Pkd2 mesenteric vessels exhibit a primary defect in endothelium-dependent vasodilatation restored by rosiglitazone

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Brookes ZL, Ruff L, Upadhyay VS, Huang L, Prasad S, Solanky T, Nauni SM, Ong AC. Pkd2 mesenteric vessels exhibit a primary defect in endothelium-dependent vasodilatation restored by rosiglitazone. Am J Physiol Heart Circ Physiol 304: H33–H41, 2013. First published October 26, 2012; doi:10.1152/ajpheart.01102.2011.—Patients with autosomal dominant polycystic kidney disease have a high prevalence of hypertension and structural vascular abnormalities, such as intracranial aneurysms. Hypertension can develop in childhood and often precedes a significant reduction in the glomerular filtration rate. The main aim of this study was to investigate whether a primary endothelial defect or a vascular smooth muscle (VSM) defect was present in murine polycystic kidney disease (Pkd2) heterozygous mesenteric vessels before the development of renal failure or hypertension. Using pressure myography, we observed a marked defect in ACh-stimulated endothelium-dependent vasodilatation in Pkd2 arterioles. In contrast, Pkd2 vessels responded normally to sodium nitroprusside, phenylephrine, KCl, and pressure, indicating unaltered VSM-dependent responses. Pretreatment with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone significantly restored ACh-dependent vasodilatation in Pkd2 mice. Isolated heterozygous Pkd2 endothelial cells displayed normal ACh-stimulated Ca2+ and nitric oxide production. However, isolated Pkd2 heterozygous VSM cells displayed basal increases in superoxide and sodium nitroprusside-stimulated peroxynitrite formation, which were both suppressed by rosiglitazone. Furthermore, we observed a defective response of Pkd2 mesenteric venules to ACh in vivo, which was more marked after ischemia-reperfusion injury. In conclusion, the results of our study suggest that the defect in vasodilatation in Pkd2 heterozygous vessels is primarily due to a reduction in nitric bioavailability secondary to increased vascular oxidative stress. The ability of rosiglitazone to correct this phenotype suggests that this defect is potentially reversible in patients with autosomal dominant polycystic kidney disease.

There is a high prevalence of hypertension and other cardiovascular abnormalities, including intracranial aneurysm rupture, in ADPKD (36). In Pkd1-null mice, embryonic lethality associated with subcutaneous oedema and vascular fragility has been described in some models, indicating a role for Pkd1-regulated responses in the vasculature (5, 18). Recently, a Pkd1 hypomorph and a Pkd1 transgenic have been reported with a high incidence of aneurysm rupture (19, 20). These studies suggest that the vascular defect may be directly related to gene dosage. Pkd2-null mice die in utero with developmental cardiac defects due to defective left-right asymmetry (31).

The pathogenesis of hypertension in ADPKD is controversial, with both renal and extrarenal vascular abnormalities described. The ADPKD proteins polycystin 1 (PC1) and polycystin 2 (PC2) are both expressed in human endothelial cells and vascular smooth muscle (VSM) cells (VSMCs) (30). Despite increased plasma levels of the vasodilator nitric oxide (NO) (24), a reduced vascular responsiveness to ACh, which causes the release of NO from the endothelium, has been reported in subcutaneous resistance vessels from normotensive ADPKD patients (38, 39). Experimentally, endothelium-dependent relaxation to NO was also impaired in mesenteric resistance vessels from Han:SPRD rats and in aortic rings from adult Pkd1 hyperphenotypic mice (27, 37). Furthermore, isolated thoracic aorta VSMCs from Pkd2 heterozygous mice demonstrate altered Ca2+ regulation (34), and haploinsufficiency of Pkd2 is associated with reduced smooth muscle contractility in Drosophila (14). Both hyporesponsiveness to endothelium-derived vasodilators (such as NO) and hypercontractility of VSM could contribute to the hypertension of ADPKD.

Peroxisome proliferator-activated receptor (PPAR-γ) agonists or thiazolidinediones (TZDs) are synthetic ligands for PPAR-γ. They are in widespread clinical use for the treatment of type 2 diabetes due to their insulin-sensitizing properties. TZDs also have multiple anti-inflammatory, antioxidant, anti-fibrotic, and vascular effects independent of blood glucose lowering (22). Of relevance to PKD, maternal administration of pioglitazone improved the postnatal survival of Pkd1-null mouse embryos, and this was associated with a reduction in renal cystic disease by uncertain mechanisms (27). In addition, adult Pkd1 heterozygous mice chronically treated with the PPAR-γ agonist pioglitazone showed a significant improvement in endothelium-dependent vasodilatation.

In this study, we sought to test the hypothesis that either an endothelial defect or a VSM defect is present in resistance vessels of normotensive Pkd2 heterozygous mice without hypertension or renal disease. In follow-up experiments, we confirmed that this defect was also present in vivo and was
reversible by pretreatment with the PPAR-γ agonist rosiglitazone, which primarily acted to increase NO bioavailability by reducing oxygen radical release and peroxynitrite production in Pkd2 VSMCs.

MATERIALS AND METHODS

Pkd2 mice. Pkd2 heterozygous founder mice were obtained from S. Somlo (Yale University). These mice carry a null allele (ws183) for Pkd2 resulting from homologous recombination at exon 1 of the Pkd2 locus (41, 42). They have been backcrossed onto a C57/B16 strain background for over 20 generations (11, 32). Wild-type (WT) male littermates were used as controls. The use of Pkd2 mouse model was approved by The University of Toledo’s Institutional Animal Care and Use Committee, entitled “Molecular Biology of Kidney Diseases” (No. N-105587). The institutional Animal Welfare Assurance Number of The University of Toledo is A3414-01, and the AALAC accreditation number is 005577.

Experimental animals. Mice were held in the animal facilities at the University of Sheffield, exposed to light on a 12:12-h cycle in a humidity- and temperature-controlled environment, maintained on a 0.3% sodium standard pelleted commercial diet, and allowed water ad libitum. Experiments were performed on animals aged 16–20 wk (n = 6 animals/group), and animals were humanely killed by cervical dislocation according to United Kingdom (UK) Home Office procedures. Rosiglitazone (gift of GlaxoSmithKline) was administered mixed with food daily at a dose of 10 mg·kg⁻¹·day⁻¹ for 4 wk before euthanization. Control animals received vehicle (DMSO) only.

Drugs and solutions. The constituents of HEPES-buffered phosphate saline solution (HEPES-PSS) were 0.2884 g/l MgSO₄, 0.245 g/l KH₂PO₄ (Sigma, Dorset, UK). On the day of use, 0.99 g/l of NaCl, 0.3504 g/l KCl, and 0.1606 g/l CaCl₂, 2.383 g/l HEPES, 8.2983 g/l NaCl, 0.3504 g/l KCl, and 0.1606 g/l KH₂PO₄ (Sigma, Dorset, UK) was added to the bath for 20 min to see if this could rescue the effect of L-NAME.

Tail-cuff blood pressure measurements. Measurements of systolic blood pressure were performed in conscious restrained mice using an automated system with an automatic tail-cuff inflator and a built-in photoelectric sensor (Visitech Systems, Apex, NC), as previously described (10).

Isolation and characterisation of murine endothelial cells and VSMCs. In our experiments, we used isolated primary VSMCs and endothelial cells from mouse femoral and mesenteric arteries in vitro. The isolation of primary cells is routinely performed in our laboratory for both endothelial cells (1) and VSMCs (17). Because the arteries were composed of only a single layer of endothelial cells, we regularly confirmed the presence of an endothelial marker (ICAM-2) using flow cytometry, as previously described (1). All primary cultures were maintained at 39°C in DMEM containing 15% FBS.

NO and Ca²⁺ measurements. Cytosolic Ca²⁺ and intracellular NO fluorescence were measured using a Nikon TE2000-U microscope connected to a photometric CoolSnap EZ 20-MHz monochrome camera and high-speed excitation wavelength changer for the DG4/DGS system controlled by MetaFluor/MetaMorph software (Molecular Devices). For better focusing, the microscope was equipped with a x-y axis motorized flat top inverted stage, Nikon automatic focusing RFA Z-axis drive, and custom-designed vibration isolation platform. For a better controlled environment, the body of the microscope was enclosed inside a custom-built chamber to control CO₂, humidity, heat, and light.

To examine cytosolic Ca²⁺, cells were loaded for 30 min at 37°C with 5 µM fura-2 AM (Invitrogen). After being washed to remove excess fura-2 AM, cells were placed and observed under the Nikon microscope. Pairs of intracellular Ca²⁺ images were captured every 5 s at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. These images were captured through a fura-2 filter kit that contains 25-mm 340/380-nm exciter filters, a dichroic mirror, and a wide-band 510-nm emission filter. A more detailed protocol has been previously described (17). To examine intracellular NO, cells were loaded for 30 min at 37°C with 20 µM 4-aminophenylendothelin-1 (Sigma). After washing to remove excess endothelin-1, cells were placed and observed under the Nikon microscope. Pairs of intracellular NO images were captured every 5 s at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. These images were captured through a filter kit that contains 25-mm 340/380-nm exciter filters, a dichroic mirror, and a wide-band 510-nm emission filter. A more detailed protocol has been previously described (17).
methylamino-2′,7′-difluorofluorescein (Invitrogen), as previously described (17). Intracellular NO was then measured every 5 s at excitation and emission wavelengths of 495 and 515 nm, respectively. The measurement was obtained from images that were captured through a 25-mm 495-nm exciter filter, a dichroic mirror, and a 25-mm 515-nm emission filter. ACh (1 μM) was applied to the cells for at least 14 s after the baseline values of Ca2+ and NO were obtained for a minimum duration of 3 min.

Superoxide and peroxynitrite measurements. After the endothelial cells or VSMCs had been grown to confluence, cells were incubated with or without 10 μM resiglitazone for 2 h. Superoxide was measured using a standard redox-sensitive, cell-permeable fluorophore dihydroethidium (DHE) dye. Cells were incubated with 10 μM DHE (Invitrogen, Molecular Probes) for 30 min at 39°C. Cells were next equilibrated for at least 30 min, and the baseline level of DHE fluorescence was measured. Cells were then challenged with 1 μM SNP. Throughout the entire experiment, fluorescence images were captured every 5 s at excitation and emission wavelengths of 520 and 610 nm, respectively. The fluorescence intensity was captured with a Nikon TiU microscope and analyzed using Metamorph software, as previously described (1, 28).

Peroxynitrite was measured using standard 5-amin-2,3-dihydro-1,4-phthalalnizedione (lumion) chemiluminescence. The culture solution was first mixed with 40 μM lumion after incubation with or without 10 μM resiglitazone for 2 h. After treatment with or without 1 μM SNP, cells were collected and mixed with an alkaline solution containing 5 mM Na2CO3 (pH 9.2). They were immediately frozen in liquid nitrogen. When the pH is raised above 9, the decomposition of peroxynitrite is greatly inhibited. We were thus able to stabilize and detect peroxynitrite by chemiluminescence using a multidetection luminometer (SpectraMax M5, Molecular Devices).

Surgical procedures to induce ischemia-reperfusion injury of mesenteric vessels. Pkd2 heterozygous or WT mice (n = 5–7 mice/group) were anesthetized with pentobarbital (intraperitoneal bolus), and an enteric vessel (20–40 μm) was exposed for epi-illumination fluorescent light microscopy and connected to a Nikon TiU microscope and analyzed using Metamorph software, as previously described (1, 28).

Measurement of macromolecular leaks. Mesenteric preparations from sham WT mice (n = 5), WT mice with ischemia-reperfusion (I/R) (n = 7), sham Pkd2 mice (n = 5), and Pkd2 mice with I/R (n = 6) were analyzed using IPPROPLUS (Media Cybernetics, Silver Spring, MD), which assigned a numeric value to fluorescence (0–255) using an eight-bit gray scale. Three 73-μm2 AOIs were assigned inside the venule, with three corresponding 73-μm2 areas just outside it. Leak was expressed as a ratio of fluorescence outside venules (AOL) compared with that inside venules (AOL), as previously described with slight modifications and after background subtraction (12): leak ratio = (AOL/AOL) × 100.

Diameters were measured during epi-illumination as previously described, 30 s before and 20 s after ACh administration (8). The percent change in diameter was determined using the diameter (in μm) measured after the addition of ACh (ΔD/Δ) compared with the diameter determined at baseline (ΔDbaseline): change in diameter (in %) = |ΔD - ΔDbaseline|/ΔDbaseline × 100.

Intraobserver error was established by obtaining 10 diameter measurements of the same point. This was determined as 1.12%. To determine interobserver error, diameters at 10 different points of a vessel were measured by 2 independent observers. A highly significant correlation between the data obtained by two separate observers was obtained using the Spearman rank test (r = 0.9451, P = 0.0001). Intracellular NO synthase (eNOS) staining on formalin-fixed paraffin embedded mesenteric tissue was performed using a rabbit polyclonal antibody raised against human eNOS (Abcam), as previously described (23). Fixed tissue was available from 6 animals/group. Semiquantitative scoring of eNOS staining was conducted in a single blinded fashion in the range of 0–3, where 0 = absent staining and 3 = highest intensity.

Statistical analysis. Statistical analysis was performed using GraphPad Prism (version 4, GraphPad Software). Values are expressed as means ± SE. Normally distributed data were compared using two-way ANOVA followed by a Bonferroni post hoc test. Data that were not normally distributed were compared using a Mann-Whitney test. Correlations within groups for different data sets were analyzed using Spearman’s rank test. P values of <0.05 were considered as statistically significant.

RESULTS

VSM responses are normal in Pkd2 heterozygous arteries. There were no significant differences in myogenic tone between WT and Pkd2 heterozygous arteries (Fig. 1A). No significant differences in constriction to either KCl (Fig. 1B) or PE (Fig. 1C) were observed. As expected, removal of extracellular Ca2+ completely abolished smooth muscle reactivity (Fig. 1, A and B).

Endothelium-dependent vasorelaxation is defective in Pkd2 mesenteric arteries. SNP-induced dilation was similar in WT and Pkd2 heterozygous arteries (EC50: 3.5 × 10−6 M in Pkd2 arteries and 1.3 × 10−6 M in WT arteries, P > 0.05; Fig. 2A). However, a striking reduction in ACh-stimulated dilation was observed in preconstricted Pkd2 heterozygous arteries compared with WT arteries (EC50: 2.5 × 10−6 M in Pkd2 arteries and 1 × 10−7 M in WT arteries, P < 0.05; Emax: 8.3% in Pkd2 arteries and 31.5% in WT arteries, P < 0.05; Fig. 2B). This...
was confirmed by denudation of the endothelial cell layer, which abolished the ACh response in both genotypes (data not shown).

**Rosiglitazone treatment partially restores the vasodilator response to ACh in Pkd2 mesenteric arteries.** Oral daily administration of rosiglitazone (10 mg·kg⁻¹·day⁻¹) for 4 wk improved the vasodilator response to ACh in Pkd2 heterozygous mesenteric arteries compared with DMSO-treated control arteries (EC₅₀: 2.5 × 10⁻⁶ M in Pkd2 arteries and 1.1 × 10⁻⁷ M in Pkd2 arteries + rosiglitazone, P < 0.05), although this effect was not significant between both genotypes. Removal of Ca²⁺ from the bath medium abolished all muscle constriction. No significant differences in myogenic responses or to KCl were observed between both genotypes. Remission of Ca²⁺ restored ACh-stimulated dilatation (EC₈₀: 4 × 10⁻⁷ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity. C: reversible inhibition of nitric oxide (NO) synthase (NOS) using L-NAME (10⁻⁴ M) or L-arginine methyl ester (L-NAME; 10⁻³ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity. C: reversible inhibition of nitric oxide (NO) synthase (NOS) using L-NAME (10⁻⁴ M) or L-arginine methyl ester (L-NAME; 10⁻³ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity. C: reversible inhibition of nitric oxide (NO) synthase (NOS) using L-NAME (10⁻⁴ M) or L-arginine methyl ester (L-NAME; 10⁻³ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity. C: reversible inhibition of nitric oxide (NO) synthase (NOS) using L-NAME (10⁻⁴ M) or L-arginine methyl ester (L-NAME; 10⁻³ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity. C: reversible inhibition of nitric oxide (NO) synthase (NOS) using L-NAME (10⁻⁴ M) or L-arginine methyl ester (L-NAME; 10⁻³ M) but not between WT and WT + rosiglitazone values at all ACh concentrations (by ANOVA). Mean values are plotted with error bars (SEs) shown in one direction for the sake of clarity.

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**H36 DEFECTIVE VASODILATATION IN PKD2 RESISTANCE VESSELS**

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**Fig. 1. Vascular smooth muscle (VSM) responses in wild-type (WT) and polycystic kidney disease 2 (Pkd2) heterozygous mesenteric arteries.** A: vessel diameter was measured for a range of intraluminal pressures (0–120 mmHg) after stabilization and expressed as a percentage of the diameter at 20 mmHg in the presence or absence [without (w/o)] of Ca²⁺. B: contractility to KCl was determined for WT and Pkd2 heterozygous arteries. Removal of Ca²⁺ from the bath medium abolished all muscle constriction. No significant differences between both genotypes: PE [EC₅₀: 3.6 × 10⁻⁶ M in Pkd2 arteries and 1.3 × 10⁻⁶ M in WT arteries, P > 0.05; EC₇₅: 64.4% in Pkd2 arteries and 70.7% in WT arteries]. Removal of the endothelial layer did not alter these responses (not shown). n = 6 for each condition and experimental group.

**Fig. 2. Endothelium-dependent responses in WT and Pkd2 heterozygous mesenteric arteries.** A: the ability of WT and Pkd2 heterozygous arteries to respond to sodium nitroprusside (SNP) was expressed as a percentage of the maximal response. There were no significant differences between both genotypes (EC₅₀: 3.5 × 10⁻⁶ M in Pkd2 arteries and 1.3 × 10⁻⁶ M in WT arteries, P > 0.05; EC₇₅: 80.4% in Pkd2 arteries and 76.4% in WT arteries). Removal of the endothelial layer did not alter these responses (not shown), n = 6 animals/group for each condition. B: the ability of WT and Pkd2 heterozygous arteries to respond to sodium nitroprusside (SNP) was expressed as a percentage of the maximal response. There were no significant differences between both genotypes (EC₅₀: 3.5 × 10⁻⁶ M in Pkd2 arteries and 1.3 × 10⁻⁶ M in WT arteries, P > 0.05; EC₇₅: 80.4% in Pkd2 arteries and 76.4% in WT arteries). Removal of the endothelial layer did not alter these responses (not shown), n = 6 animals/group for each condition.
did not completely restore the ACh response to that of WT mice ($E_{\text{max}}$: 8.3% in Pkd2 arteries and 23.6% in Pkd2 arteries + rosiglitazone, $P < 0.05$; Fig. 2B). Rosiglitazone did not significantly alter the sensitivity of the ACh response in WT arteries (EC$_{50}$: 1 × 10$^{-7}$ M in WT arteries and 3 × 10$^{-7}$ M in WT arteries + rosiglitazone) nor increase the maximal response ($E_{\text{max}}$: 35.1% in WT arteries and 42.8% in WT arteries + rosiglitazone, $P < 0.05$). Rosiglitazone had no effect on VSM responses in either genotype, including myogenic reactivity, PE, or SNP (data not shown). We found no significant difference in terminal blood pressures (WT: 111.43 ± 5.75 mmHg and Pkd2: 114.22 ± 3.76 mmHg) or body weights (WT: 31.37 ± 0.86 g and Pkd2: 29.98 ± 0.57 g) between both groups after 4 wk of treatment.

The effect of rosiglitazone is NO dependent. Responses to ACh in WT arteries were effectively blocked by prior incubation with l-NAME and restored by the presence of L-arginine, indicating that basal systemic NO production was unchanged. Treatment with rosiglutazone was associated with elevated serum nitrite levels compared with untreated mice, but this was not different between both genotypes (WT: 42.2 ± 6.3 μM, n = 6; Pkd2: 42.5 ± 4.2 μM, n = 8) and failed to reach significance compared with untreated mice.

ACh-induced Ca$^{2+}$ increase and NO production in Pkd2 endothelial cells are normal. To examine if endothelial cells play a major role in the vessel reactivity that is observed in Pkd2 heterozygous mice, we measured cytosolic production of NO. Since NO production depends on cytosolic Ca$^{2+}$ increases, we also performed Ca$^{2+}$ imaging analysis. Endothelial cells isolated from WT and Pkd2 heterozygous mice were challenged with ACh (1 μM) to induce Ca$^{2+}$ signals and NO biosynthesis (1). We did not observe significant differences in either cytosolic Ca$^{2+}$ or NO increases between WT and Pkd2 heterozygous cells (Fig. 3). In all groups, cytosolic Ca$^{2+}$ levels significantly increased from baseline after 40 s, whereas increases in NO occurred after 45 s. Our data suggest that the functionality of endothelial cells to produce NO is intact in Pkd2 heterozygous cells.

Basal superoxide levels and stimulated peroxynitrite formation are increased in Pkd2 VSMCs. Since there was no defect in total NO release detectable in Pkd2 sera, tissues, and cells, we hypothesized that the bioavailability of NO could be impaired due to superoxide production in Pkd2 VSMCs and that this might be rescued by the reported action of rosiglitazone in
reducing vascular oxidative stress. Indeed, basal superoxide production was increased in Pkd2 VSMCs, and this was clearly suppressed by pretreatment with rosiglitazone (10^(-6) M) (Fig. 4).

Of note, the basal level of superoxide in Pkd2 endothelial cells was normal, indicating that abnormal vessel reactivity in Pkd2 mice is due to abnormal superoxide levels within VSMCs. SNP (NO donor) did not induce a further increase in superoxide levels; thus, the increase in basal superoxide levels in Pkd2 VSMCs is not due to NO production in endothelial cells.

Although NO cannot contribute to the basal level of superoxide, it is known that NO and superoxide can react easily to form peroxynitrite (4). We next hypothesized that peroxynitrite is formed to scavenge NO vasodilation function. Supporting our hypothesis, the presence of a NO donor (SNP) significantly increased the production of peroxynitrite in Pkd2 VSMCs (Fig. 5). Because rosiglitazone (10 μM) could effectively decrease the basal level of superoxide in Pkd2 VSMCs, we did not observe an increase in peroxynitrite in Pkd2 VSMCs treated with rosiglitazone.

**ACh-mediated dilatation of mesenteric venules in vivo is impaired in Pkd2 heterozygous mice.** I/R injury is associated with increased oxygen radical production and vascular oxidant stress (3, 40). To examine whether oxidant stress was higher in vivo, we examined WT and Pkd2 mesenteric vessels before...
and after I/R injury. In WT venules, ACh induced vessel dilation in vivo in a dose-dependent manner (P < 0.05; Fig. 6). Of note, there was reduced venular dilation in Pkd2 heterozygous vessels between 0.001 and 1 µg/kg ACh (P < 0.05). However, contraction was often observed at the highest dose tested (10 µg/kg), suggestive of nicotinic receptor agonism at this dose.

After I/R injury, we observed a marked reduction in the ACh response in WT vessels and almost complete abolition of dilation in Pkd2 venules (P < 0.05; Fig. 6). In WT animals, simultaneous measurements of mesenteric arterial and venular diameters showed a highly significant correlation between their vasodilatory responses to ACh over the concentration range tested (0.001–1 µg/kg, P = 0.002, r = 0.93).

Macromolecular leak from Pkd2 mesenteric venules is increased after I/R injury. There were no significant differences in baseline macromolecular leaks (interstitial gray level) from postcapillary venules between sham WT (11.5 ± 2.3%) and sham Pkd2 (13.4 ± 3.5%) animals, indicating normal barrier function of the vascular endothelium. After I/R injury, however, an increase in macromolecular leak was seen in both WT (n = 7) and Pkd2 (n = 6) mice, but the extent of the leak was 1.7-fold higher in Pkd2 animals (WT: 33.7 ± 5.2% and Pkd2: 55.0 ± 3.2%, P < 0.05). This suggests that the barrier function of Pkd2 venules is more susceptible to damage after ischemia-induced injury despite apparent normal baseline function.

DISCUSSION

In this study, we demonstrated that Pkd2 homozygous mesenteric vessels have a defect in endothelium- or NO-dependent vasodilatation that is partially reversible by treatment with the PPAR-γ agonist rosiglitazone. Isolated arteries from Pkd2 heterozygous mice were relatively insensitive to the endothelium-dependent vasodilator ACh but demonstrated normal myogenic responses and dilated normally to the endo-

ACh-stimulated NO release from isolated Pkd2 endothelial cells was normal, confirming previous reports (1, 28). This implied a reduction in NO bioavailability rather than release in Pkd2 vessels. Here, we report, for the first time, that Pkd2 VSMCs have a higher basal release of superoxide, which reacts with NO to form peroxynitrite. Both superoxide and peroxynitrite release were suppressed by rosiglitazone, indicating that the beneficial effect of rosiglitazone was primarily mediated through its known action in reducing vascular oxidative stress (2, 16). In Pkd2-null cells, flow-stimulated Ca2+ and NO release mediated by endothelial cilia are lost (1, 28). Our results, however, indicate that a reduction in NO bioavailability in the heterozygous state precedes the abolition of cilia-mediated NO release observed in the Pkd2-null state. The cellular basis for increased superoxide production in this phenotype remains to be determined.

Our results confirm a previous report (27) of the beneficial effect of long-term (6 mo) administration of a different PPAR-γ agonist, pioglitazone, on endothelial function in a Pkd1 model. Pioglitazone restored ACh-dependent vasodilatation in aortic rings of aged (10 mo) Pkd1 heterozygous mice and was associated with a significant increase in urinary nitrite concentrations, indicating that an increase in NO production could be the underlying basis for restoring ACh responsiveness (27). In our study, we did not find any difference in serum nitrite concentrations between WT and Pkd2 mice, although rosiglitazone treatment increased serum nitrite concentrations in both genotypes. It has been reported that PPAR-γ agonists can stimulate NO release at multiple levels in the NO pathway, including a direct action on eNOS itself (6, 9). Of note, we observed a significant increase in the maximal response to ACh in WT vessels after rosiglitazone. This could reflect the increase in NO release seen in the WT state by the direct action of rosiglitazone on eNOS activity. In this study, we did not address the possible role of EDHF. Although a role of EDHF is not excluded, the nature of the responses studied is more consistent with a major role for NO (in view of its inhibition by l-NAME and restoration by l-arginine) as opposed to the transient action of EDHF in mesenteric vessels (15).

Consistent with our in vitro and ex vivo observations, we also demonstrated that vasodilation to ACh is significantly reduced in Pkd2 homozygous venules in vivo and that this difference is amplified in the recovery phase after I/R injury, a state characterized by increased vascular oxidant stress. We did not record the diameters of arteries, as on most occasions they were obscured from view in this mesenteric murine intravital microscopy model. Nevertheless, there was a significant correlation between the vasodilatory responses of arteries and veins to ACh in these experiments. On the other hand, macromolecular leak of albumin (FITC-BSA) from postcapillary venules, which can be used as an indicator of vascular damage...
due to the formation of gaps between the endothelial cells during inflammation (13), was similar between WT and Pkd2 heterozygous mice at baseline. Only in the presence of I/R injury did Pkd2 heterozygous mice demonstrate a greater macromolecular leak than WT mice. The latter finding supports previous observations in the Pkd2 heterozygous kidney after I/R injury, where responses to injury (proliferation, inflammation, and matrix turnover) were clearly altered despite apparent normal baseline structure and function (32). Taken together, it appears that I/R injury unmasks a defect in vascular function or repair in heterozygous mesenteric vessels that is not apparent in the basal state, probably through increased oxidative stress.

Considering the role of VSM in PKD, a previous study (33) using denudethelialized conduit (thoracic aorta) and resistance (mesenteric) arteries from Pkd2 heterozygous mice found an exaggerated response to PE compared with WT vessels. Those authors did not examine responses in the presence of a normal endothelial layer. In our study, we did not detect significant differences in VSM responses between Pkd2 heterozygous and WT vessels in response to PE, KCl, or pressure, either in the presence or absence of the endothelial layer (not shown). The reason for this difference is unclear, although genetic strain differences could have contributed.

In summary, we report here, for the first time, that a reduction in NO availability due to increased superoxide release and peroxynitrite production is the molecular basis for the reduction in ACh-induced vasodilatation in Pkd2 heterozygous mesenteric vessels. Pretreatment with rosiglitazone partially corrected this defect by reducing vascular oxidative stress. Our findings suggest that the endothelial defect in ADPKD is potentially reversible at an early stage before the onset of hypertension and could influence the development of structural abnormalities of the vessel wall.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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