Polycystin deficiency induces dopamine-reversible alterations in flow-mediated dilatation and vascular nitric oxide release in humans

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Autosomal dominant polycystic kidney disease (ADPKD) is a renal hereditary disorder associated with increased cardiovascular mortality, due to mutations in polycystin-1 and polycystin-2 genes. Endothelial polycystin-deficient cells have an altered mechanosensitivity to fluid shear stress and subsequent deficit in calcium-induced nitric oxide release, prevented by dopamine receptor stimulation. However, the impact of polycystin deficiency on endothelial function in ADPKD patients is still largely unknown. Here we assessed endothelium-dependent flow-mediated dilatation in 21 normotensive ADPKD patients and 21 healthy control subjects, during sustained (hand skin heating) and transient (postischemic hyperemia) flow stimulation. Flow-mediated dilatation was less marked in ADPKD patients than in controls during heating, but it was similar during postischemic hyperemia. There was no difference in endothelium-independent dilatation in response to glyceryl trinitrate. Local plasma nitrite, an indicator of nitric oxide availability, increased during heating in controls but not in patients. Brachial infusion of dopamine in a subset of ADPKD patients stimulated plasma nitrite increase during heating and improved flow-mediated dilatation. Thus, ADPKD patients display a loss of nitric oxide release and an associated reduction in endothelium-dependent dilatation of conduit arteries during sustained blood flow increase. The correction of these anomalies by dopamine suggests future therapeutic strategies that could reduce the occurrence of cardiovascular events in ADPKD.

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Autosomal dominant polycystic kidney disease (ADPKD) is a common hereditary disorder, affecting 1 in 400 to 1 in 1000 individuals, characterized by the development of multiple cysts in the kidneys.[1,2] ADPKD causes a progressive impairment of renal function, and it accounts for around 10% of all patients with end-stage renal disease.[1,2] Irrespective of the consequences of reduced renal function, ADPKD patients are at an increased cardiovascular risk owing to the development of hypertension at an early stage of the disease, and to the increased prevalence of cardiovascular abnormalities, including left ventricular hypertrophy, cardiac valvulopathies, intracerebral aneurysms, and aortic dissections.[2–4]

Genetic mutations in *PKD1* and *PKD2*, encoding, respectively, polycystin-1 and polycystin-2, are responsible for the disease.[1–5] Polycystin-1 is a transmembrane protein with a large extracellular domain acting as a sensor of mechanical stimuli, and with an intracellular domain that interacts with the transient receptor potential channel polycystin-2.[1–5] These proteins form a plasma membrane complex in the primary cilium, sensing flow variation and promoting calcium entry to control renal tubular cell function and proliferation.[1–5] The alteration in this pathway promotes cystogenesis in ADPKD patients.[1–5]

The cardiovascular complications of ADPKD are believed to be directly related to the presence of abnormal polycystins in cardiac, vascular endothelial, and smooth muscle cells.[2–5] Indeed, the polycystin complex is a crucial mechano-transducer in the cilia of endothelial cells, and the calcium-dependent endothelial NO-synthase (eNOS) activation in

response to experimental shear stress is abolished in both polycystin-1- and polycystin-2-deficient endothelial cells.[6,7] Whether this functional alteration, which can profoundly affect the cardiovascular protective functions of the vascular endothelium, is present in ADPKD patients remains unclear.[8–10] Interestingly, recent data show that stimulation of dopamine type 5 receptor on polycystin-deficient endothelial cells restores cilia length and shear stress–induced calcium-dependent NO release,[11] which suggests that stimulating dopamine receptors may have beneficial effects in ADPKD patients.[12]

The present study was designed to assess, using combined functional and biological approaches, whether transient and sustained flow-mediated dilatation of peripheral conduit arteries are altered at an early stage in ADPKD patients, and to investigate the mechanisms involved. In addition, pilot experiments were performed to explore the impact of local administration of dopamine on conduit artery function in ADPKD patients.

RESULTS

Twenty-one normotensive ADPKD patients without kidney dysfunction and 21 frequency-matched healthy subjects participated in the study. The diagnosis of ADPKD was made on the basis of the presence of kidney cysts and family history of ADPKD. Molecular testing was performed in 19 patients (2 patients refused the genetic testing),[13] and definitively or highly likely pathogenic *PKD1* mutations in 17 patients, a definitively pathogenic *PKD2* mutation in 1 patient, and no detectable pathogenic mutation in 1 patient were identified (Supplementary Table S1 online).

Table 1 | Baseline characteristics of control subjects and ADPKD patients

Parameters	Control subjects	ADPKD patients
Age (years)	31 ± 7	30 ± 8
Male	9 (43%)	9 (43%)
Body mass index (kg/m^2)	25.2 ± 4.8	23.8 ± 4.7
Smoking status		
Current	5 (24%)	6 (29%)
Former	4 (19%)	2 (10%)
Never	12 (57%)	13 (62%)
Pack-years smoked	3.3 (1.0–4.0)	3.2 (3.0–4.0)
Office systolic blood pressure (mm Hg)	121 ± 10	124 ± 6
Office diastolic blood pressure (mm Hg)	74 ± 8	76 ± 5
Office heart rate (b.p.m.)	68 ± 10	64 ± 12
Total cholesterol (mg/dl)	187 ± 27	178 ± 30
LDL cholesterol (mg/dl)	116 ± 38	108 ± 28
HDL cholesterol (mg/dl)	62 ± 16	50 ± 14*
Cholesterol total-to-HDL ratio	3.0 (2.6–3.6)	3.5 (2.9–4.7)
Triglycerides (mg/dl)	84 (78–120)	96 (80–114)
Fasting glucose (mg/dl)	85 ± 8	90 ± 9
Creatininemia (μmol/l)	77 ± 13	75 ± 16
GFR estimated by MDRD (ml/min per 1.73 m^2)	91 ± 13	99 ± 18
Urine albumin-to-creatinine ratio (mg/g)	3.8 (3.1–5.2)	7.6 (3.6–12.3)*
Uric acid (mg/l)	49.6 ± 14.6	47.4 ± 11.4
High-sensitive CRP (mg/l)	1.4 (0.5–4.1)	0.4 (0.3–1.1)
Total blood viscosity (cP)	3.8 ± 0.5	3.6 ± 0.3
Radial artery diameter (mm)	2.33 ± 0.32	2.20 ± 0.22
Radial artery blood flow (ml/min)	6.5 ± 2.4	7.6 ± 3.3
Radial artery mean wall shear stress (dynes/cm^2)	3.9 ± 1.5	4.2 ± 1.4

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; b.p.m., beats per minute; CRP, C-reactive protein; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease.
Data are presented as mean ± s.d., *n* (%), or median (interquartile range).
*$P < 0.05$ vs. control subjects.

Baseline clinical and biological characteristics

There was no significant difference between groups for baseline clinical and biological characteristics, except a lesser high-density lipoprotein cholesterol level, without modification in the high-density lipoprotein-to-total cholesterol ratio, and a higher urine albumin-to-creatinine ratio in ADPKD patients (Table 1). With regard to systemic hemodynamics, office blood pressures and heart rate (Table 1), as well as ambulatory blood pressure measurements, were similar between groups (Supplementary Table S2 online). Only a slight higher night-time diastolic blood pressure was observed in ADPKD patients, but without difference in the number of nondipper subjects. Furthermore, baseline plasma levels of tumor necrosis factor-α, interleukin-6 (IL-6), IL-10, active renin, the endogenous competitive inhibitor of eNOS asymmetric dimethylarginine (ADMA), and whole-blood reactive oxygen species were similar between groups (Supplementary Figure S1 online).

Assessment of endothelium-dependent flow-mediated dilatation

Radial artery endothelium-dependent flow-mediated dilatation was assessed during a progressive and sustained increase in blood flow induced by hand skin heating (34, 37, 40, and 44 °C) and during a transient flow increase induced by postischemic hyperemia (5 min of hand ischemia).[14–16] The basal radial artery diameter, blood flow measured by high-resolution echotracking and Doppler, and the calculated mean wall shear stress were similar in ADPKD patients and control subjects (Table 1). During hand skin heating, the magnitude of radial artery flow-mediated dilatation (from 34 to 44 °C) was reduced in patients (mean ± s.d.: 16.0 ± 1.2%) compared with controls (23.2 ± 1.0%, $P < 0.001$). This decreased flow-mediated dilatation was confirmed by the downward shift of the radial artery diameter–mean wall shear stress relationship obtained during hand skin heating in ADPKD patients compared with control subjects (Figure 1). The difference between groups persists when considering the 17 patients with the PKD1 mutation alone (Supplementary Figure S2A online). No difference was observed between ADPKD patients who were carriers of a truncating PKD1 mutation ($n = 6$) and carriers of a nontruncating mutation ($n = 11$; Supplementary Figure S2B online).

In contrast, the magnitude of flow-mediated dilatation in response to postischemic hyperemia was similar in patients and controls, without difference in the increase in blood flow and shear stress (Figure 2a–c).