High Density Lipoprotein-Associated Paraoxonase-1: A Possible Prognostic Biomarker for Heart Failure?

Oren Rom[1] and Michael Aviram[2*]

[1] Department of Internal Medicine, Cardiovascular Center, University of Michigan Medical Center, Ann Arbor, MI, USA

[2] The Lipid Research Laboratory, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

* Corresponding author: The Lipid Research Laboratory, Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Efron 1, Bat Galim, Haifa 31096, Israel; Tel.: +97248295278; fax: +97248520076; E-mail address: aviram@technion.ac.il

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1. Paraoxonases (PONs) overview

Located adjacently on chromosome 7 in humans, the PON gene family includes three members: PON1, PON2 and PON3. The similarities in PON genes regarding their coding regions and the locations of the exon/intron junctions, indicate that these genes arose by gene duplication (1). PON1 is expressed in various tissues, but is mainly synthesized in the liver, from which it is secreted to the circulation, where it is found in association with HDL. PON2 is expressed in nearly all human tissues, but it is not found in the circulation. PON3, like PON1, is found in the liver as well as in the serum, where it is associated with HDL, but at lower concentrations in comparison to PON1. All PONs have been shown to play a role in the attenuation of oxidative stress, however, PON1 remains the most studied of the three enzymes (2, 3). PON1 gene polymorphisms was shown to affect the blood concentrations of the enzyme and its catalytic activity. The two most common polymorphisms of PON1 consist of a glutamine-to-arginine substitution at position 192 (Q192R) and a leucine-to-methionine substitution at position 55 (L55M). PON1 gene polymorphisms have been associated with various human pathologies, including coronary heart disease (CHD), type 2 diabetes, inflammatory bowel disease (IBD) and others. (3-5). PON1 expression was shown to be regulated by various stimuli including nutritional factors (such as the polyphenol-rich pomegranate juice, red wine, etc.), oxidative status (influenced by the balance between pro-oxidants such as copper ions, iron, etc. and anti-oxidants such as flavonoids, quercetin, punicalagin and others), inflammatory conditions (IBD, pancreatitis, etc.) and several pharmacological agents, mainly those related with lipid metabolism (such as statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, peroxisome proliferator-activated receptor agonists, etc.). Indeed, sterol regulatory element-binding protein-2 (SREBP2), the key regulator of cholesterol biosynthesis genes, was shown to regulate the promoter activity of PON1 (3, 6)

2. PON1 anti-atherogenic and cardio-protective properties

PON1 is a 43 kDa calcium-dependent enzyme containing two calcium ions per molecule. One calcium ion is suggested to have a structural function, whereas the second calcium ion is predicted to be involved in the enzyme catalytic activity (6). PON1 is named so for its paraoxonase ability to hydrolyze the organophosphate paraoxon, however, PON1 also catalyzes the hydrolysis of other compounds such as arylesters (aromatic esters, like phenyl acetate) and lactones (cyclic esters, like dihydrocoumarin) (3-6). In addition, PON1 is known for its peroxidase-like activity which was demonstrated by its ability to hydrolyze hydrogen peroxides and lipid peroxides. This peroxidase-like activity of the HDL-associated PON1 contributes to the protective anti-oxidative and anti-atherogenic roles of the HDL particle which inhibits lipoprotein oxidation, stabilizes vulnerable atherosclerotic plaques, and stimulate cholesterol efflux from arterial macrophages (5-7).

In relation to PON1 polymorphism, PON1Q and PON1R were shown to possess different capabilities to hydrolyze lipid peroxides in human atherosclerotic lesions (5). Also, in patients undergoing diagnostic coronary angiography, PON1 genotype demonstrated dose-dependent associations (QQ192 > QR192 > RR192) with decreased levels of serum PON1 arylesterase and paraoxonase activities, and with increased levels of systemic oxidative stress. Moreover, the QQ192 genotype was associated with an increased risk for all-cause mortality and for major adverse cardiac events. However, the incidence of major adverse cardiac events was significantly lower in participants in the highest quartile of PON1 activity (8). Indeed, lower PON1 concentration and activity that attenuate the ability of HDL to prevent lipid peroxidation were suggested to be more important in determining the presence of CHD than PON1 genetic polymorphisms (9).

In parallel to the above studies that explored the role of PON1 genetic polymorphisms as well as its serum concentrations and activities in regard to cardiovascular risk (5, 7-9), extensive

evidence has accumulated demonstrating the mechanisms behind the protective, anti-oxidative and anti-atherogenic properties of PON1. A key role for PON1 in the attenuation of atherosclerosis development was clearly demonstrated in studies using PON1 transgenic (Tg) mice, PON1 knockout (KO) mice and in vitro transfection studies using macrophage model systems (3, 6, 10, 11). These reports indicated an anti-oxidative and anti-atherogenic role for PON1 which attenuates the development of atherosclerosis as a result of its ability to hydrolyze specific oxidized lipids in lipoproteins, in macrophages, as well as in the atherosclerotic lesions (5, 7, 10, 11). Indeed, HDL isolated from PON1 Tg mice was found to protect against LDL oxidation more effectively than HDL isolated from control mice. Moreover, the atherosclerotic lesions from PON1 Tg mice were significantly decreased in both dietary and apolipoprotein E (apoE) KO mouse models of atherosclerosis (10). In addition, PON1 KO mice demonstrated increased serum levels of lipid peroxides as well as increased oxidative stress and lipid peroxidation in arterial macrophages isolated from the mice. In accordance with these results, an increase in the atherosclerotic lesion area was observed in PON1 KO mice on an apoE KO background (11). Furthermore, at the cellular level, PON1 was shown to stimulate HDL-mediated cholesterol efflux from macrophages via the ATP-binding cassette transporter A1 (ABCA1) in association with increased HDL binding to the macrophages (3, 6).

3. PON1 activity as a possible prognostic biomarker for heart failure

Whereas PON1 protection against atherosclerosis development has been established in numerous clinical studies, experimental models and in basic research, the role of PON1 in heart failure has been understudied. In an attempt to determine the prognostic role of PON1 arylesterase activity in subjects with systolic heart failure, Tang et al. (12) has found that lower serum arylesterase activity is a strong predictor of long-term major adverse cardiac events after adjusting for possible confounders. Following this study, Hammadah et al. (13) have recently

investigated the prognostic significance of changes in PON1 arylesterase activity in 299 patients with heart failure over time. In line with their previous reports (12), Hammadah et al. (13) have found that after a mean follow up of 2.8 years, low baseline arylesterase activity was associated with increased risk of adverse heart failure events after adjustment for various confounders. Moreover, a significant drop of arylesterase activity (≥25%) during the follow up was associated with increased risk of heart failure events (13). The findings that lower baseline and follow up arylesterase activity are associated with adverse outcomes in patients with heart failure, indicate that PON1 arylesterase activity might indeed serve as a prognostic biomarker in chronic heart failure.

In contrast, Potočnjak et al. (14) have found that in acute heart failure patients, the HDL-mediated cholesterol efflux from macrophages, but not the PON1 arylesterase activity, was associated with increased risk for hospital mortality. The discrepancy between the above studies, namely, the lack of significant impact of PON1 arylesterase activity on hospital mortality in acute heart failure patient, could be explained by the possibility that PON1 arylesterase activity is useful only as a long-term prognostic marker of adverse cardiac events. Finally, in accordance with the results of Hamadah et al. (13), PON1 activity was found to be significantly lower in plasma obtained from abdominal aortic aneurysm (AAA) patients compared with controls (15). Over-expression of PON1 prevented experimental AAA in mice in association with lower oxidative stress, apoptosis and inflammation, further suggesting that strategies aimed at increasing serum PON1 activities could indeed attenuate AAA, atherogenesis and other cardiovascular diseases (15).

Oxidative stress is known to play an important role in the pathophysiology of cardiac remodeling and heart failure. Increased generation of reactive oxygen species (ROS) within the mitochondria of cardiomyocytes from failing hearts results in cellular dysfunction, protein and lipid peroxidation, accumulation of lipids leading to cellular lipotoxicity, activation of various

signaling pathways that lead to cardiomyocyte hypertrophy or apoptosis, inactivation of sarcoplasmic reticulum calcium pumps that results in impaired myocardial contractility. In addition, ROS can also promote myocardial remodeling and failure through enhanced proliferation of cardiac fibroblasts and via activation of matrix metalloproteinases (MMPs) (16). Whereas the anti-oxidative and anti-atherogenic role of PON1 was clearly demonstrated using macrophage model systems, the possible protective role of PON1 against heart failure at the cardiomyocyte model system remain unclear. Considering the marked detrimental effects of increased cardiomyocyte ROS generation in the pathophysiology of heart failure (16), together with the potent anti-oxidative roles of PON1 (3, 5-7, 10, 11), further research is warranted in order to elucidate the possible mechanisms by which PON1 may possess a protective role against heart failure at the cardiomyocyte level. Examples for such possible mechanisms are depicted in Scheme 1.

4. Conclusions

The findings by Tang et al. (12) as well as that of Hammadah et al. (13) clearly indicate that PON1 activity may serve as a prognostic biomarker to predict the outcomes of chronic heart failure patients. Progress toward the clinical implementation of PON1 as a prognostic biomarker for chronic heart failure patients requires further research in two main directions. First, confirming the findings of Tang et al. (12) and Hammadah et al. (13) in a larger cohort of chronic heart failure patients. Second, elucidating the underlying mechanisms in cardiomyocytes by which lower levels of PON1 activity increases the risk of heart failure events whereas higher levels of PON1 activity are protective.

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Scheme 1: Possible mechanisms of PON1 (and PON2) protection against cardiomyocyte oxidative stress and its consequence heart failure

The HDL-associated PON1 attenuates systemic humoral oxidative stress, whereas PON2 attenuates intracellular oxidative stress, however, their effects on cardiomyocyte oxidative stress, a key feature of the failing heart, remain unclear. Possible mechanisms by which PON1 (or PON2) may protect against cardiomyocyte oxidative stress and its consequence heart failure include attenuation of mitochondrial ROS/RNS generation and/or inactivation of ROS/RNS producing enzymes (NOX, XO or NOS) that may prevent oxidative stress-induced damage in cardiomyocytes or in the heart including: cellular dysfunction, protein and lipid peroxidation, lipid accumulation (leading to PPARα-associated cellular lipotoxicity), activation of various signaling pathways (including the PKC, the MAPKs p38, JNK, ASK1, ERK1/2, PI3K, Akt and NF-κB that lead to cardiomyocyte hypertrophy or apoptosis), altered function of calcium channels (such as SERCA or RyRs, leading to impaired myocardial contractility), and also extracellular matrix remodeling (via proliferation of cardiac fibroblasts and MMP activation).

ROS, reactive oxygen species; RNS, reactive nitrogen species; NOX, nicotinamide adenine dinucleotide phosphate-oxidase; XO, xanthine oxidase; NOS, nitric oxide synthase; $O_2^{\cdot}$-, superoxide; H_2O_2, hydrogen peroxide, $^{\cdot}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; PPARα, peroxisome proliferator-activated receptor alpha; PKC, protein kinase C; MAPKs, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinases; ASK1, apoptosis signal-regulating kinase 1; ERK1/2, extracellular signal–regulated kinases 1/2; PI3K, phosphatidylinositide 3-kinases; Akt, protein kinase B; NF-kB, nuclear factor-kB; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPas; RyRs, ryanodine receptors; MMPs, matrix metalloproteinases.