Involvement of Thromboxane Receptor in the Proatherogenic Effect of Isoprostane F$_{2\alpha}$-III
Evidence From Apolipoprotein E– and LDL Receptor–Deficient Mice

Myan Tang, PhD; Tillmann Cyrus, MD; Yuemang Yao, BSc; Luigina Vocun, BSc; Domenico Praticò, MD

Background—Atherosclerosis is a chronic inflammatory disease of the arterial wall, where it associates with oxidative stress and formation of oxidized lipids. The lipid oxidation product isoprostane iPF$_{2\alpha}$-III, also known as 8-isoPGF$_{2\alpha}$, and 15-F2t-IsoP, is elevated in patients with cardiovascular disease and present in atherosclerotic lesions. Several proatherogenic biological effects have been attributed to this isoprostane, suggesting that it could be an active factor in the pathogenesis of the disease.

Methods and Results—In this study we show that iPF$_{2\alpha}$-III directly promotes atherogenesis in 2 different mouse models (ie, apolipoprotein E [apoE]– and LDL receptor–deficient mice) by activating the thromboxane receptor (TP). This effect is mediated by potent proinflammatory vascular reactions but is independent of thromboxane A$_2$ levels, changes in blood pressure, or lipid profile. Pharmacological antagonism of TP suppresses the vascular proatherogenic effects of iPF$_{2\alpha}$-III. Endothelial cells genetically lacking TP show reduced inflammatory responses when stimulated with this product of lipid oxidation but not other oxidized lipids.

Conclusions—Our results demonstrate that in atherosclerosis iPF$_{2\alpha}$-III is not only a biomarker of oxidative stress but also an active mediator of its vascular phenotype. We conclude that in a clinical setting in which both thromboxane A$_2$ and iPF$_{2\alpha}$-III are elevated, suppression of the first alone would not provide the most benefit for patients because the coincidental presence of the isoprostane will still have a proatherogenic effect. (Circulation. 2005;112:2867-2874.)

Key Words: atherosclerosis ■ inflammation ■ lipids ■ pharmacology ■ thromboxane

Atherosclerosis is a chronic disease of the vasculature influenced by multiple exogenous and endogenous factors, which involves a complex interplay between certain blood components and the arterial wall. Inflammation plays an important role in atherogenesis and other cardiovascular diseases. On the other hand, oxidative stress is also widely recognized as a key factor in atherogenesis, where it is associated with vascular inflammatory reactions and formation of bioactive oxidized lipids. In recent years, the isoprostane iPF$_{2\alpha}$-III, one of several isoprostanes produced in vivo, also known as 8-iso-PGF$_{2\alpha}$, 8-epi-PGF$_{2\alpha}$, or 15-F2t-IsoP, has emerged as a chemically stable product of lipid oxidation and a reliable marker of oxidative stress. Its formation is increased in animal models of atherosclerosis and in patients with atherosclerotic vascular diseases, and patients with diabetes mellitus and smokers. It is present in LDL undergoing oxidative modification and in human as well as animal atherosclerotic vascular lesions. A recent case-control study showed that a high circulating level of iPF$_{2\alpha}$-III is an independent risk marker of coronary artery disease. Furthermore, its suppression coincides with reduced atherosclerosis in mice deficient for apolipoprotein E (apoE) and retarded progression of established vascular lesions in LDL receptor (LDLR)–deficient mice. Therefore, there is much associative evidence to support a role for this isoprostane in the development of atherosclerosis. Interestingly, iPF$_{2\alpha}$-III possesses several biological activities, suggesting that theoretically it is not only a marker of lipid peroxidation but could also act as mediator of the vascular effects of oxidative stress. Thus, iPF$_{2\alpha}$-III is a vasoconstrictor and affects platelets, smooth muscle cells, endothelial cells, and the interaction among these cell types, all of which have been implicated in atherogenesis.

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In the present study we examine the functional role of iPF$_{2\alpha}$-III in modulating the atherogenic vascular phenotype of 2 different mouse models, apoE-deficient and LDLR-deficient mice. Our data indicate that this product of lipid oxidation, but not others, promotes atherogenesis via a...
thromboxane receptor (TP)-mediated but thromboxane A2 (TXA2) level-independent mechanism.

Methods

Animals and Experimental Protocol

Mice were housed in a pathogen-free facility, and all of the animal procedures had the full approval of the University Institutional Animal Care and Use Committee. Unless otherwise stated, an equal number of male and female mice were used in each study. ApoE-deficient and LDLR-deficient mice, backcrossed 10 times to the C57BL/6 strain, were obtained from the Jackson Laboratory (Bar Harbor, Maine). At 10 weeks of age, mice were randomized in groups of 10 animals (5 males and 5 females) each to receive peritoneal injections of the different treatments 3 times per week. The first group received iPF2-III in PBS (1 μg/kg body wt); the second vehicle was also diluted in PBS. The final concentration of isoprostane selected was based on previous studies in which the compound has been shown to exert biological actions but not a significant acute effect on the vascular tone.1-4 A third group of mice was injected with another isoprostane, iPF3-III (1 μg/kg body wt), which does not possess vascular biological activity, and another group was treated with U46619 (1 μg/kg), a stable analogue of the endoperoxide PGH2 and a specific TP agonist.18 Finally, another group received iPF2-III (1 μg/kg) in association with a selective TP antagonist, SQ29548 (2 mg/kg), and another group received SQ28548 (2 mg/kg) alone. All the reagents were initially dissolved as stock solution in ethanol, and further dilutions were always made in PBS.

A separate group of apoE-deficient mice, while injected with iPF2-III (1 μg/kg), received aspirin in their drinking water (30 mg/L), which was replaced with fresh solution every other day. Previous studies have shown that this route of administration produces a cumulative effect in suppressing platelet cyclooxygenase-1 activity.20-21 The doses of the different compounds used in our experiments were selected on the basis of previous published studies.17-21

Another group of mice were injected intraperitoneally with [3H]iPF2-III (0.5 μCi) and then euthanized at 2 (n=4) and 24 hours (n=4). Thromboxane receptor-deficient (TP−/−) mice (a generous gift of Dr T.M. Coffman, Duke University, Durham, NC)22 and wild-type littermates (TP+/+) were injected with vehicle or the same amount of iPF2-III as described above. All the chemicals were from Cayman Chemical Co. The analyses described below were performed in a blinded fashion.

Biochemical Analyses

Plasma cholesterol and triglycerides were measured enzymatically, as previously described.13,15 Total plasma iPF2-III levels were measured by an enzyme immunoassay kit (Cayman Chemical Co); vitamin E levels were measured by a high-performance liquid chromatography assay method, as previously described.13,15 Urinary levels of 2,3-dinor-thromboxane B2 (TXB2) were measured as previously described.20 Circulating levels of soluble intercellular adhesion molecule 1 (sICAM-1), monocyte chemoattractant protein-1 (MCP-1), and endothelin-1 (ET-1) were measured by ELISA assays, as previously described.13 Serum paraoxonase 1 (PON1) activity was determined with the use of paraoxon (O’-O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co) as the substrate and measured by the increase in the absorbance at 412 nm due to the formation of 4-nitrophenol.23 Briefly, 15 μL serum was added to Tris/HCl buffer (100 mmol/L, pH 8.0) containing 1 mmol/L CaCl2 and 1 mmol/L paraoxon, and the generation of 4-nitrophenol was measured at 412 nm with an autoanalyzer. One unit of PON1 was defined as 1 nmol of 4-nitrophenol formed per minute under the aforementioned assay condition.

Blood Pressure Measurements

Systolic blood pressure and heart rate was measured 10 days before the end of the study with the use of a computerized, noninvasive, tail-cuff system, as previously described.21 One set of 10 measurements was obtained for each animal, and the mean blood pressure was calculated. Animals were habituated to the device before the blood pressures were measured to ensure accurate measurements.

Acquisition of Tissues

At the end of the injection period, mice were anesthetized, terminal blood samples were collected by puncture of the right ventricle, and mice were perfused with ice-cold PBS containing 20 μmol/L BHT and 2 mmol/L EDTA (pH 7.4), as previously described.21,24 Hearts were separated from the aorta at the base, embedded in OCT, and kept frozen at −20°C. After the removal of the surrounding adventitial fat tissue, the arch and thoracic aorta were opened longitudinally and fixed in formal/sucrose.

RNA Extraction and RNase Protection Assay

Abdominal aortas and liver were isolated and used to prepare RNA with the use of guanidium isothiocyanate, as previously described.23 Although aortas from 3 animals per group were pooled, liver from each animal was used for each RNA analysis. There was no effect of treatment on aortic weights or RNA yields. MCP-1, ICAM-1, ET-1, and GAPDH mRNA levels were assayed in the aorta, whereas PON1 and GAPDH mRNA levels were assayed in the liver. RNase protection assays were performed with 5 μg of total RNA with the use of a RiboQuant kit with a custom template set according to the manufacturer’s protocol (Pharmigen), as previously described.24 After RNase digestion, protected probes were resolved on denaturing polyacrylamide gels and quantified. The value of each hybridized probe was normalized to that of GAPDH included in each template set as internal control.

Quantification of Atherosclerotic Lesions

To evaluate the extent of atherosclerotic lesion formation, 2 vascular beds and 2 well-validated approaches were used, as previously described.13,15 The ascending aorta was used for cross-sectional microscopic analysis. Briefly, lesion size was determined from Oil Red O serial sections, cut 10 μm thick, and collected at 100-μm intervals, starting at the region where the aortic sinus becomes the ascending aorta. Lesion area was determined with the use of Image-Pro software (Media Cybernetic) on images that were captured with a color video camera connected with a dissection microscope, as previously described.13,15 Immunostaining for macrophage and smooth muscle cell content was performed in these sections. Briefly, a monoclonal antibody to mouse macrophage (MOMA-2; Accurate Chemicals) and an anti-human smooth muscle α-actin monoclonal antibody (Sigma Chemical Co) were used, respectively. Antibody reactivity was detected with the use of a Nova red substrate kit (SK-4800, Vector Laboratory). Cross sections were counterstained with hematoxylin. As control, no primary antibody was added to the same sections.21,24

Next, the aortic arch together with the descending thoracic aorta was analyzed by the en face method. Briefly, after the removal of the surrounding adventitial fat tissue, the aorta was opened longitudinally, fixed in formal/sucrose, and stained with Sudan IV. The percentage of intimal surface area covered by atherosclerotic lesions was determined by capturing these images as described above.13,15

Statistical Analysis

Statistical analysis was performed with the program Graph Pad Prism 4.0. Results were expressed as mean±SD. ANOVAs that showed P<0.05 were followed with multiple pair comparison posttests with Bonferroni correction. Results from the studies with TP−/− and wild-type mice were also analyzed by a 2-way ANOVA with genotype, treatment, and their interaction used as the predictors.

Results

Effects of iPF2-III on Biochemical Measures and its Biodistribution

Ten-week-old apoE-deficient mice, while kept on regular chow diet, were randomized to the different group treatments,
TABLE 1. Biochemical and Functional Changes After iPF2-III Treatment for 2 Months

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>iPF2-III</th>
<th>iPF3-III</th>
<th>iPF2-III + SQ29548</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>31 ± 4</td>
<td>30 ± 5.4</td>
<td>33 ± 4.1</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>717 ± 137</td>
<td>702 ± 115</td>
<td>745 ± 128</td>
<td>720 ± 134</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>75 ± 37</td>
<td>85 ± 30</td>
<td>80 ± 34</td>
<td>90 ± 38</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120 ± 21</td>
<td>125 ± 26</td>
<td>118 ± 23</td>
<td>128 ± 24</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>550 ± 54</td>
<td>570 ± 47</td>
<td>580 ± 45</td>
<td>562 ± 59</td>
</tr>
<tr>
<td>PON1, U/mL</td>
<td>78 ± 25</td>
<td>86 ± 29</td>
<td>35 ± 17</td>
<td>74 ± 26</td>
</tr>
<tr>
<td>Vitamin E, μmol/L</td>
<td>20 ± 3.6</td>
<td>22 ± 3.1</td>
<td>18 ± 4</td>
<td>19 ± 4</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. SBP indicates systolic blood pressure. n = 10 for each group.

Effects of iPF2-III on Atherogenesis

At the end of the 8-week injection experiments, aortas were removed, and the size of the atherosclerotic vascular lesion was determined by 2 independent methods. Cross-sectional analyses of the proximal aorta showed that apoE-deficient mice treated with iPF2-III had significantly larger lesion area in the aortic valve compared with animals receiving vehicle (510 ± 49 versus 981 ± 100 μm² × 10³; P < 0.01). By contrast, no effect was observed in the group of mice treated with iPF3-III (Figure 1). Similar findings were observed when the extension of the atherosclerotic lesion area was determined in the arch and thoracic aorta by the en face method. Thus, aortic lesion areas from mice receiving iPF2-III were significantly increased compared with those of control groups (Figure 1 and Figure 2). Because previous work showed that TP mediates some of the biological effects of this isoprostane, a fourth group of mice were randomized to receive iPF2-III (1 μg/kg body wt) plus SQ29548 (2 mg/kg body wt), and another group received the thromboxane mimetic U46619 (1 μg/kg body wt) alone or in combination with SQ29548 (2 mg/kg). Addition of SQ29548 completely prevented the proatherosclerotic effects of iPF2-III on the aortic tree (Figures 1 and 2). On the other hand, mice receiving U46619 showed an increase in atherosclerotic lesion area (1010 ± 120 μm² × 10³), which, as expected, was prevented by the addition of SQ29548 (530 ± 50 μm² × 10³; P < 0.01). Confirming a previous report, we observed that SQ29548 alone also reduced aortic lesion area (not shown).²⁵

Immunohistochemical analyses of aortic root sections showed no difference between vehicle- and iPF2-III-treated apoE-deficient mice in the percentage of area occupied by macrophages. However, mice receiving iPF2-III had a significant increase in this area, which was prevented by SQ29548 (Figure 1). No significant effect was observed for smooth muscle cells (not shown).

To further confirm the effect of this isoprostane on atherogenesis, we repeated the same experiments using the LDLR-deficient mice on chow. Ten-week-old mice were randomized to receive the same aforementioned treatments for the apoE-deficient mice. At the end of the study, cross-sectional analyses of the proximal aorta showed that only mice treated with iPF2-III had a significant increase in lesion area compared with controls (150 ± 30 versus 457 ± 65 μm² × 10³; n = 6 per group; P < 0.001), and this was prevented by SQ29548 (185 ± 42 μm² × 10³; n = 6). Similar to the apoE-deficient mice, no difference in lipid levels was observed among the 3 groups of mice (not shown).

Effects of iPF2-III on Platelet Activation

It is known that iPF2-III can activate platelets,⁴ which, in turn, by producing TXA₂, could then contribute to its proatherogenic effect. For this reason, we evaluated whether treatment of the apoE-deficient mice with a dose of aspirin (30 mg/L), which may completely block platelet cyclooxygenase-1-dependent TXA₂ biosynthesis,²⁰,²¹ would prevent the vascular effects of iPF2-III. As predicted, at the end of the treatment urinary levels of 2,3-dinor-TXB₂ were reduced by 95% in mice receiving aspirin (105 ± 10 versus 12 ± 3 ng/mg
creatinine; $P<0.001$). However, as shown in Figure 1, aspirin did not influence the vascular response to iPF$_{2\alpha}$-III, supporting a TXA$_2$-independent mechanism for its proatherogenic effect.

Effects of iPF$_{2\alpha}$-III on Vascular Inflammation

Because inflammation is a key factor in atherosclerosis and iPF$_{2\alpha}$-III induces in vitro cellular inflammatory reactions, we next investigated the inflammatory vascular response to this treatment. After 2 weeks of treatment, circulating levels of MCP-1 and sICAM-1 were higher in mice treated with iPF$_{2\alpha}$-III than in those receiving vehicle ($215\pm20$ versus $180\pm12$ ng/mL and $16.9\pm1.1$ versus $12\pm1.2$ ng/mL; $P<0.05$ for both). At the end of the study, both cytokine levels were still significantly higher in mice treated with iPF$_{2\alpha}$-III than in those receiving vehicle or iPF$_{3\alpha}$-III. Although aspirin treatment did not modify the levels of both markers (not shown), the addition of SQ29548 to mice receiving iPF$_{3\alpha}$-III significantly reduced both of them (Figure 3). By contrast, no change for plasma levels of ET-1 was observed among the different groups at any time point considered (not shown).

Next, we assayed the activity levels of serum PON1, an esterase associated with the HDL fraction in the plasma that has antioxidant and anti-inflammatory activities.$^{26,27}$ Serum PON1 activity was unchanged between mice receiving vehicle or iPF$_{3\alpha}$-III; however, this was significantly reduced after 2 weeks of treatment with iPF$_{3\alpha}$-III ($81\pm10$ versus $58\pm11$ U/mL). Lower levels of this marker were still observed at the end of the study, but they were normalized by SQ29548 (Table 1). By contrast, coincidental suppression of TXA$_2$ levels by aspirin did not have any effect (not shown).

Endogenous levels of another antioxidant, vitamin E, were not significantly changed among the different groups of mice (Table 1). To further confirm these observations, we examined gene expression for all these inflammatory markers. RNase protection assay results revealed that treatment with iPF$_{3\alpha}$-III significantly increased gene expression levels for MCP-1 and ICAM-1 but not ET-1 in the abdominal aorta but reduced PON1 mRNA levels in the liver of these mice compared with the mice treated with vehicle or iPF$_{3\alpha}$-III (Figure 4). These changes were prevented by pretreatment with SQ29548 (Figure 4) but not aspirin (not shown).

Involvement of TP Activation in the Vascular Effects of iPF$_{2\alpha}$-III

To further confirm the direct involvement of the TP in these biological effects, mice genetically deficient for the receptor (TP$^{-/-}$) and their wild-type littermates (TP$^{+/+}$) were injected following the same protocol. As shown in Table 3, we found that after 4 weeks of treatment, wild-type littermates (TP$^{+/+}$) but not TP$^{-/-}$ mice injected with iPF$_{3\alpha}$-III or U46619 had an increase in the levels of both MCP-1 and sICAM-1 but a decrease in PON1 activity compared with the vehicle control group. By contrast, no difference in plasma levels of ET-1 was found (Table 3). Similar results were also obtained when mRNA levels for those mediators were assayed in tissues from these 2 groups of mice (not shown). Finally, aortic rings isolated from a separate group of untreated TP$^{-/-}$ and wild-type littermate (TP$^{+/+}$) mice (n=4 per group) were incubated for 24 hours with iPF$_{3\alpha}$-III (10 $\mu$mol/L), 7$\beta$-hydroxycholesterol (10 $\mu$mol/L), or 25-hydroxycholesterol (10 $\mu$mol/L), 2 lipids derived from the oxidation of cholesterol and implicated in atherogenesis.$^{28}$ and 9-hydroxoyctadecenoic acid (9-HODE) (10 $\mu$mol/L) or 13-HODE (10 $\mu$mol/L), lipids derived from the oxidation of linoleic acid. Supernatants from wild-type animals (TP$^{+/+}$) showed a significant increase in both MCP-1 and ICAM-1 levels with all stimuli used. By contrast, whereas both oxysterols and hydroxy acids induced a somewhat similar increase in both cytokines in the absence of TP, incubation with iPF$_{2\alpha}$-III did not have any effect (Figure 5).
Discussion

Atherosclerosis is a multifactorial chronic disease, which involves a continuous interplay between the arterial wall and components of the bloodstream. Consistent evidence has shown that vascular oxidative stress is a key factor in atherogenesis, where it is associated with inflammatory reactions and formation of oxidized lipids. Thus, much attention has been focused on specific oxidized lipid molecules, which are biologically active and derive from the oxidation of LDL or membrane phospholipids. Among them, the isoprostane iPF$_{2\alpha}$-III, product of the free radical–mediated oxidation of arachidonic acid esterified to lipids, has emerged as a specific and sensitive marker of lipid peroxidation in vivo. Increased levels of this isoprostane are present in human and experimental atherosclerosis, and reduction of its levels is coincidental with decreased atherogenesis, suggesting a possible role for this oxidized lipid in the development of the disease. Interestingly, iPF$_{2\alpha}$-III also possesses several biological activities in vitro, all of which are relevant to atherogenesis. Despite this body of evidence, an in vivo and more direct role for this isoprostane in the pathogenesis of atherosclerosis has not yet been provided. Our data establish that this chemically stable lipid oxidation product accelerates the development of atherosclerosis in 2 distinct mouse models, apoE- and LDLR-deficient mice. Its effect is directly mediated by the activation of the TP and subsequent induction of inflammatory vascular responses, but it is independent of de novo TXA$_2$ biosynthesis or changes in plasma lipid levels.

Despite the fact that iPF$_{2\alpha}$-III is known to be a vasoconstrictor, surprisingly, in our study we did not observe any significant effect on systolic blood pressure. This absence of effect could be attributed to the route by which we administered the compound, ie, intraperitoneally, whereas all the studies showing an effect on vascular tone have directly infused the compound into the vascular bed. We provided several lines of evidence in support of the involvement of the TP receptor in the in vivo proatherogenic vascular actions of the isoprostane F$_{2\alpha}$-III: selective TP antagonism prevented them; a pure TP agonist reproduced them; and genetic deletion of TP abolished them. Current views regard atherosclerosis as a dynamic and progressive disease arising from the combination of endothelial dysfunction and inflammation. Inflammatory mediators appear to play a fundamental role in the initiation and progression of this chronic disease. Interestingly, in our study we found that the proatherogenic effect of iPF$_{2\alpha}$-III is secondary to the induction of specific inflammatory mediators, such as sICAM-1 and MCP-1 but not ET-1, at the site of lesion development. By using pharmacological and genetic approaches, we provided evidence in support of the hypothesis that those effects are indeed mediated via TP activation. Importantly, from our results we can exclude that the upregulation of those proinflammatory factors is a mere consequence of the increased atherosclerosis for 2 main reasons. First, we observed an increase at an early time point (2 weeks of treatment) before the development of marked differences.

Figure 2. Proatherogenic effect of iPF$_{2\alpha}$-III. Representative aortic lesion areas by en face (top) and cross-sectional preparation (bottom) of apoE-deficient mice treated with vehicle (A), iPF$_{2\alpha}$-III (B), and iPF$_{2\alpha}$-III plus SQ29548 (C).

Figure 3. Vascular inflammatory responses after iPF$_{2\alpha}$-III administration. Inflammatory cytokines in apoE-deficient mice are shown. sICAM-1 (top) and MCP-1 (bottom) levels in apoE-deficient mice treated with vehicle, iPF$_{2\alpha}$-III, iPF$_{2\alpha}$-III, and iPF$_{2\alpha}$-III plus SQ29548 (SQ) are shown. Values are mean±SD; n=10 per group. *P<0.01 vs vehicle.
biologically active product of lipid oxidation, but not others, study revisits this dogma with the novel observation that this biomarker versus a mediator of atherosclerosis. Our present and atherosclerosis was always thought to be that of a stress in vivo. In general, the link between this isoprostane and atherosclerosis was considered to be that it would then stimulate an inflammatory reaction, which could still activate the TP, inducing a proatherogenic vascular phenotype. Interestingly, reduction of F2-isoprostane formation by vitamin E in combination with the suppression of TXB2 biosynthesis has been shown to be more effective than the 2 approaches alone in experimental atherosclerosis.32

We conclude that selective pharmacological strategies aimed at preventing the activation of this receptor during chronic treatment with cyclooxygenase-1 inhibitors (aspirin-like drugs) may be rationally evaluated in the progression and evolution of human atherosclerosis.

TABLE 3. Plasma Cytokine Levels After 4-Week Injection of Vehicle, iPF2α-III, or U46619 in TP+/− or Wild-type Littermates

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>TP−/− iPF2α-III</th>
<th>TP−/− U46619</th>
<th>Vehicle</th>
<th>WT iPF2α-III</th>
<th>WT U46619</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1, ng/mL</td>
<td>85±21</td>
<td>75±34</td>
<td>80±18</td>
<td>81±18</td>
<td>155±20*</td>
<td>145±20*</td>
</tr>
<tr>
<td>ICAM-1, ng/mL</td>
<td>8±2.6</td>
<td>8.2±2.8</td>
<td>7.9±2.2</td>
<td>7.8±2</td>
<td>12.6±3*</td>
<td>13±2.7*</td>
</tr>
<tr>
<td>ET-1, pg/mL</td>
<td>4.1±1.8</td>
<td>3.8±1.9</td>
<td>4±2</td>
<td>3.9±1.6</td>
<td>3.7±1.4</td>
<td>3.8±1.9</td>
</tr>
<tr>
<td>PON1, U/mL</td>
<td>75±20</td>
<td>70±26</td>
<td>71±24</td>
<td>77±20</td>
<td>48±18**</td>
<td>40±17†</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD for each treatment genotype combination from the 2-way ANOVA model of interaction. WT indicates wild-type. n=4 mice per group.

*P<0.01, †P=0.03 vs vehicle and TP+/−.
The generous gift of the TP Institutes of Health (AG-11542, AG-22512) and the CART Fund.

0.02 vs vehicle.

bars) mice were incubated for 24 hours at 37°C with IPF2 mol/L), 7 for each group). *


CLINICAL PERSPECTIVE

Atherosclerosis is a chronic and complex disease. Consistent evidence shows that vascular oxidative stress is a key factor in its pathogenesis, in which it associates with inflammatory reactions and formation of oxidized lipids. The isoprostane iPF$_{2a}$-III, a product of lipid oxidation and specific marker of in vivo oxidative stress, is elevated in human and experimental atherosclerosis, and reduction of its levels is coincidental with decreased atherogenesis. Interestingly, this isoprostane also possesses several biological activities in vitro, all of which are relevant to atherogenesis. However, in general the link between this oxidized lipid and atherosclerosis was always thought to be that of a biomarker versus a mediator of atherosclerosis. Our present study revisits this dogma with the novel observation that this biologically active product of lipid oxidation directly promotes atherosclerosis in 2 different animal models independently of the thromboxane A$_2$ levels but via a specific thromboxane receptor–mediated mechanism that involves the induction of potent vascular proinflammatory responses. Our results have important clinical implications. Thus, in atherosclerosis in which thromboxane A$_2$ and the isoprostane iPF$_{2a}$-III are both elevated, the suppression of the first alone would not provide the most benefit for the patients because the coincidental presence of the isoprostane could still activate the thromboxane receptor, inducing a proatherogenic vascular phenotype. In conclusion, selective pharmacological strategies aimed at preventing the activation of this receptor during chronic treatment with cyclooxygenase-1 inhibitors (aspirin-like drugs) could represent a novel therapeutic approach in the secondary prevention of cardiovascular events during the progression and evolution of human atherosclerosis.


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