Paraoxonase-2 Is a Ubiquitously Expressed Protein with Antioxidant Properties and Is Capable of Preventing Cell-mediated Oxidative Modification of Low Density Lipoprotein*

Received for publication, June 19, 2001, and in revised form, September 11, 2001
Published, JBC Papers in Press, September 28, 2001, DOI 10.1074/jbc.M105660200

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The oxidation of apolipoprotein B-containing lipoproteins and cell membrane lipids is believed to play an integral role in the development of fatty streak lesions, an initial step in atherogenesis. We have previously shown that two antioxidant-like enzymes, paraoxonase (PON)-1 and PON3, are high density lipoprotein-associated proteins capable of preventing the oxidative modification of low density lipoprotein (LDL) (Reddy, S. T., Wadleigh, D. J., Grijalva, V., Ng, C., Hama, S., Gangopadhyay, A., Shih, D. M., Lusis, A. J., Navab, M., and Fogelman, A. M. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 542–547). In the present study, we demonstrate that PON2 (i) is not associated with high density lipoprotein; (ii) has antioxidant properties; and (iii) prevents LDL lipid peroxidation, reverses the oxidation of mildly oxidized LDL (MM-LDL), and inhibits the ability of MM-LDL to induce monocyte chemotaxis. The PON2 protein was overexpressed in HeLa cells using the tetracycline-inducible ("Tet-On") system, and its antioxidant capacity was measured in a fluorometric assay. Cells that overexpressed PON2 showed significantly less intracellular oxidative stress following treatment with hydrogen peroxide or oxidized phospholipid. Moreover, cells that overexpressed PON2 were also less effective in oxidizing and modifying LDL and, in fact, were able to reverse the effects of preformed MM-LDL. Our results suggest that PON2 possesses antioxidant properties similar to those of PON1 and PON3. However, in contrast to PON1 and PON3, PON2 may exert its antioxidant functions at the cellular level, joining the host of intracellular antioxidant enzymes that protect cells from oxidative stress.

Oxidation of low density lipoproteins (LDL) trapped in the arterial subendothelial space is a key process in atherosclerotic lesion development. High density lipoprotein (HDL) inhibits LDL oxidation (1–4) and prevents the synthesis and secretion of monocyte chemotactic protein-1 by artery wall cells, thereby blocking the recruitment and transmigration of monocytes through the arterial endothelial layer (5). The anti-atherogenic properties associated with HDL are due, at least in part, to the activity of HDL-associated enzymes, which interact with LDL and prevent and/or reverse its oxidation (6). The calcium-dependent ester hydrolyase paraoxonase (PON)-1 (EC 3.1.8.1) is one of several such enzymes and is found tightly associated with apoA-I in the HDL particle (7). Purified PON1 not only prevents LDL oxidation (8), but also blocks the ability of mildly oxidized LDL (MM-LDL) to induce monocyte chemotaxis and binding to endothelial cells (9). Epidemiological studies have shown that PON1 polymorphisms are correlated with variations in plasma lipoprotein levels (10) and coronary artery disease in some populations (11–15). In addition, Mackness et al. (16) showed that alloenzymes of PON1 determine the effectiveness of HDL in the protection of LDL from lipid peroxidation.

Two other members of the PON gene family, termed PON2 and PON3, have been identified (17). The three PON genes share ~65% similarity at the amino acid level and are located adjacent to each other on chromosome 7 in humans and chromosome 6 in mice. Like PON1, PON3 is also found in HDL and not only prevents the formation of MM-LDL, but also inhibits MM-LDL-induced monocyte chemotactic activity (18, 19). Although the expression of PON1 and PON3 is restricted primarily to the liver, PON2 is more widely expressed and is found in a number of tissues, including brain, liver, kidney, and testis (20). Furthermore, like PON1, PON2 polymorphisms are associated with numerous pathophysiological conditions, including variations in plasma lipoproteins (21, 22), glucose levels in fasting type II diabetics (23), and neonatal birth weight (24) and the risk of coronary heart disease (25). Although a genetic association between PON1 and these conditions has been shown, the exact function of PON2 in humans is not known. The high similarity observed in amino acid sequence between the PON proteins suggests that PON2 may possess a biochemical function similar to that of PON1 and PON3.

In this study, we demonstrate that PON2, although being widely expressed in many different human tissues, is not detectable by Western analysis in either HDL or LDL. We found that PON2 is constitutively expressed in both primary and immortalized human endothelial cells and human aortic smooth muscle cells. Furthermore, we show that PON2 has antioxidant properties. PON2 overexpression lowers the intracellular oxidative state of cells that have been treated with either hydrogen peroxide or oxidized 1,2-palmitoyl-2-arachidio-
donym-an-sn-glycerol-3-phosphorylcholine (Ox-PAPC). Cells that overexpress PON2 also oxidatively modify LDL to a lesser extent than control cells, as measured by the accumulation of LDL lipid hydroperoxides and the ability of the LDL to induce monocyte chemotactic activity in human aortic endothelial cells (HAEC). Furthermore, cells that overexpress PON2 are able to reverse the oxidative modification of MM-LDL. MM-LDL that has been incubated with cells that overexpress PON2 shows lower levels of lipid hydroperoxides and is less able to induce monocyte chemotaxis than MM-LDL that has been incubated with control cells. Our data suggest that PON2 may play an anti-atherogenic role by reducing the oxidation of LDL and/or by reducing the production of intracellular hydroperoxides.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cell culture reagents and oligonucleotides were purchased from Life Technologies, Inc. Human tissue blot containing 2 μg of poly(A)+ RNA in each lane was from Origene Technologies Inc. (Rockville, MD). t-G1-A-Palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). Ox-PAPC was prepared as described previously (26). Purified PON1 was a generous gift from B. N. La Du (University of Michigan, Ann Arbor, MI). LDL (1.019–1.063 g/ml) and HDL (1.063–1.21 g/ml) were isolated by ultracentrifugation (27) from the plasma of normal volunteers after obtaining informed consent according to the Human Research Subject Protection Committee of UCLA. LDL and HDL had endoxin levels below 20 pg/ml. 2',7'-dichlorofluorescein diacetate (H2DCFDA) was purchased from Molecular Probes, Inc. (Eugene, OR).

**Cell Culture**—HeLa “Tet-On” cells (CLONTECH) were grown in high glucose Dulbecco's modified Eagle's medium containing 10% trypsin-free fetal bovine serum. HAEC and human aortic smooth muscle cells were isolated and cultured as described previously (5) and used at passage levels 4–6. Monocytes were isolated by a modification of the “Recalde” method as previously described (28) from the blood of normal volunteers after obtaining written consent under a protocol approved by the Human Research Subject Protection Committee of UCLA. Human microcirculatory endothelial cells (HMEC) were grown in MCDR-131 (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, penicillin/streptomycin/glutamine, and endothelial cell growth medium (Life Technologies, Inc.)

**Cloning of Human PON2 from HepG2 Cells**—Based on the available sequence information in the NCBI Databases, specific primers were designed to amplify the complete coding region of PON2. PON2 specific primers along with HepG2 cell total RNA (1 μg) were subjected to reverse transcription-polymerase chain reaction using the Access RT-PCR system kit from Promega (Madison, WI). The upper and lower primers for human PON2 were 5'-ATTAGTGACGGCGATGGTGTCGCTGTGGGCTGT-3' and 5'-ATAGTTTAGCGGCCGCTTAGAGTTCACAAATTAGTCGAC-3', respectively. In each set of primers, the upper primer contained a SV40 transcription site and the lower primer had medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, penicillin/streptomycin/glutamine, and endothelial cell growth supplement.

**Southern Analysis**—To test for doxycycline inducibility, an aliquot of these cells was thawed, washed three times and then detected using an ECL Western blotting kit (Amersham Pharmacia Biotech) following the manufacturer's suggested protocol. Equal amounts (1.4 μg of cholesterol) of ultracentrifugation-isolated LDL and HDL were loaded to determine the presence of PON2 in human lipoproteins. 50 μg of protein from cell extracts was used to determine the presence of PON2 in HMEC. 0.25 μg of purified human PON1 and GST-PON2 were tested as controls (data not shown). 25 μg of HeLa-Tet-PON2 cell extracts (untreated or treated for 48 h with doxycycline at 2 μg/ml) were used to determine whether doxycycline induction of these cells resulted in an increase in PON2 protein. To isolate the plasma membrane fraction, confluent plates of HeLa-Tet-PON2 cells were scraped off the tissue culture dish using a Polytron homogenizer. Lysed cells were then spun at 5000 × g for 20 min to remove the nuclear pellet from the rest of the cell extract. This nucleus-free cell extract was subsequently spun at 20,000 × g for 30 min, and the resulting plasma membrane pellet was resuspended in Laemmli buffer. ~15 μg (by protein) of this plasma membrane sample was loaded to detect PON2 in the plasma membrane fraction. Cell supernatants were concentrated ~10-fold by filtering through a membrane, 10,000 cutoff filter (Centricon-10). 50 μg of protein was used to determine the presence of PON2 in cell supernatants.

**Dichlorofluorescein Assay**—Intracellular oxidative stress was measured by the dichlorofluorescein assay as described by Wang and Joseph (30). Briefly, HeLa-Tet-PON2 and HeLa Tet-On cells were plated onto 96-well plates and treated with or without doxycycline for 48 h. Cells were washed with Krebs-Ringer buffer and subsequently incubated in Dulbecco’s modified essential medium containing 100 μM DCFH-DA and 1% tetracycline-free fetal bovine serum in 5% CO2 and 95% air at 37°C. 1 h later, DCFH-DA was removed, and cells were washed with Krebs-Ringer buffer. 300 μM hydrogen peroxide in Krebs-Ringer buffer was added to the cells, and fluorescence was measured in a fluorescent plate reader (Spectra Max GEMini XS, Molecular Devices) with the temperature maintained at 37°C. Softmax Pro881 (Molecular Devices) and Excel (Microsoft) software were used for data analysis.

**Monocyte Chemotaxis and Quantitation of Lipid Hydroperoxides**—HeLa-Tet-PON2 and HeLa Tet-On cells were treated with or without doxycycline at 2 μg/ml for 48 h. Cells were incubated with human LDL (250 μg/ml protein) isolated by ultracentrifugation. 24 h later, this
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**Fig. 1.** Tissue distribution of human PON2. Human tissue blot containing 2 μg of poly(A)*+* RNA in each lane was hybridized to a 32P-labeled PON2 cDNA. The tissue sources are as labeled. SR, skeletal; SM, small.

**Fig. 2.** Expression of PON2 message in artery wall cells. Total RNA was harvested from cultured immortalized HMEC, primary HAEC, and primary human arterial smooth muscle cells (HASMC); and 10 μg of each RNA sample was loaded onto a 1.1% agarose gel and subjected to electrophoresis and to Northern analysis using a 32P-labeled PON2 cDNA. To control for RNA loading, this filter was stripped and reprobed with a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

**Fig. 3.** PON2 protein is not detected in HDL or LDL by Western analysis. HMEC extracts (50 μg) and human HDL and LDL (1.4 μg of cholesterol, isolated by ultracentrifugation) were subjected to SDS-polyacrylamide gel electrophoresis. Western analysis was performed using anti-PON1 antiserum as a positive control (left panel), anti-PON2 antiserum (middle panel), and preimmune serum as a negative control (right panel) as described under "Experimental Procedures." The PON1 and PON2 bands are marked by arrows.

**Fig. 4.** Doxycycline-dependent induction of PON2 message and protein in HeLa-Tet-PON2 cells and association of the PON2 protein with the plasma membrane fraction from HeLa-Tet-PON2 cells. A, total RNA was harvested from HeLa-Tet-PON2 cells that were either left untreated or treated for 48 h with 2 μg/ml doxycycline; and 2.5 μg each of the two RNA samples were loaded onto a 1.1% agarose gel and subjected to electrophoresis and to Northern analysis using a 32P-labeled PON2 cDNA. To control for RNA loading, this filter was stripped and reprobed with a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. B, doxycycline-treated HeLa-Tet-PON2 cell extracts (25 μg) were fractionated by SDS-polyacrylamide gel electrophoresis and subjected to Western analysis using the anti-PON2 antibody (left panel) or preimmune serum (right panel) as described under "Experimental Procedures." Blots were stripped and reprobed with anti-glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody as a control. C, HMEC extracts (50 μg), plasma membrane fractions (13 μg by protein), total cell extracts (50 μg), and concentrated tissue culture media supernatants (50 μg) from HeLa-Tet-PON2 cells were subjected to SDS-polyacrylamide gel electrophoresis. Western analysis was performed using anti-PON2 antiserum (upper panel) and preimmune serum as a negative control (lower panel) as described under "Experimental Procedures."

**RESULTS**

PON2 mRNA was ubiquitously expressed in nearly every human tissue tested, with the highest expression seen in the lung, placenta, testis, and heart (Fig. 1). The high PON2 expression noted in the human heart and the genetic association seen between PON2 polymorphisms and coronary artery disease suggest that PON2 may be expressed in the cells of the aortic environment, where it could play a local anti-atherogenic role. To test this hypothesis, total RNA was harvested from HAEC, HMEC, and human aortic smooth muscle cells and subjected to Northern analysis using a reverse transcription-polymerase chain reaction-amplified human PON2 cDNA as probe. We found that PON2 message was expressed in all of these cells associated with the artery wall (Fig. 2) and in primary macrophages as well (data not shown), whereas neither PON1 nor PON3 message was detectable in these cells (data not shown).

PON1 and PON3 proteins are found in HDL particles and may account for at least some of the antioxidant effects ascribed to HDL (18). Indeed, we were able to detect the PON1 protein in ultracentrifuge-isolated HDL (Fig. 3, left panel). To determine whether PON2 is associated with human plasma lipoprotein fractions, Western analysis of HDL and LDL fractions was performed using the anti-PON2 antibody. As a positive control for detection of the PON2 protein, we loaded whole cell extract isolated from HMEC. We detected an ~44-kDa protein in HMEC extracts that was not detected using preimmune antiserum. The specificity of the anti-PON2 antibody was also confirmed by immunodepletion of anti-PON2 antiserum with a PON2-specific peptide (data not shown). No PON2-specific band was detectable by Western analysis in either HDL or LDL (Fig. 3, middle panel).

To further facilitate study of the human PON2 enzyme, we constructed HeLa-Tet-PON2, an immortalized human cervical carcinoma cell line that overexpresses the tetracycline Tet-On transcriptional activator, into which a plasmid containing the human PON2 cDNA under control of the tetracycline response element was stably transfected. As would be expected given the
ubiquitous expression of PON2, HeLa cells express endogenous PON2 constitutively at a moderate level. Upon treatment for 48 h with 2 \( \mu \)g/ml doxycycline (an analog for tetracycline), the HeLa-Tet-PON2 cells dramatically overexpressed PON2 message (Fig. 4A) and protein (Fig. 4B). To determine whether PON2 is secreted in detectable amounts into the cell culture medium or remains associated with specific cellular fractions, we performed Western analysis on concentrated tissue culture medium, total cellular extract, and a plasma membrane subfraction obtained from HeLa-Tet-PON2 cell cultures. We found that PON2 protein was not detectable in concentrated tissue culture medium, whereas it was detected in both the HeLa-Tet-PON2 total cellular extract and the plasma membrane subfraction (Fig. 4C).

We have previously shown that both PON1 and PON3 have antioxidant properties and are capable of preventing the formation of MM-LDL and inactivating preformed MM-LDL (18). Given the high degree of sequence similarity seen between the three PON proteins, it is likely that PON2 possesses an enzymatic activity similar to that of PON1 and PON3. However, since PON2 is not detectable by Western analysis in HDL (unlike PON1 and PON3) and in cell culture supernatants, we hypothesized that PON2 may provide an innate cellular antioxidant activity. Wang and Joseph (30) have reported the use of DCFH-DA to measure intracellular oxidative stress. This assay quantifies the fluorescence emitted when the non-fluorescent DCFH-DA is oxidized by reactive oxygen species to the highly fluorescent dichlorofluorescein. Discontinuities in the time scale are marked (//) on the x axis.

![Graph showing PON2 protein has antioxidant properties](http://www.jbc.org/)

PON2 protein has antioxidant properties. PON2 reduced the oxidative stress induced by treatment with hydrogen peroxide (upper panel) and Ox-PAPC (lower panel). HeLa-Tet-PON2 cells with or without doxycycline (dox) treatment were incubated with DCFH-DA and treated with either 300 \( \mu \)M hydrogen peroxide (upper panel) or 15 \( \mu \)g/ml Ox-PAPC (lower panel). Intracellular oxidative stress was measured by quantifying the fluorescence emitted when the non-fluorescent DCFH-DA was oxidized by reactive oxygen species to the highly fluorescent dichlorofluorescein. Discontinuities in the time scale are marked (//) on the x axis.

We have previously shown that both PON1 and PON3 have antioxidant properties and are capable of preventing the formation of MM-LDL and inactivating preformed MM-LDL (18). Given the high degree of sequence similarity seen between the three PON proteins, it is likely that PON2 possesses an enzymatic activity similar to that of PON1 and PON3. However, since PON2 is not detectable by Western analysis in HDL (unlike PON1 and PON3) and in cell culture supernatants, we hypothesized that PON2 may provide an innate cellular antioxidant activity. Wang and Joseph (30) have reported the use of DCFH-DA to measure intracellular oxidative stress. This assay quantifies the fluorescence emitted when the non-fluorescent DCFH-DA is oxidized to the highly fluorescent dichlorofluorescein by intracellular reactive oxygen species. HeLa-Tet-PON2 cells that had been either left untreated or pretreated for 48 h with 2 \( \mu \)g/ml doxycycline were incubated with 100 \( \mu \)M DCFH-DA for 1 h and subsequently treated with hydrogen peroxide (300 \( \mu \)M) to generate reactive oxygen species. The fluorescence emitted following treatment with hydrogen peroxide was significantly lower in doxycycline-induced HeLa-Tet-PON2 cells overexpressing PON2 protein relative to control untreated HeLa-Tet-PON2 cells (Fig. 5, upper panel). The fluorescence emitted from cells treated with Ox-PAPC, which mimics the biological activity of MM-LDL (26), followed a similar trend (Fig. 5, lower panel). No significant difference in emitted fluorescence was observed between doxycycline-treated and untreated HeLa Tet-On cells following hydrogen peroxide or Ox-PAPC incubation (data not shown), indicating that the change in fluorescence resulting from PON2 overexpression was not a result of a possible cell growth difference induced by the doxycycline treatment. These experiments show that PON2 overexpression has an antioxidant effect in cells treated with hydrogen peroxide or Ox-PAPC.

We have previously shown that MM-LDL contains elevated levels of lipid hydroperoxides and is capable of inducing monocyte chemotactic activity in target cells (3, 4). In addition, we
have previously demonstrated that HDL, purified PON1, and supernatants from cells overexpressing PON1 or PON3 protein can inhibit the oxidative modification of LDL by HAEC (18). Since PON2 lowers the oxidative state of cells treated with hydrogen peroxide or Ox-PAPC, we wanted to determine whether cells that overexpress PON2 would oxidize LDL to a lower degree than control cells. We found that LDL that was incubated with doxycycline-induced HeLa-Tet-PON2 cells exhibited lower levels of lipid hydroperoxides (Fig. 6A, left panel) relative to LDL that had been incubated with untreated HeLa-Tet-PON2 cells or control HeLa Tet-On cells. Furthermore, as would be expected, this LDL was less effective in inducing monocyte chemotactic activity (Fig. 6A, right panel). PON2 not only repressed the formation of oxidative modifications on LDL, but was also able to reverse the oxidation of preformed MM-LDL. MM-LDL that had been incubated with doxycycline-induced HeLa-Tet-PON2 cells had a significantly lower level of lipid hydroperoxide modifications than MM-LDL that had been incubated with untreated HeLa-Tet-PON2 cells or control HeLa Tet-On cells (Fig. 6B, left panel). As would be expected, the ability of this MM-LDL to induce monocyte chemotactic activity was also impaired (Fig. 6B, right panel).

These results demonstrate that similar to PON1 and PON3, PON2 functions as an antioxidant enzyme. PON2 overexpression is capable of lowering the oxidative state of cells induced by hydrogen peroxide or Ox-PAPC treatment. Furthermore, PON2 overexpression prevents as well as reverses the cell-mediated oxidative modification of LDL and therefore blocks the ability of MM-LDL to induce monocyte chemotaxis.

**DISCUSSION**

We recently demonstrated that PON3, like PON1, is associated with the HDL particle and plays an anti-atherogenic role (18). We detected PON1 and PON3 proteins by Western analysis in HDL particles isolated by numerous methods (ultracentrifugation, fast protein liquid chromatography, “dextran-isolated”) and in human plasma. We were not able to detect PON2 protein by Western analysis in any of these samples. This could be a true reflection either that PON2 does not exist in the HDL particle or that PON2 may be present in HDL at such a low level that it is undetectable by this method. We are currently undertaking further approaches to definitely determine whether PON2 exists in HDL.

Both PON1 and PON3 are highly expressed in the liver, where they may associate with apoA-I and hence would likely be inserted into the forming HDL or pre-HDL particle. PON2, on the other hand, appears to be ubiquitously expressed in most human tissues. The high degree of sequence similarity between the PON proteins suggests that they share a similar enzymatic activity. However, the ubiquitous expression of PON2 and our finding that PON2 is not detectable in HDL by Western analysis suggest that PON2 may play a distinct role in vivo, separate from that played by PON1 and PON3. Although PON2 (like PON1 and PON3) does possess a putative consensus “signal sequence” in its N-terminal region, we did not detect PON2 protein by Western analysis even in concentrated cell culture supernatants from HeLa-Tet-PON2 cells. The PON2 protein may be rapidly degraded following secretion, or it may be secreted only at a very low level. Indeed, the global expression pattern of PON2 and our finding that PON2 is not detectable in HDL by Western analysis suggest that PON2 may play a distinct role in vivo, separate from that played by PON1 and PON3. Although PON2 (like PON1 and PON3) does possess a putative consensus “signal sequence” in its N-terminal region, we did not detect PON2 protein by Western analysis even in concentrated cell culture supernatants from HeLa-Tet-PON2 cells. The PON2 protein may be rapidly degraded following secretion, or it may be secreted only at a very low level. Indeed, the global expression pattern of PON2 and our finding that PON2 is not detectable in HDL by Western analysis suggest that PON2 may play a distinct role in vivo, separate from that played by PON1 and PON3. 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would therefore provide an ideal venue for the inactivation/reduction of these oxidized molecules. Indeed, our finding that PON2 overexpression is able to lower the intracellular oxidative state of cells exposed to hydrogen peroxide (which should easily diffuse through the cell membrane) or Ox-PAPC (which should at least easily interact with the cell membrane) suggests that PON2 can play just such a role.

The in vitro paraoxonase and arylesterase assays have been typically used to determine the PON activity in human and animal serum samples. We have not been able to detect significant paraoxonase activity in extracts overexpressing PON2. However, since the arylesterase and paraoxonase activities of PON1 appear to be distinct and separable from its ability to protect against LDL oxidation (32), the lack of an apparent paraoxonase activity in PON2 does not preclude it from possessing the ability to protect LDL from lipid peroxidation. Recent studies attribute a new in vitro enzymatic activity to the PON1 and PON3 proteins. Kobayashi et al. (33) have reported the cloning of a lactone hydrolase (lactonase) from Fusarium oxysporum AKU 3702 and identified sequence similarities between lactonase and PON1; and indeed, lactonase activity may be commonly shared among the three proteins (34). Nevertheless, it is unlikely that these in vitro enzymatic activities are representative of the true function of the PON proteins in vivo. Mounting evidence suggests that the PON proteins play an anti-atherogenic role through their ability to inhibit and/or reverse LDL oxidation, and the ability of PON1 to accomplish this is clearly distinct from its in vitro arylesterase and paraoxonase activities. It is possible that the antioxidant activity of PON2 is similarly distinct from any in vitro paraoxonase, arylesterase, or lactonase activities it may possess.

This study demonstrates, for the first time, that PON2 is able to lower the intracellular oxidative stress of a cell and prevent the cell-mediated oxidation of LDL. Since PON2 is ubiquitously expressed not just in cells of the artery environment, but in tissues throughout the body, it is likely that PON2 plays a role in the reduction of intracellular or local oxidative stress. Given that oxidation of LDL is thought to be a major initiator of atherosclerotic lesion development, it is likely that PON2 expression in arterial cells, in concert with the PON1 and PON3 proteins that are found in HDL, plays a key role in the body’s resistance to coronary artery disease.

REFERENCES

1. Parthasarathy, S., Barnett, J., and Fong, L. G. (1990) Biochem. Biophys. Acta 1044, 275–283
2. Maier, J. A., Barenghi, L., Pagani, F., Bradamante, S., Coni, P., and Ragnotti, G. (1994) Eur. J. Biochem. 221, 35–41
3. Navab, M., Hama, S. Y., Cooke, C. J., Anantharamaiah, G. M., Chadda, M., Jin, L., Subhanagounder, G., Faull, K. F., Reddy, S. T., Miller, N. E., and Fogelman, A. M. (2000) J. Lipid Res. 41, 1481–1494
4. Navab, M., Hama, S. Y., Anantharamaiah, G. M., Hassan, K., Hough, G. P., Watson, A. D., Reddy, S. T., Severin, A., Fonarow, G. C., and Fogelman, A. M. (2000) J. Lipid Res. 41, 1495–1508
5. Navab, M., Imes, S. S., Hama, S. Y., Hough, G. P., Ross, L. A., Bork, R. W., Valente, A. J., Berliner, J. A., Drinkwater, D. C., Laks, H., and Fogelman, A. M. (1991) J. Clin. Invest. 88, 2639–2646
6. Navab, M., Berliner, J. A., Subhanagounder, G., Hama, S., Lusis, A. J., Castellani, L. W., Reddy, S., Shih, D., Shi, W., Watson, A. D., Van Lenten, B. J., Vora, D., and Fogelman, A. M. (2001) Arteriosclerosis 21, 481–488
7. La Du, B. N., and Kulow, W. (eds) (1992) Pharmacogenetics of Drug Metabolism, pp. 51–91, Pergamon Press Inc., New York
8. Mackness, M. I., Arrol, S., and Durrington, P. N. (1991) FEBS Lett. 286, 152–154
9. Watson, A. D., Berliner, J. A., Hama, S. Y., La Du, B. N., Faull, K., Fogelman, A. M., and Navab, M. (1995) J. Clin. Invest. 96, 2882–2891
10. Panella, S., Harris, S. B., Young, T. K., Hanley, A. J., Zinman, B., Connelly, P. W., and Hegele, R. A. (2000) Clin. Chem. Lab. Med. 38, 413–420
11. Odawara, M., Tachi, Y., and Yamashita, K. (1997) J. Clin. Endocrinol. Metab. 82, 2257–2260
12. Suehiro, T., Nakauchi, Y., Yamamoto, M., Arii, K., Itoh, H., Hamashige, N., and Hashimoto, K. (1996) Int. J. Cardiol. 57, 69–78
13. Imai, Y., Morita, H., Kurihara, H., Sugiyama, T., Kato, N., Ehihara, A., Hamada, C., Kurihara, Y., Shindo, T., Oh-hashi, Y., and Yazaki, Y. (2000) Atherosclerosis 149, 435–442
14. Durrington, R. N., Mackness, B., and Mackness, M. I. (2001) Arteriosclerosis 21, 473–480
15. Shih, D., Reddy, S. T., and Lusis, A. J. in Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects (Costa, L. G., and Furlong, C. E., eds) Kluwer Academic Publishers, Norwell, MA, in press
16. Mackness, M. I., Arrol, S., Mackness, B., and Durrington, P. N. (1997) Lancet 349, 851–852
17. Primo-Parmo, S. L., Soreson, R. C., Teiber, J., and La Du, B. N. (1996) Genomics 33, 498–507
18. Reddy, S. T., Wadleigh, D. J., Grijalva, V., Ng, C., Hama, S., Gangopadhyay, A., Shih, D. M., Lusis, A. J., Navab, M., and Fogelman, A. M. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 542–547
19. La Du, B. N. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 467–468
20. Mochizuki, H., Scherer, S. W., Xi, T., Nickle, D. C., Majer, M., Huijenga, J. J., Tsui, L. C., and Prochazka, M. (1998) Gene (Amst.) 213, 149–157
21. Boright, A. P., Connelly, P. W., Brunt, J. H., Scherer, S. W., Tsui, L. C., and Hegele, R. A. (1999) Atherosclerosis 139, 131–136
22. Hegele, R. A., Harris, S. B., Connelly, P. W., Hanley, A. J., Tsui, L. C., Zinman, B., and Scherer, S. W. (1998) Clin. Genet. 54, 394–399
23. Mackness, B., Durrington, P. N., Abubashia, B., Boulton, A. J., Mackness, M. I. (2000) Clin. Sci. Lond. 98, 355–363
24. Busch, C. P., Ramdath, D. D., Ramsewak, S., and Hegele, R. A. (1999) Pharmgenetics 9, 351–356
25. Sanghera, D. K., Aston, C. E., Saha, N., and Kamboh, M. I. (1998) Am. J. Hum. Genet. 62, 36–44
26. Watson, A. D., Leitinger, N., Navab, M., Faull, K. F., Horkko, S., Wittem, J. L., Palinski, W., Schwenke, D., Salomon, R. G., Sha, W., Subhanagounder, G., Fogelman, A. M., and Berliner, J. A. (1997) J. Biol. Chem. 272, 13597–13607
27. Havel, R. J., Elder, H. A., and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345–1353
28. Fogelman, A. M., Sykes, K., Van Lenten, B. J., Territo, M. C., and Berliner, J. A. (1988) J. Lipid Res. 29, 1243–1247
29. Reddy, S. T., Winstead, M., Tischfeld, J. A., and Herschman, H. R. (1997) J. Biol. Chem. 272, 13591–13596
30. Wang, H., and Joseph, J. A. (1999) Free Radic. Biol. Med. 27, 612–616
31. Auerbach, B. J., Kiely, J. S., and Cornicelli, J. A. (1992) Anal. Biochem 201, 375–380
32. Aviram, M., Billecke, S., Brouns, R., Biaggia, C., Newton, R., Rosenblat, M., Erogul, J., Hsu, C., Dunlop, C., and La Du, B. N. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1617–1624
33. Kobayashi, M., Shimizu, M., Sakai, C., Kato, M., and Shimizu, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12787–12792
34. Dragonov, D. I., Stetson, P. L., Watson, C. E., Billecke, S. S., and La Du, B. N. (2000) J. Biol. Chem. 275, 33435–33442
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J. Biol. Chem. 2001, 276:44444-44449. doi: 10.1074/jbc.M105660200 originally published online September 28, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105660200

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