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ACE and non-ACE pathways in the renal vascular response to RAS interruption in type 1 diabetes mellitus

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Background. The enormous contribution of renin-angiotensin system (RAS) interruption with ACE (angiotensin-converting enzyme) inhibitors and angiotensin II receptor blockers (ARB) in the treatment of diabetic nephropathy has led to interest in the factors involved in angiotensin II (Ang II) generation. In normal subjects, RAS interruption using an ARB produced a 50% greater renal plasma flow (RPF) rise than with an ACE inhibitor, suggesting a substantial contribution of non-ACE pathways. Moreover, immunohistochemistry studies in kidneys of overtly proteinuric diabetic subjects showed up-regulation of chymase, an alternative Ang II-generating enzyme. Our aim was to determine the degree to which the non-ACE pathways contribute to RAS activation in type 1 diabetes mellitus (DM).

Methods. Type 1 DM patients ($N = 37$, 14 M/23 F; age 31 ± 2 years; DM duration 16 ± 1.7 years; HbA1c 7.7.0 ± 0.3%) were studied on a high-salt diet. They received captopril 25 mg po one day and candesartan 16 mg po the next day. RPF and glomerular filtration rate (GFR) were measured before and up to 4 hours after drug administration.

Results. Both captopril and candesartan induced a significant rise in RPF (baseline vs. peak <0.0001 for both), and the rise was concordant for the 2 drugs ($r = 0.77$, $P < 0.001$). However, the RPF responses were not significantly different between the 2 drugs (captopril 72 ± 11 mL/min/1.73m^2, candesartan 75 ± 12, $P = 0.841$).

Conclusion. In predominantly normoalbuminuric, normotensive type 1 DM, activation of the intrarenal RAS reflects a mechanism involving primarily the classic ACE pathway.

The enormous contribution of renin-angiotensin system (RAS) interruption with angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) in the treatment of diabetic nephropathy has led to renewed interest in the factors involved in angiotensin II (Ang II) generation and in Ang II receptor blockade.

In normal subjects, RAS interruption using an ARB produced a greater increase in renal plasma flow (RPF) than did ACE inhibition, suggesting a substantial contribution of non-ACE pathways in Ang II generation [1]. Recent immunohistochemistry studies on kidneys that showed unequivocal evidence of diabetic nephropathy, including diffuse glomerulosclerosis, nodular glomerulosclerosis, moderate to severe tubulointerstitial fibrosis, and vascular sclerosis, revealed an up-regulation of chymase—an alternative Ang II-producing enzyme—compared to normal kidneys [2]. This led us to apply a functional measure of the contribution of non-ACE pathways to RAS activation in type 1 diabetes mellitus (DM).

METHODS

We studied 37 subjects with type 1 DM, diagnosed according to accepted guidelines [3]. There were 37 subjects, all Caucasian, 14 male and 23 female. Mean age ± SEM was 31 ± 2 years, and duration of DM was 16 ± 1.7 years. The mean HbA1c was 7.7 ± 0.3%. Five had microalbuminuria and 1 had overt proteinuria. Five had retinopathy and two were hypertensive. Three were on an ACE inhibitor; none were on ARB. There was no other antihypertensive medication being used. Five had body mass indices equal to or greater than 30 kg/m^2. Baseline characteristics are seen in Table 1.

The subjects were studied during admission to a metabolic ward at the General Clinical Research Center at the Brigham and Women's Hospital, where sodium balance was achieved on a controlled diet. The protocol was approved by the Institutional Review Board, and written informed consent was obtained from each participant.

Antihypertensive medications, including ACE inhibitors, were discontinued 10 days before admission.

Key words: diabetes mellitus, renal hemodynamics, renin-angiotensin system, chymase.

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Table 1. Demographic data and baseline characteristics of the type 1 DM subjects ($N = 37$)

Parameter	
Age *year* ± *SEM*	31 ± 2
Gender *M/F*	14/23
Duration of DM *year* ± *SEM*	16 ± 1.7
Hemoglobin A1c% ± *SEM*	7.7 ± 0.3
Serum creatinine *mg/dL* ± *SEM*	0.87 ± 0.04
Nephropathy status (normo/micro/macroalbuminuric)	31/5/1
Hypertension status (normo/hypertensive)	35/2

Because a common convention in withdrawing drug therapy is to allow five half lives to go by before undertaking any other maneuver, this duration ensured more than 30 half lives for captopril and 20 half lives for other drugs influencing the renin system. All participants were placed on a high-salt isocaloric diet starting two days prior to admission and continuing throughout the hospitalization, with a daily sodium intake of 200 mmol. Daily dietary potassium (100 mmol) and fluid intake (2500 mL) were constant. Twenty-four–hour urine samples were collected daily and analyzed for sodium, potassium, creatinine, and protein.

Renal hemodynamic and hormonal responses to captopril and candesartan

Each subject participated in two experimental days. On the morning of each study day, an intravenous catheter was placed in each arm, one for infusion of para-aminohippurate (PAH), inulin, and dextrose 5% in water, and the other for blood sampling. A third intravenous line was placed for continuous infusion of insulin, which was started at 0.015 U/kg/hr. Blood glucose was measured every 30 minutes (Precision PCX; Abbott Laboratories, Chicago, IL, USA). The insulin infusion was adjusted to maintain blood glucose well below the renal threshold, but without inducing hypoglycemia, at levels of 100 to 140 mg/dL. The subjects were supine and had been fasting for at least eight hours. Each study day began with a 60-minute baseline infusion of PAH and inulin prior to drug administration to determine baseline renal plasma flow (RPF) and glomerular filtration rate (GFR), respectively. Hormonal measurements were made on blood samples obtained at baseline and at four hours, and also at eight hours on the candesartan day, after drug administration, while the subjects remained supine.

The study was designed to compare the renal hemodynamic response to captopril and to candesartan. On the first morning, the patients received captopril 25 mg orally. On the next morning, the patients received candesartan 16 mg orally. These doses were chosen because both represent the top of the relationship between dose and RPF response [4]. The order by which the drugs had been given and the time course of the blood draws had been discussed previously and reflect the shorter half-life of captopril [5].

Blood pressure was recorded during each infusion by an automatic recording device (Dinamap; Critikon, Tampa, FL, USA) at 5-minute intervals. Antihypertensive medications were resumed upon discharge.

Renal clearance studies

PAH (Merck, Sharp & Dohme, Rahway, NJ, USA) and inulin (Inutest; Fresenius Pharma Austria GmbH, Linz, Austria) clearances were assessed after metabolic balance was achieved. A control blood sample was drawn, and then loading doses of PAH (8 mg/kg) and inulin (50 mg/kg) were given intravenously. A constant infusion of PAH and inulin was initiated immediately at a rate of 12 and 30 mg/min, respectively, with a Deltec 3000 pump (St. Paul, MN, USA). This achieved a plasma PAH concentration in the middle of the range in which tubular secretion dominates excretion. At this plasma level of PAH, clearance is independent of plasma concentration and represents approximately 90% of RPF when corrected for individual body surface area. Likewise, at the level of plasma inulin achieved, inulin clearance reflects GFR. RPF and GFR determinations were made at baseline (3 values taken 5 minutes apart) and at 45-minute intervals thereafter for 225 minutes (∼4 hrs) while the subjects were supine.

Laboratory procedures

Blood samples were collected on ice, spun immediately, and the plasma was frozen and stored at −70°C until assay. Urinary sodium and serum potassium levels were measured using the ion selective electrode. PAH and inulin were measured using an autoanalyzer technique. Plasma renin activity (PRA) was determined by radioimmunoassay. Hemoglobin A1C (HbA1C) was measured by high-performance liquid chromatography. The normal range is 4.4% to −6.3%.

Statistical analyses

Standard error of the mean (SEM) was used as the index of dispersion. For renal hemodynamic data, the baseline value taken was the average of the three pre-drug determinations, and the peak response was the average of the two highest consecutive values. *T* test was used to compare the renal vasodilatory responses to captopril and candesartan. Paired *t* test was used to compare baseline and peak responses for each drug. Pearson's correlation was used to test the association of the renal response to candesartan with the response to captopril, the association between baseline RPF and RPF response to the two drugs, and the association between RPF response and GFR response. A subgroup analysis comparing the

Table 2. Baseline renal hemodynamics and the responses to captopril and candesartan (mean ± SEM)

	Captopril	Candesartan	*P* value Captopril vs. candesartan
Renal plasma flow *mL/min/1.73m²*			
Baseline	590 ± 18	612 ± 21	0.418
Peak	662 ± 25	686 ± 27	0.509
Delta	72 ± 11	75 ± 12	0.841
Glomerular filtration rate *mL/min/1.73m²*			
Baseline	122 ± 3.2	119 ± 3.0	0.576
Peak	121 ± 3.2	123 ± 3.4	0.589
Delta	−1.1 ± 2.0	3.8 ± 1.9	0.072

responders to captopril versus the responders to candesartan was carried out using *t* test and chi-square test.

RESULTS

Captopril and candesartan both induced a significant increase in RPF of 72 ± 11 and 75 ± 12 mL/min/1.73m^2, respectively (baseline vs. peak <0.0001 for both). The baseline and peak renal plasma flow values were similar for the captopril and the candesartan day (Table 2). The RPF response was highly concordant for the two drugs ($r = 0.77$, $P < 0.001$) (see Fig. 1). Baseline and peak GFR did not significantly differ between the captopril and candesartan days (Table 2). However, comparison of baseline versus peak GFR response to candesartan showed a trend toward significance ($P = 0.051$); the GFR response to captopril was not significant (baseline vs. peak $P = 0.567$).

Baseline PRA was not significantly different on the two days (captopril day 0.50 ± 0.08, candesartan day 0.71 ± 0.12 ng Ang I/mL/hr, $P = 0.136$). There was also no significant difference in the average blood glucose levels during the study for the captopril and candesartan days (133 ± 6 vs. 142 ± 5 mg/dL, $P = 0.258$).

We evaluated the subset of 6 subjects with micro- ($N = 5$) or macroalbuminuria ($N = 1$). Again, the RPF response to captopril was not significantly different from the RPF response to candesartan (32 ± 11.3 vs. 48 ± 18.7 mL/min/1.73m^2, $P = 0.436$) in these six subjects.

We then compared subjects who had a greater RPF response to candesartan with subjects who had a greater RPF response to captopril to see if any variable distinguished them. Eleven subjects had a RPF response to candesartan that was greater than their response to captopril by 30 mL/min/1.73m^2 or more, and eight subjects had a RPF response to captopril that was greater than their response to candesartan by 30 mL/min/1.73m^2. The following variables were similar between these two groups: age (24 vs. 31 years, $P = 0.177$), duration of DM (12 vs. 10 years, $P = 0.351$), HbA1c (7.8 vs. 7.6%, $P = 0.801$), BMI (25.5 vs. 24.9 kg/m^2, $P = 0.598$), average blood glucoses during the captopril study (135 vs. 158 mg/dL, $P = 0.340$) and the candesartan study (165 vs.158 mg/dL, $P = 0.372$), and baseline PRA during the captopril day (0.51 vs. 0.76 ng Ang I/mL/hr, $P = 0.336$) and the candesartan day (0.77 vs. 1.00, $P = 0.539$). Likewise, gender, hypertension status, and nephropathy status were not significantly different between these two groups.

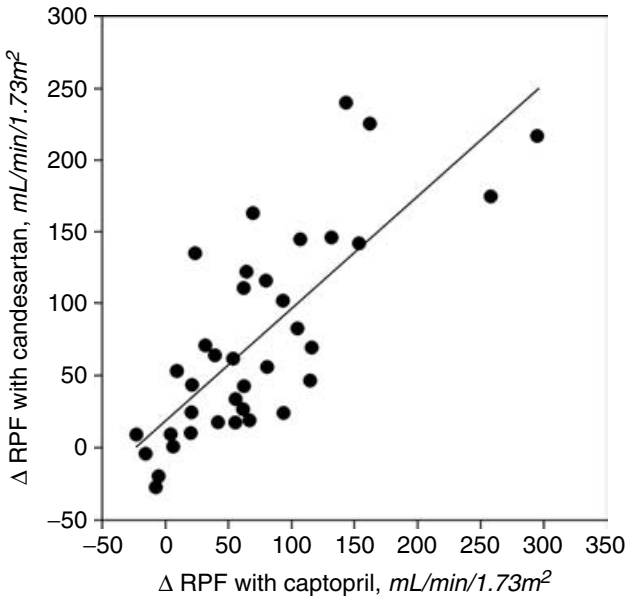

Fig. 1. Correlation between renal plasma flow (RPF) response to captopril and RPF response to candesartan.

DISCUSSION

The benefits of ACE inhibition in the management of nephropathy in type 1 DM are well known [6]. More recently, landmark studies conducted with ARBs showed a reduced incidence of doubling of serum creatinine and end-stage renal disease, and decreased albuminuria levels in type 2 DM [7–9]. Blockade of Ang II generation or action is key in management of diabetic nephropathy.

In our study, captopril and candesartan induced a similar rise in RPF to our surprise, suggesting that non-ACE pathways do not seem to play a major role in type 1 DM. In nondiabetic subjects, the non-ACE pathway seems to play an important role as RAS interruption with the ARB candesartan produced a significantly larger RPF response than the ACE inhibitor captopril [1]. Thus, an alternate, non-ACE pathway for Ang II generation is operative in nondiabetic subjects, accounting for 40% of the conversion of Ang I to Ang II, and is not fully blocked by an ACE inhibitor. Multiple observations underscore the importance of this pathway [10, 11].

In the study by Hollenberg et al on normal subjects, an ARB produced a greater increase in RPF than did an ACE inhibitor, suggesting a substantial contribution of

non-ACE pathways in Ang II generation [1]. Why would the physiology in normal subjects, as reported by Hollenberg et al, be different from our type 1 DM subjects? Our patients with type 1 DM had a similar age compared to the normal subjects in Hollenberg et al's study. The HbA1c in our study on type 1 DM averaged 7.7%, and the average blood glucose level during the studies was in the 130 to 145 mg/dL range, showing some degree of glucose dysregulation. In Hollenberg et al's study on healthy subjects, blood glucose was not measured during the study but the fasting blood glucoses were normal. It has been shown in multiple studies that hyperglycemia activates the RAS [12–14]. The mechanism involves intracellular effects of glucose rather than the physical osmolar effect of hyperglycemia, per se [15]. Why hyperglycemia tips the balance in favor of the ACE pathway remains unclear. It is also possible that enzymes other than chymases are responsible for the greater RPF response to ARBs in healthy subjects.

Is our finding that the non-ACE pathway does not play a prominent role compatible with the immunohistochemistry study of Huang et al in diabetes [2]? Their study showed up-regulation of both ACE and chymase expression. Chymase up-regulation correlated with blood pressure increase, severity of proteinuria, and development of diabetic glomerulosclerosis, tubulointerstitial fibrosis, and arterial sclerosis, while ACE up-regulation correlated with serum creatinine increase. There was greater chymase up-regulation in their hypertensive DM versus normotensive DM group; ACE expression did not show the same correlation. What might be the crucial difference is that their study involved kidneys from type 2 DM subjects with more advanced nephropathy, with a mean proteinuria of 2.8 g/24 hours in the normotensive DM group and 5.1 g/24 hours in the hypertensive DM group. Racial breakdown was not reported and was unavailable (personal communication with Dr. Hui Y. Lan). Our subjects were mostly normotensive, normoalbuminuric, Caucasian type 1 DM, with normal creatinine levels.

Is there a subset of type 1 DM where the non-ACE pathway contributes? We evaluated a small number of subjects with microalbuminuria or overt proteinuria ($N = 6$). There was no difference between the renal hemodynamic response to captopril and the response to candesartan in this subgroup, suggesting that the non-ACE pathway also is not favored in this group, but this group was small, enhancing the possibility of a type II error. Comparison between subjects with a greater RPF response to candesartan with the subjects that responded better to captopril, likewise, did not uncover a feature that predisposed to either direction.

Why would the classic (ACE) pathway be preferentially active in type 1 DM? Part of the predilection for the classic pathway may lie in the ACE gene polymorphisms in diabetes. The insertion/deletion (I/D) polymorphism of the ACE gene has been linked with the development of diabetic nephropathy in Caucasian type 1 DM [16–19] and Asian type 2 DM [20, 21], but association studies in type 2 Caucasian DM were largely negative [20, 22, 23].

A possible limitation of this study involves the fact that the sequence of drug administration was not randomized. We had addressed this in greater detail in a previous study [5]. In brief, captopril administration results in a renal hemodynamic response that fades rapidly after two to three hours [24], and PRA and RPF levels have returned to baseline by 24 hours, prior to candesartan administration.

Because the RPF responses to the two drugs were highly correlated, the action of ACE inhibition reflected primarily blockade of Ang II generation rather than the generation of bradykinin and prostaglandins [25, 26]. Thus, not only does the ACE pathway dominate in the conversion of angiotensin I to Ang II, but the action of ACE inhibitors is mainly through blockade of this pathway in type 1 DM.

CONCLUSION

In this group of type 1 DM subjects who are predominantly normotensive, normoalbuminuric, and with normal creatinine levels, activation of the intrarenal RAS reflects a mechanism primarily involving the classic ACE pathway.

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