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SHORT COMMUNICATIONS

Monoaminoguanidine inhibits aldose reductase

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Presently non-enzymatic protein glycosylation mediated generation of 'advanced glycosylated end product' and aldose reductase mediated intracellular accumulation of sorbitol have been considered as two major pathways underlying molecular basis of complications in diabetes [1]. Many inhibitors of aldose reductase have been found to arrest the pathological sequalae, e.g. cataracts, retinal capillary basement membrane thickening, corneal wound healing, nerve conduction velocity deficits and microalbuminuria, in diabetic animals [2]. Most potent inhibitors currently being studied, are sorbinil (CP 45,634) and tolrestat (AY 22,273). Ca^{2+}, free radicals and *in situ* antioxidant changes have also been implicated in cataract formation [3–4].

Recently monoaminoguanidine has been reported to inhibit advanced glycosylated product formation *in vitro* and in experimental diabetic animals with chronic hyperglycemia [6]. These authors have suggested that inhibition of advanced glycosylated protein accumulation by daily intraperitoneal administration of monoaminoguanidine (25 mg/kg body wt) to diabetic rats for 16 weeks, may lead to inhibition of diabetes associated complications.

The present communication provides evidence for inhibition of rat eye lens aldose reductase activity by monoaminoguanidine *in vitro* and *in vivo*.

Materials and Methods

DL-Glyceraldehyde, sorbitol and sorbitol dehydrogenase were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and monoaminoguanidine from Ferrak (Berlin, F.R.G.). All other chemicals used were of AR grade.

Adult male Sprague–Dawley rats from the CDRI animal colony, housed in air conditioned quarters with a light cycle of 12 hr and fed *ad lib*. Hindustan Lever pellet diet with free access to water, were used in the present study. Chronic experimental hyperglycemia was launched in the animals by intravenous injection of alloxan (50 mg/kg body wt). Diabetes was evaluated by blood sugar and rats with blood sugar ≥ 250 mg/dL were considered as diabetic. The diabetic rats were placed into two groups. One group received monoaminoguanidine (25 mg/kg body wt) solution in normal saline daily, intraperitoneally for 98 days and the other was administered equal volume of normal saline.

The animals were killed by decapitation. Their eyes excised and lenses removed. Homogenate (5% w/v) of eye lens was prepared in 10 mM phosphate buffer pH 7.0. The homogenate was centrifuged at 9000 *g*. The supernatant was used as a source of aldose reductase activity. Aldose reductase activity was determined according to Lee *et al.* [7]. The enzyme reaction mixture contained 0.1 M HEPES buffer (pH 7.0), 0.4 M ammonium sulfate, 10 mM DL-glyceraldehyde and 0.12 mM NADPH in 3.0 mL final volume. Monoaminoguanidine solution was prepared in water just before use. The enzyme reaction was followed spectrophotometrically at 340 nm and its activity was expressed as nmoles of NADPH oxidized per minute. The sorbitol level in the rat eye lens was measured in perchloric acid supernatant (from which potassium perchlorate had been precipitated out) of the lens tissue homogenate by spectrophotometric enzyme assay [8].

Results and Discussion

Aldose reductase activity was linear for at least 5 min under the present experimental conditions and monoaminoguanidine inhibited the enzyme activity. Optimum concentration vs velocity plot was found to be 50 mM. In order to rule out the effect of the inhibitor on NADPH absorbance, appropriate controls were used in the experiment. The compound did not elicit any spectral change on coincubation with NADPH solution in the present experiment. For a Lineweaver–Burk plot, the rate of enzyme reaction was determined at different concentrations of NADPH as well as DL-glyceraldehyde with and without two concentrations of monoaminoguanidine. The inhibition was non-competitive with respect to NADPH (Fig. 1a) and DL-glyceraldehyde (Fig. 1b) and exhibited a K_i value of 27 mM for NADPH and 25 mM for DL-glyceraldehyde.

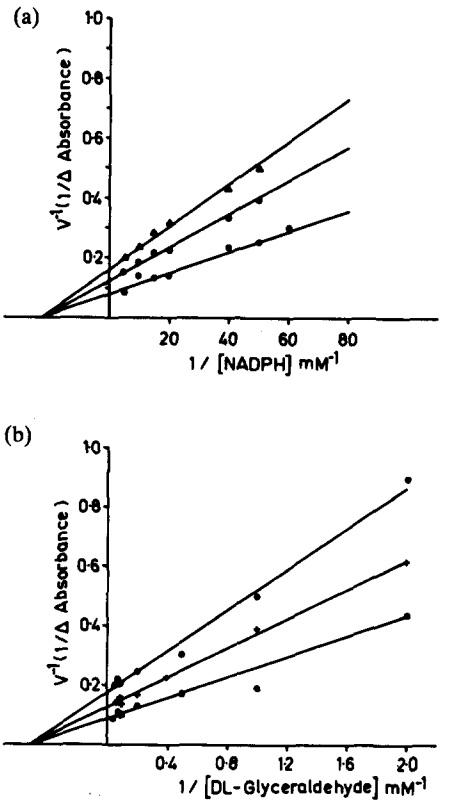

Fig. 1. (a) Lineweaver–Burk plot of rat eye lens aldose reductase activity with varying concentrations of NADPH (○—○) and the effect of 25.0 mM (●—●) and 50.0 mM (△—△) monoaminoguanidine, respectively, on the V_{max} and K_m of the enzyme. (b) Lineweaver–Burk plot of rat eye lens aldose reductase activity with varying concentrations of DL-glyceraldehyde (○—○) and the effect of 25.0 mM (×—×) and 50.0 mM (●—●) monoaminoguanidine, respectively, on the V_{max} and K_m of the enzyme.

Table 1. Effect of preincubation of rat eye lens aldose reductase with monoaminoguanidine at 37° on aldose reductase activity

Addition	Aldose reductase activity (units/assay sample) after (preincubation time)			
	0 min	15 min	30 min	60 min
None	85	81	80	80
12.5 mM MAG	75 (12%)	61 (25%)	55 (31%)	50 (37%)
25.0 mM MAG	65 (23%)	49 (40%)	45 (44%)	40 (50%)
50.0 mM MAG	42 (51%)	29 (64%)	22 (72%)	16 (80%)

Per cent inhibition is shown within parentheses.
MAG, monoaminoguanidine.

Preincubation of the enzyme with monoaminoguanidine increased the degree of inhibition of the enzyme by the inhibitor at all the three concentrations tested (Table 1). Dilution of the enzyme–50 mM monoaminoguanidine mixture (preincubated for 1 hr) to 2.5-fold and 5-fold did not produce any change in extent of the inhibition (70%) observed in the undiluted sample. Similarly 73% inhibition of aldose reductase activity was recorded in the samples preincubated with 50 mM MAG for 1 hr before dialysis and 74% after dialysis. From the foregoing experiments we conclude that monoaminoguanidine binds irreversibly with the enzyme leading to non-competitive inhibition of the enzyme activity.

Daily intraperitoneal administration of monoaminoguanidine solution in normal saline at a dose of 25 mg/kg body weight to alloxan diabetic rat for 98 days reduced its eye lens sorbitol level from 1.75 ± 0.04 μmol/g wet weight to 0.59 ± 0.05 μmol/g wet weight, thereby causing 66% reduction in eye lens sorbitol content. The diminution in eye lense sorbitol due to monoaminoguanidine is consistent with aldose reductase inhibitory activity of the compound.

Brownlee *et al.* [6] demonstrated inhibition of non-enzymatic glycosylation mediated advanced glycosylated product formation by monoaminoguanidine. They did not investigate the effect of monoaminoguanidine on aldose reductase activity. The present observation, a first report on aldose reductase inhibitory activity of monoaminoguanidine, is interesting since it would appear that the compound inhibits both the independent pathways responsible for development of diabetes associated complications. Monoaminoguanidine is known to inhibit formation of glucose mediated homo- and heteropolymer adducts of proteins by its nucleophilic attack on the carbonyl groups of primary glycosylation products of proteins, thereby arresting their progression to advanced glycosylation end products [6]. However, we do not know how monoaminoguanidine inhibits aldose reductase activity. Non-competitive inhibition of aldose reductase activity by monoaminoguanidine suggests that the compound does not compete with either NADPH or aldose binding site of the enzyme. Its nucleophilicity may tend to favour its interation with carbonyl group of aldose. However, due to lack of information, it is premature to speculate on the possible mechanism of inhibition of AR activity by MAG.

The present study opens an exciting possibility of aminoguanidine and other non-toxic nucleophiles as lead compounds to design drugs for arresting or preventing diabetic complications on the one hand and as antiaging compounds on the other, for recently Kohn [9] has demonstrated that severe complications of aging that occur in collagen rich tissues developed at an earlier age in diabetes.

In summary, Monoaminoguanidine, reported to be an inhibitor of advanced glycosylated end product formation, inhibited rat eye lens aldose reductase activity, *in vitro* non-competitively with K_i = 27 mM with regard to NADPH and K_i = 25 mM for DL-glyceraldehyde. Intraperitoneal administration of monoaminoguanidine to diabetic rats, daily for 98 days, resulted in significant lowering of eye lens sorbitol compared with untreated diabetic rats.

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