Human PON3, Effects Beyond the HDL
Clues From Human PON3 Transgenic Mice

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The article by Shih et al in this issue of Circulation Research is the first report of transgenic expression of human Paraoxonase 3 (PON3) in mice and its ability to decrease significantly atherosclerotic lesion formation and adiposity. These effects were, interestingly, observed only in male but not in female mice on either C57BL/6J or LDLR KO backgrounds. Noteworthy, no human or mouse PON3 were detected in mouse HDL and plasma and therefore the effects of human PON3 were derived from PON3 in the tissues, not in the blood.

PON3 is a member of the PON gene family that includes PON1, PON2, and PON3. PON enzymes are well conserved in mammals: at the amino acid level, the orthologs share 79% to 95% and the paralogs ~65% identity. PON1, PON2 and PON3, have different cell and tissue distribution as well as different regulation of expression, suggesting distinct physiological roles for each of them. These roles, however, remain largely unknown. Phylogenetic, structural and biochemical data demonstrate that all three PONs are primarily lactone hydrolizing enzymes, albeit with different substrate specificity. PON1 is by far the most studied member of the family, and much of our understanding of the PON enzymes is derived primarily from studies involving PON1 protein.

When PON3 proteins were detected in rabbit and human serum associated with the HDL fraction, they were first tested in systems in which PON1 had demonstrated an effect: purified rabbit PON3 protected LDL against copper induced oxidation in vitro; stably transfected cells overexpressing human PON3 activity was secreted, and no PON3 protein could be detected by Western blotting in the medium. What is the HDL indeed the place where PON3 acts physiologically, at least for all mammalian species?

Here is a brief review of the interspecies differences in PON3 expression and tissue distribution. PON3 protein has been isolated from and/or detected in the livers or liver-derived cell lines from rabbit, rat, mouse and human. Human PON3 is expressed throughout the entire gastrointestinal tract, with highest message and protein levels in the esophagus and stomach. In mouse gastro-intestinal tract, PON3 has highest expression in jejunum and ileum and was not detected in colon. PON3 cDNA was detected in cultured airway epithelial from wild type and at even higher levels from PON1-knockout mice. PON3 is expressed in murine, but not in human macrophages.

Shih and colleagues report the presence of PON3 message in other mouse organs and tissues such as kidney, lung, brain, adipose tissue, and, interestingly, relatively high levels in the aorta. No human or mouse PON3 was detected in mouse HDL or in plasma of nontransgenic and human PON3-transgenic mice. The authors speculate that mouse HDL may not be a good acceptor for either human or mouse PON3. However, an additional/alternative explanation can be provided.

Human PON3 may exert its action primarily inside rather than outside cells. We have compared the secretion of human and rabbit PON3 and human PON1 after transient expression in heterologous cells. Approximately 80% of the total PON3 activity was secreted, and no PON3 protein could be detected by Western blotting in the medium. What accounts for this striking difference in PON localization?

The distal N-terminal sequence of rabbit PON3, unlike the human, mouse and other mammalian PON3 sequences, is very similar to the PON1 N-termini. We hypothesized that the N-terminus in PON1 and rabbit PON3 determines their secretion. Indeed, human PON1/human PON3 and rabbit PON3/human PON3 chimeras were secreted into culture medium to a less extent than control cells and were able to retard biological effects of preformed mildly oxidized LDL. Thus, PON3 became well thought-out like another HDL protein with antioxidant/antiatherogenic properties despite the fact that in rabbit and human sera PON3 is at least 2 orders of magnitude less abundant than PON1.

So, is the HDL indeed the place where PON3 acts physiologically, at least for all mammalian species?
physiological process. Thus, human PON3 protein is expected to exert its physiological roles primarily in the liver and in other tissues, but not in serum. The current work of Shih and colleagues supports this hypothesis.

All 3 human PON isoforms appear to be membrane-bound proteins, but except for the plasma membrane localization of PON1, little is known about the subcellular distribution of PON2 and PON3. In cultured Caco-2 cells, a colon carcinoma-derived cell line, PON2 protein was detected in the apical (luminal side), PON1 in the basolateral (circulatory side), and PON3 in both locations, but more abundant in the basolateral side. Recently, the presence of all 3 PONs has been demonstrated in murine tracheal epithelial cells with PON2 and PON3 expressed at the highest levels. This article provides evidence for intracellular and not a cell membrane localization of PON2. It is not clear at present if the differences in the cell localization are species and/or tissue specific. Microsomal localization for PON3 has been demonstrated in rabbit and rat livers. Interestingly, in rabbit liver, PON3 (dubbed microsomal paraoxonase) was consistently copurifying with the microsomal triacylglycerol transfer protein (MTP). MTP is essential for the proper assembly of the apolipoprotein B containing lipoproteins, very low density lipoprotein and chylomicrons, by the liver and small intestine. MTP has been identified also in adipocytes with proposed role in lipogenesis and lipolysis. It is tempting to speculate that PON3 may regulate lipid metabolism through its interaction with MTP and that this interaction may account for the decreased adiposity and lower circulating leptin levels in male PON3 Tg mice.

The sexual dysmorphism in the effect of human PON3 overexpression in 2 independent lines of transgenic mice is certainly an interesting observation. Male mice are more likely than female mice to become obese and to develop atherosclerosis when put on atherogenic diet and thus the modest effect of human PON3 overexpression may be diluted and not obvious in the females. Sex differences in PON1 activity and expression (higher in females than in males) have been reported in both human and nonhuman animals, and warrant further investigations of the interactions between gender, sex hormones and PON proteins.

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References


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