 481-487.

Rees, D.D., Palmer, R.M, Moncada, S. (1989).Role of endothelium-derived NO in the regulation of blood pressure. *Proceedings of National Academia of Sciences USA*, **86**, 3375-3378.

Rosen, G.M., Tsai, P., Weave,r J., Porasuphatana, S., Roman, LJ., Starkov, A.A., Fiskum, G, Pou, S. (2002). The role of tetrahydrobiopterin in the regulation of neuronal nitric-oxide synthase-generated superoxide. *Journal of Biological Chemistry*, **277**,40275-80.

Sampaio, R.M., Fernandes, N., da Silva, N.P. Sato, E.I. (2012). Increased myeloperoxidase plasma levels in rheumatoid arthritis. *Rheumatology International*, **32**, 1605-1609.

Schmidt, H. H. H. W. & Walter, U. (1994). NO at work. *Cell*, **79,** 919–925.

Shishehbor M.H., Hazen S.L. (2004). Inflammatory and oxidative markers in atherosclerosis: relationship to outcome. *Current Atherosclerosis Report*, **6**, 243-50.

Stone, J.R., Marletta M.A. (1996). Spectral and kinetic studies on the activation of soluble guanylate cyclase by nitric oxide. *Biochemistry* **35,**1093-9.

Stuehr, D.J (1997) Structure-function aspects in the nitric oxide synthasis. *Annual Reviews Pharmacoal. Toxical* **37**, 339-359.

Stuehr, D.J., Ikeda-Saito, M. (1992) Spectral characterization of brain and macrophage nitric oxide synthases. Cytochrome P-450-like hemeproteins that contain a flavin semiquinone radical. *Journal of Biological Chemistry*, **267,** 20547-20550.

Tang, W. H., Brennan, M. L., Philip, K., Tong, W., Mann, S., Van Lente, F., Hazen, S. L. (2006). Plasma

myeloperoxidase levels in patients with chronic heart failure. *American Journal of Cardiologists*, **98**, 706-799.

Vincent, S. R. (1994) Nitric oxide: A radical neurotransmitter in the central nervous system. *Progress in Neurobiology*. **42,** 129–160.

van der Vliet A, Eiserich, J.P., Halliwell, B, Cross, C.E. (1997). Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *Journal of Biological Chemistry*, **272**,7617-7625.

Wang, J., Rousseau, D.L, Abu- Sound, H.M, Stuehr, D.J (1994). Heme coordination of **NO** in **NO** synthase. *Proceedings of National Academia of Sciences USA*, USA **91,** 10512-10516.

Weinberger, B.,Heck, D.L., Laskin, D.L., Laskin, J. D. (1999) Nitric oxide in the lung : therapeutic and cellular mechanism of action. *Pharmacology Therapies*, **84**, 401-411.

White, K. A, Marletta, M. A. (1992). Nitric Oxide synthase is a cytochrome P-450 type hemoprotein. *Biochemistry*, *81*, 6627-6631.

Zgang, C., Yang, J, Jennings, L.K., (2004). Leukocyte-derived myeloperoxidase amplifies high-glucose-induced endothelial dysfunction through interactions with high-glucose stimulated vascular non-leukocyte derived oxygen species. *Diabetes*, **53**, 2950-9.

Summary/Sažetak

Fagocitni enzim mijeloperoksidaze (MPO) igra bitnu ulogu u inflamatornom procesu pri čemu katalizira stvaranje reaktivnih vrsta uključenih u ubijanju mikroba generiranjem hipokloričnе kiseline (HOCl) s H_2O_2 pri fiziološkim ($\geq$ 100 mm) Cl^- koncentracijama.

Međutim, povećana MPO aktivnost je povezana s nizom patoloških stanja bilo u inicijaciji i/ili progresiji upalnih procesa. Na primjer, nivoi leukocita i serumske MPO su povišeni kod pacijenata sa koronarnom bolesti srca, te se MPO može marker za kardiovaskularne događaje. MPO proizvedeni oksidansi su povezani sa neurodegenerativnim poremećajima, kancerogenezom, bolestima pluća i disajnih puteva reumatoidnim artritisom, oštećenjima bubrega i aterosklerozom. Nedavni podaci pokazuju vezu između povećanja razine MPO i razvoja dijabetesa, implicirajući enzim kao katalizator oksidativnih reakcije u vaskularnom zidu. Jedan od važnih molekula izravno modulirana sa MPO je nitrogen oksid, čija bioraspoloživost igra središnju ulogu u razvoju različitih bolesti. Nakon analize dosadasnjih istraživanja predloženi su sveobuhvatni reakcijski mehanizmi koji povezuju upalno djelovanje MPO i bioraspoloživosti NO što rezultira poremećajima normalnih fizioloških funkcija.