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Polychlorinated Biphenyl 77 Augments Angiotensin II-Induced Atherosclerosis and Abdominal Aortic Aneurysms in Male Apolipoprotein E Deficient Mice

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Abstract

Infusion of angiotensin II (AngII) to hyperlipidemic mice augments atherosclerosis and causes formation of abdominal aortic aneurysms (AAAs). Each of these AngII-induced vascular pathologies exhibit pronounced inflammation. Previous studies demonstrated that coplanar polychlorinated biphenyls (PCBs) promote inflammation in endothelial cells and adipocytes, two cell types implicated in AngII-induced vascular pathologies. The purpose of this study was to test the hypothesis that administration of PCB77 to male apolipoprotein E (ApoE) ^{-/-} mice promotes AngII-induced atherosclerosis and AAA formation. Male ApoE^{-/-} mice were administered vehicle or PCB77 (49 mg/kg, i.p.) during week 1 and 4 (2 divided doses/week) of AngII infusion. Body weights and total serum cholesterol concentrations were not influenced by administration of PCB77. Systolic blood pressure was increased in AngII-infused mice administered PCB77 compared to vehicle (156 ± 6 vs 137 ± 5 mmHg, respectively). The percentage of aortic arch covered by atherosclerotic lesions was increased in AngII-infused mice administered PCB77 compared to vehicle (2.0 ± 0.4 vs 0.9 ± 0.1 %, respectively). Lumen diameters of abdominal aortas determined by in vivo ultrasound and external diameters of excised suprarenal aortas were increased in AngII-infused mice administered PCB77 compared to vehicle. In addition, AAA incidence increased from 47 to 85% in AngII-infused mice administered PCB77. Adipose tissue in close proximity to AAAs from mice administered PCB77 exhibited increased mRNA abundance of proinflammatory cytokines and elevated expression of components of the renin-angiotensin system (angiotensinogen, angiotensin type 1a receptor (AT1aR)). These results demonstrate that PCB77 augments AngII-induced atherosclerosis and AAA formation.

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Keywords

Aryl Hydrocarbon receptor ligand; polychlorinated biphenyl; angiotensin; aneurysm; atherosclerosis

Introduction

Abdominal aortic aneurysms (AAAs), influencing over 1.1 million Americans (Lim *et al.*, 2011; Nordon *et al.*, 2011), are gradual expansions of the aorta that rupture more frequently as aneurysms increase in size (Diehm *et al.*, 2005). Mortality rates from ruptured AAAs exceed 80%, and there are no medical therapies that have been proven to limit aneurysm expansion. Risk factors for AAA development and growth include family history, male gender (Aggarwal *et al.*, 2011), smoking (Kent *et al.*, 2010; Stolle *et al.*, 2010) and obesity (Police *et al.*, 2009; Long *et al.*, 2010; Nordon *et al.*, 2011). Previous studies in our laboratory demonstrated that infusion of angiotensin II (AngII) to male hypercholesterolemic mice augments atherosclerosis and causes formation of AAAs (Daugherty *et al.*, 2000; Daugherty and Cassis, 2002; Daugherty *et al.*, 2006). Longitudinal studies examining AAA pathology in male apolipoprotein E (ApoE) deficient mice demonstrated that early after the onset of AngII infusion macrophages localize to medial smooth muscle of the suprarenal aorta, with macrophages present in the adventitia (Saraff *et al.*, 2003) and perivascular adipose tissue (Police *et al.*, 2009) of formed AAAs. These results suggest that vascular inflammation contributes to AngII-induced AAAs. Similar pathologic features have been demonstrated in human AAAs (Kaneko *et al.*, 2011). In addition to the ability of AngII to modulate vascular diseases such as atherosclerosis or AAAs, this peptide has been studied extensively as a contributor to the development of hypertension (for review see Yiannikouris *et al.*, 2010).

It is well recognized that exposure to environmental pollutants has a negative impact on cardiovascular disease (Puga, 2011; Wu *et al.*, 2011). The majority of studies examining effects of environmental toxins on cardiovascular disease have focused on smoking or air pollution as contributors to atherosclerotic lesion formation. By comparison, relatively little is known regarding environmental toxin exposures as risk factors for AAAs. Smoking (Stolle *et al.*, 2010), male sex (Henriques *et al.*, 2004; Kent *et al.*, 2010) and obesity (Police *et al.*, 2009; Long *et al.*, 2010) are positive risk factors for human as well as AngII-induced AAAs, suggesting that the AngII infusion model of AAA formation in hypercholesterolemic ApoE^{-/-} mice is suitable for studies investigating environmental risk factors impacting AAA development.

Polychlorinated biphenyls (PCBs) are environmental pollutants that were commercially manufactured for use in dielectrics in transformers and capacitors, as lubricants, flame retardants and plasticizers (Ludewig *et al.*, 2007; WHO, 1976). Due to the stability of these compounds, they have persisted worldwide in the environment (Jensen, 1966). Humans are exposed to PCBs through food, in the water, and by inhalation (Schneider *et al.*, 2001; Hornbuckle *et al.*, 2006). Increasing evidence suggests that exposures to PCBs through these routes may adversely impact hypertension (Goncharov *et al.*, 2011), as well as serum lipids and cardiovascular disease (Goncharov *et al.*, 2008). In addition, results demonstrated that PCB77, a coplanar PCB, increased expression of pro-inflammatory genes in endothelial cells (Majkova *et al.*, 2011) and adipocytes (Arsenescu *et al.*, 2008), cell types that have been implicated in atherosclerosis and AAAs. Moreover, previous studies in our laboratory demonstrated that PCB77, a coplanar ligand of the aryl hydrocarbon receptor (AhR), promoted the development of atherosclerosis in ApoE deficient mice (Arsenescu *et al.*, 2008). Since inflammation is a key feature of AngII-induced and human AAAs, in this study

we tested the hypothesis that exposures of male ApoE^{-/-} mice to PCB77 would augment AngII-induced atherosclerosis and AAA formation.

Methods

Animals

Male ApoE^{-/-} mice (2 months of age, bred in house to C57BL/6 mice from stock originally purchased from The Jackson Laboratory, Bar Harbor, ME) were housed in a pathogen free environment with water and normal laboratory diet ad libitum. Mice were implanted with osmotic minipumps (Model 2004, Durect Cor, Cupertino, CA) for infusion of AngII (1,000 ng/kg/min) as described previously (Daugherty et al., 2000). Mice were injected (i.p.) with vehicle (safflower oil, 0.4 ml) or PCB77 (49 mg/kg) four times, with the first two injections during the first week (two days apart) and the final two injections during the last week of AngII infusion. The dosing regimen for administration of PCB77 was based on previous results from our laboratory demonstrating that administration of PCB77 at 49 mg/kg in four divided doses increased atherosclerosis in ApoE^{-/-} mice (Arsenescu et al., 2008). In addition, in the current study this dosing regimen of PCB77 of 49 mg/kg resulted in plasma PCB77 levels of 0.37 ± 0.17 ug/ml, equivalent to 2.78 μ M. A concentration of 3.4 μ M of PCB77 was demonstrated in previous studies to promote inflammation in endothelial cells (Hennig et al., 2002), intestinal cells (Choi et al., 2010) and adipocytes (Arsenescu et al., 2008). All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Measurement of systolic blood pressure

Systolic blood pressure was measured on conscious restrained mice by tail cuff during week 3 of AngII infusion as described previously (Cassis et al, 2007). Mice were acclimated to the restraining device for 3 days, and then measurements were taken every day for 3 days.

Measurement of plasma and serum components

Serum cholesterol concentrations were measured using an enzymatic assay (Wako Pure Chemical, Richmond, VA) as described previously (Arsenescu et al., 2008). Plasma concentrations of renin were measured by incubating plasma with an excess concentration of partially purified angiotensinogen with radioimmunoassay of angiotensin I as described previously (Owens et al., 2010). Plasma and tissue concentrations of PCB77 were measured using a Dionex ASE 200 system (Dionex Corporation, Sunnyvale, CA) for assisted solvent extraction and gel permeation chromatography/mass spectrometry as described previously (Arsenescu et al., 2008).

Quantification of vascular pathologies

Atherosclerosis was quantified en face in the aortic arch and aortic sinus as described previously (Daugherty et al., 2004; Daugherty and Rateri, 2005). Maximal diameters of abdominal aortas were measured on cleaned, formalin-fixed aortas by ImagePro Plus 5.1 software (Media Cybernetics, Bethesda, MD). Three observers blinded to the experimental design evaluated AAA incidence. Abdominal aortic lumen diameter measurements (during diastole) were made using high-resolution ultrasound (VisualSonics, Vevo 660, Toronto, CA) as described previously (Barisione et al., 2006).

Quantification of mRNA abundance by real-time PCR

Adipose tissue surrounding the abdominal aorta was removed and RNA extracted using the Ambion Recover AllTM Total Nucleic Acid Isolation Kit (Ambion, Inc., Austin, TX) The embedded tissue was deparaffinized by a short incubation with 100% Xylene at 50° C,

followed by washing steps with 100% ethanol. The resulting tissue pellet was then incubated with digestion buffer and protease at 50° C for 30 minutes. Total mRNA was isolated using proprietary additive and washing solutions. In the final purification steps, samples were incubated with RNase mix for 30 min. The mRNA was reverse transcribed to generate cDNA template using the High Capacity cDNA Reverse Transcription Kit (Ambion, Inc., Austin, TX). Abundance of mRNA was quantified by real-time PCR using an iCycler (Bio-Rad, Hercules, CA), IQ SYBR Green Supermix (Bio-Rad) and using primers indicated in Table II. GAPDH reference gene was used to normalize mRNA abundance for test genes according to the $2^{-\Delta\Delta CT}$ method. The formula used was $[2^{-(CT_{test} - CT_{reference})}] \times 100\%$.

Fluorescent Immunohistochemistry

Formalin-fixed, paraffin embedded AAA sections (5-7 μ m) placed on coated slides were sequentially deparaffinized and rehydrated using xylene and ethanol. Slides were then treated for 10 min with a citric acid-based antigen-unmasking solution (Vector Laboratories, Burlingame, CA). Next, slides were incubated for 1 hour at room temperature in normal blocking serum (1% in PBS) and incubated overnight at 4°C with the primary antibody (dilution 1:100): CD68 rabbit anti-mouse monoclonal antibody (Dako, Glostrup, Denmark). On the second day, after washing, sections were incubated for 1 h with a FITZ - conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Sections were counterstained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Molecular Probes Invitrogen, Eugene, OR) to visualize nuclei, and mounted with VECTASHIELD® medium (Vector Laboratories, Burlingame, CA). Images were taken with an Olympus BX51 microscope, using a 20x and 40 x objectives. We generated the composite images using Image-ProPlus 5.0 (Media Cybernetics, Inc.) software.

Statistical analysis

Data are represented as mean $\pm$ SEM. Comparison of AAA incidence between groups was analyzed using Fisher's exact test. Unpaired Student's t test was used to compare parametric data between groups. Analyses were performed using SigmaPlot 11 (Systat Software, Inc., San Jose, CA). Statistical significance was defined as $P < 0.05$.

Results

PCB77 promotes AngII-induced atherosclerosis

Body weights, adipose tissue mass and serum cholesterol concentrations were not significantly different between mice administered vehicle or PCB77 (Table 1). In mice administered vehicle, PCB77 levels were not detected in plasma or adipose tissue. In contrast, PCB77 levels were 56 ± 7 ug/g in visceral adipose tissue and 0.37 ± 0.17 ug/ml in plasma of PCB77-treated mice at study endpoint. As reported previously (Arsenescu *et al.*, 2008), liver weight was significantly increased by administration of PCB77 (Figure 1A; $P < 0.05$). In addition, even though serum cholesterol levels were not significantly influenced by administration of PCB77, lipid deposition was visible within hepatocytes (Figure 1B). Previous studies demonstrated that infusion of AngII to hypercholesterolemic mice increased systolic blood pressure (Cassis *et al.*, 2009). AngII-induced elevations in systolic blood pressure were significantly increased by exposure of ApoE^{-/-} mice to PCB77 (Table 1; $P < 0.05$). En face quantification of atherosclerosis on the intima of the aortic arch demonstrated that administration of PCB77 significantly increased (2-fold) atherosclerotic lesion surface areas compared to vehicle controls (Figure 2A; $P < 0.05$). In addition, administration of PCB77 promoted lesion areas in aortic sinus sections stained with Oil Red O, with effects more pronounced in sections of aorta that are proximal to the transition between the sinus and ascending aortic arch (Figure 2B,C; $P < 0.05$). Atherosclerotic lesions were visualized by Oil Red O staining of tissue sections from the aortic sinus of mice in

each group. Representative lipid-stained lesions from mice administered PCB77 were larger compared to those from ApoE^{-/-} mice administered vehicle (Figure 2D).

PCB77 augments the incidence and severity of AngII-induced AAAs

Ultrasound was used to measure the in vivo diameter of the suprarenal aorta of mice in each treatment group following 28 days of AngII infusion. Previous results demonstrated that lumen diameters of abdominal aortas from saline-infused ApoE^{-/-} mice quantified by ultrasound are typically 0.8-1.0 mm (Rateri *et al.*, 2011). Following infusion of AngII, lumen diameters of the suprarenal portion of the abdominal aorta increased to 2.0 mm in ApoE^{-/-} mice administered vehicle (Figure 2A). In addition, lumen diameters of abdominal aortas from AngII-infused mice administered PCB77 were significantly increased compared to vehicle controls (Figure 3A; $P < 0.05$). To quantify AAA size, we measured external diameters of cleaned suprarenal aortas from mice in each group at study endpoint. External diameters of the suprarenal aorta were also significantly increased by administration of PCB77 (Figure 3B; $P < 0.05$). These results suggest that the size and/or severity of AngII-induced AAAs was increased in mice administered PCB77. AAA incidence was calculated from mice in treatment group (% of mice exhibiting an AAA) and increased from 47 to 85% in vehicle versus PCB77-treated mice, respectively, (Figure 3C; $P < 0.05$). Moreover, since previous studies demonstrated that approximately 10% of AngII-infused mice die from aneurysmal rupture (Cassis *et al.*, 2007), we quantified the percent of mice surviving the AngII infusion protocol which decreased in mice administered PCB77 (from 80 to 60%; Figure 3D).

PCB77 increased macrophage recruitment within tunica adventitia of the abdominal aorta

Previous studies demonstrated that macrophages are readily apparent in early and advanced stages of AngII-induced AAAs (Saraff *et al.*, 2003). Moreover, macrophage immunostaining extended to peri-adventitial adipose tissue surrounding AngII-induced AAAs (Saraff *et al.*, 2003; Police *et al.*, 2009). Thus, we examined macrophage immunostaining in tissue sections of AAAs from mice in each treatment group. Using fluorescent immunohistochemistry with an anti-CD68 antibody in AAA tissue sections, administration of PCB77 induced periaortic inflammation and macrophage recruitment in the abdominal aortic adventitia (Figure 4).

PCB77 activates the renin angiotensin system (RAS) and promotes expression of inflammatory cytokines in periaortic adipose tissue from AngII-infused mice

We previously demonstrated increased inflammation in periaortic adipose tissue surrounding AngII-induced AAAs from obese C57BL/6 mice (Police *et al.*, 2009). These results suggest that inflammation in peri-adventitial tissue surrounding the abdominal aorta may contribute to increased AAA formation and/or severity. Thus, we examined mRNA abundance of components of the RAS and inflammatory cytokines in periaortic adipose tissue surrounding the abdominal aorta of AngII-infused mice administered either vehicle or PCB77. Periaortic adipose tissue from AngII-infused mice administered PCB77 exhibited significantly increased mRNA abundance of RAS components (angiotensinogen (Ao), angiotensin type 1 receptors (AT1aR)), inflammatory cytokines (TNF α , IL1 β) and chemokines (MCP-1) compared to vehicle controls (Figure 5). In contrast, expression of the anti-inflammatory adipokine, adiponectin (adipoq), was decreased in periaortic adipose tissue from mice administered PCB77.

Discussion

Results from this study demonstrate that exposure of male hypercholesterolemic mice to PCB77 augmented two distinct vascular diseases induced by infusion of AngII,

atherosclerosis and AAAs. In addition to its effects on vascular diseases, PCB77 also increased the blood pressure response to infused AngII. Perivascular adipose tissue surrounding the abdominal aorta, the site of AAA formation, exhibited increased expression of inflammatory cytokines and components of the RAS in response to PCB77. These results demonstrate that short-term exposures to moderate levels of PCB77 promote two common vascular diseases induced by infusion of AngII, atherosclerosis and AAA formation.

Previous studies in our laboratory demonstrated that PCB77 administration to hypercholesterolemic ApoE^{-/-} male mice increased atherosclerosis (Arsenescu *et al.*, 2008). In agreement, results from this study demonstrate similar effects of PCB77 to promote AngII-induced atherosclerosis. However, in contrast to previous findings, in the present study PCB77 promoted AngII-induced atherosclerosis in the absence of an effect on sera cholesterol concentrations. Infusion of AngII in the present study may have influenced effects of PCB77 on sera cholesterol. Regardless, these results suggest that effects of PCB77 to increase AngII-induced atherosclerosis were unrelated to changes in sera cholesterol.

Coplanar PCBs, ligands of the AhR, have been reported to have several effects that would predictably increase atherosclerotic lesion formation. In endothelial cells, PCB77 disrupted barrier function (Toborek *et al.*, 1995), and promoted oxidative stress and the expression of inflammatory cytokines (Toborek *et al.*, 1995; Hennig *et al.*, 2002). PCBs exposure increased phosphorylation of eNOS (Lim *et al.*, 2007), and promoted VCAM expression (Han *et al.*, 2010) in aortas from mice. These effects of PCB77 would predictably contribute to endothelial dysfunction and lesion development. Previous studies in our laboratory demonstrated that administration of a similar dose of PCB77 to ApoE^{-/-} mice increased atherosclerosis (Arsenescu *et al.*, 2008). Thus, PCB77 has the potential to increase atherosclerosis in the absence or presence of infusion of AngII. However, it is unclear from the present study whether the effects of PCB77 to increase atherosclerosis are additive to those induced by infusion of AngII (Daugherty *et al.*, 2000).

We observed an increase in systolic blood pressure in AngII-infused mice administered PCB77. Previous investigators demonstrated cardiac hypertrophy and elevated blood pressure in AhR deficient mice at modest altitude (1632m) that was associated with increased systemic concentrations of AngII (Lund *et al.*, 2003; Lund *et al.*, 2008). However, follow-up studies by this group demonstrated that AhR deficient mice are hypotensive at low altitude (225m) and respond minimally to blockade of the RAS for blood pressure control, suggesting that activation of the AhR stimulates the RAS and increases blood pressure (Lund *et al.*, 2008; Zhang *et al.*, 2010). Further studies demonstrated that sub-chronic exposures of mice to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased blood pressure (Kopf *et al.*, 2008). Our results extend previous findings by demonstrating that administration of an AhR ligand, PCB77, increases the blood pressure response to exogenously infused AngII. However, given that previous results have demonstrated that AngII-induced hypertension is not a major contributor to the peptides effects on atherosclerosis and AAA (Cassis *et al.*, 2009) it is unlikely that increased blood pressure from PCB77 exposure contributed to the toxins ability to promote AngII-induced vascular diseases.

To our knowledge this is the first report demonstrating an effect of PCBs to promote the formation and severity of experimentally induced AAAs. The alarming increase in AAA rupture in AngII-infused mice administered PCB77 is of concern since aneurysmal rupture causes significant mortality in humans (Catalano and Siani, 2005). Previous studies suggested that mechanisms for AngII-induced atherosclerosis and AAA formation may be distinct, as manipulations have been demonstrated to have opposing effects on these AngII-induced vascular pathologies (Daugherty *et al.*, 2010; Henriques *et al.*, 2004). Longitudinal

assessment of vascular pathology in mice infused for different durations with AngII demonstrated the presence of macrophages in medial smooth muscle and adventitia of the abdominal aorta early in AAA evolution (Saraff *et al.*, 2003). Recent studies from our laboratory demonstrated that infusion of AngII to obese mice increased macrophage immunostaining in periaortic adipose tissue surrounding the site of AngII-induced AAAs (Police *et al.*, 2009), suggesting a role for perivascular inflammation as a contributor to AAA formation. We previously demonstrated that PCB77 promoted expression of inflammatory cytokines in 3T3-L1 adipocytes (Arsenescu *et al.*, 2008). In the present study, administration of PCB77 to AngII-infused mice increased expression of inflammatory cytokines and RAS components in adipose tissue surrounding a formed aneurysm. Moreover, while levels of PCB77 in plasma were very low, adipose tissue had much higher levels of PCB77. These results suggest that sequestration of PCB77 to adipose tissue in close proximity to the abdominal aorta may have contributed to localized inflammation and augmented AngII-induced AAAs.

Macrophages play an important role in atherosclerosis and lipoprotein metabolism (Linton and Fazio, 2003). Scavenger receptor mediated transport of oxidized cholesterol coupled with abnormal intracellular cholesterol efflux results in “foamy cell” formation within the arterial intima. Aryl hydrocarbon receptor agonists can induce this abnormal macrophage phenotype (Vogel *et al.*, 2004; Wu *et al.*, 2011). We found that PCB77 treatment enhanced MCP-1 expression within the perivascular adipose tissue from AngII-infused mice. This likely led to vascular homing of CCR2 receptor bearing monocytes. AngII can also induce MCP-1 in certain cell types (Kanno *et al.*, 2005), suggesting combined influences of AngII and PCB77 to promote expression of this chemoattractant. Previous studies from our laboratory suggested that perivascular adipose can serve as a source of inflammatory mediators (Police *et al.*, 2009). In addition, results from our laboratory demonstrated that low concentrations of PCB77 induced adipocyte differentiation as well as enhanced TNF α secretion from these cells. In the present study, data suggest that PCB77 promotes pro-inflammatory cytokine expression in perivascular adipose tissue, potentially through effects at adipocytes or infiltrating macrophages. Interestingly in a mouse model of inflammatory bowel diseases, we recently found that attenuated AhR expression reduced proinflammatory cytokine expression in colon (Arsenescu *et al.*, 2011).

In conclusion, short term exposures to moderate levels of PCB77 resulted in an increase in AngII-induced hypertension, atherosclerosis and AAA formation in male ApoE^{-/-} mice. These effects were associated with increased inflammation in periaortic adipose tissue from mice administered PCB77. The ability of moderate exposures to PCB77 to markedly augment blood pressure, atherosclerosis and AAAs suggests that therapies that limit or detoxify PCB exposures may effectively lower the burden of these common life-threatening vascular diseases.

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Highlights

- Polychlorinated biphenyl 77 (PCB77) augments angiotensin II (AngII)-induced hypertension.
- PCB77 augments AngII-induced atherosclerosis
- PCB77 augments AngII-induced abdominal aortic aneurysm formation (AAA) and rupture
- Effects of PCB77 are associated with enhanced inflammation in periaortic adipose tissue surrounding formed AAAs.

Figure 1.

Effect of PCB77 on liver weight (A) and structure (B) in ApoE^{-/-} mice infused with AngII. A, Liver weight (expressed as a % of body weight) in mice from each group. Administration of PCB77 increased liver weights of AngII-infused mice. Data are mean $\pm$ SEM from n = 10 mice/group. B, Representative liver sections of mice infused with AngII that were administered either vehicle (Control) or PCB77. Liver sections from mice administered PCB77 exhibited vacuole structures suggesting increased lipid deposition. *, P<0.05 compared to vehicle.

Figure 2.

PCB77 increases AngII-induced atherosclerosis. A, Lesion surface areas (%) were quantified on the aortic arch from AngII-infused mice administered vehicle (Control) or PCB77. Administration of PCB77 significantly increased lesion surface areas on the aortic arch. B, Lesion area (mm²) of aortic sinus sections stained with Oil Red O. Lesions were quantified from Oil Red O staining in sections spanning from -240 to +240 µm distance from the transition, which is between the sinus and ascending aortic arch and depicted as 0. In aortic sinus sections proximal to the transition, lesion areas were increased in mice administered PCB77 (n = 5 mice/group). C, Average of lesion areas in sections spanning the entire distance of B. D, Oil Red O staining of representative aortic sinus sections; scale bar = 200 µm. Data are mean ± SEM from n = 6/group. *, P<0.05 compared to vehicle.

Figure 3.

PCB77 promotes the incidence and severity of AngII-induced AAAs. A, Lumen diameters of the suprarenal aorta measured by ultrasound were significantly increased in AngII-infused mice administered PCB77. B, External diameters of excised cleaned suprarenal aortas were significantly increased in AngII-infused mice administered PCB77. C, AAA incidence (% of mice exhibiting an AAA) was significantly increased in AngII-infused mice administered PCB77. D, Percent survival of mice in each treatment group over 28 days of AngII infusion. Survival rate decreased from 80 to 60% in mice administered PCB77. Data are mean $\pm$ SEM from $n = 6-10$ /group. *, $P < 0.05$ compared to vehicle.

Figure 4.

Effect of PCB77 on aortic wall inflammation. The upper panels are representative abdominal aortic aneurysm sections from mice infused with AngII or AngII $\pm$ PCB77. Inserts (lower panels) depict perianeurysmal adipose tissue at 40x magnification. Fluorescent immunohistochemistry for anti-CD68 antibody (green) detected macrophage infiltration located to perianeurysmal adipose tissue from mice administered PCB77 (right insert) compared with control mice (left insert). Nuclei were identified by DAPI staining (blue).

Figure 5.

PCB77 promotes mRNA abundance of RAS components and inflammatory cytokines in the perianeurysmal adipose tissue. Top, mRNA abundance of angiotensinogen (Ao), angiotensin type 1a receptors (AT1a), angiotensin converting enzyme (ACE) or adiponectin (Adipoq) in perianeurysmal adipose tissue from AngII-infused mice administered vehicle (control) or AngII. Bottom, mRNA abundance of inflammatory cytokines in perianeurysmal adipose tissue from mice in each group. Data are mean $\pm$ SEM from n = 9/group. *, P<0.05 compared to vehicle.

Table 1Characteristics of AngII-infused ApoE^{-/-} mice administered vehicle or PCB77.

Parameter/Group	Vehicle	PCB77
Body weight (g)	29.8 ± 0.6	29.2 ± 0.8
Epididymal fat (g)	0.22 ± 0.02	0.18 ± 0.02
Liver (g)	4.3 ± 0.2	7.0 ± 0.5*
Sera cholesterol (mg/dL)	327 ± 33	360 ± 52
Systolic blood pressure (mmHg)	137 ± 5	156 ± 6*

Data are mean ± SEM from n = 8-10/group.

Table 2

List of primers used for qPCR.

GENE	5'-SEQUENCE-3'	PRODUCT SIZE (bp)
ACE	GCCTTGGCTTCATCAGTCTC GGAGTACTTCCAACCGGTCA	193
ADIPOq	GTTGCAAGCTCTCCTGTTCC ATCCAACCTGCACAAGTTC	120
Ao	TCTCTTTACCCCTGCCCTCT GAAACCTCTCATCGTTCCTTG	147
AT1a	CGAAGCGATCTTACATAGGTG GACCAACTCAACCCAGAAAAGC	340
GAPDH	TGAAGCAGGCATCTGAGGG CGAAGGTGGAAGAGTGGGAG	102
MCP1	CCTGCTGCTACTCATTACCC TGTCTGGACCCATTCTTCT	158
TNF α	CCCACTCTGACCCCTTTACTC TCACTGTCCCAGCATCTGT	158