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Aminoguanidine ameliorates albuminuria in diabetic hypertensive rats

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Summary. We studied the effect of aminoguanidine, an inhibitor of advanced glycation product formation, on albuminuria in chronically diabetic spontaneously hypertensive rats. At the time of killing, there was no statistically significant difference in blood glucose concentration between the treated and untreated diabetic animals (18.2 ± 0.69 mmol/l), nor was there any difference among the non-diabetic, diabetic untreated, and diabetic treated rats with respect to blood pressure (169 ± 6.9 mm Hg). However, non-diabetic hypertensive animals had a mean quantitative 24-h urinary albumin excretion of 28 ± 2 mg albumin/24-h, while untreated diabetic hypertensive animals averaged nearly four times that amount (106 ± 3 mg albumin/24 h). Without affecting blood pressure, aminoguanidine treatment of diabetic hypertensive animals decreased the diabetic-associated elevation in urinary albumin excretion by 75% (48 ± 2 mg/24 h). These data suggest than inhibition of advanced glycation product formation ameliorates the glomerular dysfunction caused by chronic hyperglycaemia.

Key words: Nephropathy, diabetes, aminoguanidine, glycation, hypertension.

The development of diabetic nephropathy is closely correlated with the duration and magnitude of antecedent hyperglycaemia [1]. Prolonged hyperglycaemia has been postulated to cause glomerular dysfunction by a variety of biochemical mechanisms, including excessive polyol pathway flux, altered redox state of pyridine nucleotides, an increased rate of de novo diacylglycerol synthesis with activation of protein kinase C, and accumulation of advanced glycation products [2]. However, the primary mechanism operative in vivo is not known.

The rate of decline in diabetic renal function is also profoundly influenced by blood pressure. Unilateral renal artery stenosis markedly decreases the severity of nephropathy on the affected side [3], and treatment of hypertension dramatically slows the progression of glomerular filtration rate loss [4], although hypertension is neither necessary nor sufficient to produce the lesions of diabetic glomerulosclerosis.

Reasoning from this that prevention of diabetic glomerular dysfunction by pharmacologic inhibition of a hyperglycaemia-induced abnormality in the presence of unaltered hypertension would constitute strong evidence for the in vivo primacy of that abnormality, we evaluated the effect of aminoguanidine, an inhibitor of advanced glycation product accumulation [5], on albuminuria in chronically diabetic SHR rats. Aminoguanidine, a virtually non-toxic nucleophilic hydrazine (LD_{50} = 1800 mg/kg in rodents) prevents advanced glycation product formation by reacting with fragmentation products of sugar-derived ketoamines [2].

Materials and methods

Male SHR rats (Charles River Laboratories, Wilmington, Mass, USA), with blood pressures of 170–180 mm Hg were used in this study. This Wistar-derived strain develops spontaneous hypertension of considerable severity. Rats weighing between 75 and 90 g at 6 weeks of age were injected with 60 mg/kg body weight i. v. streptozotocin (Sigma, St. Louis, Mo., USA) after an overnight fast. Only animals with blood glucose levels greater than 15 mmol/l 1 week after injection were included as diabetic animals in this study. Diabetic animals were randomized to receive either no treatment, or 50 mg/kg aminoguanidine HCl (Aldrich, Rochester, NY, USA) injected i. p. once daily. All rats were caged individually and fed a normal diet. Animals were weighed at weekly intervals over the 24-week study. At monthly intervals blood glucose was determined by an automated glucose oxidase technique using a Beckman Glucose Analyser, and systolic blood pressure was measured by indirect tail-cuff plethysmography in a Gould apparatus as previously described [6]. Before being killed, animals were housed in individual metabolic cages for collection of 24-h urine samples for measurement of albuminuria.

Urinary albumin content was measured by rocket immunoelectrophoresis using a high affinity anti-rat albumin antibody. The inter-

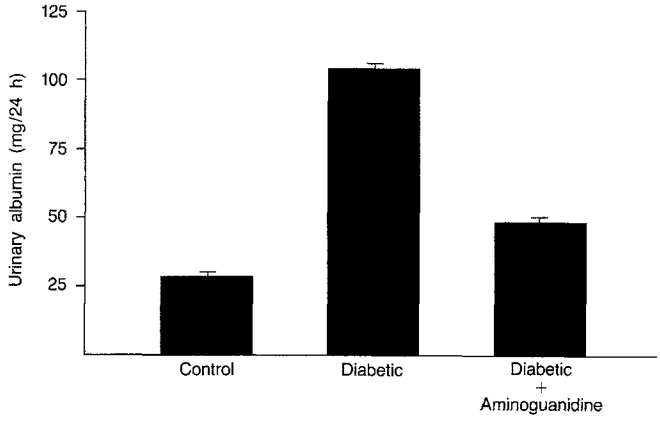

Fig. 1. Urinary albumin excretion in non-diabetic control, diabetic, and aminoguanidine-treated diabetic hypertensive (SHR) rats. Data are shown as the mean ± SEM of 11, 7, and 5 separate determinations

assay coefficient of variation was 2–10% ($n = 8$) and the lowest detection limit of the assay was 10 ng/μl.

Statistical analysis

Albumin excretion data were analysed by analysis of variance after logarithmic transformation using the SYSTAT program on an IBM AT personal computer.

Results and discussion

At the time of killing, there was no statistically significant difference in blood glucose concentration between the treated and untreated diabetic animals (18.2 ± 0.69 mmol/l) nor was there any difference among the non-diabetic, diabetic untreated, and diabetic treated rats with respect to blood pressure (169 ± 6.9 mm Hg). However, there were significant differences in quantitative 24-h urinary albumin excretion, as shown in Figure 1. Non-diabetic hypertensive animals had a mean ± SEM of 28 ± 2 mg albumin/24 h. In contrast, untreated diabetic hypertensive animals averaged nearly four times that amount (106 ± 3 mg albumin/24 h). Without affecting blood pressure, aminoguanidine treatment of diabetic hypertensive animals decreased the diabetes-associated elevation in urinary albumin excretion by 75% (48 ± 2 mg/24 h).

These data demonstrate that chronic administration of an inhibitor of advanced glycation product formation to diabetic animals prevents most of the increase in urinary albumin excretion associated with hyperglycaemia, even in the presence of persistent hypertension. Thus, since increased urinary albumin excretion has been associated with clinically significant glomerular pathology in man, aminoguanidine may have potential in the treatment of diabetic nephropathy. Morphologically, aminoguanidine treatment decreases glomerular basement membrane advanced glycation product content [7] and prevents basement membrane thickening in diabetic animals [8].

Although a direct pharmacologic vasodilator effect of aminoguanidine on glomerular vessels cannot be ruled out, the recent demonstration that aminoguanidine did not alter nerve blood flow or microvascular nerve resistance in normal rats makes this possibility less likely [9]. Rather, an indirect effect on chronic hyperglycaemia-induced alterations in glomerular haemodynamics is suggested by aminoguanidine's ability to normalize the marked reduction in nerve blood flow and increased microvascular resistance during chronic administration to diabetic rats [9].

These data are consistent with the speculation that chronic hyperglycaemia causes permanent changes in microvascular blood flow due to the formation of advanced glycation products [10], thereby contributing to the development of diabetic microvascular complications in the glomerulus.

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