Biological role, protein expression, subcellular localization, and oxidative stress response of paraoxonase 2 in the intestine of humans and rats

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Abstract

The number of studies suggesting the protective properties of PON2 is, however, limited, and there has been little insight into its role in animal and human physiology. In particular, only limited data are available on the intestine, even though this organ is exposed on a daily basis to high levels of oxidative stress and requires strong antioxidants, which may preserve important endocrine, metabolic, immunological, and absorptive functions. The major aims of the present investigation are to determine the protein expression, subcellular localization, and oxidative stress response of paraoxonase 2 (PON2), a member of a powerful antioxidant family in human and rat intestine. Biochemical and ultrastructural experiments all showed a substantial expression of PON2 in human and rat intestine. Western blot analysis disclosed higher levels of PON2 in the jejunum than in the duodenum, ileum, and colon. Cell fractionation revealed a predominant PON2 association with microsomes and lysosomes in the human jejunum, which differed from that in rats. PON2 was detected in the intestine as early as week 15 of gestation and was significantly increased by week 20. Iron ascorbate-mediated lipid peroxidation induced a marked decrease in PON2 expression in intestinal specimens coincidental to an abundant rise in malondialdehyde (MDA). On the other hand, preincubation of permeabilized Caco-2 cells with purified PON2 led to a protection against iron-ascorbate-induced lipid peroxidation. These observations demonstrate that the human intestine is preferentially endowed with a marked PON2 expression compared with the rat intestine and this expression shows a developmental and intracellular pattern of distribution. Furthermore, our observations suggest PON2 protective effects against prooxidant stimuli in the small intestine.

REDOX BALANCE, USUALLY OBSERVED in healthy subjects, is maintained by an equilibrium between prooxidants and antioxidants (21, 38). A decrease in antioxidant protection, an excess of reactive oxygen species (ROS), as well as a failure to repair oxidative damage lead to redox imbalance (21). The resulting accumulation of free radicals can have deleterious effects by reacting and oxidizing key organic substrates, such as polyunsaturated fatty acids, proteins, and DNA (6, 12, 50). This process, called oxidative stress, disturbs normal functioning and is involved in a wide spectrum of pathologies: cancer, atherosclerosis, cystic fibrosis, Alzheimer, and Parkinson (11, 26, 30, 39, 53, 55). Importantly, a variety of gastrointestinal diseases are also associated with ROS and oxidative stress (19, 41, 42). In fact, the gastrointestinal mucosa is repetitively exposed to luminal oxidants from ingested foods (13, 20, 41) and, despite the antioxidant properties of its mucus lining, there is a continuous generation of oxidative stress (20). Clearly, the ingestion and/or occurrence of peroxides may have implications for human health, particularly in the long term.

Antioxidants play a crucial role in preventing damage induced by oxidative stress through the neutralization of free radicals. Alimentary nonenzymatic oxidants, such as α-tocopherol, vitamin C, and retinoids, possess a chemical structure that allows the quenching of singlet oxygen and peroxides (25). In addition, humans are endowed with endogenous enzymatic antioxidants that include superoxide dismutase, which catalyzes the conversion of superoxides into oxygen and hydrogen peroxide and is less reactive toward organic molecules (36); catalase, a heme-containing enzyme, which allows the dismutation of hydrogen peroxide into water and oxygen (37); and glutathione peroxidase, which detoxifies activated oxygen through the catalysis of hydrogen peroxide reduction (1, 37). Recently, numerous investigators have focused on the paraoxonase (PON) protein family (2). Its distinct members (PON1, PON2, and PON3) are believed to be powerful attenuators of oxidative damage and highly atheroprotective (17, 32, 40). PON1 and PON3 circulate attached to high-density lipoprotein particles (15). They inhibit atherogenesis by hydrolyzing lipid hydroperoxides and by preventing low-density lipoprotein (LDL) oxidative modification (15). The distribution of PON2 in many tissues suggests the possibility of its playing an antioxidant role (15). PON2 is increased in cells harvested from 4-mo-old apolipoprotein (apo) E(−/−) animals exhibiting signs of oxidative stress (46). Additionally, the administration of PON2 to macrophages from apo E(−/−) mice reduced lipid peroxide content (46). Similarly, overexpression of PON2 in Hela cells prevents the formation of oxidized LDL (40, 45). Overall, these observations are indicative of the antioxidant properties of PON2.

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were to examine the protein expression, subcellular localization, ontogeny, role, and response to oxidative stress of PON2 in the human and rat intestine. Experiments were also carried out to explore whether powerful antioxidants may prevent the manifestation of oxidative stress and the depletion of PON2 following the exposure of microsomal fractions to iron-ascorbate.

MATERIALS AND METHODS

Small intestine and large bowel tissues were obtained from fetuses ranging from 15 to 20 wk following legal or therapeutic abortion with informed patient consent. No tissues were collected from cases associated with known fetal abnormalities or fetal death. Intestinal samples were also obtained from patients (50–60 yr of age) who had undergone surgical resection of different areas of the digestive tract. Normal tissue found adjacent to the resected pathological tissues was used in all cases, as ascertained by routine hematoxylin-eosin staining. The human study protocol was approved by the Ethics Committee of Sainte-Justine Hospital and the Institutional Review Committee for the use of human material from the Centre Hospitalier Universitaire de Sherbrooke/Faculté de Médecine. All experimental animal procedures were authorized by the Institutional Animal Care Committee.

Preparation and specificity of PON2 antibody. The PON2 polyclonal antibody (Ab) was prepared by Invitrogen (Carlsbad, CA). It was generated against the PON2 region spanning amino acids 94–112. The specificity of the antibody was evaluated by various methods, including ELISA that recognized only PON2 among various proteins, Western blotting following the incubation of the Ab in the presence or absence of the 19-amino acid antigen, the omission of the primary PON2 Ab in Western blot, and the identification of the PON2 sequences following immunoprecipitation and SDS-PAGE. Noteworthy is the KEEKPRARELRISRGFDL epitope that allows cross-reaction between humans and rats. It is also important to note that pilot studies were initiated to demonstrate 1) the specificity of the PON2 polyclonal Ab in humans and rats and 2) the equivalent response to PON2 Ab from human and rat tissues, which were simultaneously tested by PAGE and Western blot.

Preparation of microsomes and subcellular fractions. Intestinal specimens were rinsed, homogenized, and centrifuged for 15 min at 12,000 g at 4°C to prepare microsome fractions, a technique described earlier (8, 9). The supernatant fraction was then centrifuged for 60 min at 100,000 g. The pellet was centrifuged for 60 min at 4°C. The washed microsomal pellets were quick frozen and stored at −80°C for later use. To isolate subcellular fractions, tissue homogenate was subjected to differential and discontinuous sucrose density gradient centrifugation.

Western blots. To assess the presence of PON2 and evaluate its mass, intestinal tissues were homogenized and adequately prepared for Western blotting as described previously (29). The Bradford assay (Bio-Rad), was used to estimate protein concentration. Proteins were denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 4–20% gradient SDS-PAGE, and electroblotted onto nitrocellulose membranes. Nonspecific binding sites of the membranes were blocked with defatted milk proteins followed by the addition of primary antibodies directed against PON2. The relative amount of primary antibody was detected with species-specific horse-radish peroxidase-conjugated secondary antibody. Even if identical protein amounts of tissue homogenates were applied, the β-actin protein was used to confirm equal loading on SDS-PAGE (results not shown). Blots were developed and the mass of PON2 was quantitated using an HP Scanjet scanner equipped with a transparency adapter and software.

Effect of antioxidants. To determine whether lipid peroxidation was responsible for alterations in PON2 protein expression, the antioxidants butylated hydroxytoluene (BHT) (0.5 mM), N-acetylcysteine (NAC) (5 mM), and Trolox (0.5 mM) were added to microsomes or to the apical compartment of Caco-2 cells for 1 h before incubation with iron-ascorbate.

Estimation of lipid peroxidation. The amount of free malondialdehyde (MDA) formed during the reaction was determined by HPLC, as we previously described (6). Proteins were first precipitated with a 10% sodium tungstate (Na2WO4) solution (Aldrich, Milwaukee, WI). The protein-free supernatants were then reacted with an equivalent volume of 0.5% (wt/vol) thiobarbituric acid solution (TBA; Sigma, St. Louis, MO) at 90°C for 60 min. After cooling to room temperature,
the pink chromogene [(TBA) 2-MDA] was extracted with 1-butanol and dried over a stream of nitrogen at 37°C. The dry extract was then resuspended in a potassium dihydrogen phosphate (KH₂PO₄)-methanol mobile phase (70:30, pH 7.0) before MDA determination by HPLC with fluorescence detection.

Permeabilization of Caco-2 cells by digitonin. Caco-2 cells were obtained from the American Type Culture Collection (Manassas, VA). They were grown in minimum essential medium (MEM; GIBCO-BRL, Grand Island, NY) and supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and L-glutamine (4 mM). Medium was changed every 2–3 days. Cells (4.5 × 10⁵ cells) were used after 15 days of confluence and permeabilized in Krebs-Ringer bicarbonate solution containing 10 mM magnesium dichloride (MgCl₂), 0.1 potassium chloride (KCl), 1 mM Na-free EDTA, 1 mM ATP, 50 pg/ml digitonin, and a mixture of antiproteases (25 μg/ml phenylmethanesulfonyl fluoride, 1 μg/ml pepstatin, 10 μg/ml aprotinin, 10 μg/ml leupeptin S) for 15 min. Then, Caco-2 digitonized cells were extensively washed and preincubated for 30 min with 10 μg PON2 (purified from plasma by chromatography on DEAE-sepharose CL-6B) before they were cul-

Fig. 2. Immunogold labeling of PON2 on human intestinal tissue. The labeling by gold particles is present over the microvilli (mv) and is particularly associated with their limiting membrane and the endosomal vesicles (end) (A). Intracellularly, the labeling is present over the rough endoplasmic reticulum (RER), the Golgi apparatus (G), and the lysosomes (Lys), as well as in mitochondria (M) and nuclei (N) (B, C, and D). Lipid droplets (L), however, are devoid of any labeling (D). Protein A-golds are also noted on the basolateral membrane (blm) (D).
tured in MEM in the presence or absence of iron-ascorbate (100 μM/200 μM) for 24 h. Cells and media were collected and MDA was determined as described above.

Statistical analysis. All values were expressed as means ± SE. The data were evaluated by ANOVA, when appropriate, and the differences between the means were assessed by Student’s two-tailed t-test.

RESULTS

First we evaluated PON2 protein mass in intestinal homogenates. As illustrated by the profiles reported in Fig. 1, PON2 protein mass was higher in humans than in rats for the same quantities of homogenate proteins loaded on SDS-PAGE. Additionally, its expression was revealed at varying degrees in the

Fig. 3. Subcellular localization of PON2 in jejunum from humans and rats. Subcellular fractions were prepared from homogenates by sucrose density gradient centrifugation. Samples of each fraction were subjected to SDS-PAGE and Western blotting. Values represent means ± SE for 4 separate experiments. *P < 0.005 vs. homogenates.

Fig. 4. Protein expression of PON2 in intestinal microsomes. Microsomes were obtained by subcellular fractionation and PON2 profile was examined by SDS-PAGE and Western blotting. Values represent means ± SE for n = 4 in each group of experiments. *P < 0.05 vs. humans; **P < 0.05 vs. duodenum and ileum.

Fig. 5. Developmental pattern of PON2 expression in the human intestine. Proximal and distal small intestine (A) and colon (B) were analyzed with similar protein amounts at the various periods of gestation. PON2 profile was examined by SDS-PAGE and Western blotting. Values represent means ± SE for n = 4 in each group of experiments. *P < 0.05 vs. proximal segment; **P < 0.005 vs. proximal region; ***P < 0.01 vs. 20 wk of gestation.
different regions of the small intestine. PON2 levels in the gastrointestinal tract predominated in the jejunum. In fact, the protein expression of PON2 in the different human regions of the intestine (duodenum, jejunum, ileum) was found to be 2.7-, 3.1-, and 2.7-fold greater than corresponding rat tissue (Fig. 1A). We have also assessed PON2 gene expression in human tissues to determine whether it parallels PON2 protein distribution. PCR evaluation showed that the profile of PON2 mRNA transcripts corroborated the pattern of the PON2 protein expression in the intestinal regions (Fig. 1B).

Subsequently, immunolabeling for PON2 was carried out on human intestine tissue using specific anti-PON2 antibody in combination with the protein A-gold complex. Labeling by gold particles was obtained over cellular membranes as well as intracellular compartments. In human intestinal tissues (Fig. 2), the labeling was associated with microvilli as well as along the basolateral membrane. Apical membrane-derived endosomal vesicles also displayed gold particles. Additionally, the rough endoplasmic reticulum-Golgi complex secretory pathway as well as lysosomes and mitochondria nuclei displayed some labeling, whereas lipid droplets were devoid of gold particles. Under the control condition in which the primary antibody was omitted, the labeling was totally abolished (results not shown).

We then examined the cellular localization of PON2 in the adult jejunum by conventional fractionation. As shown in Fig. 3, the pattern of PON2 distribution was different in rats and humans. In rats, PON2 was mostly associated with nuclei, mitochondria, lysosomes, and microsomes, whereas its expression in humans was predominant in lysosomes and microsomes. The question arose as to whether microsomal PON2 expression pattern paralleled the homogenate profile displayed in Fig. 1. Western blotting of microsomes exhibited similar PON2 observations (Fig. 4), and PON2 was also preeminent in the jejunum compared with the duodenum and ileum.

We also explored the ontogeny of PON2 in the human small and large intestine using equal protein amounts of tissue homogenates. An increase in PON2 was noted only in the proximal regions of the small intestine and colonic tissue as a function of fetal age (Fig. 5). The proximal small intestine and colon exhibited a significant progressive rise in PON2 protein content: 145 and 123%, respectively, at the end of week 20 compared with the value (100%) noted at the end of week 15.

![Fig. 6. Analyses of PON2 content in subcellular fractions. PON2 expression in cellular fractions obtained from fetal small intestine. The organelles were isolated from the proximal intestine (17 wk of gestation) using sucrose density gradient centrifugation. They were resolved by SDS-PAGE, electroblotted onto nitrocellulose membranes, and incubated with primary and secondary antibodies. Values represent means ± SE for n = 4 in each experimental group. *P < 0.05 vs. homogenates; **P < 0.005 vs. homogenates.](image)

![Fig. 7. Effect of iron-ascorbate-induced lipid peroxidation on PON2 protein expression in rat jejunum microsomes. Microsomal fractions were incubated in the presence or absence of iron-ascorbate (Fe/Asc). A: oxidative stress was assessed by measuring malondialdehyde (MDA) as an index of lipid peroxidation. To determine whether lipid peroxidation was largely responsible for PON2 changes, various antioxidants [butylated hydroxytoluene (BHT), N-acetylcysteine (NAC), and Trolox] were added separately to the reaction mixture before incubation with iron-ascorbate. B: then PON2 mass was quantitated by SDS-PAGE and Western blotting. Values are expressed as means ± SE for n = 4 for each experimental group. *P < 0.05 vs. control; **P < 0.005 vs. control.](image)
No marked ontogenic differences in PON2 protein expression were recorded in the other intestinal segments. We next performed cell fractionation to examine the subcellular localization of PON2 in human fetal jejunum. This experimental approach revealed a PON2 distribution similar to that in rats with evidently less enrichment in lysosomes (Fig. 6).

Given the growing importance of PON as a cardiovascular disease risk factor and its potential involvement in protection against free radicals, we expected the status of PON2 to be significantly decreased under oxidative stress. To test this hypothesis, we exposed rat jejunum microsomes to iron-ascorbate-mediated lipid peroxidation. Incubation with iron-ascorbate at the concentration of (100 μM/200 μM) resulted in a significant increase in MDA levels in rat (Fig. 7A). Preincubation with strong antioxidants, such as BHT, NAC, and Trolox, markedly suppressed the production of MDA, providing direct evidence for the ability of the iron-ascorbate system to provoke profound lipid peroxidation (Fig. 7B). Similar findings were noted in human jejunal microsomes (Fig. 8). It is important to note that the addition of iron-ascorbate to microsomes never decreased the protein expression of protein disulfide isomerase (results not shown), a microsomal marker, which suggests a selective response of PON2 to oxidative stress.

Similar studies were repeated with fetal proximal intestinal microsomes (Fig. 9) and integral Caco-2 cells (Fig. 10) and corroborated the capability of iron-ascorbate to reduce PON2 protein expression. Preincubation with BHT at a concentration of 0.5 mM prevented the PON2 protein decline observed with iron-ascorbate, which suggests the direct involvement of oxidative stress.

Finally, studies were undertaken to assess the direct role of PON2 in oxidative stress. To this end, plasma membranes of Caco-2 cells were permeabilized with digitonin to introduce purified PON2 before the addition of iron-ascorbate. As illustrated in Fig. 11, permeabilized Caco-2 cells had more PON2 protein content compared with normal cells not exposed to...
digitonin. Furthermore, Table 1 indicates that MDA production was markedly diminished in permeabilized cells and their media in the presence of iron-ascorbate. On the other hand, MDA levels were raised in permeabilized Caco-2 cells incubated with PON2 antibody (results not shown). Taken together, these findings reasonably suggest the protective action of PON2 against iron-ascorbate-induced lipid peroxidation.

**DISCUSSION**

The PON family consists of the three PON1, PON2, and PON3 members that share structural properties. Most of the information available on the PON family function derives from studies of PON1. We therefore initiated a comparative investigation of rats and humans with special respect to subcellular localization and ontogeny as well as the status of PON2 in response to oxidative stress. Our data clearly established that PON2 is 1) localized in different regions of the intestine, including the duodenum, jejunum, ileum, proximal colon, and distal colon; 2) present at higher concentrations in the human intestine than in rat counterparts; 3) distributed in various cellular organelles, such as nuclei, mitochondria, lysosomes, and microsomes; 4) more associated with mitochondria and lysosomes in rat jejunum and with lysosomes and microsomes in human jejunum; and 5) decreased in the presence of iron-ascorbate-induced lipid peroxidation and restored with antioxidants. In addition, our findings emphasize the protective antioxidant role of PON2 in permeabilized Caco-2 cells.

ROS are abundantly released by activated neutrophils that infiltrate the intestinal wall during the cascade of immunological events that produce intestinal inflammation. Moreover, their luminal concentrations can dramatically increase owing to environmental factors, such as dietary oxidant ingestion, intraluminal catalase-negative bacteria, and desquamated cell

![Fig. 10](image1.png) Effect of iron-ascorbate-induced lipid peroxidation on PON2 status in microsomes and Caco-2 cells. Iron ascorbate (100 μM/200 μM) was administered to Caco-2 cell microsomes (A) as well as to differentiated Caco-2 cells (B) for 30 min at 37°C. BHT was added in some experiments at the concentration of 0.5 mM to neutralize the oxidative stress. Cells were then harvested, washed, homogenized, and tested for PON2 mass by SDS-PAGE and Western blotting. Values are expressed as means ± SE for n = 4 for each experimental group. *P < 0.05 vs. control.

![Fig. 11](image2.png) Effect of digitonin on PON2 uptake in Caco-2 cells. Digitonin (50 pg/ml) was added to Caco-2 cells as described in MATERIALS AND METHODS. Following the incubation period, cells were washed, cultured in medium containing purified PON2 protein, homogenized, and tested for PON2 mass by SDS-PAGE and Western blotting. Control cells (Cont) were cultured in the same conditions, but without the addition of PON2. Values are expressed as means ± SE for n = 3 for each group. *P < 0.01 vs. control.

**Table 1. Lipid peroxidation in Caco-2 cells challenged with purified PON2**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No Addition</th>
<th>+ Iron/Ascorbate, pmol/mg cellular protein</th>
<th>+ Iron/Ascorbate and PON2, pmol/mg cellular protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>78 ± 5</td>
<td>296 ± 12*</td>
<td>105 ± 14†</td>
</tr>
<tr>
<td>Medium</td>
<td>6 ± 1</td>
<td>47 ± 4*</td>
<td>15 ± 1†</td>
</tr>
</tbody>
</table>

Caco-2 cells were preincubated for 30 min with 10 μg paraoxonase 2 (PON2) purified from plasma by chromatography on DEAE-sepharose CL-6B. Thereafter, iron/ascorbate (100 μM/200 μM) was added for 24 h. The cells and media were collected and malondialdehyde was determined as described in the MATERIALS AND METHODS section. *P < 0.05 vs. controls (No Addition); †P < 0.05 vs. iron/ascorbate.
oxidases. Therefore, important cellular detoxification systems may adequately control the amplified generation of ROS, terminate radical injury, and repair damaged cellular elements. As documented in the present study, PON2 is abundantly expressed along the intestine. We speculate that PON2 may be a potentially important antioxidant that could prevent impairment or breakdown of membrane integrity, ensuring the maintenance of cellular homeostasis and functions. In line with this assumption, several workers have suggested that PON2 may possess a biochemical function similar to that of PON1 and PON3, given its genetic association with pathophysiological conditions, including variations in plasma lipoproteins (7, 22), glucose levels in fasting type 2 diabetics (35), neonatal birth weight (10), the risk of coronary heart disease (47), and decreased oxidative state in human endothelial cells (40).

To delineate the role of PON2, we decided to introduce purified PON2 protein in permeabilized Caco-2 epithelial cell monolayers to assess its protective action against iron-ascorbate-induced lipid peroxidation. Cell permeabilization has been extensively used to allow access of extracellular reagents to intracellular targets (18, 23, 33). Digitonin opens pores in the membrane that allow the diffusion of large molecules, such as antibodies or enzymes, into the cytoplasm (14, 27, 48, 51). The results shown in the present investigation reveal the potential of PON2 in reducing the production of MDA in digitonin-permeabilized cells, indicating its antioxidant role. Confirmation was obtained with PON2 antibodies that favored the induction of lipid peroxidation. These permeabilization experiments constitute a direct evidence for the antioxidant function of PON2 since most of the accessible studies have only been suggestive compared with the substantial information available on PON1. In agreement with our observations, a quite new article (published during the preparation of the present manuscript) has reported that PON2 represents an endogenous defense mechanism in vascular cells (24). In this work, overexpression of PON2 markedly reduced oxidative stress, whereas its knockdown increased ROS in human umbilical vein endothelial cell-derived EA.hy 926 cells.

Of considerable interest is the detection of PON2 in a number of intracellular compartments. Subcellular fractionation analyses revealed the distribution of PON2 in nuclei, mitochondria, lysosomes, and microsomes. If the role of PON2 truly lies in counteracting oxidative stress, its subcellular localization may suggest its active participation in the protection of organelle integrity and function during lipid peroxidation.

This work provides evidence that PON2 expression is higher in the jejunum than in the duodenum and ileum in adult humans and rats. Previous studies emphasized that the jejunum is the preferential site of fatty acid uptake, lipid resynthesis, and chylomicron assembly (28). Since the jejunal wall is continuously exposed to free radicals present in the diet (13, 20, 41), PON2 likely plays a significant role in neutralizing the effects of prooxidant excess that trigger dramatic changes in intestinal epithelial cells, including the collapse of the cytoskeleton, disruption of tight junctions, and loss of mucosal barrier integrity (3, 34, 44). Very little information is available concerning the antioxidant status of the enterocyte following prooxidative attack directed toward the brush-border membrane. Using iron-ascorbate oxygen-radical-generating system, we showed that oxidative stress did not induce changes in the Caco-2 cells antioxidant enzyme activity of superoxide dismutase, catalase, glutathione peroxidase, and glutathione transferase (6). In the present investigation, iron-ascorbate-mediated lipid peroxidation induced a diminished level of PON2 protein expression, pointing out the direct implication of this protein in diminishing lipid peroxidation. Other proteins may counteract oxidative stress capable of provoking the loss of enterocyte function. Tso’s team (43, 52) has provided evidence of the powerful antioxidant capacity of apo A-IV, a protein produced by the small intestine. Shamir et al. (49) have shown that the gut could be the site of synthesis of PON family members. Collectively, all these antioxidant proteins may protect against oxidative stress that arises from dietary oxidized lipids, circulating oxidized lipoproteins, luminal flora, or inflammatory bowel diseases, such as Crohn’s disease and ulcerative colitis.

Crohn’s disease and ulcerative colitis are two severe disorders characterized by chronic intestinal inflammation with increased risks of developing colorectal carcinoma (16). Intestinal damage in inflammatory bowel diseases has been related to increased free radical production resulting from impaired antioxidant defense, respiratory burst of infiltrating phagocytic cells, and pathologic flora (19, 30, 54). Thus scavenging ROS is considered to be critical for regulating intestinal inflammation. Additional work is needed to highlight the status and elucidate the role of PON2 in these chronic inflammatory diseases.

Rats have largely been used to exploit their physiological uniqueness in addressing biomedical and nutritional research issues. Very often, findings in lipid metabolism and lipid peroxidation in rats are inadequately extrapolated to humans. Since many aspects relative to these fields in rats are not identical to humans (5), we have addressed the pattern of distribution in the intestine, the intracellular localization, and the response to oxidative stress of PON2 in rats and humans. Our findings could document various quantitative and qualitative changes in the two species; thus the rat may not represent an appropriate model for the prediction of PON2 localization and antioxidative effects in humans.

In conclusion, our data demonstrated the presence of PON2 in all the regions of the intestine and in different subcellular compartments. Microsomal PON2 protein expression was reduced by iron-ascorbate-induced lipid peroxidation whereas pretreatment with powerful antioxidants abolished PON2 decline. According to our experimental data, the cell-associated PON2 probably acts to decrease ROS that could otherwise cause deleterious effects on various intracellular organelles.

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GRANTS

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