

Role of Endothelin and Nitric Oxide in the Pathogenesis of Arterial Hypertension in Autosomal Dominant Polycystic Kidney Disease

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Summary

The pathogenesis of arterial hypertension in autosomal dominant polycystic kidney disease (ADPKD) is complex and likely dependent on interaction of hemodynamic, endocrine and neurogenic factors. We decided to evaluate the role of endothelin (ET1) and nitric oxide (NO) in the regulation of arterial blood pressure (BP) and to determine plasma levels of ET1 and NO in the group of patients with ADPKD. The ADPKD group (18 patients, 6 men + 12 women, mean age 44.6 ± 11.7 years, with creatinine clearance_{corr} > 1.1 ml/s) was compared with a control group of 27 healthy volunteers of comparable age. Plasma levels of ET1 assessed by direct RIA determination in the group of ADPKD patients (11.03 ± 1.8 fmol/ml) were significantly increased ($p < 0.001$) in comparison with the control group (2.66 ± 0.58 fmol/ml), while no significant differences were observed between normotensive and hypertensive patients in the ADPKD group. Serum levels of NO were evaluated according to the determination of serum levels of their metabolites – nitrites/nitrates. Serum levels of NO in the group of ADPKD patients (39.85 ± 6.38 μ mol/l) were significantly higher ($p < 0.05$) in comparison with the control group (22.7 ± 1.20 μ mol/l), whereas in the ADPKD group no significant differences were observed between normotensive and hypertensive patients. Thus, our study supports the concept of complex alteration of both vasoconstrictor and vasodilator systems in the pathogenesis of arterial hypertension in ADPKD.

Key words

Endothelin • Nitric oxide • Autosomal dominant polycystic kidney disease • Arterial hypertension • Chronic renal failure

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the commonest hereditary kidney disease, with an inherited abnormality of the tubular wall, leading to its cystic dilatation. Arterial hypertension (AH) ranges

among early clinical manifestations of ADPKD and markedly influences its prognosis. AH in ADPKD is characterized by an increased intrarenal production of renin in the tubulocystic epithelium and an abnormal tubular excretion of sodium (Torres *et al.* 1992).

Only a few studies have been focused on the role of ET1 in ADPKD. Increased plasma levels of ET1 were observed in a group of 21 patients with ADPKD (hypertensive and normotensive) and with normal values of the glomerular filtration rate (GFR) in comparison not only with the group of healthy subjects, but also in comparison with a group of patients with essential AH (Giusti *et al.* 1995).

Endothelins form a family of peptide hormones, whose synthesis and secretion take place predominantly in endothelial cells and which play an important role in the regulation of vasoconstriction. The best documented is the effect of endothelin-1 (ET1), and recently the role of endothelin-3, with its effects closely related to ET1. Basal levels of endogenous endothelin lead to systemic and/or renal vasoconstriction. ET1 induces vasoconstriction of renal vasculature *via* binding to the receptors ET_A and ET_B and mediates vasodilatation through acting on endothelial ET_B receptors. The mechanism of action of ET1 was also studied at the kidney tissue level (such as in glomerulonephritis) by using hybridization techniques; the observation of increased levels of ET1 at ET_B receptors *in situ* seems to suggest their possible role in the pathogenesis of this disease. The research of the biology and pathogenetic role of endothelins and especially of ET1 is related to a number of different clinical situations. In patients with arterial hypertension, increasing interest is devoted to the inhibition of ET1 by drugs blocking ATII receptors. Under study is the contribution of ET1 to the progression of chronic nephropathies to chronic renal failure (Benigni and Remuzzi 2001). Other studies deal with the possible role of ET1 in the pathogenesis of acute renal failure (particularly after a load of the contrast medium), tend to clarify the possible effect of ET1 in the episodes of hypotension during hemodialysis sessions or to disclose the role of ET1 in rejection processes after renal transplantation. Our contemporary knowledge concerning endothelins, with emphasis on their renal and cardiovascular role, is summarized in some recent reviews (Horký 1998, Naicker and Bhoola 2001).

Nitric oxide (NO) plays a key role in the regulation of kidney blood flow, in the regulation of glomerular hemodynamics and in the control of sodium excretion. NO induces vasodilation, inhibits the aggregation of platelets and participates in the modulation of inflammatory and immune processes. NO produces a wide spectrum of effects resulting from its interactions with reactive oxygen species (ROS), with some metals (iron) and thiols. ROS by reaction with NO form peroxynitrate anions; by their decomposition develop NO₂

and hydroxyl radicals. NO thus participates by a dual mechanism in the peroxidation of lipids – prooxidatively *via* the generation of peroxynitrates and/or antioxidatively *via* chelation of hydroxyl radicals. The role of NO was studied in different clinical and pathologic situations, as well as under basal conditions and in response to different stimuli. Some overviews concerning the NO role in AH and renal diseases were published recently (Klahr 2001).

Some studies focused on the production of NO in ADPKD. Wang *et al.* (1999) studied the expression and localization of NO synthase in the model of Han:Sprague-Dawley (SPRD) polycystic rats in context with the development of renal cystic lesions. During the growth of renal cysts they observed a decrease of different isoforms of NO synthase in the cystic epithelium. In another study, these authors studied the possible contribution of NO to the development of vascular changes in ADPKD (Wang *et al.* 2000). Using myography they evaluated the difference in vascular reactivity in the presence of the substrate and the inhibitor of NO synthase in the group of ADPKD patients and in control healthy subjects; the vascular response in the former group was markedly influenced by changes of NO synthase. It can thus be speculated that endothelial dysfunction in ADPKD can be partly regulated by the activity of NO synthase.

In a previous study, we demonstrated increased activity of the process of lipoperoxidation and partly a decreased protective activity of antioxidative enzymes in patients with ADPKD and preserved GFR (Merta *et al.* 1995). In extending our prior investigations, we aimed to assess the plasma levels of NO and ET1 in ADPKD patients with preserved GFR in attempt to elucidate the role of these important vasoactive factors in the pathogenesis of AH/cystic lesions in ADPKD.

Methods

The ADPKD group consisted of 18 patients with diagnosis of ADPKD (6 males and 12 females with a positive family history and with ultrasound (US) findings of multiple cystic kidney involvement, age range 20-70 years, mean age 44.6±11.7 years, GFR was within normal ranges or slightly lower (clearance creatinine_{corrig} >1.1 ml/s). Control group (C) consisted of 27 healthy volunteers of corresponding age.

Patients were considered as hypertensive (ADPKD-H subgroup) in case of repeatedly increased blood pressure (BP) readings above the normal values during ambulatory examinations according to WHO

criteria, or were considered as hypertensive if treated by antihypertensive therapy, the remaining ADPKD patients formed the ADPKD normotensive (ADPKD-N) subgroup.

In all patients the extent of renal cystic involvement was assessed semi-quantitatively by ultrasound (degree 1 = 0 to one cyst in one or both kidneys, degree 2 = 2-5 cysts in both kidneys, degree 3 = more than 5 cysts in both kidneys).

Plasma ET1 levels. Every investigated subject has been laid in a stabilized position for a period of 30 min before withdrawal of a blood sample for direct RIA determination. Patients with AH on antihypertensive therapy were maintained on their ACE inhibitor and beta-blocker drugs; calcium channels blockers were withdrawn for a period of at least 2 weeks before sample withdrawal.

NO. Production of NO, its concentration and degradation were assessed by determination of NO metabolites (production of nitrites and nitrates correlating with NO production). A modified method based on enzymatic reduction of nitrates by nitrate-reductase and subsequent determination of nitrites by the Griess reaction with sulphanilamide and naphthylendiamine was used (Crkovská and Štípek 1998).

The differences in plasma levels of ET1 and NO between the ADPKD group and the C group, the differences in plasma levels of ET1 and NO between ADPKD-H and ADPKD-N subgroups as well as the relationship between ET1 and NO plasma levels were statistically tested. Furthermore, the extent of kidney cystic involvement was assessed by ultrasound (ANOVA test, tests of correlation analysis).

Results

In the group of ADPKD patients. Twelve subjects were normotensive (ADPKD-N) and 6 subjects were hypertensive (ADPKD-H). The distribution of the extent of cystic lesions determined by ultrasound was as follows: degree 1 – four patients. (22 %), degree 2 – 8 patients (44 %), degree 3 – 6 patients (33 %). Most ADPKD patients were normotensive, while a minority exhibited cystic lesions of a lesser degree (degree 1).

ET1. Table 1 presents the values of plasma levels of ET1 in the whole group of ADPKD patients and in patients of the control group; the comparison of ET1 plasma levels between normotensive and hypertensive ADPKD patients. is given in the Table 2.

NO. Table 3 presents the values of plasma levels of NO in the whole group of ADPKD patients and in patients of the control group; a comparison of NO plasma

levels between normotensive and hypertensive ADPKD patients is given in Table 4.

Table 1. Plasma levels of ET1 in the ADPKD group and the control group (C).

Group	n	ET1 _{plasma} (fmol/ml)	
ADPKD	18	11.03 ± 1.80 (3.1 – 17.7)	p<0.001
C	27	2.66 ± 0.58 (1.3 – 4.4)	

Data are mean ± S.D.

Table 2. Plasma levels of ET1 in normotensive (ADPKD-N) and hypertensive (ADPKD-H) patients.

Group	n	ET1 _{plasma} (fmol/ml)	
ADPKD-N	12	11.13 ± 2.20	n.s.
ADPKD-H	6	9.92 ± 1.90	

Data are mean ± S.D.

Table 3. Plasma levels of NO in patients with ADPKD and the control group (C).

Group	n	NO _{plasma} (μmol/l)	
ADPKD	18	39.85 ± 6.38	p<0.05
C	27	23.6 ± 1.45	

Data are mean ± S.D.

Table 4. Plasma levels of NO in normotensive (ADPKD-N) and hypertensive (ADPKD-H) patients.

Group	n	NO _{plasma} (μmol/l)	p
ADPKD-N	12	40.13 ± 7.42	n.s.
ADPKD-H	6	37.00 ± 5.66	

Data are mean ± S.D.

Discussion

Plasma ET1 levels in our ADPKD patients were significantly elevated in comparison with the control group. This finding could be interpreted as supportive evidence documenting the abnormal situation in the vasoactive (i.e. vasoconstrictive) system in ADPKD patients. This issue is in good accordance with some previous observations (Giusti *et al.* 1995). It is not easy to explain the reason for the increase of ET1 plasma levels in ADPKD patients. One of the putative mechanisms

leading to increased ET1 levels could be the secretion of ET1 by tubulocystic epithelial structures – a hypothesis stressed by some authors (Schichiri *et al.* 1989), though not supported by others (Munemura *et al.* 1994). For this reason, we analyzed the relationship between the extent of the cystic lesions (semiquantitative distribution of patients in dependence upon US lesions) and plasma levels of ET1, but no significant relationship was found (ANOVA, correlation analysis). It is worthwhile to note that the increase of plasma ET1 was present in ADPKD subjects who were not affected by arterial hypertension. Giusti *et al.* (1995) made a similar observation. It is very likely, that some disturbances of vasoactive system in ADPKD patients (e.g. intrarenal secretion of renin and possibly the increased production of ET1) can take place even before the manifestation of AH (Giusti *et al.* 1995). Using noninvasive 24-h ambulatory blood pressure monitoring (ABPM) for detection of AH in young subjects with ADPKD, some studies suggest that AH is diagnosed as an early finding in many ADPKD patients who were asymptomatic so far (Seeman *et al.* 1999). The elevation of BP occurs in parallel with distinct activation of the renin-angiotensin aldosterone system (increased plasma renin activity). In our study only a minority of patients was reexamined (in parallel with a standard BP examination) by ABPM. Thus, it cannot be excluded that the activation of the endothelin system corresponds (or is time-related) to these early hormonal changes. This remains matter of controversy, and it cannot be concluded on the basis of our study, whether the changes in the endothelin system are primary or represent a reaction to changes in other vasoactive systems. Yet, our study supports the issue of some recent experimental studies emphasizing the importance of the activation of the endothelin system in the pathophysiology of AH in ADPKD (Hoher *et al.* 1998a,b). An attempt to clarify the pathogenetic role of the endothelin system could be of some clinical significance as documented by the effect of pharmacological blockade of the endothelin system on the progression of renal insufficiency in Heyman nephritis model and/or low-renin model of renal damage (Amann *et al.* 2001).

The fact that plasma levels of nitrites/nitrates in ADPKD patients were significantly increased in comparison with the control group of healthy subjects

could be considered as a supportive argument testifying the onset of some processes occurring during stress situations, pathological processes and/or inflammatory changes even in the early phases of this disease (e.g. in patients with preserved GFR). The production of NO is a relatively nonspecific mechanism applying to patients with kidney involvement of different origin and relevance. Increased production of NO was found during hemodialysis treatment or kidney transplantation, but was also demonstrated in the course of other renal diseases. Particular attention has been paid to the relationship between NO production and kidney damage (Yokozawa *et al.* 1999). NO production could be mediated directly by some inborn connective tissue disorder and/or tubular wall abnormality (e.g. at the polycystine level, in the earliest phases of the disease). However, the concept of a production potentiated by some stimuli at subsequent phases of the development of renal cystic lesions appears to be more probable. This concept is not in agreement with the fact that no relationship was found between the extent of the cystic lesions (semiquantitative distribution of patients in dependence on US lesions) and plasma levels of NO and that the same NO plasma levels were found in hypertensive and normotensive ADPKD patients (ANOVA, correlation analysis).

Nonspecific changes of NO production in ADPKD should be considered as possible factors involved in further progression of cystic lesions in ADPKD and/or associated complications, including arterial hypertension. New insights could be gained from animal model studies (Yoshida *et al.* 2000) as well as from clinical studies (Reiterová *et al.* 2002) addressed to determine the role of NO synthase in polycystic kidney disease.

It may thus be concluded that our study supports the view about the relatively early disturbance of vasoactive/vasoconstrictive system in patients with ADPKD and preserved GFR, documented by an increased level of production of ET1/NO in hypertensive and normotensive patients in comparison with healthy subjects.

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References

- AMANN K, SIMONAVICIENE A, MEDWEDEWA T, KOCH A, ORTH S, GROSS ML, HAAS C, KUHLMANN A, LINZ W, SCHOLKENS B, RITZ E: Blood pressure-independent additive effects of pharmacologic blockade of the renin-angiotensin and endothelin systems on progression in a low-renin model of renal damage. *J Am Soc Nephrol* **12**: 2572-2584, 2001.

- BENIGNI A, REMUZZI G: How renal cytokines and growth factors contribute to renal disease progression. *Am J Kidney Dis* **37** (Suppl 2): S21-S24, 2001.
- CRKOVSKÁ J, ŠTÍPEK S: Factors influencing the determination of nitrites and nitrates in serum using nitrate reductase and Griess reagent (in Czech). *Klin Biochem Metab* **6**: 82-87, 1998.
- GIUSTI R, NERI M, ANGELINI D, CARLINI A, FIORINI I, BIGONGIARI P, ANTONELLI A: Plasma concentration of endothelin and arterial pressure in patients with ADPKD. *Contrib Nephrol* **115**: 119-121, 1995.
- HOCHER B, ZART R, BRAUN N, SCHWARZ A, VAN DER WOUDE F, ROHMEISS P, KOPPENHAGEN K: The Endothelin system in polycystic kidneys of Han:SPRD rats. *J Cardiovasc Pharmacol* **31** (Suppl 1): S342-S344, 1998a.
- HOCHER B, ZART R, SCHWARZ A, VOGT V, BRAUN C, THONE-REINEKE C, BRAUN N, NEUMAYER HH, KOPPENHAGEN K, BAUER C, ROHMEISS P: Renal endothelin system in polycystic kidney disease. *J Am Soc Nephrol* **9**: 1169-1177, 1998b.
- HORKÝ K: Patophysiological role of endothelin in cardiovascular diseases and the possibilities of its blockade (in Czech). *Remedia* **8**: 408-413, 1998.
- KLAHR S: The role of nitric oxide in hypertension and renal disease progression. *Nephrol Dial Transplant* **16** (Suppl 1): 60-62, 2001.
- MERTA M, ZIMA T, ŠTÍPEK S, CRKOVSKÁ J, PLÁTENÍK J: Lipid peroxidation and activity of superoxide dismutase in ADPKD. *Contrib Nephrol* **115**: 109-112, 1995.
- MUNEMURA C, UEMASU J, KAWASAKI H: Epidermal growth factor and endothelin in cyst fluid from autosomal dominant polycystic kidney disease cases: possible evidence of heterogeneity in cystogenesis. *Am J Kidney Dis* **24**: 561-568, 1994.
- NAICKER S, BHOOLA KD: Endothelins: vasoactive modulators of renal function in health and disease. *Pharmacol Ther* **90**: 61-88, 2001.
- REITEROVÁ J, MERTA M, TESAŘ V, ŠTEKROVÁ J: Endothelial nitric oxide synthase affects the progression of autosomal dominant polycystic kidney disease. *Kidney Blood Press Res* **25**: 87-90, 2002.
- SEEMAN T, VONDRICHOVA H, STRIZOVA J, SIKUT M, JANDA J, SCHARER K: Blood pressure, functional and morphological study of kidneys in children with autosomal dominant polycystic kidney disease. *Cas Lek Ces* **138**: 178-184, 1999.
- SCHICHIRI M, HIRATA Y, EMORI T: Secretion of endothelin and related peptides from renal epithelial cell lines. *FEBS Lett* **253**: 203-206, 1989.
- TORRES VE, DONOVAN KA, SCICLI G, HOLLEY KE, THIBODEAU SN, CARRETERO OA, INAGAMI T, MCATEER JA, JOHNSON CM: Synthesis of renin by tubulocystic epithelium in autosomal dominant polycystic kidney disease. *Kidney Int* **42**: 364-373, 1992.
- WANG D, IVERSEN J, STRANDGAARD S: Contractility and endothelium-dependent relaxation of resistance vessels in polycystic kidney disease in rats. *J Vasc Res* **36**: 502-509, 1999.
- WANG D, IVERSEN J, STRANDGAARD S: Endothelium-dependent relaxation of small resistance vessels is impaired in patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* **11**: 1371-1376, 2000.
- YOKOZAWA T, CHUNG HY, KIM DW, GOTO H: Involvement of superoxide and/or nitric oxide in renal tissue injury. *Exp Toxicol Pathol* **59**: 517-521, 1999.
- YOSHIDA I, BENGAL R, TORRES VE: Gender-dependent effect of L-NAME on polycystic kidney disease in Han:SPRD rats. *Am J Kidney Dis* **35**: 930-936, 2000.

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