Monoaminoguanidine prevents sorbitol accumulation, nonenzymatic protein glycosylation and development of kidney lesions in diabetic rats[1]

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Summary. Monoaminoguanidine administration (25 mg/kg b.wt, i.p. for 14 weeks) to alloxan diabetic rats (blood glucose $\geq$ 250 mg/dl) decreased the nonenzymatic protein glycosylation and sorbitol levels. It prevented development of Armanni-Ebstein tubular lesions, pathological changes in the glomerular capillary tufts and glomerular basement membrane thickening in the kidney.
Key words. Monoaminoguanidine; alloxan diabetes; nephropathy; sorbitol; nonenzymatic protein glycosylation.

Hyperglycemia has been recognized as the major risk factor for the development of diabetic nephropathy[3]. Accumulation of end-products of nonenzymatic glycosylation (NEG), advanced glycosylation end products, on long-lived tissue proteins such as collagen and elastin, has recently been implicated in the pathogenesis of capillary basement membrane changes in diabetes[4,5]. Recently, monoaminoguanidine (MAG) has been reported to inhibit the NEG-mediated accumulation of advanced glycosylation end products in the aorta of diabetic rats and on albumin and collagen in vitro[6]. The efficacy of MAG in preventing the development of diabetic renal pathology, however, remains to be determined.

Recent studies also indicate that increased aldose reductase activity and the resultant excessive accumulation of sorbitol might play an important role in the development of various diabetic complications such as cataract, neuropathy, retinopathy[7,8] and nephropathy[9]. We have recently demonstrated that MAG-administration inhibits aldose reductase activity in the lens and delays the development of lens opacity in alloxan-diabetic rats[10]. The present study was undertaken, therefore, to investigate whether monoaminoguanidine, because of its unique property of inhibiting both NEG-mediated accumulation of advanced glycosylation end products and aldose reductase activity, could delay or prevent the development of kidney lesions in diabetic rats.

Materials and methods

Male Sprague-Dawley rats (180–200 g) were used. They were maintained in temperature- and humidity-controlled quarters in the animal house of the Central Drug Research Institute, Lucknow, and had free access to water and a pellet diet (Hindustan Lever, India). Diabetes was produced in the rats by a single intraperitoneal injection of alloxan (180 mg/kg b.wt) dissolved in physiological saline. Blood glucose levels of the rats were monitored every week after ascertaining the development of frank hyperglycemia. Blood glucose was measured by the glucose oxidase method[11], using whole blood obtained from the retrorbital plexus. Only those rats which showed blood sugar levels $\geq$ 250 mg/dl and which did not require exogenous insulin supplementation for their survival were included in this study.

Monoaminoguanidine (Ferak, Berlin), dissolved in normal saline and sterilized by millipore-filtration, was administered daily for 14 weeks at a dose of 25 mg/kg b.wt/day i.p. to half of the diabetic rats. The other half of the hyperglycemic rats received an equal volume of normal saline for the same period and served as controls.

All the rats were sacrificed under nembutal anesthesia after 14 weeks of treatment. The right kidney from each animal was used for histological study while the left kidney was used for estimation of non-enzymatic protein glycosylation and sorbitol concentration. For histology, 3-mm thick slices of kidney were fixed in Carnoy's fluid, embedded in paraffin (Paraplast-plus, Lancer, USA), and sectioned.

Serial sections (6 µm thick) were stained by periodic acid Schiff-haematoxylin[12] with or without prior treatment with α-amylase (0.5% in 0.004 M acetate buffer, pH 5.5; incubation for 40 min, at 37 °C[13]). Representative sections were also stained by Jones' silver impregnation method[14] for the demonstration of basement membranes. The stained sections were code-numbered and evaluated microscopically by two investigators who had no prior knowledge of the treatments. Morphometric quantitation of the tissue pathology was also conducted but is not included in the present communication.

Nonenzymatic protein glycosylation was determined according to the method of Elder and Kennedy[15]. Briefly, trichloroacetic acid (TCA) precipitated kidney protein was autoclaved in 1.0 M oxalic acid at 121 °C for 1.0 h, then allowed to react with thiobarbituric acid (0.5 M) and the color read spectrophotometrically (443 nm). This method did not detect any lipid peroxidation products in the tissue. Glycosylation was expressed as nmoles of fructose/mg protein. Protein was estimated according to Lowry[16]. Sorbitol was estimated fluorimetrically in neutralized perchlorate tissue extract, using sorbitol dehydrogenase[17], and expressed as µmoles sorbitol/g of kidney.

Results and discussion

The severe ketotic diabetic condition which alloxan is known to produce is associated with a poor survival rate, especially over the long term. Therefore, only those rats in which alloxan induced a moderate diabetic condition with blood sugar levels of around 250 mg/dl were used in the present experiments. Over the observation period of 14 weeks, the body weight of the diabetic rats decreased significantly. Characteristically for the diabetic condition, there was an increase in the relative kidney weight (per 100 g b.wt) of the diabetic rats (table). Monoaminoguanidine treatment for 14 weeks did not alter the body and kidney weights or the hyperglycemia (table).

In the present study, sorbitol concentration (table), which is central to the polyol pathway, was found to be increased in the diabetic rat kidney. This increase was to 2-fold that of normal rat kidney. Monoaminoguanidine treatment markedly reduced the sorbitol concentration by more than 60% of untreated control values. Similarly, the diabetic rat kidney showed a 2-fold increase in glycosylated protein content as compared to normoglycemic controls (table), and monoaminoguanidine treatment reduced these levels by more than 60% compared with the untreated controls (table). Although the mechanism by which MAG brings about a reduction in sorbitol levels is not readily apparent, our previous studies indicate that it could be via inhibition of kidney aldose reductase activity [10]. On the other hand, the reduction in nonenzymatic glycosylation by MAG could be directly due to its competition with amino functions of the proteins [6].

In order to find out whether the changes observed in renal sorbitol concentration and nonenzymatic protein glycosylation are associated with any renal pathology, the kidney was subjected to histopathological examina-

Effect of monoaminoguanidine treatment for 14 weeks on alloxan diabetic rats[a]

Group	Body wt (g)	Kidney wt/100 g body wt	Blood glucose (mg/dl)	Total protein glycosylation (nmoles of fructose/ mg kidney protein)	Sorbitol concentration (μmoles/g wt of kidney)
Normoglycemic	345 ± 15	0.45 ± 0.009	59 ± 6.0	3.24 ± 0.44	0.23 ± 0.014
Diabetic – untreated	181 ± 17[b]	0.71 ± 0.011[b]	248 ± 8.5[b]	6.63 ± 0.53[b]	0.58 ± 0.016[b]
Diabetic – treated with monoaminoguanidine	173 ± 23	0.70 ± 0.012	256 ± 11	4.59 ± 0.38[c]	0.30 ± 0.013[c]

[a] Values are mean ± SD of 6–8 animals in each group; statistical analysis by Student's t-test; [b] vs normoglycemic group, $p < 0.001$; [c] vs untreated diabetic group, $p < 0.001$.

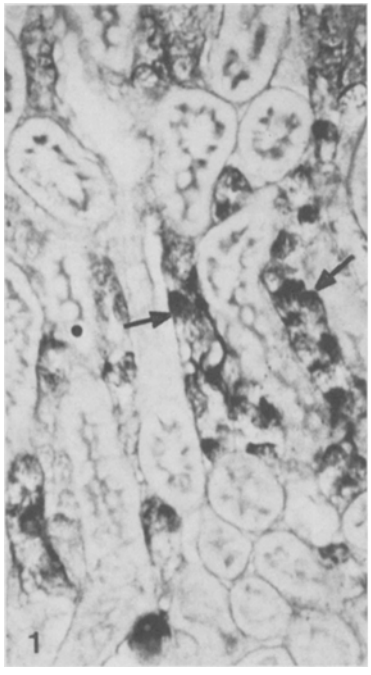

Figure 1. Section of diabetic rat kidney showing glycogen deposition in distal tubules (arrows). PAS without counterstain × 320.

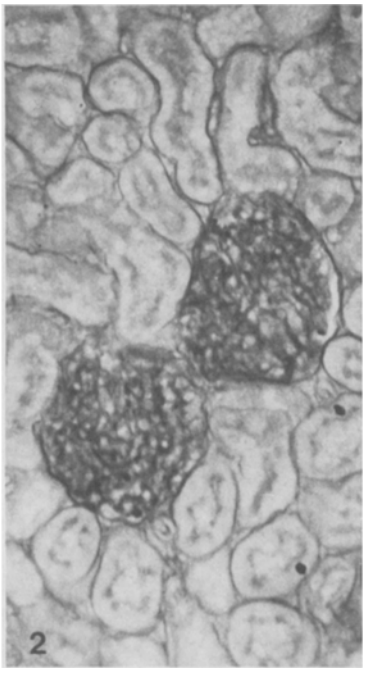

Figure 2. Two cortical glomeruli in diabetic rat kidney showing gross reduction in their size, and collapsed capillary tufts with 'diffuse' PAS-positive thickening of the capillary basement membrane. PAS without counterstain × 320.

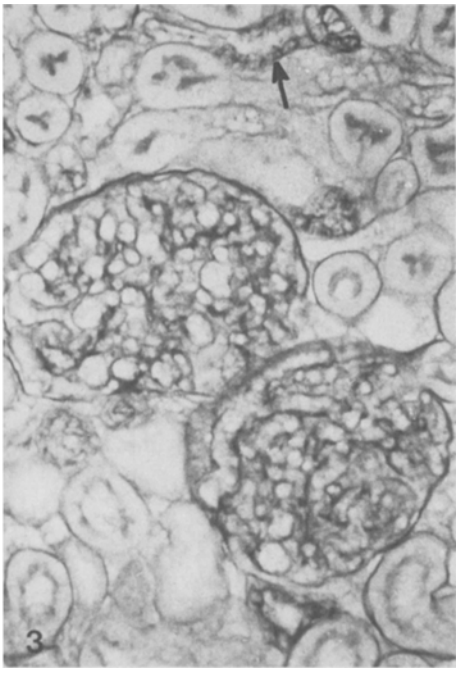

Figure 3. Kidney from a diabetic rat treated with monoaminoguanidine for 14 weeks showing normal well-dilated capillary loops in the glomeruli, and minimal glycogen deposits in distal tubules (arrows). PAS without counterstain × 320.

tion. In the diabetic rats PAS-stained kidney sections revealed Armanni-Ebstein tubular lesions (fig. 1), collapsed or occluded glomerular capillary tufts, a marked reduction in the size of the glomeruli (fig. 2), and diffuse glomerular basement membrane thickening, as well as exudative deposits in the tubules and in glomerular subcapsular space. The Armanni-Ebstein lesions characteristically showed deposition of glycogen in distal tubules, especially the ascending limbs of the loop of Henle and those forming the juxta-glomerular apparatus. On the other hand the PAS-positive exudative deposits in tubules and glomeruli were α-amylase-resistant, which indicated their glycoprotein nature.

Treatment with MAG markedly reduced these tubular and glomerular lesions (fig. 3). The glomeruli were almost normal in appearance with open capillary tufts and were devoid of any noticeable PAS-positive thickening in the mesangial areas or the capillary walls. However, a mild form of tubular Armanni-Ebstein lesions was still evident but the tubules showed very little glycogen deposition (fig. 3). To our knowledge, this is the first definitive evidence that at least some of the diabetic kidney lesions can be prevented or reduced by MAG. This effect of monoaminoguanidine on renal pathology was, however, not associated with any significant reduction in the blood glucose levels, or with renal hypertrophy, which indicates that MAG-induced reversal of pathology is not via amelioration of hyperglycemia. We have recently observed that monoaminoguanidine inhibits nonenzymatic protein glycosylation and protein cross-linking in vitro [18]. Earlier, Brownlee et al. [6] also reported that MAG administration prevents the formation of fluorescent advanced nonenzymatic glycosylation products and the cross-linking of arterial wall connective tissue protein in diabetic rats. Similar changes in glomerular macromolecules are thought to be involved in diabetic nephropathy [5].

The results of the present study, therefore, suggest that the prevention of renal pathological lesions in the diabetic rat by monoaminoguanidine could be due to inhibition both of nonenzymatic protein glycosylation and of the accumulation of sorbitol.

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4-Aminopyridine and barium chloride attenuate the anti-epileptic effect of carbamazepine in hippocampal slices

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Summary. The exact mode of action of the anti-epileptic agent carbamazepine is unknown. In hippocampal slices in which epileptiform discharges were induced by addition of penicillin to the perfusion medium, the depressant effect of carbamazepine was attenuated by the potassium-channel blockers barium chloride (0.1 mM) and 4-aminopyridine (200 μM), which suggested that potassium fluxes might be involved in the mechanism of action of carbamazepine.
Key words. Carbamazepine; potassium; hippocampus; epilepsy; electrophysiology; 4-aminopyridine.