Nephrol Dial Transplant (2000) 15: 827–832

Nephrology Dialysis Transplantation

Original Article

Duplex sonographic registration of age and diabetes-related loss of renal vasodilatory response to nitroglycerine

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Abstract

Background. Nitroglycerine effects dilatation of the vas afferens in the kidney by its active metabolite nitric oxide (NO). In diabetic nephropathy, NO appears to cause hyperfiltration and proteinuria. The aim of this study was to investigate the potential of duplex Doppler ultrasonography in recording changes in resistive indices following nitroglycerine and whether the extent of changes in diabetic nephropathy is reduced as a result of the arteriolar dilatation mediated by NO.

Methods. Fifty-three subjects made up three groups: group 1, 12 young healthy volunteers (5 male, 7 female, 28 ± 6 years); group 2, 21 older healthy volunteers (7 male, 14 female, 62 ± 8 years); group 3, 20 patients (13 male, 7 female, 56 ± 18 years) with mild diabetic nephropathy. The resistive index (RI) was measured in the interlobar arteries before, and 1, 3 and 5 min after administration of 0.8 mg sublingual nitroglycerine.

Results. The initial RI of 0.592 decreased in group 1 by 10.8% ($P<0.01$) after nitroglycerine, in group 2 the initial figure of 0.631 decreased by 5.9% ($P<0.01$), and in group 3 the initial figure of 0.669 decreased by 3.4% ($P<0.01$). Initial RI values differed significantly between groups 1 and 3 ($P<0.01$) and groups 1 and 2 ($P<0.01$), as did values between all healthy volunteers (groups 1 and 2, $n=33$) and patients with nephropathy ($P<0.005$). The extent of ΔRI differed significantly between groups 1 and 3 ($P<0.01$), 1 and 2 ($P<0.02$), and between all volunteers and group 3 ($P<0.003$). In groups 1 and 2 the initial RI was dependent on age ($P<0.03$), in group 3 on age and creatinine clearance ($P<0.02$ and $P<0.05$ respectively). ΔRI correlated with age in the healthy subjects ($P<0.01$) and with duration of diabetes in diabetic nephropathy ($P<0.03$).

Conclusions. Haemodynamic changes in renal perfusion caused by nitroglycerine can be detected using duplex ultrasonography. In healthy subjects, ΔRI declines with increasing age. In diabetic nephropathy patients, the response to nitroglycerine is reduced. The increased initial RI in such patients suggests fixed arteriolar damage.

Keywords: age; diabetic nephropathy; duplex Doppler ultrasonography; nitric oxide; nitroglycerine; resistive indices

Introduction

Sublingual nitroglycerine is a common and widespread treatment for acute attacks of angina pectoris. In contrast to the mechanisms underlying the effect on the heart, relatively little is known about the effects of nitroglycerine on renal haemodynamics [1].

In addition to its effects on the heart, nitroglycerine causes vasodilatation of the arterioles, with reduction of peripheral resistance and a drop in systolic and diastolic blood pressure [2]. In recent years, there have been many reports of experimental and clinical trials regarding nitric oxide (NO) or endothelium-derived relaxing factor, the mediator of the action of nitroglycerine to the blood vessels [3]. One hypothesis, that in the early stages of diabetic nephropathy an NO-mediated dilatation of the vas afferens results in glomerular hyperfiltration followed by proteinuria, is worth noting [4,5].

For some years, duplex ultrasonography has provided an easily applicable and non-invasive method for investigating renal haemodynamics, which could, in principle, be appropriately used for evaluation of the effect of nitrate on the kidneys. The method is well established for investigating renal artery stenosis and for measuring vascular resistance in the renal parenchyma using various resistive indices [6,7]. The resistive indices determined using duplex ultrasonography are also used for diagnosis of dysfunction following kidney transplantation [8]. Pathologically high resistive indices also appear to be of negative prognostic significance with respect to recovery or improvement of the blood pressure in cases of renal revascularization procedures [9]. Increased intrarenal resistive indices have recently

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been shown as a function of creatinine clearance, age, and duration of diabetes in cases of diabetic nephropathy [10].

The aim of this study was threefold: firstly to find out whether duplex ultrasonography could be used to detect the changes in renal haemodynamics after administration of nitroglycerine, secondly to ascertain the parameters on which any change in these resistive indices is dependent, and thirdly to determine whether the change in resistance is less evident in patients with diabetic nephropathy because of the existing arteriolar dilatation caused by NO, mentioned above.

Patients and methods

Patients

The study, which was approved by the Ethics Commission, included 53 healthy volunteers and patients between October 1996 and December 1999. The volunteers and patients were divided into three different groups.

The following inclusion and exclusion criteria were used. For all groups, written declaration of consent and current good health was required.

Group 1, young, healthy volunteers: aged between 16 and 40 years, no history of or clinical indications of kidney disease. Exclusion criteria were arterial hypertension (values detected before, during and 24 h after duplex ultrasonography of over 150 mmHg systolic and 95 mmHg diastolic), smoking, diabetes mellitus, taking any medication except for hormonal contraceptives, and a kidney size of less than 9 cm. Twelve volunteers were finally included, the relevant characteristics of whom are listed in Table 1.

Group 2, older, healthy volunteers: aged between 50 and 85 years, no history or clinical indications of kidney disease. Exclusion criteria were arterial hypertension, smoking, diabetes mellitus, any current medication, with the exception of hormone replacement therapy, serum creatinine >120 μmol/l, urea >8.5 mmol/l, pathological urinanalysis (Combur 10®, Boehringer Mannheim, Germany), and kidney size of less than 9 cm, or morphological abnormalities detectable with duplex ultrasonography. Three volunteers were excluded from this group. Twenty-one volunteers were included and the patient characteristics are again shown in Table 1.

Group 3, patients with diabetic nephropathy: diabetes mellitus type 1 or 2 [11], age 17–81 years, mild to moderate proven nephropathy (serum creatinine <120 μmol/l, serum urea <8.5 mmol/l), combined with proteinuria, either detected as macroalbuminuria >0.04–1.0 g/24 h (Preciset® U/CSF Protein, photometric determination of protein after precipitation with trichloroacetic acid, Boehringer Mannheim, Germany), or, if the Preciset test was negative, as microalbuminuria, inclusion value >20 mg/l (Turbiquant®, photometric determination of the albumins on immunochemical reaction with specific antibody, detection limit 6 mg/l, Behring Diagnostik GmbH, Germany). Exclusion criteria were unstable diabetic metabolic status with blood glucose values of <4 or more than 15 mmol/l in the 48 h immediately prior to duplex ultrasonography, arterial hypertension, treatment with antihypertensives (exception, diuretics for mild heart failure), treatment with ACE inhibitors, prostaglandin synthesis inhibitors (exception: acetylsalicylic acid, 100 mg/day), smoking, creatinine clearance below 50 ml/min/1.73 m^2 (determined by the Cockcroft formula [12]), kidney size less than 9 cm, or morphological abnormalities detected with duplex ultrasonography. An orientational preliminary selection yielded primarily 36 possible patients, 20 of these were included in the study. The chronic medication prescribed for seven patients (platelet aggregation inhibitors, diuretics, beta blockers) was stopped at least 24 h prior to the duplex ultrasonographic investigation and were not given again until after the ultrasonography. The patients' characteristics are shown in Table 1.

Duplex ultrasonographic measurements before and after administration of nitroglycerine

The measurements were commenced after 10 min rest in a horizontal position. All the examinations were performed by

Table 1. Patient data

	Group 1	Group 2	Group 3
Number	12	21	20
Men	5	7	13
Women	7	14	7
Age (years)	28.4 ± 5.99	61.9 ± 7.95	55.6 ± 18.42
range	20–40	50–83	17–81
BMI (kg/m^2)	22.0 ± 1.7	25.7 ± 3.9	25.8 ± 5.0
Diabetes mellitus type 1			6 (30%)
Diabetes mellitus type 2			14 (70%)
Duration of diabetes in years (range)			9 ± 10.5 (0.1–40)
insulin-dependent			13 (65%)
Oral antidiabetic medication			8 (40%)
Other late complications (neuropathy, retinopathy, etc.)			6 (30%)
Serum creatinine (μmol/l) (normal up to 110)		82.8 ± 10.4	80.5 ± 10.3
Serum urea (mmol/l) (normal 3.0–7.4)		5.4 ± 1.2	5.2 ± 2.0
Creatinine clearance (ml/min)			88.21 ± 23.0
Proteinuria (g/day) ($n=13$)			0.300 ± 0.2
Microalbuminuria only (mg/l) ($n=7$)			74.1 ± 87.9
Glycohaemoglobin A_{1c} (%)			8.7 ± 2.8
BP systolic (mmHg)	125.7 ± 8.6	139.8 ± 15.7	143.2 ± 20.1
BP diastolic (mmHg)	80 ± 4.6	86 ± 5.9	84 ± 9.2

Data are expressed as the mean ± SD.

two experienced investigators (BF, PN) with an Acuson 128 XP 10 ultrasound unit (Acuson Corporation, Mountainview, CA, USA) using a 3.5 MHz or 4 MHz vector array transducer with wall-filter setting of 50 Hz. After recording pulse and blood pressure, the first measurement was the size of the left and right kidney. For orientation purposes, perfusion in the whole of the left and right kidneys was then checked using colour duplex ultrasonography and the main trunk of the renal artery was displayed. If the orientational duplex ultrasonography did not reveal any abnormalities in size or perfusion, three measurements each were taken within 5 min in the vicinity of the interlobar artery at the boundary of the centre of the kidney and the upper pole on the right side, using pulsed Doppler. To this end, the pulsed Doppler measured volume was located in the interlobar artery and the Doppler angle corrected in accordance with the course of the artery (a maximum of 60°). The maximum systolic and minimum diastolic flow rate was recorded in centimetres per second.

Subsequent to these first three measurements, the volunteers or patients were given two puffs of sublingual nitroglycerine (2×0.4 mg glyceryl trinitrate, Nitrolingual® Spray, Pohl-Boskamp, Germany). A first Doppler measurement was taken 1 min after administration and repeated after 3 and 5 min at the same place. Pulse and blood pressure were measured again 3 min after administering the drug. Any adverse reactions were noted.

Calculation of the resistance parameters

The resistive index (RI) according to Pourcelot [13] was calculated on the basis of the following formula:

$$1 - \frac{\text{minimal diastolic velocity}}{\text{peak systolic velocity}}$$

Since all the pulse measurements were between 52 and 90 beats per minute, the correction recommended by Mostbeck *et al.* for bradycardic or tachycardic values was not implemented [14].

Accuracy

To determine the accuracy of the RI measurements in our laboratory, we performed on 10 healthy volunteers an intra- and interobserver variability test with two blinded investigators. The two measurements were taken within 1 week and revealed an intraobserver variability of 3.5 and 5.1% respectively, and an interobserver variability of 4.7%.

Statistical analysis

Data input, basic evaluations, and graphic representations were carried out using Excel 97 table calculation program (Microsoft® Corporation, Bellevue, WA, USA). The Statistical Package for the Social Sciences (SPSS® Inc. Chicago, IL, USA) was used for more detailed statistical calculations. The data were checked for normal distribution (Q-Q plot) and combined as mean $\pm$ SD. One-way ANOVA was used to calculate the differences between groups 1, 2 and 3. The paired *t*-test was used for analysis of the values before and after administration of nitroglycerine within the same group. Correlation calculations were performed using Pearson's test and multiple linear regression. The null hypothesis was tested at the significance level $P < 0.05$.

Results

RI before and after administration of nitroglycerine

Table 2 shows an overview of all values of all groups.

Differences between groups 1, 2 and 3

The starting RI and ΔRI values differed significantly between groups 1 and 3 ($P < 0.01$ and. $P < 0.001$ respectively). RI and RI reduction differed significantly also between groups 1 and 2 ($P < 0.01$ and $P < 0.02$ respectively) but not between groups 2 and 3 ($P = 0.071$ and $P = 0.077$ respectively). If all healthy volunteers were compared to all DN patients ($n = 33$ *vs* 20, age 50 ± 18 *vs* 56 ± 18 years, n.s.) again significant differences for RI and ΔRI were found (0.616 ± 0.042 *vs* 0.669 ± 0.086 before, ΔRI 7.7 *vs* 3.4% after nitroglycerine, $P < 0.005$ for initial RI and $P < 0.003$ for ΔRI). Figure 1 illustrates the different response to nitroglycerine in diabetic nephropathy patients compared to the healthy volunteers.

The starting RI and ΔRI as a function of various parameters

Table 3 shows the different factors influencing starting RI and ΔRI. Significant correlations exist between age and RI and ΔRI in healthy subjects, between age and RI and creatinine clearance and RI in diabetic nephropathy, and between duration of diabetes and ΔRI.

Pulse and blood pressure behaviour

As was expected, the systolic blood pressure in group 1 fell slightly but significantly by 3.4% after administration of nitroglycerine ($P < 0.01$). Among the healthy older subjects, the decrease in blood pressure was slightly more marked with respect to systolic pressure, at 4.3% ($P < 0.01$); the diastolic decrease was not significant. In the patients with nephropathy, nitroglycerine caused a decrease in blood pressure of 3.0%, n.s.; the decrease in diastolic pressure of 0.3% was also not significant. In none of the groups was pulse statistically affected.

Discussion

Our study shows that haemodynamic changes in renal perfusion occur after sublingual administration of nitroglycerine, which can be detected using duplex ultrasonography. The differences in this reaction to medication found between young and older volunteers and patients with diabetic nephropathy are remarkable.

The parameter used in this paper to grade the intrarenal resistance, resistive index, or RI, represents the intrarenal resistance downstream of the measuring site and is the easiest of all the known resistance parameters to record [8]. The reproducibility of the RI measurements is demonstrated by our own intra- and interobserver variability results and data in the

Table 2. RI (mean ± SD) before and 1, 3 and 5 min after nitroglycerine administration

Group	RI before	RI 1 min after (% reduction)	P*	RI 3 min after (% reduction)	P*	RI 5 min after (% reduction)	P*	RI 1 min, 3 min, 5 min mean value (% reduction)	P*	ΔRI mean value
1 ($n = 12$)	0.592 (±0.033)	0.536 ± 0.055 (−9.5 ± 7.4)	<0.01	0.529 ± 0.039 (−10.5 ± 4.3)	<0.01	0.515 ± 0.041 (−13.0 ± 4.1)	<0.01	0.528 ± 0.036 (−10.8 ± 2.8)	<0.01	0.064 (±0.016)
2 ($n = 21$)	0.631 (±0.040)	0.615 ± 0.052 (−2.4 ± 6.6)	NS	0.577 ± 0.058 (−8.6 ± 7.3)	<0.01	0.579 ± 0.047 (−8.1 ± 6.2)	<0.01	0.593 ± 0.046 (−5.9 ± 5.1)	<0.01	0.038 (±0.033)
3 ($n = 20$)	0.669 (±0.086)	0.662 ± 0.092 (−0.9 ± 5.8)	NS	0.642 ± 0.102 (−4.3 ± 4.8)	<0.01	0.634 ± 0.091 (−5.3 ± 5.1)	<0.01	0.647 ± 0.091 (−3.4 ± 3.0)	<0.01	0.022 (±0.020)

*Compared with value prior to nitroglycerine administration.

literature [6,15]. Despite the small absolute values of ΔRI, the relative reduction of 50% and more between our different groups yields clear statistical results.

The starting values we recorded in our healthy volunteers compare well with figures from the literature. A statistically significant correlation between age and RI was found in this population. This relationship is known [16,17]. Blood pressure also correlates with age in these subjects. This fact was recently confirmed by Jacquet *et al.* in a larger population [18]. It is possible that the increased resistance parameter already represents arteriolar dysfunction or minimal arteriolar damage, even in older subjects who are still normotensive by definition.

The phenomena observed after administration of sublingual nitroglycerine are particularly interesting. We know of no study to date which has used duplex ultrasonography to investigate the influence on renal haemodynamics of this drug, which is frequently administered for cardiac indications. The rapid change in RI corresponds to the known rapid cardiac effect, which can be expected within 1–3 min [1].

The action measured by the fall in RI can probably be explained by a reduction in the tension of the resistive vessels, i.e. the afferent arterioles. The direct effects of this dilatation on the vas afferens are only partially known. In the rat kidney, nitroglycerine leads to increased renal plasma flow with a non-significant increase in glomerular filtration rate [19]. Inhibition of sodium transport by nitric oxide also results in increased elimination of sodium in the urine [20]. Bank and Aynedjian, in 1993, showed that inhibition of nitric oxide synthesis in healthy rat kidneys results in both a decrease in renal blood flow and in a reduction in the glomerular filtration rate [21]. Although it remains hypothetical in the final analysis, it could well be assumed that by lowering resistance at the vas afferens, nitroglycerine probably contributes to increased renal blood flow, increased glomerular filtration, and increased natriuresis. The clinical significance of this mechanism remains unknown at present.

In comparison with the healthy subjects, patients with mild diabetic nephropathy exhibit a limited reactivity to nitroglycerine, which depends on the duration of the diabetes. However, in addition the starting RI values are to be higher, thus confirming the results obtained by Ishimura *et al.* [10]. We presume that the higher starting RI as well as the decreased ΔRI in patients at an early stage of nephropathy are influenced more by ultrastructurally probable glomerular damage and perhaps arteriolosclerosis than by pre-existing submaximal vasodilatation by nitric oxide [10,22]. It is worth noting that the RI decrease in the diabetic subjects does not depend on chronological age but on the duration of the diabetes. This is a further indication of a disease-specific restriction of responsiveness to nitroglycerine.

The existing figures in the literature for resistive indices in healthy volunteers and also in cases of diabetic nephropathy were confirmed. Although the clinical significance is actually unknown, the study

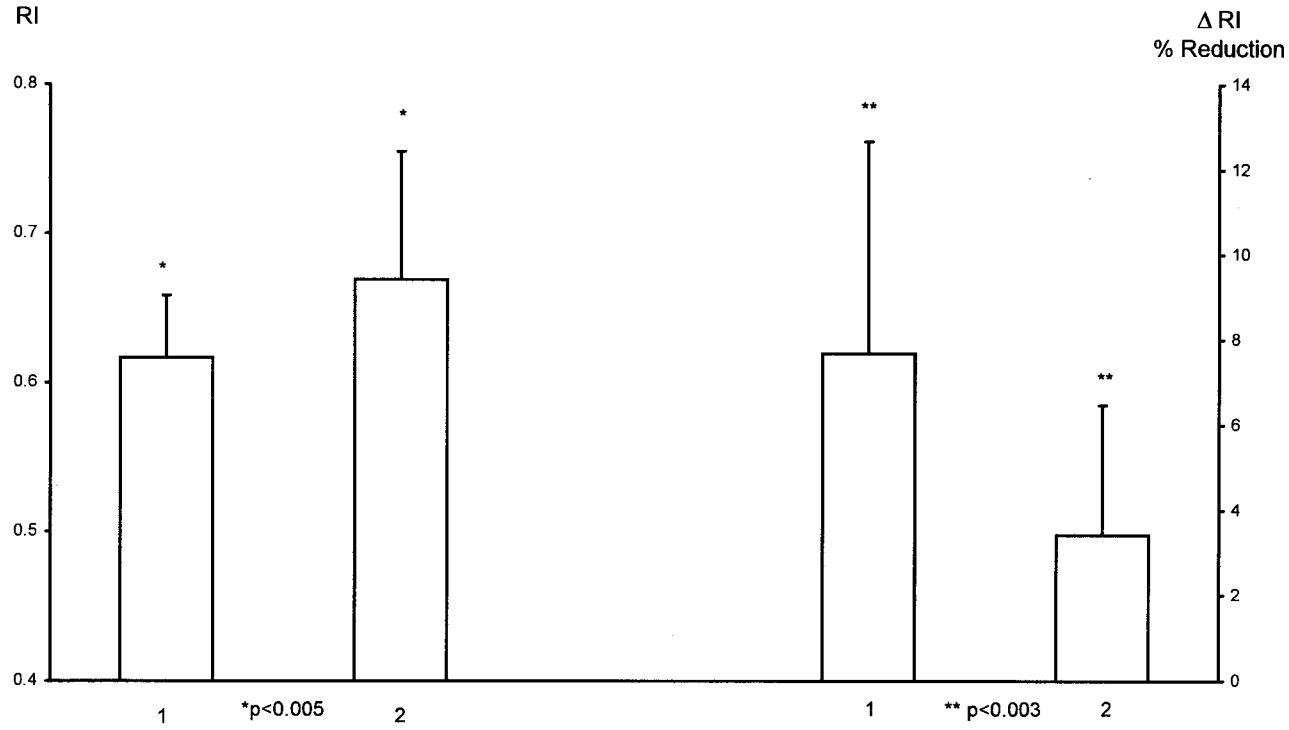

Fig. 1. Comparison of initial RI (left) and ΔRI (right) in all healthy volunteers (1, $n=33$) and in patients with nephropathy (2, $n=20$).

Table 3. Dependency of RI and ΔRI on different factors

Factor	RI		ΔRI	
	k*	*P**	k*	*P**
Healthy volunteers ($n=33$)				
Age	0.418	0.024	−0.548	0.004
Systolic blood pressure	0.179	0.316	0.007	0.97
Difference of syst. blood pressure before and after nitroglycerine			0.042	0.797
Patients with nephropathy ($n=20$)				
Age	0.553	0.017	−0.568	0.102
Systolic blood pressure	0.002	0.991	−0.014	0.963
Difference of syst. blood pressure before and after nitroglycerine			0.316	0.178
Creatinine clearance	−0.401	0.047	−0.366	0.204
Duration of diabetes	−0.028	0.848	−0.542	0.024

*Multiple linear regression.

reveals additional interesting phenomena of renal haemodynamics induced by nitroglycerine. Furthermore, it proves that duplex ultrasonography is also suitable for recording changes during therapy or in physiological studies.

Acknowledgements. We would like to offer our sincere thanks for the secretarial work of Esther Brandner.

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Received for publication: 26.7.99
Accepted in revised form: 1.2.00