Inhibition of Prostaglandin E\(_2\) Synthesis in Abdominal Aortic Aneurysms

Implications for Smooth Muscle Cell Viability, Inflammatory Processes, and the Expansion of Abdominal Aortic Aneurysms

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Background—There is no treatment proven to limit the growth of abdominal aortic aneurysms, in which the histological hallmarks include inflammation and medial atrophy, with apoptosis of smooth muscle cells and destruction of elastin.

Methods and Results—Aneurysm biopsies were used for explant cultures, the preparation of smooth muscle cell cultures, and isolation of macrophages. Tissue macrophages stained strongly for cyclooxygenase 2. Prostaglandin E\(_2\) (PGE\(_2\)) concentrations in aneurysm tissue homogenates, conditioned medium from explants, and isolated macrophages were 49±22 ng/g, 319±38 ng/mL, and 22±21 ng/mL, respectively. PGE\(_2\) inhibited DNA synthesis and proliferation in normal aortic smooth muscle cells (IC\(_{50}\) 23.2±3.8 and 23.6±4.5 ng/mL, respectively). In smooth muscle cells derived from aneurysmal aorta, PGE\(_2\) also caused cell death, with generation of oligonucleosomes. Conditioned medium from the mixed smooth muscle and monocyte cultures derived from explants also had potent growth-inhibitory effects, and fractionation of this medium showed that the growth-inhibitory molecule(s) coeluted with PGE\(_2\). In explants, indomethacin 10 \(\mu\)mol/L or mefenamic acid 10 \(\mu\)mol/L abolished PGE\(_2\) secretion and significantly reduced IL-1b and IL-6 secretion. In a separate case-control study, the expansion of abdominal aortic aneurysms was compared in 15 patients taking nonsteroidal anti-inflammatory drugs and 63 control subjects; median growth rates were 1.5 and 3.2 mm/y, respectively, \(P=0.001\).

Conclusions—The adverse effects of PGE\(_2\) on aortic smooth muscle cell viability and cytokine secretion in vitro and the apparent effect of anti-inflammatory drugs to lower aneurysm growth rates suggest that selective inhibition of PGE\(_2\) synthesis could be an effective treatment to curtail aneurysm expansion. (Circulation. 1999;100:48-54.)

Key Words: aorta • aneurysm • prostaglandins • indomethacin • cyclooxygenase

The specific risk factors that cause the abdominal aorta to become aneurysmal in later life have not been identified. Smoking, hypertension, and hypercholesterolemia, identified in epidemiological studies as risk factors for abdominal aortic aneurysm (AAA), are also risk factors for atherosclerosis. No specific causative genes have been identified, although there is a strong familial predisposition to AAA. The 2 histopathological features that discriminate AAA from aortic atherosclerosis without dilatation are (1) medial attenuation, with loss of elastic lamellae, elastin, and smooth muscle cells, and (2) adventitial inflammation.1-3 Proteolytic disruption of the tunica media is considered to be an important cause of aortic dilatation.4-6

The importance of inflammation as a factor contributing to the expansion of AAAs has recently been appreciated.4-7,9 Separate studies have shown that the magnitude of inflammation in the adventitia and the serum concentration of interferon-\(\gamma\) appear to relate to aneurysm diameter and growth, respectively.4,6 Inflammatory cells in the adventitia and media, together with the cytokines and proteolytic enzymes they elaborate, stimulate the continued proteolysis, with weakening of the aortic wall and aneurysm expansion. The therapeutic implication of interrupting these processes is considerable. For instance, in fibroblasts, interleukin (IL)-1\(\beta\) increases the expression of matrix metalloproteinases (MMPs), including MMP-9, and decreases collagen synthesis.10,11 Cyclooxygenase 2, but not cyclooxygenase 1, is widely expressed in the aneurysm wall, with concomitant synthesis of prostaglandin E\(_2\) (PGE\(_2\)), which may have additional effects to decrease collagen synthesis.11,12

Screening studies have shown that 4% to 5% of men >60 years old have small AAAs (3 to 5 cm in diameter).13,14 Even in these small aneurysms, the inflammatory process appears to be well established.4,8 Most of these small AAAs continue expanding, with annual increases in diameter ranging from 2 to
6 mm/y.\(^{15}\) It is important to find a medical therapy to prevent the expansion of small aneurysms to a size at which prophylactic AAA repair is considered (>5.5 cm in diameter).\(^{16}\) After the success of propranolol in limiting aortic root dilatation in patients with Marfan syndrome,\(^{17}\) the efficacy of propranolol to attenuate AAA growth is being evaluated in clinical trials. Therapy targeted at dampening the inflammatory process in the aneurysm wall might provide an alternative approach.

Our own investigations of inflammatory mediators in the AAA wall were initiated by the observation that when explants of tunica media from AAA were cultured, mononuclear cells appeared to restrict the outgrowth and proliferation of smooth muscle cells. By a process of elimination, our attention became focused on prostanoid metabolites.

### Methods

Patients taking nonsteroidal anti-inflammatory drugs, with AAAs 4.0 to 5.5 cm in diameter at presentation, entered locally into the UK Small Aneurysm Study\(^ {18}\) and followed up for aneurysm growth for >12 months were identified. These patients (cases) included 12 men and 3 women. Other patients (n=63) matched for age, sex, initial aneurysm diameter, and smoking status were selected as control subjects (controls). Aneurysm diameters were measured by ultrasonography every 3 to 6 months, and growth rates, from a minimum of 3 separate diameter evaluations, were calculated by linear regression analysis. The repeatability of diameter measurement was ±2 mm.

Aneurysm biopsies were obtained from 21 patients (17 male), mean age 70±4 years, at the time of surgical repair from the anterior aneurysm wall of large aneurysms (>5.5 cm) opposite the inferior mesenteric artery and were transported at room temperature to the laboratory in DMEM. After removal of adherent thrombus, full-thickness explant cultures were established with the intima uppermost in 10-cm\(^2\) dishes, using 10 mL DMEM/g tissue wet wt. After the tissue had equilibrated for 24 to 48 hours, the medium was replaced and harvested every 48 hours. The first conditioned medium harvested at 96 hours was used for cytokine assays.

Medial explant cultures were established by dissection of the biopsy to obtain the media, which was then cut into 1- to 2-mm\(^2\) pieces and placed in explant culture on type I collagen–coated dishes in Cascade medium 231 (Cascade Inc). Culture medium was changed after 7 days and every 2 to 3 days thereafter. Within the first week, mixed cultures comprising smooth muscle cells with varying numbers of adherent mononuclear cells were established. The smooth muscle cells were characterized by staining with anti–smooth muscle cell actin and the mononuclear cells by staining for CD45 (antibodies from Dako). Conditioned medium from these cultures was harvested every third day during the 3- to 4-week period needed to attain confluence. When confluent, smooth muscle cells were transferred (at passage 2 or 3) onto uncoated 24-well or 96-well plates for assessment of metabolic activity, proliferation, and apoptosis.

Macrophage isolation from aortic aneurysm biopsies was performed by enzymatic dispersion and immunomagnetic separation. Pieces of tissue (~1 g) were diced into small pieces, ~1 mm\(^3\), and washed twice in PBS. The washed pieces were incubated in physiological salt solution (20 mL) containing (in mmol/L) NaCl 130, KCl 6, CaCl\(_2\) 0.01, MgCl\(_2\) 1.2, glucose 14, and HEPES 10.7, buffered to pH 7.2 with NaOH, plus 2 mg/mL BSA, 1 mg/mL collagenase type 2, 1 mg/mL collagenase type 4 (both from Worthington Biochemical Corp), and 5 mmol/L dithiothreitol, for 12 to 16 hours at 4°C followed by 2 hours at 37°C. Cells were dispersed by agitation and washed twice in cold PBS containing 2% FCS before washed anti-CD14–coated Dynabeads (Dynal Ltd), 7.5 \(\mu\)L, were added to each tube and incubated with gentle mixing at 4°C for 30 minutes. The rosseted CD14-positive cells were isolated by placing the tube in a magnetic particle concentrator, before being resuspended and washed 4 times in PBS containing 2% FCS before final suspension in Cascade medium 231 and establishment of cultures ±10 \(\mu\)mol/L indomethacin for 72 hours. The yield of macrophages ranged from 90 000 to 190 000/g tissue wet wt; nonspecific esterase staining indicated that ~85% of the isolated cells were viable macrophages.

Smooth muscle cells from normal aorta (an 18-year-old male donor) were purchased from Cascade Biologics Inc, cultured in Cascade medium 231, and used at the fifth or sixth passage. Saphenous vein endothelial cells at passage 3 were a gift from Suzanne Harley of this laboratory.

MTT assays for mitochondrial activity and 5-bromo-2′-deoxyuridine (BrdU) assays to measure DNA synthesis were performed in 96-well tissue culture plates, using cells at 75% confluence, with triplicate wells for each condition. For MTT assays, cells were treated with test or control medium (200 \(\mu\)L), and MTT in PBS (20 \(\mu\)L, 5 mg/mL) was added to each well for 4 hours. Medium was removed by aspiration, the purple crystalline deposit was dissolved in dimethyl sulfoxide (100 \(\mu\)L), and the absorbance at 570 nm was recorded. For BrdU assays, according to the manufacturer’s instructions (Boehringer Mannheim), cells were deprived of serum overnight before addition of test or control medium (200 \(\mu\)L) for the final 24 hours. Results were expressed as a percentage ratio of absorbances for test condition/new medium, and comparisons were made by Student’s paired \(t\) test.

Cell numbers were determined by counting and acid phosphatase assay. For counting, smooth muscle cells from explant cultures (n=4) were plated onto 6-well tissue culture plates at 6×10\(^4\) cells/well. The next day, cells were treated with conditioned (n=8 for each experiment) or control medium for 24, 48, and 72 hours.
after which, cells were trypsinized and counted in a hemocytometer. Alternatively, cells in 96-well plates were washed twice with cold PBS before the addition of 10 mmol/L p-nitrophenylphosphate in 0.1 mol/L sodium acetate buffer, pH 5.5, containing 0.1% Triton X-100 (125 μL). Plates were incubated at 37°C for 2 hours, the reaction was stopped with 1 mol/L sodium hydroxide (10 μL), and the absorbance was read at 405 nm. Trypsinized cells were used to construct a standard curve.

Apoptosis was assessed by oligonucleosome ELISA (Calbiochem-Novabiochem) using cells grown in 24-well plates. Oligonucleosomes were harvested and assayed according to the manufacturer’s instructions. Smooth muscle cells treated with a cytokine cocktail (tumor necrosis factor [TNF]-α, interferon [IFN]-γ, and IL-1β) provided a positive control.

Other assays included ELISAs for PGE_2 and MMP-9 (Amersham International), IL-1, IL-6, and IFN-γ (Pelikine, CLB). Monocyte chemotactic protein (MCP)-1 and TNF-α were assayed by in-house ELISAs. Neutralizing antibodies to IL-6, TNF-α, and MCP-1 were obtained from R&D Systems, antibodies to cyclooxygenases from Santa Cruz and Affiniti, and prostaglandins from Sigma.

Prostanoid metabolite isolation was performed by solid-phase extraction followed by high-performance liquid chromatography (HPLC). Nonpolar solid-phase bonded silica columns (Amprep octadecyl C18, Amersham International) were used to separate prostanoids from conditioned media into a methanol phase. Columns (Amprep Nova-Pak C18 column (3.9×150 mm, Waters), and developed with a 10% to 28% gradient of a vol/vol solution of acetonitrile containing 0.04% trifluoroacetic acid, applied to a 150 mm, Waters), and developed with a 10% to 28% gradient of a vol/vol solution of acetonitrile containing 0.04% trifluoroacetic acid. Prostanoids from conditioned media were tested varied from 4 to 13. Cells cultured in control medium showed no evidence of oligonucleosome generation after 48 hours.

### Results

**Aortic Smooth Muscle Cell Proliferation Is Limited by Mononuclear Cells**

Mixed cultures of smooth muscle cells, always with adherent mononuclear cells, were derived from the medial layer of aneurysm biopsies (Figure 1, top). Proliferation of the smooth muscle cells appeared to be limited by the mononuclear cells, which stained positively with antibodies to CD68 (data not shown).

Incubation of normal aortic smooth muscle cells (24 hours) in the medium harvested from these mixed cultures (conditioned medium), compared with control medium (conditioned by normal human aortic smooth muscle cells), caused a 30% reduction in mitochondrial activity (MTT test at 4 hours, medium from 13 separate biopsies, P=0.0001) and almost halved the rate of DNA synthesis (incorporation of BrdU at 24 hours, n=10, P<0.0001), Table 1. After 48 hours of culture in conditioned medium, the cell number (acid phosphatase assay) also was reduced (n=7, P=0.028), and low concentrations of oligonucleosomes were detected (Table 1).

Assays were repeated using smooth muscle cells cultured from aneurysm biopsies, with similar but often accentuated findings (Table 1). For example, BrdU incorporation was reduced by 60±20% (n=10, P=0.0001). After 48 hours, the conditioned medium, compared with control medium, led to net loss of viable cells (acid phosphatase assay, Table 1). Cell count experiments, established with 60 000 cells/well, showed cell counts in the controls (n=4) of 95 000±11 000 at 48 hours and 124 000±12 000 at 72 hours, compared with 87 000±15 000 at 48 hours and 43 000±12 000 at 72 hours in the conditioned medium (n=10). After 48 hours of culture in conditioned medium, oligonucleosomes were detectable (Table 1).

The conditioned medium did not reduce BrdU or MTT uptake at 24 hours in human saphenous vein endothelial cell cultures (Table 1). These data suggested that the mononuclear cells of the cultures secreted component(s) that selectively repressed DNA synthesis and proliferation of smooth muscle cells and provoked apoptosis in some cells.

### Treatment of Explant Cultures With Indomethacin Abolishes the Damaging Effects of Conditioned Medium on Cultured Smooth Muscle Cells

Conditioned media were treated by dialysis or heat inactivation (80°C, 1 hour) or were prepared in the presence of indomethacin 10 μmol/L, N^6^-monomethyl-L-arginine (L-NMMA) 1 mmol/L, or glutathione 0.1 mmol/L. The capacity to diminish BrdU uptake was lost after dialysis or preparation of conditioned

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**TABLE 1. Effects of Conditioned Medium on Cultured Vascular Cells**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Endothelial Cells From Human Saphenous Vein (Passage 3)</th>
<th>Smooth Muscle Cells From Normal Aorta (Passage 5/6)</th>
<th>Smooth Muscle Cells From AAA Biopsies (Passage 2/3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrdU uptake at 24 h, % control</td>
<td>95±4 (n=4)</td>
<td>51.2±18.4* (n=10)</td>
<td>39.6±20.3* (n=10)</td>
</tr>
<tr>
<td>MTT uptake at 4 h, % control</td>
<td>106±8 (n=5)</td>
<td>69.4±17.9* (n=13)</td>
<td>76.0±11.5* (n=8)</td>
</tr>
<tr>
<td>Acid phosphatase activity at 48 h</td>
<td>ND (n=7)</td>
<td>84±10† (n=6)</td>
<td>72±6* (n=6)</td>
</tr>
<tr>
<td>Oligonucleosomes at 48 h, U/mL</td>
<td>ND (n=6)</td>
<td>0.44±0.18* (n=6)</td>
<td>0.85±0.23* (n=4)</td>
</tr>
</tbody>
</table>

The conditioned medium was harvested from tunica media explants from aneurysm biopsies. The results (mean±SD) are from paired experiments in which results for control medium (conditioned by normal human aortic smooth muscle cells) are set at 100%.

Student’s paired t test, *P<0.01, †P<0.05, or values not determined (ND); the number of different patient biopsies from which conditioned medium was tested varied from 4 to 13. Cells cultured in control medium showed no evidence of oligonucleosome generation after 48 hours.
medium in the presence of indomethacin (n=7) or glutathione (n=4). L-NMMA and heat treatment had no effect on the toxic component(s) (Table 2). Neutralizing antibodies to IL-6, MCP-1, TNF-α, or IFN-γ did not alter the effect of the conditioned medium to reduce BrdU uptake in cultured smooth muscle cells. These data suggested that the toxic component(s) secreted by mononuclear cells was an arachidonic acid metabolite.

### Cyclooxygenase 2 Is Abundantly Expressed in Aneurysm Tissue, Which Secretes High Concentrations of PGE₂, IL-6, and MCP-1 in Explant Culture

Aneurysm biopsies stained strongly for cyclooxygenase 2, particularly in macrophages (Figure 2) and activated endothelium; staining for cyclooxygenase 1 was negligible. Homogenates of aneurysmal tissue also contained large amounts of PGE₂, 49±22 ng/g tissue wet wt (n=8), whereas homogenates of normal aorta contained <2 ng/g tissue wet wt (n=4). Full-thickness explant biopsies (n=7) secreted high concentrations of PGE₂ (319±38 ng/mL), MCP-1 (71±28 ng/mL), and IL-6 (210±18 ng/mL), with smaller amounts of IL-1β (0.166±0.022 ng/mL), Table 3; TNF-α and IFN-γ were not detected.

### Macrophages Isolated From the Aneurysmal Wall Secrete PGE₂

Macrophages were isolated from aneurysm biopsies by immunomagnetic separation (Figure 1, bottom). The isolated macrophages stained strongly for cyclooxygenase 2 but not for cyclooxygenase 1 (data not shown). Macrophage cultures (n=6) secreted PGE₂ (5 to 35 ng/mL), this secretion being abolished when cells were cultured in the presence of indomethacin 10 μmol/L or mefenamic acid 10 μmol/L. Incubation of normal aortic smooth muscle cells with the macrophage-conditioned medium, prepared in the presence of indomethacin, had no effect on BrdU or MTT uptake. In contrast, when prepared in the absence of indomethacin, the conditioned medium reduced oligonucleosome formation at 48 hours in AAA SMCs from 0.92±0.19 to 0.28 U/mL, n=4, P<0.05.

### PGE₂ Affects Aortic Smooth Muscle Cell Proliferation

PGA₂, PGI₁, and PGF₂α 1 to 1000 ng/mL or indomethacin 10 μmol/L had no effect on DNA synthesis in normal aortic smooth muscle cells, whereas PGE₂ caused a concentration-
dependent reduction in BrdU incorporation after 24 hours (IC$_{50}$, 23.2 ± 3.8 ng/mL) (Figure 3A). PGE$_2$ also limited cell proliferation over a period of 72 hours as measured by acid phosphatase assay in 4 separate experiments (Figure 3B), but oligonucleosomes were never detected.

Similarly, PGE$_2$ (but not PGA$_2$, PGE$_1$, or PGF$_2$) caused a concentration-dependent inhibition of BrdU uptake at 24 hours in smooth muscle cells derived from aneurysm biopsies (IC$_{50}$, 6.0 ± 1.6 ng/mL) (Figure 3A). After 72 hours of incubation with PGE$_2$, cell proliferation was reduced (acid phosphatase assay: IC$_{50}$, 21.8 ± 4.4 ng/mL), with a net decrease in cell numbers at the highest PGE$_2$ concentrations (300 to 1000 ng/mL), in cells from 5 different patients (Figure 3B). PGE$_2$ increased the number of oligonucleosomes present in cultures of smooth muscle cells derived from aneurysm biopsies, with oligonucleosome concentrations after 48 hours (72 hours) increasing from undetectable levels in the absence of PGE$_2$ to 0.09 ± 0.05 (0.13), 0.25 ± 0.06 (0.39), and 0.52 ± 0.10 (0.88) U/mL at PGE$_2$ concentrations of 3, 30, and 300 ng/mL, respectively. Insufficient cells were available from any single patient to conduct a full concentration-response curve; however, the proportion of apoptotic cells, even at high concentrations of PGE$_2$, was only a fraction (10% to 15%) of that induced by the cytokine cocktail IL-1β, IFN-γ, and TNF-α.

Therefore, PGE$_2$ appears to selectively repress DNA synthesis and cell proliferation in aortic smooth muscle cells cultured from either normal or aneurysmal aorta. In cells derived from aneurysmal aorta, PGE$_2$ may also initiate apoptosis.

Fractionation of Conditioned Medium Suggests That a Prostanoid Related to PGE$_2$ Represses DNA Synthesis in Aortic Smooth Muscle Cells

The prostanoids and isoprostanes in the conditioned media were fractionated by HPLC, and the separate fractions were tested for their ability to diminish BrdU uptake by normal aortic smooth muscle cells. The fractions that inhibited the uptake of BrdU eluted at the same position as [3H]PGE$_2$ in conditioned medium from 4 separate aneurysm biopsies (Figure 4).

Use of Nonsteroidal Anti-Inflammatory Drugs Is Associated With Slower Aneurysm Growth Rates

Suppression of cyclooxygenase 2 activity by indomethacin or other anti-inflammatory drugs may have other beneficial effects in the wall of AAAs. First, in full-thickness aortic explants, indomethacin or mafenamic acid abolished the very high secretion of PGE$_2$, and significantly reduced the secretion of IL-1β and IL-6, although MMP-9 release was unchanged (Table 3). When explants were cultured in the presence of

<p>| TABLE 3. Effect of Indomethacin on Secretion From Full-Thickness Aneurysm Explants |
|---------------------------------|------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Explants Cultured for 96 h</th>
<th>Basal</th>
<th>+10 μmol/L Indomethacin</th>
<th>+10μmol/L Indomethacin</th>
<th>+10 μmol/L Mefenamic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE$_2$, ng/mL</td>
<td>319±38</td>
<td>3.3±0.3</td>
<td>65±22</td>
<td>4.5±1.6</td>
</tr>
<tr>
<td>MCP-1, ng/mL</td>
<td>71±28</td>
<td>48±30</td>
<td>65±22</td>
<td>39±27</td>
</tr>
<tr>
<td>IL-1β, pg/mL</td>
<td>166±22</td>
<td>52±19*</td>
<td>108±12</td>
<td>62±15*</td>
</tr>
<tr>
<td>IL-6, ng/mL</td>
<td>210±18</td>
<td>99±12‡</td>
<td>182±16</td>
<td>85±22*</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>22±7</td>
<td>25±8</td>
<td>ND</td>
<td>18±3</td>
</tr>
</tbody>
</table>

ND indicates not determined. Results show mean±SEM of 7 experiments.

*Significant reduction compared with untreated explant, $P<0.001$; ‡significant reduction compared with either untreated explant or explant cultured in the presence of indomethacin and PGE$_2$, $P<0.001$, ANOVA with Bonferroni correction.

Figure 3. PGE$_2$ inhibits DNA synthesis and proliferation in aortic smooth muscle cells. A, Bromodeoxyuridine uptake; B, acid phosphatase assay. Open symbols show results for cells from aneurysmal aorta; closed symbols, for cells from normal aorta. Curves were fitted with a power logistic incorporating Hill coefficients. Acid phosphatase assay was calibrated with known numbers of aortic smooth muscle cells. 100% represents 20 000 to 22 000 cells. Assay commenced with 7500 cells (35% to 38%) 12 to 24 hours before addition of conditioned medium. Therefore, after 72 hours, high concentrations of PGE$_2$ led to a net loss of smooth muscle cells derived from aneurysmal aorta.

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both indomethacin and exogenous PGE$_2$ 500 ng/mL, the secretion of IL-1\(\beta\) and IL-6 increased again (Table 3).

Second, aneurysm growth rates were measured in 2 groups of patients with small aneurysms, cases (n = 15) taking nonsteroidal anti-inflammatory drugs (mainly for joint problems) and controls (n = 63) not taking anti-inflammatory drugs; see Table 4. Systolic blood pressures were slightly higher in the group taking anti-inflammatory drugs and plasma cholesterol concentration was slightly higher in the controls, but these differences were far from significant. The usage of other drugs, including diuretics, calcium channel blockers, other antihypertensive drugs, and lipid-lowering drugs, was not different between the 2 groups. Low-dose aspirin (75 mg/d) was taken by 3 of 15 patients taking anti-inflammatory drugs and 21 of 63 patients not taking these drugs. Only 11 patients took \(\beta\)-blockers, 3 of these among the cases; no patient took propranolol. The median aneurysm growth rate in the cases was 1.5 mm/y compared with 3.2 mm/y in controls, \(P=0.001\) (Mann-Whitney U test), Figure 5.

**Discussion**

PGE$_2$, which is synthesized at high concentration in the walls of AAAs, appears to have adverse effects in vitro on both proliferation of aortic smooth muscle cells and cytokine secretion by the aneurysm wall. The possibility that PGE$_2$ has a pivotal role in the dilatation of AAAs is accentuated by our finding, from a small case-control study, that usage of nonsteroidal anti-inflammatory drugs was associated with slower aneurysm growth rates.

We have shown that outgrowth of smooth muscle cells from explants of AAA biopsies appears to be limited by an arachidonate metabolite, probably macrophage-derived PGE$_2$. PGE$_2$ also inhibited DNA synthesis and proliferation of smooth muscle cells cultured from normal and aneurysmal aorta. Other prostaglandins tested were ineffective. Specific receptors for PGE$_2$ in aortic smooth muscle cells may mediate these effects, with EP$_1$ receptors being described as having higher affinity for PGE$_2$ than PGE$_1$.\(^2^1\) The differential expression of EP receptors in smooth muscle cells from young healthy aorta and cells from AAAs could be sufficient to explain why PGE$_2$ caused cell death, probably by apoptosis, only in cells derived from aneurysmal aorta. The apparent concentration of PGE$_2$ in homogenates of aneurysmal aorta (49 ± 22 ng/mL) was of the same order of magnitude as its IC$_{50}$ for inhibiting cell proliferation (24 ± 5 ng/mL). In culture, however, as in vivo, PGE$_2$ may be metabolized to isopros-
tanes, cross-reacting with antibodies used to assay PGE.
Therefore, either PGE or its metabolic products may exert
adverse effects on aortic smooth muscle cell proliferation
and viability in vivo.

Macrophages in and from the AAA wall stain strongly for
cyclooxygenase 2 but not cyclooxygenase 1, which confirms
previous findings.2 Therefore, the effects of indomethacin or
mefenamic acid (which is relatively more selective for
cyclooxygenase 2 than cyclooxygenase 1 in vivo) on
explant and cell cultures must be attributed to inhibition of
cyclooxygenase 2. The low concentrations of drugs used
(10 μmol/L) and the reversal of their effects by exogenous
PGE diminished the possibility that these drugs were acting
as PPAR-

In short-term explant cultures, both
indomethacin and mefenamic acid rapidly repressed the
secretion of PGE and diminished the secretion of IL-1β and
IL-6. Alterations in MMP-9 expression and release may be a
later event, which unfortunately cannot be studied during the
limited period of explant viability. The effects of nonsteroidal
anti-inflammatory drugs to suppress cytokine secretion could
have long-term benefits by reducing both inflammation and
proteolysis in the AAA wall. These mechanisms are likely to
contribute to the apparent effect of nonsteroidal anti-
inflammatory drugs to halve the rate of aneurysm expansion.
This observation requires confirmation from a randomized
trial using selective cyclooxygenase 2 inhibitors.

These studies have highlighted both the detrimental effects
that excessive production of PGE could have in the aneu-
rysm wall and the potential benefits of inhibition of cycloo-
oxynase 2 inhibition for the patient with a small AAA.

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