Advanced glycation end products in the plasma and renal tissues in experimental model of unilateral renal artery stenosis

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ABSTRACT

Purpose: To evaluate whether advanced glycation end products (AGE) levels are increased in the plasma and renal tissues of rats after unilateral renal artery stenosis (RAS).
Materials and methods: AGE levels were measured using commercially available ELISA kit in plasma and renal tissue samples obtained from 16 rats with experimental induced RAS for 3 and 28 days and from 6 respective sham-operated control rats. We also analyzed by HPLC the concentration of 4-hydroxynonenal (4-HNE), a known inducer of AGE formation and accumulation.
Results: Plasma concentrations of 4-HNE and AGE were significantly increased ($p<0.05$) after 28 days of RAS. At this time point, the concentration of AGE was markedly increased in the clipped atrophic kidney (by about 10-fold; $p<0.05$), but it was unchanged in the contralateral kidney of the same rats. No differences in plasma and renal AGE levels were detected at day 3 of RAS. Sham operation did not affect the levels of AGE and 4-HNE at each time point.
Conclusions: Increased accumulation of AGE both in the plasma and in the ischemic atrophic kidney suggest that AGE levels can be used as a reliable biomarker for monitoring the development of ischemic nephropathy caused by renal artery stenosis.
Key words: advanced glycation end products, kidney, oxidative stress, unilateral renal artery stenosis, rats.

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INTRODUCTION

Advanced glycation end products (AGE) are heterogeneous group of compounds that are formed in a complex and sequential non-enzymatic reactions, collectively called the Maillard reaction [1,2].

This reaction occurs between the carbohydrates/reducing sugars or reactive carbonyl compounds (e.g. glyoxal, methylglyoxal) and the free amino groups of amino acids, peptides and proteins [1]. AGE are formed in response to oxidative stress and can be detoxified by specific enzymes, e.g. reductases in the liver, degraded and excreted by the kidneys or may accumulate in the tissues [2-4]. Increased plasma and tissue AGE levels have been reported during normal aging processes [5] and in a variety of common pathologies, including diabetes mellitus, chronic renal failure, atherosclerosis, arterial hypertension, Alzheimer's disease, and rheumatoid arthritis [6-8].

It has also been demonstrated that in diabetes, renal AGE formation and accumulation are closely associated with progressive nephropathies such as glomerulosclerosis, interstitial fibrosis, and tubular atrophy [8-10]. Therefore, circulating AGE constitute an important biomarker for monitoring the development of diabetic nephropathy, and other nondiabetic renal diseases [9,11,12].

In this study, we evaluated whether AGE levels are increased in plasma and renal tissues during the development of ischemic nephropathy in experimental model of unilateral renal artery stenosis.

MATERIALS AND METHODS

The experimental procedures involving animals and their care were approved by local authorities and conducted in conformity with national and international regulations and Guidelines for the Use of Animals in Biochemical Research.

The study included archived plasma and kidneys (clipped and unclipped) obtained from 16 rats with induced unilateral renal artery stenosis (RAS) for 3 days (n=8) and 28 days (n=8) and from their respective sham-operated control rats (n=6).

AGE levels in plasma samples (50 µg of proteins) and renal homogenates (20 µg of proteins) were measured using commercially available ELISA kit (OxiSelect^{TM} Advanced Glycation End Product (AGE) ELISA Kit, Cell Biolabs, Inc, USA) according to the manufacturer's protocol.

The concentration of 4-hydroxynonenal (4-HNE), a marker of oxidative stress, was assayed as a fluorometric derivative with 1, 3-cyclohexandione (CHD) separated on RP C_{18} column by HPLC with spectrofluorometric detection (excitation λ = 380 nm, emission λ = 445 nm) [13].

Total protein concentration in blood plasma and tissues extracts was determined by the method of Bradford [14] using the Bio-Rad assay reagent with bovine serum albumin as a standard.

All results are presented as mean percentage changes ± SD of three independent measurements. All data were analyzed by ANOVA. A p value < 0.05 was considered statistically significant.

RESULTS

Figure 1 demonstrates the concentrations of 4-hydroxynonenal (4-HNE) and AGE in the plasma samples from rats with induced renal artery stenosis (RAS) and sham-operated controls. At day 3 of RAS, the plasma concentrations of 4-HNE and AGE did not significantly vary from the levels seen in the plasma of normal sham-operated rats ($p > 0.05$ for both).

By contrast, both parameters were significantly increased after 28 days of RAS ($p< 0.05$). There were no significant differences in the levels of 4-HNE and AGE between control plasma at each time point.

Renal AGE levels with respect to the kidney weight to body weight ratio of rats are presented in Figure 2.

After 28 days of RAS, the weights of the left clipped kidneys were significantly ($p<0.05$) reduced (by about 10 – 15 %), whereas the weights of the right unclipped kidneys were increased (by about 20 – 25 %), as compared to the control kidneys from sham-operated rats (Fig.2b).

At this time point, the concentration of AGE was markedly increased in the clipped kidneys (by about 10-fold; $p<0.05$), but it was unchanged in the contralateral kidneys of the same rats.

No differences in the kidney weights and AGE levels were detected 3 days after the induction of RAS. Sham operation did not affect the levels of AGE in the kidneys at any time point.

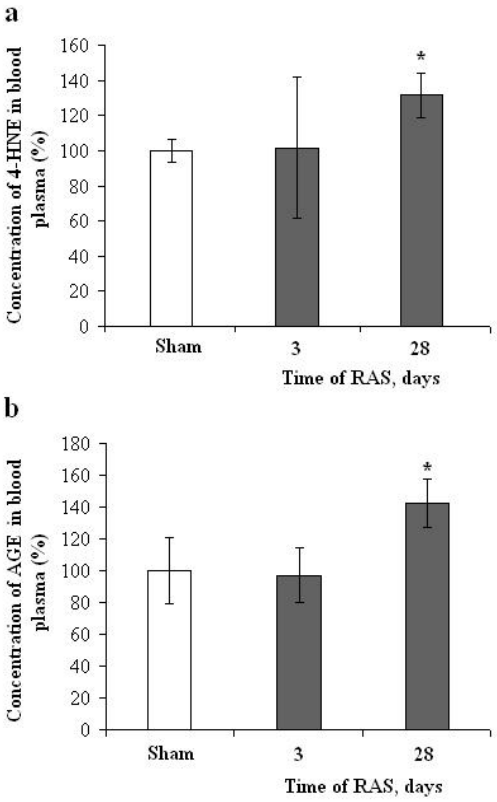

Figure 1. The concentration of 4-hydroxynonenal (a) and advanced glycation end products (AGE) (b) in blood plasma at 3 and 28 days after the induction of unilateral renal artery stenosis (RAS) in rats. All values are presented as mean percentage changes ± SD of three independent measurements for eleven rats from each group. *Denotes significant ($p<0.05$) differences between RAS and sham-operated rats.

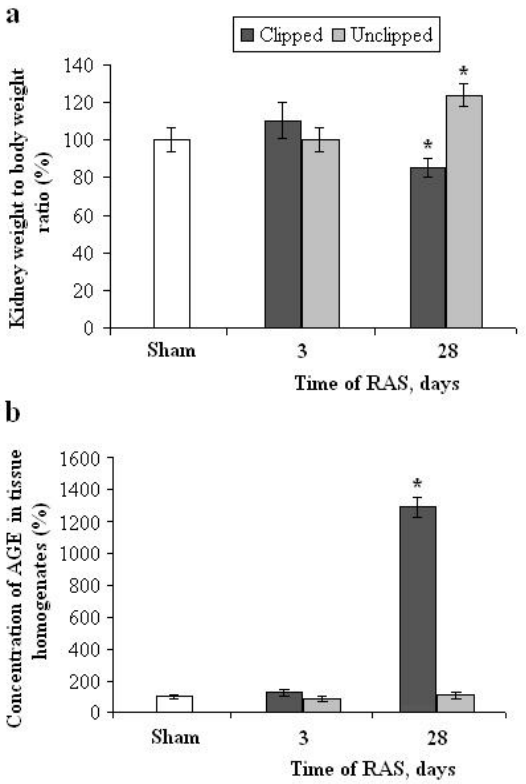

Figure 2. Kidney weight to body weight ratio (a) and advanced glycation end products (AGE) (b) in renal tissue homogenates at 3 and 28 days after the induction of unilateral renal artery stenosis (RAS) in rats. All values are presented as mean percentage changes ± SD of three independent measurements for eleven rats from each group.* Denotes significant ($p<0.05$) differences between RAS and sham-operated rats.

DISCUSSION

The kidneys play a key role in clearance and metabolism of advanced glycation end products (AGE) and therefore, the loss of renal function can lead to increased AGE formation and accumulation, both in the plasma and in the tissues. Among others it has been documented that circulating AGE levels are significantly increased in patients with diabetic nephropathy [7,9], end-stage renal disease (ESRD) [15,17] and acute and chronic renal failure (CRF) [16-18].

In our study, we demonstrate for the first time that unilateral renal artery stenosis (RAS) also leads to a significant increase in the AGE levels both in the plasma and in the clipped atrophic kidney. Moreover, increased concentration of AGE becomes tightly associated with increased level of 4-hydroxynonenal (4-HNE), a known marker of oxidative stress [19]. It is well-established that renal artery stenosis leads to increased generation of angiotensin II that via AT1 receptors promotes the production of various reactive oxygen species in the stenotic kidney [19,20]. There is also substantial evidence for a direct link between renin-angiotensin system and advanced glycation end products [21-24]. It has been demonstrated that angiotensin II receptor (AT1R) antagonists or angiotensin-converting enzyme (ACE) inhibitors, by lowering the oxidative stress, reduce the accumulation of AGE *in vitro* [22] in experimental diabetic nephropathy [23] and in patients with diabetic and non-diabetic nephropathies [24,25].

It is suggested that AGE accumulation may arise from ineffective removal of oxidatively modified proteins by the cellular proteolytic systems, such as the proteasomes [26]. The proteasomes play a key role in the degradation of damaged proteins both in the cytoplasm and nucleus, while their activity is suppressed during aging and in a variety of oxidative stress-induced pathologies [27,28]. In addition, we have previously reported that proteasome activity is decreased in the stenotic kidney of rats with angiotensin II-dependent renal hypertension [29, 30]. Therefore, we cannot exclude the possibility

that increased accumulation of AGE in the clipped atrophic kidney reported in this study may be due, in part, to a decline in the ability of proteasomes to degrade oxidatively-modified proteins

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CONCLUSIONS

Our research results clearly indicate that unilateral renal artery stenosis causes increased accumulation of AGE levels both in the plasma and in the ischemic atrophic kidney. Therefore, we submit and propose that AGE levels can be used as a reliable biomarker for monitoring the development of ischemic nephropathy. Further studies would be required to investigate and clarify the molecular mechanisms of the metabolism of AGE in the ischemic kidney.

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Conflicts of interest

We declare that we have no conflicts of interest.

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