Paraoxonase-2 Reduces Oxidative Stress in Vascular Cells and Decreases Endoplasmic Reticulum Stress–Induced Caspase Activation

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Background—In the vascular system, elevated levels of reactive oxygen species (ROS) produce oxidative stress and predispose to the development of atherosclerosis. Therefore, it is important to understand the systems producing and those scavenging vascular ROS. Here, we analyzed the ROS-reducing capability of paraoxonase-2 (PON2) in different vascular cells and its involvement in the endoplasmic reticulum stress pathway known as the unfolded protein response.

Methods and Results—Quantitative real-time polymerase chain reaction and Western blotting revealed that PON2 is equally expressed in vascular cells and appears in 2 distinct glycosylated isoforms. By determining intracellular ROS, we show that overexpression of PON2 markedly reduced ROS, whereas its knockdown increased ROS levels significantly. Using microscopic and biochemical methods, we found PON2 mainly in the nuclear membrane and endoplasmic reticulum. Furthermore, PON2 expression was induced at both the promoter and protein levels by endoplasmic reticulum stress pathway unfolded protein response. This pathway may promote both apoptotic and survival mechanisms. Functionally, PON2 reduced unfolded protein response–accompanying oxidative stress and unfolded protein response–derived caspase activation.

Conclusion—We suggest that PON2 represents an endogenous defense mechanism against vascular oxidative stress and unfolded protein response–induced cell death, thereby contributing to the prevention of atherosclerosis. (Circulation. 2007;115:2055-2064.)

Key Words: apoptosis ■ atherosclerosis ■ oxidative stress ■ paraoxonase
ing PON1 developed fewer atherosclerotic lesions. Experimental studies suggest that vascular protection is associated with the ability of PON1 to hydrolyze specific lipid peroxides in oxidized lipoproteins. Accordingly, it has been claimed that PON1 protects against coronary artery diseases.

Compared with the substantial information available on PON1, little is known about PON2. Genetic polymorphisms of PON2 have been reported to be associated with cardiovascular diseases and diabetic complications. Potential antiatherosclerotic effects of PON2 can be hypothesized because the enzyme has been shown to reduce ROS in HeLa cells, reverse the oxidation of oxidized low-density lipoprotein, and inhibit the ability of oxidized low-density lipoprotein to induce monocyte chemotaxis. In agreement with these data, in vivo antiatherogenic properties of PON2 have recently been shown in PON2-deficient and PON2-overexpressing mice with an apolipoprotein E/H11002/H11002 background.

The present study was designed to investigate the expression, regulation, and potential antioxidant functions of PON2 in 3 major human vascular cell types. PON2 was localized mainly in the ER of these cells, stimulated by the UPR pathway, and protected against oxidative stress, apoptosis, and probably atherosclerosis.

Figure 1. Western blot of native and overexpressed PON2. A, Lysates (25, 50, 100 μg) of naïve EA.hy 926 endothelial cells or EA.hy 926 cells stably overexpressing the larger isoform of PON2 fused to GFP (PON2-iso1-GFP) were analyzed by Western blot with purified anti-PON2 antibody (final dilution, 1:2000). B, The same EA.hy 926 cell extracts (100 μg) were analyzed with the preimmune serum (1:100 dilution) of the anti-PON2 antibody. C, Lysates (25 μg) of naïve EA.hy 926 cells or those overexpressing PON2-iso1-GFP or PON2-iso1 fused to hemagglutinin (PON2-iso1-HA) were analyzed by Western blot with anti-PON2 antibody. Lysates were left untreated or were treated for 4 hours with 2.5 U N-glycosidase-F. In lanes 3 and 4, ~65-kDa signals represent PON2-iso1-GFP; the upper bands in lanes 5 and 6 represent PON2-iso1-HA. D, EA.hy 926 total RNA was prepared and digested with DNase-I, and RT-PCRs were performed with primers designed to amplify full-length PON2 mRNA (lane 1), a fragment of either PON2 isoform 1 or 2 mRNA (lanes 2 and 3, respectively), or both (iso 4). Lane 5 is water control. Schematics of primer positions relative to putative splice site are shown on the right (see Methods); size markers (M; bp) are shown on the left. The 2 bands appearing in lane 4 (asterisks) were eluted and sequenced. E, Lysates (50 μg) of EA.hy 926 cells overexpressing PON2-iso1-GFP (left) or PON2-iso2-GFP (right) with or without N-glycosidase-F treatment were analyzed by Western blot using anti-PON2 antibody. Molecular weight markers (kDa) are given on the left. Each blot is representative of 2 to 3 others.

Methods
See the online Data Supplement for detailed descriptions of methods applied and materials used.

Cell Culture and Cell Transfection
Human umbilical vein endothelial cell–derived EA.hy 926 cells, primary human coronary artery smooth muscle cells (SMCs), and primary human aortic adventitial fibroblasts (AoAFs) were cultured...
as reported elsewhere or as described in the Data Supplement. PON2-hemagglutinin (HA) or PON2 enhanced green fluorescent protein (GFP) fusion proteins were stably overexpressed in EA.hy 926 cells. For reporter gene assays, EA.hy 926 cells were transfected with a firefly luciferase construct cloned before a 2249-bp PON2 promoter fragment.

RNA Interference
Stealth small interfering RNA (siRNA) oligos (Invitrogen, Carlsbad, Calif) targeting the human PON2 mRNA were transfected into EA.hy 926 cells. A stealth-siRNA oligo with a scrambled sequence but with a similar G/C content served as negative control.

Quantitative Real-Time Polymerase Chain Reaction
Quantitative real-time polymerase chain reaction was performed as reported elsewhere. Relative expression of PON2 was calculated by the $2^{-\Delta\Delta C(T)}$ method.

Western Blotting
SDS-PAGEs, electrophoretic transfer of proteins, and Western blotting were done as described elsewhere.

Immunocytochemistry
Cells were grown on glass-bottomed chamber slides. Staining for nuclei and ER was done with 4',6-diamidino-2-phenylindole (DAPI; Roche, Basel, Switzerland) and ER tracker red dye (Invitrogen), respectively. PON2 was stained with a rabbit polyclonal antibody to a PON2 peptide fragment (residues 94 to 108 of human PON2).

Detection of ROS
ROS was detected with the luminol derivative L-012 (Wako Chemicals, Neuss, Germany) or 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H$_2$DCFDA, Invitrogen).

Determination of Caspase Activity
Caspase activity was assessed in cells with the caspase-Glo 3/7 system (Promega, Madison, Wis).

Figure 2. PON2 localizes mainly to the nuclear envelope and the ER. A, Live EA.hy 926 cells overexpressing PON2 iso1-GFP were stained with ER tracker red dye; nuclei were stained DAPI; cells were analyzed by confocal microscopy. B, Partial magnification of the lower cell from A. C, Fixed naïve EA.hy 926 cells were stained with DAPI and primary antibodies against PON2 and calnexin and secondary Cy3- and Cy2-labeled antibodies, respectively. D, EA.hy 926 cells were transiently transfected with a plasmid encoding for a GFP-tagged plasma membrane protein and fixed the next day. Endogenous PON2 and nuclei were stained as before; cells were examined by confocal microscopy. Scale bars = 10 μm (A, B) and 20 μm (C, D).

Figure 3. Biochemical analyses confirm intracellular localization of PON2. A, EA.hy 926 PON2 iso1-GFP cells were mock treated or treated with 25 or 50 μg/mL proteinase K for 10, 20, or 30 minutes; lysed; and subjected to Western blotting with antibodies against angiotensin II receptor 1 (AT$_1$), α-tubulin, or GFP. Equal amounts of protein (75 μg) were loaded. Positions of molecular weight markers (kDa) are indicated on the left. B, EA.hy 926 cells (6×10$^6$) were fractionated by differential and density gradient centrifugation. Protein (20 or 11 μg) of each fraction (gradient fractions 1 [bottom] to 7 [top]) was subjected to Western blotting with antibodies against PON2, GRP78, and α-tubulin. Each blot is representative of 3 others. NP indicates nuclear pellet; MP, mitochondrial pellet; S100, 100 000 g supernatant; and P100, 100 000 g pellet.
Statistical Analysis
In Figures 4 and 5, nonlinear regression curve fits were used, and maxima were analyzed for statistically significant differences. In Figures 7 and 8, statistical significance was tested with 2-way ANOVA.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Isoforms and Subcellular Localization of PON2
In EA.hy 926 cells, the anti-PON2 antibody detected both endogenous PON2 and overexpressed PON2-GFP (Figure 1A). For overexpressed PON2-GFP, identical signals were obtained with an anti-GFP antibody (not shown). When preimmune serum of the anti-PON2 antibody was used, no such signal was detected (Figure 1B). At higher electrophoretic resolution (40 kDa), the anti-PON2 antibody regularly detected 2 bands, which showed only small differences in molecular weight (Figure 1C). This suggests the presence of 2 isoforms of PON2, which is in agreement with the 2 PON2 mRNA splice variants found in public databases. Expression of the 2 splice variants in EA.hy 926 cells was validated with different RT-PCR approaches (Figure 1D). Fragments of both mRNAs were enriched, purified, and sequenced, thereby confirming the expression of 2 different PON2 mRNAs. The respective proteins are referred to here as PON2-iso1 (the larger protein) and PON2-iso2 (the smaller protein). Treatment of the lysate with N-glycosidase-F enhanced the electrophoretic mobility of PON2, generating 2 bands with lower apparent molecular weights (Figure 1C). To determine whether both PON2 isoforms are glycosylated, we cloned and expressed the putative PON2-iso1-GFP and PON2-iso2-GFP in EA.hy 926 cells. As depicted in Figure 1E, deglycosylation altered the electrophoretic mobility of both isoforms. Glycosylation also is shown for overexpressed PON2-iso1-hemagglutinin (PON2-iso1-HA; Figure 1C, lanes 5 and 6).

We next determined the subcellular localization of PON2. As shown in Figure 2A and 2B, low nucleoplasmic signals were obtained by confocal microscopy when ana-
alyzing overexpressed PON2-iso1-GFP, in contrast to substantial amounts in the nuclear envelope and the ER. ER association is assumed on the basis of the colocalization with an ER labeling dye. Similar results were obtained with other cell lines (HeLa, HEK293T, Huh7, U2OS; data not shown). We also analyzed the localization of endogenous PON2 by immunocytochemistry and subsequent confocal microscopy. Figure 2C shows a predominant staining of the nuclear envelope. Additionally, PON2 immunoreactivity largely colocalized with signals obtained with an antibody against the ER resident protein calnexin. Interestingly, nucleoplasmic endogenous PON2 is markedly increased in contrast to signals derived from PON2-iso1-GFP (compare Figure 2A with Figure 2C). Because staining for endogenous PON2 detects both isoforms, this might indicate a differential localization, particularly for the nucleoplasm (analyzed separately). As control, plasma membrane localization of endogenous PON2 was excluded (Figure 2D).

To verify our findings with independent biochemical methods, EA.hy 926 cells were treated with different concentrations of proteinase K, which degrades proteins associated with the plasma membrane. Subsequent analysis of total protein extracts by Western blot shows efficient disintegration of the angiotensin II receptor 1 (Figure 3A), an integral membrane protein. The integrity of intracellular proteins such as α-tubulin was not affected by proteinase K. Likewise, PON2-iso1-GFP remained unchanged, suggesting an intracellular localization. If parts of PON2-iso1-GFP were to face the extracellular space, partial digestion by proteinase K would result, which was not observed. The same results were obtained for endogenous PON2 (not shown).

The subcellular localization of PON2 also was confirmed by differential and density gradient centrifugation. Figure 3B shows that a large fraction of endogenous PON2 was found in the 1000g fraction representing the nuclear pellet. PON2 was also detected in the 13 000g pellet, representing a fraction of several organelles such as mitochondria, lysosomes, and Golgi membranes. Finally, PON2 was absent from the 100 000g supernatant containing soluble proteins but was detected in various fractions of the ER. To verify correct subcellular fractionation, samples were probed for α-tubulin and glucose-regulated protein 78 (GRP78; Figure 3B).
PON2 Reduced Intracellular ROS in Vascular Cells

Further experiments addressed the question of whether PON2 controls intracellular ROS in EA.hy 926 cells. Oxidative stress was induced with 2,3-dimethoxy-1,4-naphthoquinone (DMNQ; 10 μmol/L). To validate the system, EA.hy 926 cells were treated with the antioxidant vitamin C or the water-soluble vitamin E derivative Trolox, both of which reduced DMNQ-induced ROS production (Figure 4A and 4B). Then, oxidative stress was induced in EA.hy 926 cells stably overexpressing PON2-iso1-GFP or PON2-iso1-HA (Figure 4C). PON2-overexpressing cells had markedly reduced ROS levels in response to DMNQ compared with indigenous EA.hy 926 cells (Figure 4C). Results obtained with the ROS reporters L-012 or carboxy-H2DCFDA were interchangeable, and overexpression of GFP did not affect intracellular ROS (not shown). Next, we tested EA.hy 926 cells, in which PON2 had been knocked down by RNA interference (RNAi). RNAi reduced PON2 mRNA to ~10% of control 1 day after application (not shown). Efficient reduction of both PON2 protein isoforms was followed by a delay of 2 or 3 days (Figure 4D). Importantly, ROS levels in response to DMNQ increased after PON2 knockdown relative to control or untreated cells (Figure 4E). Similar results were obtained with another PON2-specific siRNA (not shown). Although levels of ROS induction varied somewhat between experiments (Figure 4C and 4E), the relative effects of PON2 overexpression or suppression remained unchanged.

Expression and Antioxidant Function of PON2 in Different Vascular Cell Types

In the vascular wall, ROS production occurs in the intima, media, and adventitia. Therefore, we analyzed EA.hy 926 endothelial cells and primary human umbilical vein endothelial cells, SMCs, and AoAFs for PON2 expression. Quantitative real-time polymerase chain reaction revealed no differences in PON2 mRNA levels between these cells (Figure 5A). Likewise, PON2 protein expression and glycosylation appeared comparable (Figure 5B). Thus, between the cell types tested, no evidence was found for specific variations in PON2 expression.

We next analyzed whether PON2 also controls ROS in SMCs and AoAFs. Figure 5C and 5D shows the efficient downregulation of endogenous PON2 by RNAi in these cells. As in EA.hy 926 cells, intracellular ROS levels in response to DMNQ were markedly increased after PON2 knockdown (Figure 5E and 5F). As before, results were confirmed with another PON2-specific siRNA (not shown).
PON2 Is Induced by the UPR

Considerable amounts of PON2 were found in the ER. Because PON2 may reduce ROS that accompanies ER stress, an enhancement of its expression by ER stress pathway UPR appears reasonable. This was assessed by UPR induction in EA.hy 926 cells by dithiotreitol (DTT; 2.5 mmol/L), which increased GRP78 expression (Figure 6A and 6B), which is considered characteristic of UPR induction. Simultaneously, PON2 expression, especially PON2-iso2, also was enhanced by DTT in a time-dependent fashion (Figure 6A and 6B). Interestingly, PON2 induction preceded that of GRP78. Another compound, tunicamycin, also enhanced GRP78 expression (Figure 6C). Tunicamycin is an inhibitor of protein N-glycosylation, thereby activating the UPR by a different mechanism. In addition, tunicamycin induced PON2; however, because of the blockade of N-glycosylation, induced PON2 isoforms appear as new bands comigrating with the deglycosylated PON2 isoforms (Figure 6C, lanes 2 and 3). Both of these bands were obliterated by cycloheximide (Figure 6C, lane 5), indicating that they represent newly synthesized proteins.

To investigate the mechanism underlying this enhanced PON2 expression, we cloned a PON2 promoter fragment and used it to perform reporter gene assays. These assays revealed that the stimulator of the UPR, tunicamycin, produced a concentration-dependent stimulation of PON2 promoter activity (Figure 6D). Similar stimulations of PON2 promoter activity were observed with the UPR stimulators DTT (0.01 to 10 mmol/L) and thapsigargin (0.01 to 10 mmol/L; n = 4 each; data not shown). This indicates that induction of PON2 expression through the UPR is a transcriptional event.

PON2 Reduces ROS Production Stimulated by the UPR

Because of its ER association, it seemed reasonable that PON2 could reduce overwhelming ROS during UPR. Exposure of naïve EA.hy 926 cells to tunicamycin (1 μg/mL) induced oxidative stress, which after 24 hours was even more pronounced than that induced by DMNQ (10 μmol/L; Figure 7A). In PON2-iso1-GFP– or PON2-iso1-HA–overexpressing EA.hy 926 cells, ROS production in response to tunicamycin (and DMNQ) was almost abolished (Figure 7A). This demonstrates that PON2 can reduce ROS generated by ER stress.

The effect of PON2 on ER-associated oxidative stress was verified in a different model. SMCs treated with 50 nmol/L of either nonspecific (control) siRNA or PON2-specific siRNA were exposed to transforming growth factor-β1 (TGFβ1; 10 ng/mL) for 6 or 24 hours. Then, total RNA was prepared and subjected to quantitative real-time polymerase chain reaction. Nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) mRNA amplification rates were normalized to RNA polymerase IIa mRNA values obtained within the same preparation. Columns represent mean ± SEM of 3 independent measurements. B, SMCs were treated with TGFβ1 (10 ng/mL) for 6 or 24 hours. Then, total RNA was prepared and subjected to quantitative real-time polymerase chain reaction. Nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) mRNA amplification rates were normalized to RNA polymerase IIa mRNA values obtained within the same preparation. Columns represent mean ± SEM of 3 independent measurements. C, Untreated SMCs or SMCs treated with 50 nmol/L nonspecific (control) or PON2-specific siRNA were treated with TGFβ1 (10 ng/mL) for 24 hours starting 3 days after RNAi and analyzed for intracellular ROS with carboxy-H2DCFDA. Columns represent mean ± SEM of 3 independent measurements. Differences were tested for statistical significance with 2-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 7. PON2 decreases ER-generated ROS. A, Naïve or PON2-iso1-GFP– or PON2-iso1-HA–overexpressing EA.hy 926 cells were treated for 24 hours with dimethyl sulfoxide (DMSO; 1%; solvent control), DMNQ (10 μmol/L), or tunicamycin (1 μg/mL). ROS was determined with L-012. Columns represent mean ± SEM of 3 independent measurements. B, SMCs were treated with TGFβ1 (10 ng/mL) for 6 or 24 hours. Then, total RNA was prepared and subjected to quantitative real-time polymerase chain reaction. Nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) mRNA amplification rates were normalized to RNA polymerase IIa mRNA values obtained within the same preparation. Columns represent mean ± SEM of 3 independent measurements. C, Untreated SMCs or SMCs treated with 50 nmol/L nonspecific (control) or PON2-specific siRNA were treated with TGFβ1 (10 ng/mL) for 24 hours starting 3 days after RNAi and analyzed for intracellular ROS with carboxy-H2DCFDA. Columns represent mean ± SEM of 3 independent measurements. Differences were tested for statistical significance with 2-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001.

we tested the potential effect of PON2 on UPR-induced apoptosis. Treatment of naïve EA.hy 926 cells for 16 hours with the UPR-inducing reagent tunicamycin stimulated caspase 3/7 activity in a concentration-dependent manner (Figure 8A and 8B). EA.hy 926 cells stably overexpressing PON2-iso1-HA showed significantly less caspase 3/7 activation in response to tunicamycin (Figure 8A). Conversely, EA.hy 926 cells treated with PON2-specific siRNA (Figure 4D) showed significantly more caspase 3/7 activation in response to tunicamycin than control-treated cells (Figure 8B). Vitamin C and Trolox, which had an ROS-reducing effect (Figure 4A and 4B) did not decrease the ER stress–induced caspase 3/7 activation (Figure 8C and 8D).
Vascular oxidative stress and excessive ROS generation are an important triggering mechanism of atherogenesis.1,2 We demonstrate that PON2 is similarly expressed in 3 major vascular cell types and show that PON2 represents a further defense system against oxidative stress in addition to other established enzymes.3

Unlike high-density lipoprotein–associated PON1 and PON3, PON2 is a cell-based enzyme found to be equally expressed in vascular cells. Interestingly, in Western blotting experiments, PON2 appeared in 2 bands with apparently small differences in molecular weight, which suggests the presence of 2 as-yet undescribed isoforms. Indeed, 2 PON2 mRNA splice variants can be found in public databases. In the present study, their expression was confirmed for human endothelial cells. When calculated from their deduced amino acid sequences, the isoforms differ by 3 kDa. This may be overlooked in gel electrophoreses unless optimized for separation of 40-kDa proteins (compare Figure 1A with Figure 1C). Furthermore, we present data suggesting that both isoforms of PON2 were glycosylated.

In earlier reports that used nonvascular cells such as Caco-230 and HeLa cells,5 PON2 had been described as a plasma membrane–associated protein. This could not be confirmed here for EA.hy 926 cells. Both confocal microscopy and biochemical cell fractionation demonstrated a prominent enrichment of PON2 in the nuclear envelope and the ER. Results were confirmed by microscopy of overexpressed PON2-iso1-GFP in several other cell types (SMCs, AoAFs, HeLa, HEK293T, and U2OS; not shown). Furthermore, experiments with proteinase K, which effectively digests extracellular proteins, did not alter PON2, again indicating an intracellular localization of PON2.

So far, the physiological functions of PON2 are still largely unclear. Genetic polymorphisms of PON2 have been found to be associated with cardiovascular diseases,31 and an ROS-reducing activity of PON2 has been reported in HeLa cells.5 When low-density lipoproteins accumulate in the subendothelial space, one of their most important modifications in early atherogenesis is lipid oxidation, probably as a consequence of exposure to ROS produced by vascular cells. Hence, a reduction in oxidative stress at these sites appears beneficial for the reduction of atherogenesis. Experiments performed in the present study in 3 major vascular cell types clearly demonstrate that PON2 is capable of reducing oxidative stress. Because overproduction of ROS may cause the depletion of vascular nitric oxide, endothelial dysfunction, and the initiation and promotion of atherogenesis,1,2 PON2 can be considered an additional element of antioxidant and antiatherosclerotic vascular defense. In line with this conclusion, antiatherogenic properties have recently been attributed to mouse PON2 in a PON2-deficient apolipoprotein E−/− mouse model.16 However, it remains to be determined whether the reduction in ROS levels in response to PON2 represents a direct or an indirect effect.

Because of its ER association, it seemed reasonable to assume that PON2 could reduce ROS in this organelle. ER

![Figure 8.](https://circ.ahajournals.org/doi/10.1161/01.CIR.105.05.0194)
resistant proteins are major targets of oxidative stress, and ER stress has been implicated in the pathogenesis of many diseases. Furthermore, ER stress can induce the UPR, which may result in both proapoptotic and antiapoptotic mechanisms. However, the exact downstream signaling pathways that counteract ER stress–stimulated apoptosis remain unclear. Here, we present evidence that the expression of PON2 protein is enhanced by ER stress caused by stimulation of PON2 promoter activity as the underlying mechanism. Concordantly, an ER stress element–like sequence can be found in the putative promoter region of PON2. Moreover, PON2 controls oxidative stress in the ER and reduces ER stress–associated cell death. Thus, PON2 may be one of those elements that limit ER stress–stimulated apoptosis.

Functionally, PON2 reduced ROS generated as a response to ER stress. UPR can induce apoptosis through different pathways, and all of them will ultimately result in activation of caspase 3. PON2 seems to interfere with at least one of these pathways because PON2 overexpression reduced UPR-derived caspase activity, whereas PON2 knockdown enhanced this activity. Thus, the antioxidant capacity of PON2 may not necessarily be its only important function; it may also reduce apoptosis of vascular cells after ER stress. Whether the control of caspase 3 activity by PON2 is related to its antioxidative effect cannot be decided from the present experiments. Figure 8C and 8D suggests that it may be unrelated because the antioxidants vitamin C and E could not reduce the UPR-induced caspase 3 activation.

Conclusions

The cell-associated PON2 probably acts in concert with the serum-associated enzymes PON1 and PON3 to decrease vascular ROS. PON2 is likely to reduce ROS that could otherwise cause endothelial nitric oxide synthase uncoupling, thereby promoting endothelial dysfunction and death of endothelial cells and SMCs. Given that oxidative stress is thought to be a major initiator of atherosclerotic lesion development, it is likely that PON2 expression in vascular cells, in concert with the serum-associated PON1 and PON3 proteins, plays a key role in the body’s resistance to coronary artery disease. Dietary or pharmacological interventions to increase cellular (PON2) and/or humoral (PON1 and 3) paraoxonase activity may be a useful approach for preventing atherosclerotic vascular disease.

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Clinical Perspective

In Western societies, cardiovascular diseases are a major cause of morbidity and mortality, numerically more important than all kinds of cancers combined. Consensus exists that many cardiovascular risk factors are associated with increased oxidative stress, defined as an excess production of reactive oxygen species. Oxidative stress causes adverse effects in the vasculature that include lipid/lipoprotein modifications, inflammatory responses, and a reduction in nitric oxide bioavailability. As a consequence, oxidized lipids and inflammatory cells accumulate in the vascular wall, thereby promoting plaque formation and atherogenesis. To balance and combat oxidative stress, the human vasculature contains antioxidative enzyme systems such as superoxide dismutase and glutathione peroxidase. Here, we show that an additional enzyme, paraoxonase 2 (PON2), is likely to contribute to antioxidative and potentially antiatherogenic defense. The present study was designed to investigate the expression, regulation, and potential antioxidative functions of PON2 in 3 major cell types of the human vasculature. We show that PON2 is expressed at similar levels in human endothelial cells, smooth muscle cells, and adventitial fibroblasts. We provide evidence that PON2 significantly controls intracellular levels of reactive oxygen species. Furthermore, we demonstrate that PON2 is cytoprotective and involved in an intrinsic stress signaling pathway that is activated, for example, during atherogenesis. Given that oxidative stress is thought to be a major initiator of atherosclerosis, PON2 expression in vascular cells is likely to contribute to the body’s resistance to cardiovascular disease.
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