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Original Article

# Paraoxonase-1 deficiency in mice predisposes to vascular inflammation, oxidative stress, and thrombogenicity in the absence of hyperlipidemia

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**Abstract**

**Background:** Paraoxonase-1 is a polymorphic enzyme that is strongly associated with circulating high-density lipoproteins. The absence of paraoxonase-1 in mice has been shown to promote diet-induced atherosclerosis. As paraoxonase-1 has been recently shown to be a lactonase, its functional role remains to be fully elucidated. We explored additional vascular changes in *Pon1* knockout mice in the absence of atherogenic diet challenge. **Methods:** Early steps in atherogenesis, namely, leukocyte rolling and firm adhesion, were measured using intravital microscopy. Vascular oxidative status was determined by lucigenin-derived chemiluminescence. Arterial thrombosis was determined by in vivo carotid thrombosis assay. Gene expressions were determined by reverse transcription polymerase chain reaction. **Results:** We observed a twofold increase in leukocyte adhesion, but no significant change in leukocyte rolling in $Pon1^{-/-}$ mice versus wild-type controls. This finding is correlated with a 1.6-fold increase in aortic mRNA levels of *P-selectin* ($P<.016$), a 1.3-fold up-regulation in *Vcam1* ($P=.096$), and a 1.5-fold up-regulation in *Icam1* ($P=.016$). Aortic *Tnfα* mRNA expression was unchanged. $Pon1^{-/-}$ mice were also found to show a threefold increase in aortic superoxide production rate ($P=.04$). Furthermore, carotid thrombosis assay revealed a 57% reduction in time to occlusion in $Pon1^{-/-}$ mice ($P<.001$). In spite of such vascular proinflammatory phenotypes, we observed no change in plasma levels of inflammatory cytokines or in hepatic mRNA expression of serum amyloid A. **Conclusion:** Our data revealed significant vascular changes in adhesion, oxidative stress, and thrombotic tendencies in $Pon1^{-/-}$ mice in the absence of hyperlipidemia and systemic inflammation. 

*Keywords:* Paraoxonase; Leukocyte adhesion; Thrombosis; Oxidative stress; Atherosclerosis

## 1. Introduction

Atherothrombosis remains the most common cause of morbidity and mortality in the Western world. Acute thrombus formation in vulnerable atherosclerotic plaques represents the most common form of acute occlusive arterial disease and is associated with high morbidity and mortality rates. Emerging experimental evidence continues to support the doctrine that atherosclerosis is an inflammatory process [1]. Expression of adhesion molecules on the endothelial surface of the arterial wall occurs early, and recruitment of circulating leukocytes and monocytes through rolling, adhesion, and subsequent transmigration represents the initiating phase of the inflammatory cascade [2].

Hypercholesterolemia has long been recognized as one of the major risk factors that promote the initiation and propagation of the atherosclerotic process. Deposition of circulating proatherogenic apolipoprotein (apo) B-containing

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lipoproteins [e.g., low-density lipoproteins (LDL) and intermediate-density lipoproteins (IDL)] and their subsequent oxidative modification promote atherosclerosis, whereas circulating high-density lipoproteins (HDL) confer protection. Studies on rodent models of hyperlipidemia, namely, apoE knockout (*apoE* KO) mice and LDL receptor knockout (*Lldr* KO) mice, have provided compelling evidence that elevated circulating LDL or IDL promote the expression of adhesion molecules in the absence of any cellular infiltrates to the arterial wall [3,4]. These changes are further corroborated with early activation of one of the proinflammatory mediators, NFκB, in the aortae of *Lldr* KO mice. Likewise, increased circulating levels of $F_2$-isoprostane, a well-characterized marker for systemic oxidative stress, has also been reported in *apoE* KO mice from a very young age onward [5].

The role of HDL in the attenuation of atherogenesis continues to attract intense interest. In addition to its well-established role in mediating cholesterol efflux from lipid-laden cells, including foam cells in atherosclerotic plaques, HDL have also been shown to have antioxidative and anti-inflammatory functions and, in many incidences, to demonstrate antithrombotic activities [6].

Paraoxonase-1 (PON1) is a polymorphic enzyme that is strongly associated with circulating HDL. The enzyme was originally noted for its ability to catalyze the hydrolysis of organophosphate insecticides and nerve gases [7]. In recent years, several lines of clinical and experimental evidence strongly support a potential role for PON1 in protection against atherosclerosis. *Pon1*$^{-/-}$ mice have been shown to be prone to diet-induced atherosclerosis, in association with elevated circulating levels of oxidized lipoproteins. Similarly, peritoneal macrophages in *Pon1*$^{-/-}$ mice after stimulation manifested a higher level of oxidative stress [8] in conjunction with elevated levels of oxidized LDL, suggestive of early development of proatherosclerotic factors. Although convincing evidence for direct enzymatic action of PON1 in the removal of oxidized lipids remains elusive [9,10], these observations suggest a role for PON1 in the attenuation of the early stages of atherosclerosis. We report in vivo evidence for the role of PON1 in the initiation phase of atherothrombosis in the arterial wall of *Pon1*$^{-/-}$ mice fed a normal chow diet.

## 2. Experimental procedures

### 2.1. Animals

*Pon1*$^{-/-}$ mice backcrossed into a C57Bl/6 background were kind gifts of Dr. Diane Shih [11]. Study mice were between 6 and 9 months of age. Age- and gender-matched wild-type (WT) C57Bl/6 mice from Jackson Laboratory were used as controls. Approximately equal numbers of male and female mice were used in each study. All experimental mice were fed a regular chow diet, as previously described [12]. All experimental procedures were approved by the Animal Care Committee at St. Michael's Hospital.

### 2.2. Plasma lipid analyses

Plasma lipid analyses were performed on mice aged 3–4 months. Plasma was obtained as described previously [13]. Fast protein liquid chromatography (FPLC) fractionation on total plasma was performed on a Superose 6HR column (10 mm×30 cm; Amersham Biosciences). Plasma and Superose fractions were analyzed using enzymatic assays for total cholesterol.

### 2.3. Intravital microscopy

The method was adapted from Ley and Tedder [14]. Briefly, male mice were preanesthetized with intraperitoneal ketamine (125 mg/kg), xylazine (12.5 mg/kg), and atropine (0.25 mg/kg). A tracheal tube was inserted, and the mice were maintained at 37°C on a thermocontrolled rodent blanket. To maintain anesthesia, Nembutal was administered through a cannulus placed in the jugular vein. Blood pressure measurement and blood sampling were possible via a cannulus placed in the carotid artery. After the scrotum had been incised, the testicle and surrounding cremaster muscle were exteriorized onto an intravital microscopy tray. The cremaster muscle was stretched and pinned across the intravital microscopy stage. Cremaster preparation was superfused with thermocontrolled (36°C) and aerated (95% $N_2$ and 5% $CO_2$) bicarbonate-buffered saline throughout the experiment. Microvessel data were obtained within an hour of scrotal incision using an Olympus BX51WI microscope with water-immersion objectives (40× or 60×).

Rolling leukocytes were identified as visible cells passing through a plane perpendicular to the vessel axis, counted, and expressed as the number of leukocytes rolling per minute. To compensate for differences in systemic leukocyte count, rolling leukocytes were reported as the rolling leukocyte flux fraction—the number of rolling leukocytes in a vessel as a percentage of the total leukocyte flux [15]. Adherent leukocytes were counted and expressed as the number of leukocytes per unit vessel wall area: $2\pi(D_v/2)\times$(length of vessel analyzed). Up to 10 venules were observed in each mouse.

### 2.4. Aortic superoxide production assay by lucigenin-derived chemiluminescence (LDCL)

Aortic superoxide production assay has been described previously [16]. Briefly, mice were anesthetized before isolation of the full-length aorta from the aortic root to the femoral bifurcation (wet weight, 11–16 mg). Each segment was cut into 5-mm rings before placement in an opaque 96-well microtiter plate in phosphate-buffered saline (pH 7.5) with 100 μM NADH, followed by incubation at 37°C under 95% $O_2$ and 5% $CO_2$ for 30 min before counting