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# Inhibition of cyclooxygenase with indomethacin phenethylamide reduces atherosclerosis in apoE-null mice

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**Abstract**

Non-selective inhibition of cyclooxygenase (COX) has been reported to reduce atherosclerosis in both rabbit and murine models. In contrast, selective inhibition of COX-2 has been shown to suppress early atherosclerosis in LDL-receptor null mice but not more advanced lesions in apoE deficient (apoE$^{-/-}$) mice. We investigated the efficacy of the novel COX inhibitor indomethacin phenethylamide (INDO-PA) on the development of different stages of atherosclerotic lesion formation in female apoE$^{-/-}$ mice. INDO-PA, which is highly selective for COX-2 in vitro, reduced platelet thromboxane production by 61% in vivo, indicating partial inhibition of COX-1 in vivo. Treatment of female apoE$^{-/-}$ mice with 5 mg/kg INDO-PA significantly reduced early to intermediate aortic atherosclerotic lesion formation (44 and 53%, respectively) in both the aortic sinus and aorta en face compared to controls. Interestingly, there was no difference in the extent of atherosclerosis in the proximal aorta in apoE$^{-/-}$ mice treated from 11 to 21 weeks of age with INDO-PA, yet there was a striking (76%) reduction in lesion size by en face analysis in these mice. These studies demonstrate the ability of non-selective COX inhibition with INDO-PA to reduce early to intermediate atherosclerotic lesion formation in apoE$^{-/-}$ mice, supporting a role for anti-inflammatory approaches in the prevention of atherosclerosis.


*Keywords:* Atherosclerosis; Prostaglandins; Cyclooxygenase; COX inhibition; ApoE$^{-/-}$ mice; Aorta

## 1. Introduction

Atherosclerosis has features of an inflammatory disease and is the leading cause of death in industrialized nations [1]. Cyclooxygenase (COX) plays a key role as an inflammatory mediator in virtually all diseases involving inflammation [2]. COX exists as two isoforms, which are coded for by two separate genes [2,3]. COX-1 is found in most tissues and mediates normal physiology requiring prostaglandin production. COX-2 is induced rapidly at sites of inflammation and is expressed in atherosclerotic lesions of humans [4,5], and mice [6] by macrophages, smooth muscle cells and endothelial cells.

Eicosanoids produced by COX-1 and COX-2 have been ascribed a variety of functions in the promotion of cardiovascular health and disease. The beneficial effect of low dose aspirin in reducing cardiovascular events has been largely attributed to inhibition of platelet thromoxbane production, a COX-1 mediated process [7]. In contrast, COX-2 has been proposed to play both beneficial and deleterious roles in cardiovascular health [8–11]. Recent evidence from the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial indicating that selective COX-2 inhibition with rofecoxib results in increased cardiovascular events after 18 months compared to placebo has resulted in removal of rofecoxib from the market (www.vioxx.com). Yet studies in animal

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models and humans support roles for COX-2 in promoting endothelial dysfunction [12], early atherosclerotic lesion formation [6] and plaque instability [13,14]. The dramatic removal of rofecoxib from the market highlights our need for a better understanding of the roles of COX-1 and COX-2 in atherosclerosis, plaque rupture, and cardiovascular events.

Non-selective inhibition of COX has been reported to reduce atherosclerosis both in cholesterol fed rabbit models [15] and genetically altered murine models of atherosclerosis [6,16]. Belton et al. have reported that selective inhibition of COX-1 attenuates atherosclerosis in apoE deficient mice [9]. However, reports on the impact of selective COX-2 inhibition on the development of atherosclerosis in murine models have been mixed with decreased, increased or unchanged atherosclerotic lesion area [6,16–19]. The divergence in the results may be the consequence of differences in experimental design, including efficacy and selectivity of the inhibitors, gender of the mice and stage of atherosclerotic lesions.

A new class of COX-2 selective inhibitors has been developed by derivatization of the conventional non-steroidal anti-inflammatory drugs (NSAIDs) indomethacin, resulting in >1100 times more selectivity for COX-2 than COX-1 when tested in vitro [20]. In the current studies, we examined the impact of this novel amide derivative of indomethacin, designated INDO-PA, on the development of different stages of atherosclerosis in apoE$^{-/-}$ mice. Interestingly, INDO-PA was found to produce a 61% reduction in platelet thromboxane, indicating partial inhibition of COX-1 in vivo. Treatment of apoE$^{-/-}$ mice with INDO-PA dramatically reduced aortic prostaglandin levels and early and intermediate aortic atherosclerotic lesion formation. These studies demonstrate the ability of non-selective COX inhibition with INDO-PA to reduce early and intermediate atherosclerotic lesion formation in apoE$^{-/-}$ mice, supporting the efficacy of anti-inflammatory approaches in the prevention of atherosclerosis.

## 2. Methods

### 2.1. Animal procedures

Female apoE$^{-/-}$ mice were at the 10th backcross into the C57BL/6 background and originally purchased from Jackson Laboratories (Bar Harbor, ME). Mice were maintained on a rodent chow diet containing 4.5% fat (PMI No. 5010, St. Louis, MO) and autoclaved acidified (pH 2.8) water. Animal care and experimental procedures were carried out in accordance with the regulations and under the approval of Vanderbilt University's Animal Care Committee.

### 2.2. COX inhibition

The dose of INDO-PA used in our study was chosen based on oral dosing in acute studies of carageenan-induced footpad edema plethysmometry in rats in which the oral $ED_{50}$ for this assay in rats is 1.5 mg/kg [20]. Treatment of apoE$^{-/-}$ mice with 5 mg/kg INDO-PA intraperitoneal (IP; 3.33-fold over $ED_{50}$ in rats) was well-tolerated and did not produce any gastric ulceration and toxicity even at a dose of 30 mg/kg of INDO-PA (data not shown). In contrast, apoE$^{-/-}$ mice were able to tolerate daily doses of 2.5-mg/kg indomethacin by the IP route but higher doses (3 mg/kg) of it resulted in gastrointestinal hemorrhage with 100% mortality by 1 week (data not shown). Drugs were administered daily based on the body weight by IP injection (100 μl) in a sterile mixture of 1% DMSO, 5% ethanol, 5% Tween-80 and 89% PBS

### 2.3. Serum cholesterol and triglyceride analysis

Mice were fasted for 4 h and blood was collected under isoflurane anesthesia. Serum was separated by centrifugation and lipoprotein integrity was preserved by using 1 mM phenylmethylsulfonyl fluoride (Sigma). The concentration of total cholesterol and triglycerides was determined using Sigma kits adapted for 96-well plate assay as described [21].

### 2.4. Platelet thromboxane level measurement

Nine-week-old apoE$^{-/-}$ mice were given vehicle ($n = 10$) or 5 mg/kg INDO-PA ($n = 9$) for 1 week. Ninety minutes after the final injection, blood samples were collected in the presence of 25 units of heparin sodium (Sigma) and 1.25 μl 10 μM A23187 $Ca^{++}$ ionophore (Calbiochem). Blood was placed in a 37 °C water bath for 30 min. Plasma was isolated by centrifugation at 1100 rpm for 10 min at 4 °C. Platelet thromboxane $A_2$ metabolite, thromboxane $B_2$ ($TxB_2$) was quantified by gas chromatography/mass spectrometry (GC/MS) as described [22].

### 2.5. Aortic prostaglandin levels analysis

Seven-week-old apoE$^{-/-}$ mice were given daily vehicle ($n = 4$) or 5 mg/kg INDO-PA ($n = 5$) for 9 weeks. Ninety minutes after the last dose administration, mice were sacrificed by cervical dislocation. Aortas were dissected free of adjacent adventitial tissue and snap-frozen in liquid nitrogen. Prostacyclin metabolite 6-keto $PGF_{1\alpha}$ and $PGE_2$ were purified as described and quantified by GC/MS by the Eicosanoid Analysis Core at Vanderbilt University [23].

### 2.6. Analysis of Aortic Lesions

ApoE$^{-/-}$ mice were sacrificed and flushed with PBS through the left ventricle. The aorta was dissected and pinned out in an en face preparation as described previously [24]. In the first experiment, a subset of the distal aortas ($n = 3$) in each group were snap-frozen in liquid nitrogen for prostaglandin determinations. The heart with

the proximal aorta was embedded in OCT and snap-frozen in liquid nitrogen. Fifteen alternate cryosections of 10-μm thickness were collected from the proximal aorta starting from the beginning of the aortic sinus and extending 300 μm distally as described [25]. The sections were stained with Oil-Red-O and lesion area was quantified using a Kontron computer system [24].

### 2.7. Data analysis

Data are expressed as mean ± S.E.M. Total serum cholesterol, triglycerides, $PGE_2$, 6-keto $PGF_{1\alpha}$, $TxB_2$ and aortic lesion areas between the groups were determined using the SigmaStat V.2 Software (SPSS Inc., Chicago, IL) by Student's *t*-test and the Mann–Whitney rank sum test, respectively.

## 3. Results

### 3.1. INDO-PA inhibits platelet thromboxane production in apoE$^{-/-}$ mice

INDO-PA has been reported to be a highly selective COX-2 inhibitor in vitro [20]. The structures of indomethacin and the amide derivative used in the treatments, INDO-PA, are shown in panels A and B of Fig. 1.

To test the COX-2 selectivity of INDO-PA, we measured platelet thromboxane production in apoE$^{-/-}$ deficient mice (Fig. 1C). Surprisingly, INDO-PA inhibited platelet thromboxane $A_2$ metabolite $TxB_2$ production by 61% compared to vehicle (25.7 ± 3.0 ng/ml versus 65.9 ± 2.4 ng/ml, respectively; $p = 0.001$). Thus, in contrast to its behavior in vitro, INDO-PA significantly inhibited COX-1 expression in apoE$^{-/-}$ mice in vivo.

### 3.2. INDO-PA does not affect plasma lipid levels in apoE$^{-/-}$ mice

To examine the impact of treatment with INDO-PA on lipid metabolism and atherosclerosis, three independent studies were designed to allow the development of fatty streak, intermediate and advanced atherosclerotic lesions in female apoE$^{-/-}$ mice. These mice were treated for different periods: from ages 7 to 16 weeks, from 9 to 18 weeks and from 11 to 21 weeks. However, serum lipids remained unchanged throughout the course of treatment in all three studies (Tables 1–3).

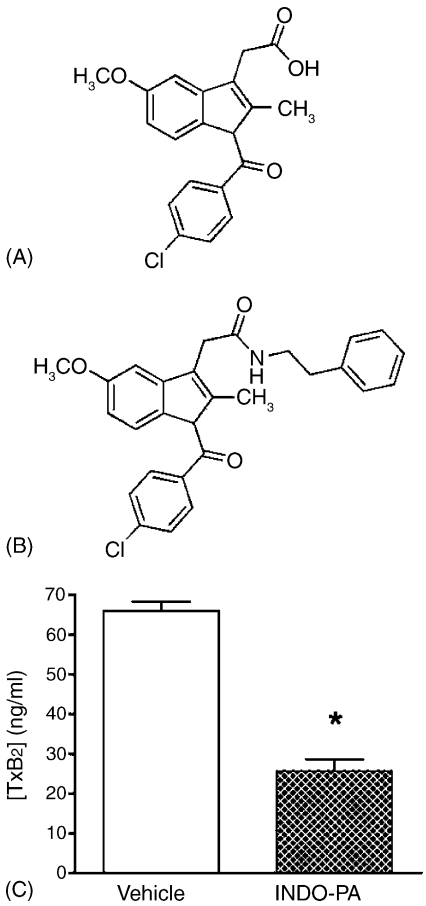


Fig. 1. Inhibition of $Ca^{2+}$ ionophore stimulated platelet thromboxane production: (A) chemical structure of indomethacin, (B) chemical structure of indomethacin amide derivative INDO-PA and (C) apoE$^{-/-}$ mice were given vehicle (clear bar) or INDO-PA (hatched bar) for a week. Blood was collected and stimulated using A23187 $Ca^{2+}$ ionophore. Platelet production of the thromboxane metabolite $TxB_2$ was analyzed by GC/MS.

### 3.3. INDO-PA reduces atherosclerosis in apoE$^{-/-}$ mice

Treatment of 7-week-old apoE$^{-/-}$ mice with INDO-PA for 16 weeks significantly reduced atherosclerotic lesion formation in the proximal aorta by 44% (29620 ± 4148 μm$^2$ versus 52525 ± 6007 μm$^2$; $p = 0.013$) and by 47% in the en face analysis of the aortas (0.43 ± 0.04% versus 0.81 ± 0.08%; $p = 0.033$) compared to mice treated with vehicle, respectively (Fig. 2A and B). Representative Oil-Red-O stained sections from the proximal aorta of mice treated with vehicle (Fig. 3A) or INDO-PA (Fig. 3B) indicate fatty streak lesions consisting predominantly of foam cells.

Table 1
Serum lipid levels in apoE$^{-/-}$ mice treated with vehicle or INDO-PA from 7 to 16 weeks of age

| Animal group | Baseline | | 6 weeks | | 10 weeks | |
|---|---|---|---|---|---|---|
| | Chol. | Trigl. | Chol. | Trig. | Chol. | Trig. |
| Vehicle ($n = 10$) | 320 ± 13 | 55 ± 3 | 276 ± 10 | 62 ± 2 | 311 ± 9 | 69 ± 5 |
| INDO-PA ($n = 10$) | 333 ± 16 | 54 ± 6 | 260 ± 6 | 69 ± 3 | 322 ± 6 | 72 ± 4 |

Values are in mg/dl (mean ± S.E.M.). The number of animals in each group is indicated by *n*.

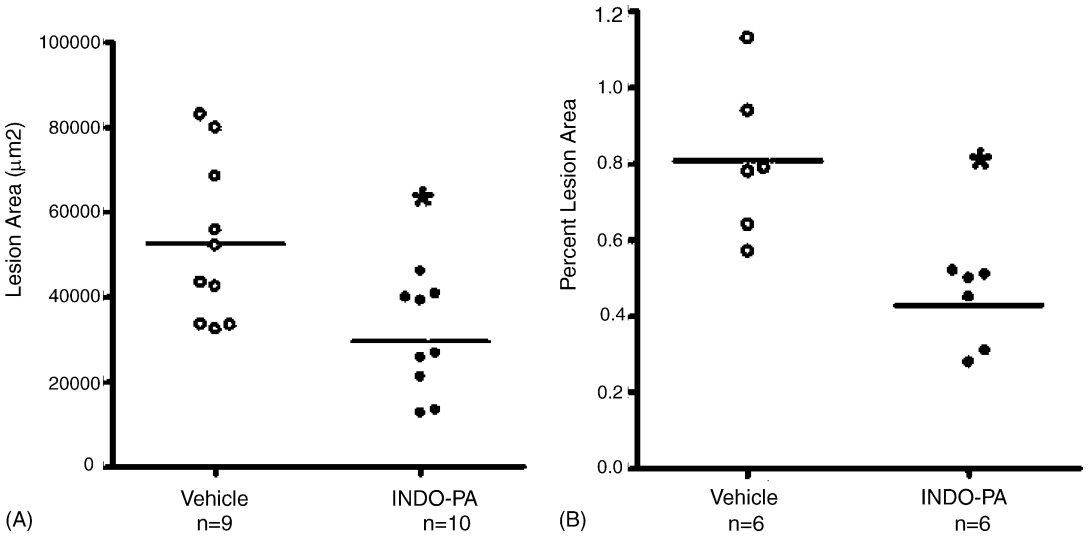


Fig. 2. Reduced atherosclerosis in apoE$^{-/-}$ mice treated with INDO-PA from 7 to 16 weeks of age. (A) The extent of atherosclerotic lesions in female apoE$^{-/-}$ after treatment with vehicle (open circles) or INDO-PA (filled circles) was quantified using Oil-Red-O stained sections. Values are in $\mu m^2$ with horizontal bar representing the mean of each group. (B) En face preparation of whole aortas were stained with Sudan IV and analyzed by a video imaging system. Data are represented as the percent of lesion area for each mouse and the horizontal bar represents the mean for each group.

Table 2
Serum lipid levels in apoE$^{-/-}$ mice treated with vehicle or INDO-PA from 9 to 19 weeks of age

| Animal group | 2 weeks | | 9 weeks | |
|---|---|---|---|---|
| | Chol. | Trig. | Chol. | Trig. |
| Vehicle ($n$ = 13) | 352 ± 24 | 101 ± 8 | 396 ± 30 | 72 ± 5 |
| INDO-PA ($n$ = 10) | 343 ± 9 | 110 ± 5 | 408 ± 27 | 89 ± 6 |

Values are in mg/dl (mean ± S.E.M.). The number of animals in each group is indicated by $n$.

To examine the impact of INDO-PA on the production of two aortic prostaglandins, $PGE_2$ and $PGI_2$, apoE$^{-/-}$ mice were treated with INDO-PA or vehicle for 9 weeks. As shown in Fig. 4, INDO-PA inhibited production of $PGE_2$ by 88% compared to vehicle (5.13 ± 1.01 ng/mg versus 43.79 ± 14.31 ng/mg tissue, respectively; $p = 0.001$). INDO-PA also inhibited production of the $PGI_2$ metabolite by 87% compared to vehicle (29.13 ± 9.21 ng/mg versus 229.22 ± 61.98 ng/mg tissue, respectively; $p = 0.002$).

In the next study, INDO-PA treatment of 9-week-old apoE$^{-/-}$ mice for 9 weeks significantly reduced atherosclerosis by 53% in the proximal aorta (60997 ± 12280 $\mu m^2$ versus 129808 ± 18926 $\mu m^2$; $p = 0.023$; Fig. 5A) and by 64% in the en face analysis of the aorta (0.40 ± 0.05% versus 1.12 ± 0.22%; $p = 0.021$; Fig. 5B) compared to the vehicle treatment group. The atherosclerotic lesions in these mice consisted of both fatty streaks and intermediate lesions in the proximal aorta in the vehicle group (Fig. 3C) and INDO-PA treated group (Fig. 3D).

In contrast, treatment of 11-week-old apoE$^{-/-}$ mice with INDO-PA for 10 weeks produced only a trend for a 19% ($p = 0.38$) reduction in the extent of atherosclerosis in the proximal aorta that did not achieve statistical significance compared to mice treated with vehicle (Fig. 6A). The atherosclerotic lesions in the proximal aortas of these mice were intermediate to advanced in stage both in the vehicle-treated (Fig. 3E) and INDO-PA-treated mice (Fig. 3F) with evidence of connective tissue. Interestingly, there was a dramatic 76% reduction (Fig. 6B) in the en face analysis of the extent of aortic atherosclerosis in the apoE$^{-/-}$ mice treated with INDO-PA compared to the vehicle-treated group (0.61 ± 0.18% versus 2.5 ± 0.39%, respectively; $p = 0.022$).

## 4. Discussion

We examined the impact of a novel amide derivative of indomethacin, INDO-PA, on the development of atherosclerosis in female apoE$^{-/-}$ mice. Although INDO-PA is highly selective for COX-2 enzyme in vitro [20], we have found that INDO-PA inhibits platelet thromboxane in vivo. Treatment of apoE$^{-/-}$mice with INDO-PA dramatically reduced early to intermediate atherosclerotic

Table 3
Serum lipid levels in apoE$^{-/-}$ mice treated with vehicle or INDO-PA from 11 to 21 weeks of age

| Animal group | Baseline | | 2 weeks | | 9 weeks | |
|---|---|---|---|---|---|---|
| | Chol. | Trig. | Chol. | Trig. | Chol. | Trig. |
| Vehicle ($n$ = 9) | 292 ± 9 | 64 ± 3 | 242 ± 15 | 98 ± 6 | 271 ± 18 | 89 ± 3 |
| INDO-PA ($n$ = 9) | 282 ± 16 | 58 ± 5 | 257 ± 23 | 99 ± 9 | 306 ± 29 | 96 ± 4 |

Values are in mg/dl (mean ± S.E.M.). The number of animals in each group is indicated by $n$.

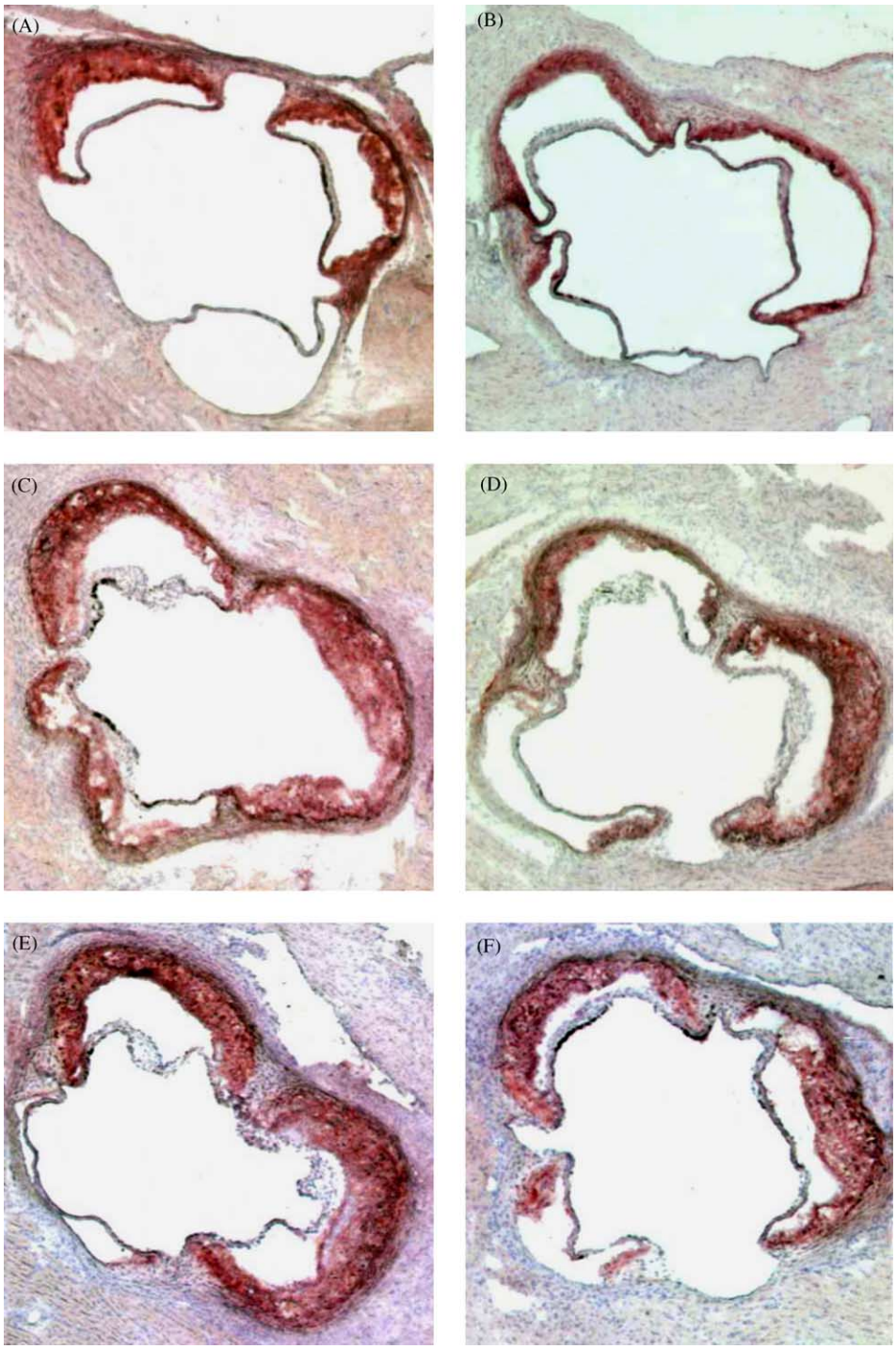


Fig. 3. Representative Oil-Red-O stained aortic root sections from groups treated with vehicle and INDO-PA. (A and B) Early stage atherosclerotic lesions in apoE$^{-/-}$ mice treated with vehicle (A) and INDO-PA (B) from 7 to 16 weeks of age. (C and D). Intermediate stage atherosclerotic lesions in apoE$^{-/-}$ mice treated with vehicle (C) and INDO-PA (D) from 9 to 18 weeks of age. (E and F) Advanced stage atherosclerotic lesions in apoE$^{-/-}$ mice treated with vehicle (E) and INDO-PA (F) from 7 to 16 weeks of age.

lesion formation. In addition, INDO-PA inhibited $PGE_2$ and $PGI_2$ metabolite production in the aorta by 88 and 87%, respectively, demonstrating efficacy of the INDO-PA in inhibiting prostaglandins in the artery wall in vivo. Thus, non-selective inhibition of COX with INDO-PA reduces the development of atherosclerosis in apoE$^{-/-}$ mice, supporting the potential for COX inhibition and anti-inflammatory approaches in the prevention of atherosclerosis.

Treatment with 5 mg/kg INDO-PA (3.33-fold over $ED_{50}$ for oral dosing in rats [20]) was well-tolerated and did not produce gastric ulceration in apoE$^{-/-}$ mice. In these mice at steady state of INDO-PA, we observed a significant but incomplete (61%) inhibition of platelet thromboxane pro-

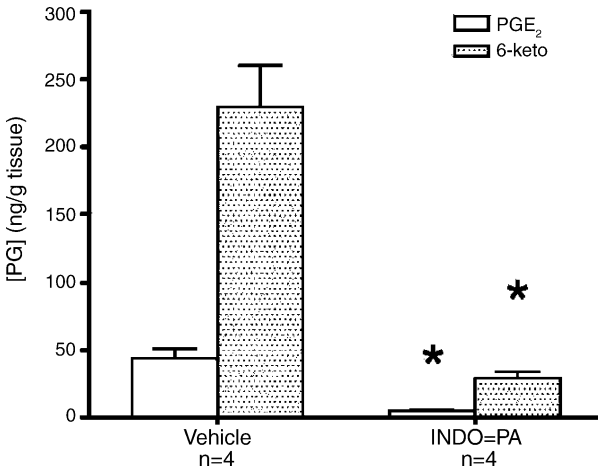


Fig. 4. Inhibition of prostaglandin production in aortic tissue of apoE$^{-/-}$ mice. ApoE$^{-/-}$ mice were given vehicle or INDO-PA beginning at 7 weeks of age for 9 weeks. Aortas were analyzed by GC/MS for $PGE_2$ and $PGI_2$ metabolite 6-keto-$PGF_{1\alpha}$.

duction indicating partial inhibition of platelet COX-1. Further studies in rats have verified that a small percentage of INDO-PA (5–10%) is converted into indomethacin in vivo (R.P. Remmel and L.J. Marnett, unpublished results). Although these data indicate that INDO-PA is only partially selective, previous data demonstrating that >90% inhibition of platelet thromboxane is required to inhibit platelet aggregation [26,27] suggests that inhibition of thromboxane-mediated-platelet aggregation is unlikely to have contributed significantly to the reduction in atherosclerosis. Three decades ago, non-selective inhibition of COX was reported to reduce atherosclerosis in cholesterol fed rabbits [15]. We and others have shown that non-selective inhibition of COX with indomethacin associated with 90–95% reductions in platelet thromboxane reduces early and intermediate atherosclerotic lesions in LDLR$^{-/-}$ mice fed a western diet [6,16]. In contrast, Egan et al. have recently reported that treatment of 6-week-old western diet fed apoBec-1/LDLR DKO mice with indomethacin for 13 weeks was associated with only a 70% reduction in platelet thromboxane and caused a 12.9% reduction in complex atherosclerotic lesions [28]. Thus, differences in these studies include the mouse model used, the extent of atherosclerosis and the efficacy of the indomethacin. Data with regard to the impact of aspirin on murine models have been conflicting, with a study by Cayatte et al. showing no effect [29] and studies in high-fat diet fed apoE$^{-/-}$ [30] and LDLR$^{-/-}$ mice [31] demonstrating significant reductions in lesion formation. However, Cayette et al. reported that a thromboxane receptor antagonist, which inhibited serum thromboxane activity by only 39%, reduced atherosclerosis in apoE$^{-/-}$ mice [29]. The authors interpreted these results as indicating that eicosanoids other than thromboxane are involved in promoting atherosclerosis. Recently, Belton et al. have reported that selective inhibition of COX-1, which reduced urinary 2,3-dinor-$TxB_2$, by around 50% reduced atherosclerotic lesion formation in apoE deficient mice [9]. Thus, it is possible that inhibition of thromboxane may have contributed to the reduction in atherosclerosis seen with INDO-PA by partially offsetting potentially negative effects of reducing prostacyclin. However, it is also possible that reductions of other eicosanoids due to inhibition of COX-1 and/or COX-2 may have contributed to the reduction in atherosclerosis.

Reports on the impact of selective COX-2 inhibition on the development of atherosclerosis in murine models have been mixed indicating decreased, increased or unchanged atherosclerotic lesion area [6,16–19]. The differences in results of these studies may be explained by variability in

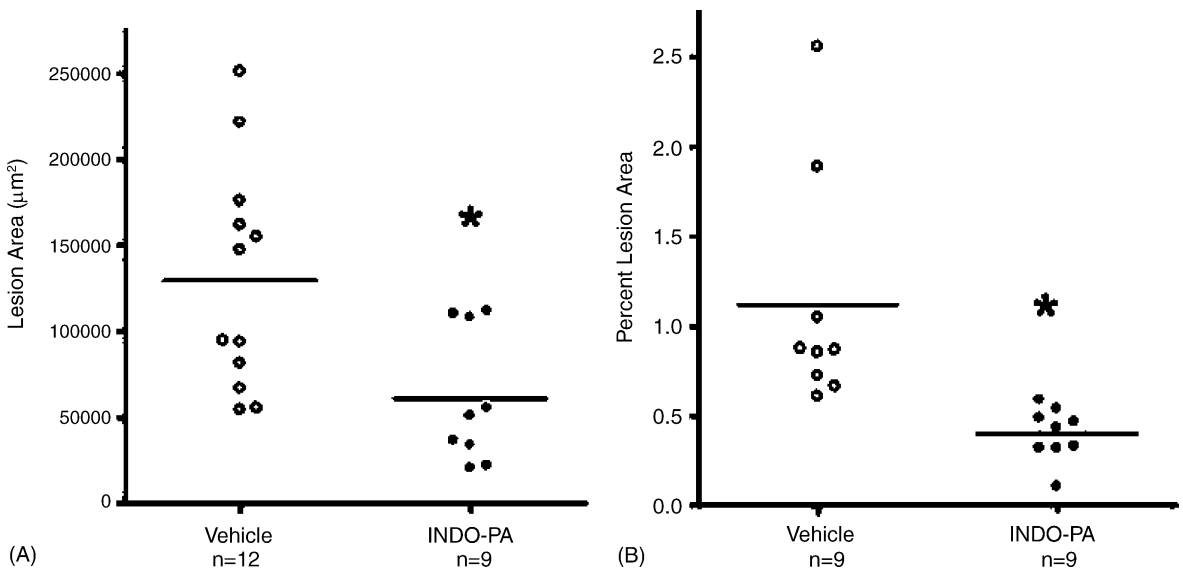


Fig. 5. Reduced atherosclerosis in apoE$^{-/-}$ mice treated with INDO-PA from 9 to 18 weeks of age. (A) The extent of atherosclerotic lesions in the proximal aorta of apoE$^{-/-}$ mice after treatment with vehicle (open circles) or INDO-PA (filled circles) was quantified using Oil-Red-O stained sections. (B) En face preparation of whole aortas were stained with Sudan IV and analyzed by a video imaging system. Data are represented as the percent of lesion area for each mouse and the horizontal bar represents the mean for each group.

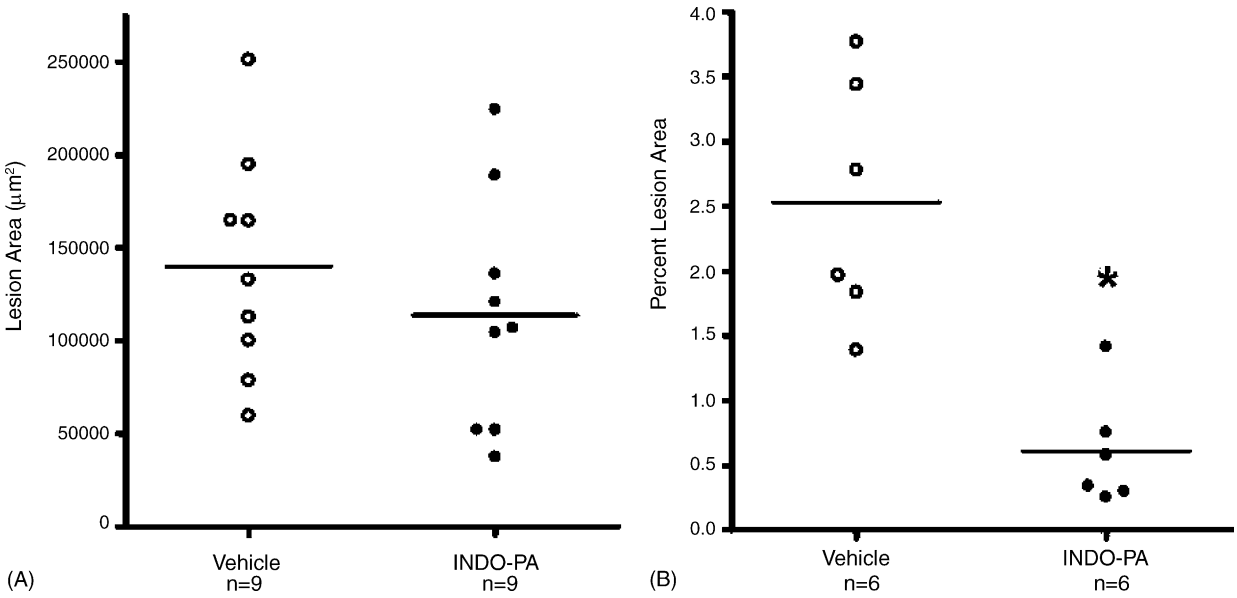


Fig. 6. Impact of INDO-PA treatment on atherosclerosis in apoE$^{-/-}$ mice from 11 to 21 weeks of age. (A) The extent of atherosclerotic lesions in the proximal aorta of apoE$^{-/-}$ after treatment with vehicle (open circles) or INDO-PA (filled circles) was quantified using Oil-Red-O stained sections from 300 μm of the proximal aorta. (B) En face preparation of whole aortas were stained with Sudan IV and analyzed by a video imaging system.

experimental design, including efficacy and selectivity of the inhibitors, gender of the mice and atherosclerotic lesion stage. We have previously reported that rofecoxib reduces early atherosclerotic lesion formation in LDLR$^{-/-}$ mice [6]. Consistent with our results, Krul et al. have presented data that treatment of apoE$^{-/-}$ mice with a selective COX-2 inhibitor, celecoxib, results in a significant reduction in aortic atherosclerosis [32]. Using bone marrow transplantation studies, we have demonstrated that macrophage COX-2 expression promotes early atherosclerotic lesion formation in LDLR$^{-/-}$ mice [6], providing genetic evidence consistent with COX-2 inhibition reducing early atherosclerotic lesion formation. In contrast, the ability of selective inhibition of COX-2 to impact atherogenesis appears to be limited in the setting of advanced atherosclerotic lesions [9,17,18], perhaps due to LXR-mediated downregulation of COX-2 in macrophage-derived foam cells [33] and the inhibition of anti-proliferative effects of COX-2 expression in smooth muscle cells [34]. Thus, the impact of COX-2 on atherosclerosis is complex and may vary according to the cell type and lesion stage.

Our current results demonstrate that non-selective inhibition of COX with INDO-PA reduces the formation of early and intermediate atherosclerotic lesions in female apoE$^{-/-}$ mice. Interestingly, we saw a non-significant trend for a reduction of atherosclerosis in the proximal aortas of apoE$^{-/-}$ mice with advanced stage lesions, whereas the extent of atherosclerosis in the en face aortas was dramatically reduced by 76%. In murine models, atherosclerosis develops first in the proximal aorta and then progresses distally [24,25]. These results are reminiscent of the findings that treatment with the selective COX-2 inhibitor, nimesulide, produced a non-significant trend for a reduction in atherosclerosis in LDLR$^{-/-}$ mice with intermediate stage lesions, whereas treatment with indomethacin produced a significant reduction in atherosclerosis [16]. Although INDO-PA partially inhibits COX-1, we believe that it is acting largely as a COX-2 inhibitor, given the relatively low rate of conversion to indomethacin in vivo, the incomplete inhibition of platelet COX-1, and the much improved safety profile of INDO-PA compared to indomethacin. These results suggest that as the disease progresses from intermediate to advanced lesion stage, COX-2 inhibition appears to have less of an effect on modulating progression of atherosclerosis. Interestingly, INDO-PA virtually eliminated the progression of atherosclerosis in the en face aortas, as can be seen by the similar lesion burdens in all three treatment groups.

Although atherosclerosis is the pathological substrate underlying heart attack and stroke, plaque rupture and thrombosis are responsible for precipitating acute cardiovascular events. Mounting evidence supports the critical involvement of eicosanoids in the processes of plaque rupture and thrombosis. Inhibition of COX-1 mediated production of platelet thromboxane by aspirin reduces the risk for myocardial infarction and stroke [7]. In contrast, rofecoxib, a highly selective COX-2 inhibitor, has recently been taken off the market due to evidence from the APPROVe trial demonstrating increased cardiovascular events after 18 months (www.vioxx.com). The mechanism responsible for the increased cardiovascular events in patients on rofecoxib remains to be elucidated. Concerns have been raised that COX-2 inhibition may promote cardiovascular events by inhibiting prostacyclin and promoting a prothrombotic state [11]. However, the impact of a prothrombotic state might be expected to cause an increase in cardiovascular events sooner than the

18 months seen in the APPROVe trial, suggesting that other mechanisms may be responsible.

Although three published studies have reported an increase in cardiovascular events in patients taking rofecoxib, principally at doses >25 mg a day [10,35,36], other studies found no evidence for increased risk of cardiovascular events with rofecoxib [37,38] or celecoxib [39]. Several important questions remain to be answered. Is the increase in cardiovascular events seen with rofecoxib a class effect that pertains to all other COX-2 inhibitors? Does the presence of COX-1 inhibition in addition to COX-2 inhibition, as seen with non-selective COX inhibitors, eliminate this risk of increased cardiovascular events due to chronic COX-2 inhibition alone? Recently, Pfizer has announced an increase in cardiovascular events associated with valdecoxib in patients in two small studies of patients undergoing coronary artery bypass grafting [19], and no increase in cardiovascular events based on clinical trial database of nearly 8000 patients treated with valdecoxib for durations ranging from 6 to 52 weeks (http://www.pfizer.com/are/news_releases/2004pr/mn_2004_1015.html). Although our current studies do not address the issues of plaque rupture and thrombosis, our results support the ability of non-selective COX inhibition to reduce atherosclerosis. Thus, non-selective inhibition of COX has the potential to favorably impact atherosclerosis, plaque rupture and thrombosis. A better understanding of the complex roles of COX-1 and COX-2 in atherogenesis and plaque stability may lead to new therapeutic approaches to the prevention of cardiovascular disease.

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