

ALBUMINURIA AS A THERAPY TARGET FOR RENAL-CV PROTECTION

The effects of valsartan on the accumulation of circulating and renal advanced glycation end products in experimental diabetes

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Background. Blockade of the RAS with the ACE inhibitor ramipril prevents the accumulation of advanced glycation end products (AGEs) in experimental diabetes. Although AT₁ receptor antagonists may inhibit AGE formation in vitro, their effect in normotensive animals with type 1 diabetes has not been established.

Methods. Streptozotocin-induced diabetic and control animals were randomized ($N = 10/\text{group}$) to receive the AT₁ antagonist valsartan at a dose of 30 mg/kg/day by oral gavage for 24 weeks, or no intervention. Renal and plasma AGE accumulation was correlated with renal functional parameters.

Results. Valsartan reduced the albumin excretion rate consistent with its renoprotective effects. Renal and skin collagen accumulation of the non-fluorescent AGE carboxymethyllysine (CML) were increased in animals with diabetes, but normalized following treatment with valsartan. Renal fluorescence and skin collagen pentosidine levels were also increased by diabetes. However, valsartan only provided a modest attenuation of these parameters. In addition, diabetes was associated with increased plasma fluorescence, which was unaffected by AT₁ antagonism.

Conclusion. Renoprotective doses of valsartan are associated with a significant reduction in the accumulation of tissue and plasma CML. These effects were not the same for all AGEs, suggesting combination approaches will be required to optimize renoprotection in diabetes.

Blockade of the renin-angiotensin system (RAS) is one of the most important interventions in patients with diabetic kidney disease [1]. Its benefits have been attributed to interruption of both hemodynamic and non-hemodynamic pathways that lead to progressive nephropathy [2, 3]. In particular, recent studies have suggested that blockade of the RAS may be able to inhibit the formation of advanced glycation end products (AGEs). AGEs are known to be major independent contributors to diabetic nephropathy because blockade of their for-

mation has proven beneficial in experimental diabetic nephropathy in the presence of ongoing hyperglycemia [4–6]. Both ACE inhibitors and angiotensin receptor type 1 (AT₁) antagonists are able to inhibit AGE formation in vitro in the absence of a functioning RAS. Furthermore, recent studies by our group using the ACE inhibitor ramipril have suggested that these agents may be equally potent in vivo [7]. This study aims to determine whether renoprotective therapy with the AT₁ receptor antagonist valsartan has similar effects on AGE accumulation in an experimental model of type 1 diabetes.

METHODS

Experimental rodent model

Experimental diabetes was induced in male Sprague-Dawley rats (200–250 g) by injection of streptozotocin (50 mg/kg) [7]. Diabetic and control animals were randomized ($N = 10/\text{group}$) to receive no treatment (D and C), or the AT₁ antagonist valsartan (CAT₁ and DAT₁) at a dose of 30 mg/kg/day by oral gavage for 24 weeks. Two units of Ultralente insulin (Ultratard HM; Novo Industries, Bagsvaerd, Denmark) were administered daily to diabetic animals. Systolic blood pressure (SBP) was assessed by tail cuff plethysmography, albumin excretion rate (AER) by radioimmunoassay, and glycated hemoglobin (HbA_{1c}) were measured every eight weeks. All animal procedures were in accordance with guidelines set by the Austin Hospital Animal Ethics Committee.

Plasma low-molecular-weight AGE fluorescence and renal fluorescence

LMW-AGE fluorescence (Ex 370 nm, Em 440 nm) was assayed in deproteinated, delipidated plasma samples as described previously [8]. Renal fluorescence was determined as above in acid hydrolyzed cortical tissue as previously described [8].

Key words: CML, diabetic nephropathy, albuminuria.

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Table 1. Renal functional and structural parameters

	Control N = 10	Control + AT1a N = 10	Diabetes N = 10	Diabetes + AT1a N = 10
Plasma Glucose mmol/L	6.9 ± 0.3	6.0 ± 0.2	27.3 ± 2.5 ^a	26.0 ± 2.5 ^a
Glycated Hb %	3.1 ± 0.1	3.0 ± 0.1	14.3 ± 0.8 ^a	14.5 ± 0.7 ^a
Mean SBP mmHg	127 ± 3	135 ± 11	147 ± 4 ^a	136 ± 8 ^{b,d}
AER mg/ 24 hr	3.2 × /÷ 0.8	2.1 × /÷ 0.4	18.7 × /÷ 10.0 ^a	4.4 × /÷ 0.9 ^c
KW:BW ratio × 10 ⁻³	5.8 ± 0.5	5.8 ± 0.5	13.5 ± 3.8 ^a	12.2 ± 2.0 ^a

Abbreviations are: SBP, systolic blood pressure; AER, 24-hour albumin excretion rate.

^aP < 0.001 vs. C.

^bP < 0.05 vs. C

^cP < 0.001 vs. D.

^dP < 0.05 vs. D.

Isolation of skin collagen and analysis of AGEs

Rat skin collagen was prepared as described previously [5]. The AGE, N^ε(carboxymethyl)lysine (CML), was quantified by isotope dilution, selected ion monitoring gas chromatography-mass spectrometry (SIM-GC/MS) [9], and normalized to the parent amino acid lysine. Pentosidine was analyzed by RP-HPLC and was also normalized to lysine content [9].

Immunohistochemistry for renal CML

Immunohistochemistry on formalin-fixed sections with anti-CML antibody (4G9, 1:500; Alteon, Inc., Ramsay, NJ, USA) was performed and quantified by computer-aided densitometry with Optimas 6.2 software (Optimas 6.2–Video Pro-32; Bedford Park, SA, Australia) as described previously [10]. Results are expressed as the percentage area of positive immunostaining.

RESULTS AND DISCUSSION

Diabetes was associated with a marked increase in plasma and tissue AGEs. Renoprotective therapy with the AT₁ receptor antagonist valsartan reduced both renal (Table 1) and skin levels of the non-fluorescent AGE, CML to levels seen in control animals (Fig. 1). This is consistent with recent studies demonstrating similar effects on CML accumulation using AT₁ antagonists in a model of type 2 diabetes [12]. The mechanism of this effect remains to be established. As this activity may be demonstrated in vitro, it is possible that these agents interfere with the formation of carbon-centered radicals and hydroxyl radicals, possibly by metal chelation and the scavenging of reactive oxygen species [11]. Certainly, in vivo blockade of the RAS with ramipril reduces the formation of ROS, predominantly generated by NAD(P)H oxidase in diabetic nephropathy [8]. Indeed, oxidation is a key component in the generation of CML, which is considered to be a product of glycooxidation, rather than purely as a result of glycation alone.

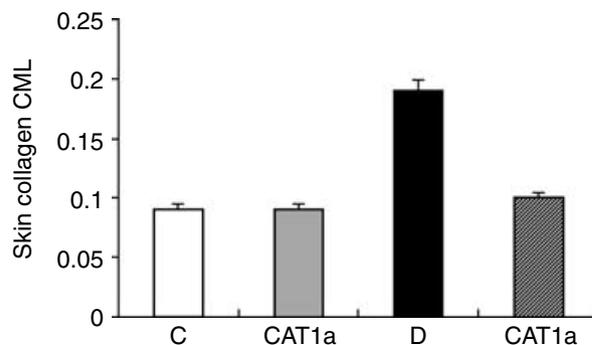


Fig. 1. Skin collagen carboxymethyllysine as measured by SIM-GC/MS. *P < 0.001 vs. C, †P < 0.001 vs. D.

Table 2. Tissue and plasma AGE parameters measured

	Control N = 10	Control + AT1a N = 10	Diabetes N = 10	Diabetes + AT1a N = 10
Renal CML %	3.25 ± 0.51	2.85 ± 0.36	9.1 ± 0.39 ^a	3.27 ± 0.4 ^a
Renal Fluorescence AU/OD 280 nm	4.2 ± 0.1	4.5 ± 0.6	7.0 ± 0.3 ^a	5.5 ± 0.3 ^{a,b}
LMW-AGE fluorescence AU/OD 280 nm	11.0 ± 0.4	11.4 ± 0.5	17.4 ± 1.0 ^a	17.1 ± 0.8 ^a
Skin pentosidine pmol/mg Hydroxyproline	1.37 ± 0.25	1.08 ± 0.12	2.42 ± 0.23 ^a	1.9 ± 0.52 ^a

CML, carboxymethyllysine.

^aP < 0.001 vs. C.

^bP < 0.001 vs. D.

The beneficial effect of valsartan may also be partly conferred via blood pressure reduction. For example, antihypertensive treatment with either olmesartan or hydralazine was able to equally reduce AGE-accumulation in experimental type 2 diabetes [12]. However, hypertension may be a more important driving factor for renal injury in that model. In addition, hydralazine may have independent AGE-lowering effects, since it is chemically related to aminoguanidine, a potent inhibitor of AGE formation [4]. Furthermore, the synergy between blood pressure lowering and inhibition of advanced glycation also make this effect more difficult to interpret. Indeed, specific AGE inhibitors may also reduce blood pressure [8].

Although valsartan reduced CML accumulation, its effects on the fluorescent AGEs, including pentosidine, were more modest (Table 2). In addition, the present study demonstrated no attenuation of AGE-fluorescence attributed to circulating LMW-AGEs in diabetic animals treated with valsartan (Table 2). The difference between fluorescent and non-fluorescent AGEs (e.g., CML) in response to valsartan treatment is consistent with this agent not influencing all AGEs in a similar manner. This may not be surprising given that pentosidine can be generated from nonoxidation pathways that may not be

blocked by AT₁ antagonism [13]. In addition, although blockade of the RAS slows the progression of renal disease in diabetes, it does not completely prevent it. Ultimately, the only way to prevent nephropathy in those with diabetes will be to disrupt all of the pathways that lead from hyperglycemia to renal injury. It therefore seems likely that a combination of approaches will be required to achieve persistent renoprotection [15].

The molecular identity of the AGEs that most contribute to the development of diabetic complications, including nephropathy, has not been clearly determined. In recent studies, Miura et al found that fluorescent AGEs better correlated with complications in patients with type 1 diabetes than levels of pentosidine or non-fluorescent AGEs like CML [16]. However, in another study of patients with type 1 diabetes, Beisswenger et al reported that non-fluorescent CML-AGE levels were better associated with the presence of complications, including retinopathy and nephropathy [17]. That renoprotection was still observed in our study despite no apparent effect on fluorescent AGE formation also suggests that the dominant pathogenic AGE ligands in diabetes may be linked to the pathways that lead to the production of CML. Ultimately, precise chemical modification may be of less consequence than its binding affinity and ability to activate pathogenic pathways.

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