INHIBITION OF MYELOPEROXIDASE: A NEW THERAPEUTIC TARGET

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The oxidative modification of LDL is a key event in the process of atherosclerosis. Oxidized derivatives of lipoproteins accumulate in the vascular wall and promote local inflammatory lesions which trigger the progression of the atheromatous plaques.

Myeloperoxidase (MPO) is a heme-containing enzyme of the peroxidase family that produces hypochlorous acid (HOCl) in the presence of hydrogen peroxide (H₂O₂) and chloride anions (Cl⁻), leading to oxidative modifications of protein and cellular structures. MPO has indeed been reported as a contributory factor in many inflammatory syndromes such as rheumatoid arthritis, end-stage renal diseases, and atherosclerosis.

In respect with atherogenesis, MPO which is a highly cationic protein (isoelectric point > 10) binds easily to LDL. This phenomenon contributes to the oxidation of these lipoproteins. Various data demonstrate that HOCl produced by the MPO system mainly acts on the protein part of LDL. We developed an ELISA test involving specific monoclonal antibody for the quantification of the oxidative modifications of apo B [1]. By this method, we also demonstrated that the oxidation can partly take place at the surface of endothelial cells (EC), constituting an additional mechanism to subendothelial oxidation in atheromatous lesions [2]. This enzyme therefore appears as a potential therapeutic target. Many studies investigated the inhibition of MPO measuring either the scavenging of the oxygen species produced by the enzyme or the direct inhibition of the synthesis of HOCl.

In this regard, the non-steroidal anti-inflammatory drugs (NSAID) have been studied by many authors because of the presence of such compounds at the site of MPO activity. However, NSAID have a poor antioxidant effect towards H₂O₂ and hydroxyl radical (·OH). Therefore, we investigated the interaction with HOCl and the MPO/H₂O₂/Cl⁻ system and demonstrated the efficiency of flufenamic acid which specifically inhibits the synthesis of HOCl [3]. In other experiments, flufenamic acid always had the best inhibiting effect among the different NSAIDS tested towards the MPO/H₂O₂/Cl⁻-system. This inhibition is characterized by an oxidation of the drug which behaves like an electron donor, giving rise to the formation of the 5-hydroxy-derivative but also to a 5-chloro-derivative, another potential inhibitor of the MPO/H₂O₂/Cl⁻ system. These results could lead to the synthesis of new MPO inhibitors, derived from the structure of flufenamic acid.

Based on these observations with several anti-inflammatory and thiol-containing drugs, a new study was designed to test the hypothesis that anti-hypertensive agents from the angiotensin converting enzyme (ACE) inhibitors group, such as captopril, lisinopril, ramipril, enalapril maleate, and sodium fosinopril or of their active counter-parts, inhibit the oxidative modification of Apo B-100 caused by MPO [4]. The inhibition was examined in the absence and in the presence of LDL using a human recombinant MPO produced by Chinese hamster ovary cell line. The procedure included: a. the measurement of the interaction with HOCl; b. the direct interaction with MPO; and c. the dose-response relationships between the inhibition of LDL oxidation and several drug concentrations (for the quantification of MPO oxidized ApoB-100) [1-3].
The inhibition of the MPO chlorinating activity can be measured by assessing the chlorination of taurine in the presence of molecules under investigation. In order to be able to compare the different molecules in a system involving a chemical competition towards the MPO/H₂O₂/Cl⁻ system, methionine was used as a competitor. A molecule which reacts with the MPO/H₂O₂/Cl⁻ system required a higher amount of methionine to inhibit its oxidation. Captopril was more efficient than the other ACE-inhibitors, probably as a consequence of the presence in the molecules of a thiol function, which has already been described as highly reactive towards HOCl and is able to compete with methionine. The other ACE inhibitors have an amine function and do not react with HOCl in presence of methionine. The action of these drugs is essentially due to a single chemical reaction with HOCl, without any interactions with the enzymatic system.

The inhibition of the enzymatic system was observed by the measurement of the accumulation of compound II, a derivative form of MPO. Captopril was the sole molecule to reduce compound II by behaving as an electron donor. If the thiol function is a key parameter in this mechanism, our group like others has also demonstrated that the size of the molecule could play an important role. Others ACE inhibitors that were unable to scavenger compound II have a larger size that is unfavorable for this purpose.

The results in Figure 1 corroborate those described above. Indeed captopril was able to reduce the amount of Ox-LDL. On the other hand, the other ACE inhibitors were unable to inhibit LDL oxidation, probably as a consequence of their inability to inhibit MPO or to efficiently scavenger HOCl. Captopril, which is an efficient antihypertensive drug inhibiting ACE, could also protect against the atherosclerotic process by inhibiting the oxidative modifications of ApoB-100 in LDL. More studies are needed to assess the contribution of this mechanism in the anti-atherosclerotic effects of captopril.

References


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Figure 1. LDL oxidation (%) in relation to different concentrations of thiol containing molecules. Results are the means +/- SD.