Editorial
Atherosclerosis, oxidative stress and glutathione peroxidase-1: a new kid on the block

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Atherosclerosis is a chronic disease of the arterial vessel wall and as such the leading cause of death in western societies nowadays. In recent years a number of hypotheses, which have been reviewed extensively, have been put forward to explain the pathogenesis of atherosclerosis[1-5]. In spite of the differences in the various models, they are not mutually exclusive. Especially the inflammatory nature of the intimal response has attracted the interest of researchers, and it is perceived that an understanding of the mechanisms leading to inflammation is central to an overall understanding of atherogenesis.

Along these lines, oxidation of low-density lipoproteins (LDL) has been implicated as a major proinflammatory stimulus critically involved in atherogenesis[6]. The evidence that oxidation of LDL is indeed relevant in atherogenesis has been summarized by Steinberg[7]. First, oxidized LDL have been isolated from plaques of experimental animals as well as of patients. Second, epitopes cross-reactive with antibodies against oxidized LDL have been detected in lesions. Third, autoantibodies reactive with oxidized LDL have been found in patients and experimental animals. And finally, antioxidants can slow the progression of atherosclerosis in experimental animals. Even though animal studies and *in vitro* experiments have provided overwhelming evidence for the oxidation hypothesis, no successful trial has shown a protective effect of antioxidant treatment in humans[8,9]. The reasons for this lack of protection in human beings remain elusive. Clearly, it is not related to insufficient statistical power of the trials. For instance, the HOPE trial had more than a 90% power to detect a 13% relative reduction in the risk of the primary outcome in the vitamin E arm[10].

Does the recent discovery that a low activity of a key cellular antioxidant enzyme, namely glutathione peroxidase-1 (GPx-1), is associated with an increased risk for cardiovascular events in patients with documented coronary artery disease provide new support for the oxidation hypothesis[11]? In a prospective analysis of 636 patients with documented coronary artery disease, who were followed for a median of over 4.5 years, the association of GPx-1 with future cardiovascular events was strong and fully independent of traditional vascular risk factors. In fact, an increase in GPx-1 activity of one standard deviation decreased the relative risk by more than 30%. This association is comparable to or even stronger than that of high-density lipoproteins (HDL), which is considered to be one of the strongest known risk factors on a population basis.

Most compelling, high GPx-1 activity appears to interact with the smoking status such that smoking was predominantly associated with future cardiovascular risk in patients with low GPx-1 activity. Thus, a high antioxidative capacity as reflected by a high GPx-1 activity may be especially beneficial in patients who are exposed to increased oxidative stress. The combined effect of low antioxidative capacity and environmental oxidative stress leads to an exponential increase in the cardiovascular risk.

GPx-1 represents the first example of a genuine antioxidant enzyme associated with human cardiovascular disease in a large prospective cohort. Paraoxonase-1,

which can degrade lipid peroxides and has recently been shown to be inversely correlated with the cardiovascular risk[12], is probably involved in the protection against lipid peroxides but is not a typical antioxidant enzyme. Interestingly, another antioxidant enzyme, Cu/Zn superoxide dismutase, was not associated with cardiovascular events in the Athero*Gene* study[11]. This underscores that the relevant mechanisms for the observed effects may imply pathways other than just the neutralization of reactive oxygen species (ROS).

How may GPx-1 protect from cardiovascular events? Glutathione peroxidases constitute a family of currently four known seleno-cysteine containing enzymes, GPx-1 to 4[13,14]. Selenium is incorporated as seleno-cysteine into the enzymes. This process requires a seleno-cysteine specific tRNA complementary to a stop-codon (UGA) and in addition a specific loop structure in the 3' untranslated region of the mRNA[15]. Glutathione peroxidases are able to convert peroxides to their respective alcohols; however, their individual substrate range varies slightly. While GPx-1 may reduce fatty acids within phospholipids only in the presence of phospholipase A2, GPx-4 and, to a lesser extent, GPx-3 may use phospholipids as substrates (Table I). The general reaction mechanism is shown in figure 1. All the glutathione peroxidases rely on reduced glutathione as their major reducing substrate, but GPx-4 can also use other reducing substrates.

Guo et al.[16] reported that oxidation of LDL with aortic segments or smooth muscle cells from GPx-1 knock-out mice was significantly increased compared to wild type mice, while it was reduced in the presence of tissue from mice overexpressing Cu/Zn superoxide dismutase. This observation is compatible with the notion that the cellular content of ROS determines lipoprotein oxidation in the vicinity of such cells. It should be noted that Cu/Zn superoxide dismutase, which inhibits LDL oxidation in this *in vitro* model, was not associated with events in coronary patients[11].

Therefore, perhaps other functions of GPx-1 might be more relevant in terms of its vascular protective effects. GPx-1 knock-out mice have been shown to have an endothelial dysfunction caused by a deficiency in bioactive nitric oxide[17,18]. Dayal et al.[18] showed that hyperhomocysteinemia aggravates or even precipitates endothelial dysfunction. This would constitute a link of the GPx-1 activity to another well-known cardiovascular risk factor, namely homocysteine. Besides their impaired endothelial function, GPx-1 knock-out mice showed structural alterations in the arterial vessel wall, with prominent neointima formation and periadventitial inflammation[17]. This process is apparently age-dependent, since it has not been seen in young GPx-1 knock-out mice.

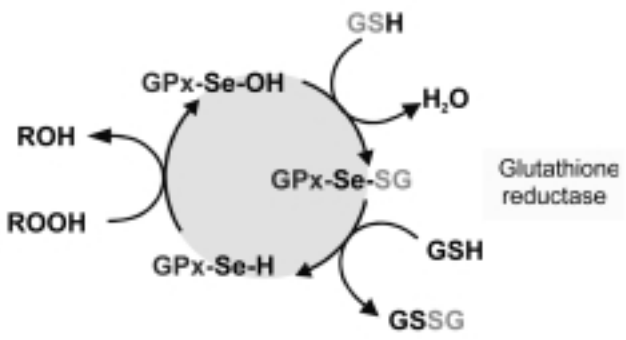

Figure 1. *Reaction mechanisms of glutathione peroxidases (GPx). In a first step, selenol (Se-H), contained in the catalytic seleno-cysteine molecule, is oxidized to selenic acid (Se-OH). Two equivalents of reduced glutathione (GSH) are oxidized to the glutathione disulfide (GSSG) to recycle the active selenol. The recycling of Se-H is strongly dependent on the availability of GSH or, in the case of GPx-4, other reducing molecules.*

Both the effects of GPx-1 deficiency described above are extracellular and therefore perhaps indirect, because GPx-1 is a cytosolic enzyme. With regard to the intracellular functions of GPx-1 with potential relevance in atherogenesis, two areas deserve consideration. First, glutathione peroxidases inhibit leukotriene and prostanoid synthesis in various cells including endothelial cells, platelets, and leukocytes. Lipid hydroperoxides have been shown to convert the active site iron of 5-lipoxygenase from the ferrous to the ferric state, which is required for its enzymatic activity[19]. A reduced availability of lipid hydroperoxides in the presence of glutathione peroxidases is a candidate mechanism for the observed inhibition of 5-lipoxygenase. While GPx-4 seems to be a major inhibitor of leukotriene synthesis in cells of lymphocytic origin, GPx-1 specifically inhibits 5-lipoxygenase in monocytic cells[20,21]. With the recent observation that 5-lipoxygenase is an important proatherogenic enzyme, this

Table I. Mammalian glutathione peroxidases (GPx).

	Protein	Tissue of synthesis	Site of action	Substrates
GPx-1	Tetramer, 22-23 kD subunit	Ubiquitous	Cytosolic	Hydrogen peroxide, fatty acid peroxides*, other organic peroxides, peroxynitrite
GPx-2	Tetramer, 24 kD subunit	Liver, large intestine	Cytosolic	Similar to GPx-1
GPx-3	Tetramer, 25 kD subunit	Kidney, others	Extracellular	Similar to GPx-1, phospholipid hydroperoxides
GPx-4	Monomer, 19 kD	Testis, most others	Cytosolic, mitochondrial	Phospholipid hydroperoxides

* phospholipid hydroperoxides can only be reduced in the presence of phospholipase A2.

function of GPx-1 is perhaps one of the most appealing in terms of its antiatherogenic potential[22,23].

Just as the ability of GPx-1 to inhibit 5-lipoxygenase, its ability to inactivate ROS such as hydrogen peroxide has been shown to modulate ROS-dependent cellular signaling and in particular the activity of ROS-dependent transcription factors, which include nuclear factor-κB (NF-κB) and peroxisome proliferator activating receptors. The effect of ROS has probably been best analyzed in the NF-κB system, which is strongly activated by ROS, and probably by the oxidant tone within the cell[24]. Interestingly, in various cell lines overexpression of GPx-1 suppresses NF-κB activation by hydrogen peroxide and/or tumor necrosis factor-α[25,26]. Many studies have shown that NF-κB is central to the activation of a whole range of supposedly proatherogenic factors, including cytokines, chemokines, adhesion molecules and many others. Therefore, it has been postulated that this transcription factor may initiate a proatherogenic response in the vessel wall[27]. In fact, activated NF-κB has been found only in atherosclerotic lesions, while in the normal arterial wall NF-κB is mostly localized within the cytoplasm, i.e. it is inactive[28].

The effects on 5-lipoxygenase and NF-κB have in common that they are both mediated by the intracellular peroxide scavenging activity of GPx-1. They have apparently little to do with lipoprotein oxidation. Could it be that the observed beneficial effects of GPx-1 activity are related to its ability to control the oxidant tone within cells? In this case, GPx-1 could be considered as a master regulator of a broad range of ROS-triggered cellular responses. This would fit well with the concept of atherosclerosis as an inflammatory disorder, because these responses are mainly proinflammatory (Fig. 2).

The GPx-1 knock-out mouse[29] will be an interesting animal model to test the various proatherogenic pathways controlled by GPx-1. At present, no data on the atherosclerosis susceptibility of this strain are available, but it is likely that we will see more rapid lesion development. This will then permit more detailed studies on the cellular events in the arterial wall under the conditions of GPx-1 deficiency. Until then, we may only speculate on the precise mechanisms that underlie the inverse correlation of GPx-1 and cardiovascular risk in humans.

At this point we would like to move on to the clinical implications of GPx-1. The question arises whether or not GPx-1 activity should be determined in patients with coronary artery disease. Even though it may be too early for a final statement in response to this question, red cell GPx-1 activity apparently contributes to the overall risk of patients with coronary artery disease. Thus, the determination of GPx-1 activity will help to estimate the individual risk of a patient. At present, this information does not immediately translate into a specific treatment. The main conclusion that may be drawn is that in case of a low GPx-1 activity, i.e. an additional risk factor, more rigorous treatment of the known and treatable risk factors, e.g. LDL cholesterol, would be indicated. Since we do not know whether the association observed in patients with preexisting coronary artery disease also applies to healthy individuals, the measurement of GPx-1 in a primary prevention setting cannot be recommended until valid epidemiological data become available.

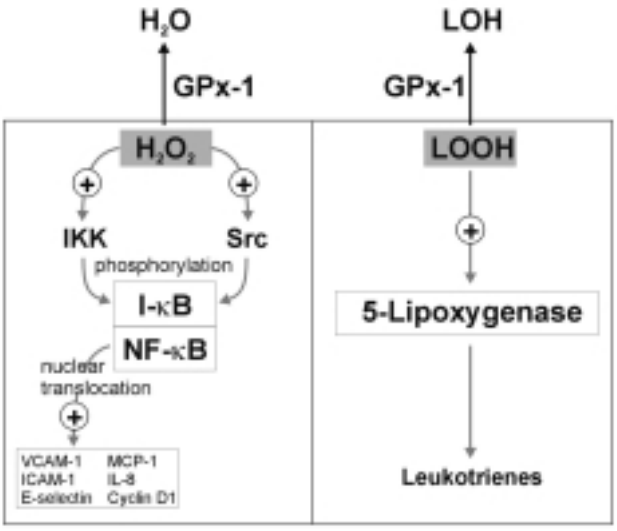

Figure 2. *Peroxide activation of atherogenic/inflammatory mediators. Hydrogen peroxide* (left side) *activates the I-κB kinase complex (IKK) and/or Src kinase, which phosphorylate I-κB. I-κB dissociates from nuclear factor-κB (NF-κB) that is translocated into the nucleus and activates several supposedly proatherogenic genes. Lipid hydroperoxides (LOOH) are required for full activity of 5-lipoxygenase and subsequent leukotriene production* (right side). *Glutathione peroxidase-1 (GPx-1) scavenges both activators and converts them into inactive compounds.*

Finally, it is tempting to speculate that the association of selenium deficiency with atherosclerosis[30,31] is mediated by a low GPx-1 activity. If this is correct, individuals with a low GPx-1 activity may benefit from selenium supplementation. It is long known that correction of selenium deficiency increases platelet glutathione peroxidase activity, which plateaus at selenium levels of approximately 100 ng/ml[32]. This is still above the average selenium level observed in the Athero*Gene* population[11]. While protection from atherosclerosis by selenium supplementation has been observed in animal models[33], no rigorously controlled data that would support an effect of selenium supplementation in human beings are available.

In summary, the recent observation that low GPx-1 activity in red blood cells is associated with an increased risk for cardiovascular events in patients with coronary artery disease opens up several new perspectives. In terms of the pathophysiology of atherosclerosis, GPx-1 may regulate the threshold for the inflammatory responses in the vessel wall by controlling intracellular hydroperoxides and ROS. Moreover, due to its dependence on selenium availability, GPx-1 is potentially amenable to therapeutic intervention. Therefore, we would not be surprised if the determination of

the GPx-1 levels develops into a routine diagnostic test for risk assessment as well as into a therapeutic target. Clearly, this requires that the open questions addressed above be answered by further research.

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