J. Pharm. Pharmacol. 1991, 43: 337–341
Received November 12, 1990

An Investigation into the Effects of Aminoguanidine Treatment on the Plasma and Blood of Free-fed and Dietary-restricted Rats

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Abstract—An investigation has been made into the effect of oral aminoguanidine (50–60 mg kg^{-1} day^{-1}) on the blood biochemistry of male Wistar rats which either had free access to food or were dietary-restricted (50% of the food consumed by the free access group). In control rats (i.e. without aminoguanidine treatment) three weeks of food restriction caused significant increases in plasma sodium and albumin and the erythrocyte count, haematocrit and haemoglobin. There were reductions in plasma calcium, phosphate, alkaline phosphatase activity, urea, triglycerides, creatinine, glucose and the red cell volume. Similar effects of food restriction were observed in aminoguanidine-treated rats. Aminoguanidine ingestion in free-fed animals caused a reduction in plasma creatinine concentration. In dietary-restricted rats, aminoguanidine ingestion reduced plasma sodium and total plasma proteins (largely as a result of a decline in albumin), and increased plasma urea concentrations. Aminoguanidine was added to plasma of control rats in-vitro to determine whether it interfered with the assay of urea and creatinine. At concentrations of 0·1 to 10 mg mL^{-1}, aminoguanidine had no effect on urea determinations. However, aminoguanidine significantly reduced the apparent concentration of plasma creatinine by between 7 to 81%. The changes in plasma analytes in aminoguanidine-treated rats may be indicative of minor hepatic perturbations or kidney function, but the data also imply that prior nutritional state is a determinant of aminoguanidine effects.

Aminoguanidine, a potent inhibitor of diamine oxidase activity (Tamura et al 1989), also has other properties. For example, Baylin et al (1975) showed that aminoguanidine minimized nitrogen loss in clinical cancer cachexia and also promoted the growth of laboratory rats. Aminoguanidine, is thus a potentially interesting therapeutic agent and this premise is supported by observations showing that it has other pharmacological effects. It has the ability to inhibit the non-enzymatic glycosylation of serum albumin and advanced glycosylation end-products in renal basement membranes (Khatami et al 1988; Nicholls & Mandel 1989). Moreover, aminoguanidine also increases the proportion of soluble collagens and diminishes total collagens in skin and bones (Dabrowski & Szczepanowska 1984). Other studies have shown that aminoguanidine can alter hepatic and blood levels of histamine after partial hepatectomy (Moulinoux et al 1977). However, there is little published information on its toxicological effects. We therefore investigated its influence in normal laboratory rats (i.e. free-fed) and those subjected to dietary restriction (laboratory chow administered as 50% of normal intake). We measured those plasma electrolytes, enzyme activities and metabolites, and haematological parameters which are considered to be sensitive indicators of metabolic stress and organ dysfunction.

Materials and Methods

Source of materials

Aminoguanidine hydrochloride was a gift from Dr M. A. Yamin (The Rockefeller University, New York City, USA).

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Male Wistar rats were purchased from Bantin and Kingman (Alderborough, Hull, UK). Other chemicals for analysis were purchased from either the Sigma Chemical Company (Poole, Dorset, UK) or BDH (Poole, Dorset, UK) and were of the optimum purity.

Treatment of animals

Rats (n = 24) were obtained at initial weights of 55–66 g (mean ± s.e.m., 60·8 ± 0·7 g) and housed in an approved animal house that was humidified and temperature controlled. The lighting was maintained on a 12 h light: 12 h dark cycle commencing at 0700 h. The rats had free access to a standard laboratory diet (LAD2 diet, Labsure, Manea, Cambridgeshire, UK) until the commencement of the experiment and free access to tap-water. At between 73 and 85 g (mean ± s.e.m., 78·2 ± 0·9 g) the rats were ranked in order of weight and divided into four groups of identical mean weights (n = 6 rats in each group). The four groups were as follows: Group 1: rats were allowed free access to the laboratory diet and tap-water; Group 2: rats were allowed free access to the laboratory diet and also to tap-water that contained aminoguanidine at a final concentration of 350 mg L^{-1}, equivalent to a daily intake of 50–60 mg kg^{-1}; Group 3: rats were administered the laboratory diet in amounts equivalent to 50% of that weight consumed by the free access rats in Group 1. Rats had free access to tap-water; Group 4: rats were treated as for Group 3 except that the tap-water contained aminoguanidine (350 mg L^{-1}).

The aminoguanidine solutions in Groups 2 and 4 were prepared fresh each day. Body weights, water and food intakes were monitored throughout the study. At the end of three weeks treatment, rats were decapitated and blood collected into ice-cold heparinized tubes for either extraction

of plasma by centrifugation (2000 *g*, 10 min, 4°C) or analysis of haematological indices as described below. All plasma samples were frozen at −70°C until analysis.

Treatment of plasma with aminoguanidine

To determine whether aminoguanidine interfered with the assay of urea and creatinine, plasma was obtained from four free-fed control rats (i.e. Group 1) and divided. The plasma (0·225 mL) was mixed with equal quantities of distilled water (0·025 mL) containing aminoguanidine at concentrations of 0, 1, 10 and 100 mg mL^{-1}. The final concentrations were therefore 0, 0·1, 1 and 10 mg mL^{-1}. Plasma samples were assayed for urea and creatinine as described below.

Blood haematology

Blood was analysed on a Coulter counter, model ZF (Coulter Electronics Ltd, Luton, Beds) immediately after the rats were decapitated. Haemoglobin was measured by reaction with cyanide and subsequent spectrophotometric measurements at 525 nm, and was assayed by an automated technique on the Coulter counter, model ZF.

Blood biochemistry

The analytical methods used were:

Electrolytes. Plasma sodium and potassium were determined as reported by Rao et al (1972) with ion-selective electrodes; calcium was measured by Gitelman (1967), and was also corrected for plasma protein binding; magnesium was determined according to Forbes et al (1985) and phosphate by the method of Daly & Ertingshausen (1972).

Enzymes. Plasma enzyme activities were determined as follows: alkaline phosphatase [EC 3.1.3.1.] by the method of Bowers & McComb (1975); γ-glutamyl transferase [EC 2.6.2.2.] according to Gowenlock et al (1988); alanine aminotransferase [EC 2.6.1.2.] after Bergmeyer & Horder (1980); and lactate dehydrogenase [EC 1.1.1.27.] according to Gowenlock et al (1988).

Metabolites. The assays used were: total plasma proteins, Gornall et al (1949); albumin, Webster (1977); urea, Talke & Schubert (1965); triglycerides, Fossati & Prencipe (1982); creatinine, Lustgarten & Wenk (1972); and glucose, Cooper (1973).

Statistical evaluation

All in-vivo data (Tables 1–4) are presented as means ± s.e.m. of 5–6 observations in each group. Differences between means were assessed by Student's *t*-test using the pooled estimate of variance. All in-vitro data (Table 5) are presented as mean ± s.e.m. of four observations in each group. Differences between means were assessed by Student's *t*-test for paired samples. Significance taken at $P \leqslant 0{\cdot}05$.

Results

There were no significant effects of aminoguanidine treatment on body weights, food intakes or water intakes (results not presented). By virtue of the experimental design, dietary restriction reduced body weights by approx. 50%, as was expected (results not presented).

Table 1. The effect of aminoguanidine treatment and dietary restriction on plasma electrolytes. Male Wistar rats had free access to food or had dietary restriction with or without aminoguanidine treatment as described in the text. All data are shown as mean ± s.e.m. of 5–6 observations. Differences between means were assessed by Student's *t*-test using the pooled estimate of variances.

	Free-fed rats	
Electrolyte (mmol L^{-1})	Control	+Aminoguanidine
Sodium	139 ± 1	139 ± 1
Potassium	7·3 ± 0·3	6·9 ± 0·2
Magnesium	0·77 ± 0·02	0·71 ± 0·03
Calcium	2·72 ± 0·03	2·72 ± 0·03
Corrected calcium	2·91 ± 0·03	2·92 ± 0·02
Phosphate	3·13 ± 0·16	3·13 ± 0·14
	Dietary-restricted rats	
Electrolyte (mmol L^{-1})	Control	+Aminoguanidine
Sodium	141 ± 1*	139 ± 1#
Potassium	7·7 ± 0·2	7·7 ± 0·2
Magnesium	0·78 ± 0·02	0·80 ± 0·01
Calcium	2·51 ± 0·02**	2·49 ± 0·03
Corrected calcium	2·67 ± 0·02**	2·69 ± 0·02
Phosphate	2·70 ± 0·08*	2·84 ± 0·05

Differences between free-fed controls and dietary-restricted controls *$P < 0{\cdot}025$; **$P < 0{\cdot}001$. Differences between free-fed controls and free-fed aminoguanidine-treated rats, or between dietary-restricted controls and dietary-restricted aminoguanidine-treated rats, #$P < 0{\cdot}025$.

Effect on plasma electrolytes (Table 1)

The results show that dietary restriction causes a small (i.e. 2%) but statistically-significant increase in plasma sodium concentrations. Plasma calcium and inorganic phosphate were decreased by 8–14%, but neither potassium nor magnesium were altered. Dietary restriction of the aminoguanidine-treated rats also caused decreases in plasma calcium levels, but there was no significant change in plasma sodium concentrations. In aminoguanidine-treated rats dietary restriction caused significant increases in potassium (12%) and magnesium (13%), though plasma phosphate concentrations were unaltered. Aminoguanidine treatment

Table 2. The effect of aminoguanidine treatment and dietary restriction on the activities of plasma enzymes.

	Free-fed rats	
Enzyme activities (int. units L^{-1})	Control	+Aminoguanidine
Alkaline phosphatase	470 ± 30	438 ± 38
γ-Glutamyltransferase	0·50 ± 0·34	0·60 ± 0·40
Alanine aminotransferase	66·0 ± 4·0	55·4 ± 4·1
Lactate dehydrogenase	1770 ± 190	1580 ± 220
	Dietary-restricted rats	
Enzyme activities (int. units L^{-1})	Control	+Aminoguanidine
Alkaline phosphatase	305 ± 15*	314 ± 23
γ-Glutamyltransferase	0·33 ± 0·33	0·80 ± 0·49
Alanine aminotransferase	55·8 ± 3·9	53·7 ± 4·6
Lactate dehydrogenase	2150 ± 320	2260 ± 260

All data are presented as means ± s.e.m. of 5–6 observations. Differences between free-fed controls and dietary-restricted controls, *$P < 0{\cdot}001$. Differences between free-fed controls and free-fed aminoguanidine-treated rats, or between dietary-restricted controls and dietary-restricted aminoguanidine-treated rats were not significant.

itself had no effect on plasma electrolytes in free-fed rats, but significantly reduced plasma sodium by 2% in dietary-restricted rats.

Effect on enzyme activities (Table 2)

Dietary restriction in control rats significantly decreased alkaline phosphatase activities by 35%, but had no effect on either γ-glutamyl transferase, alanine aminotransferase or lactate dehydrogenase activities. Similar changes due to dietary restrictions were obtained in aminoguanidine-treated rats. Aminoguanidine treatment itself had no significant effect on the activity of any plasma enzymes, either in control or dietary-restricted rats.

Effect on plasma proteins and metabolites (Table 3)

Dietary restriction caused a small, but significant, increase in plasma albumin concentration (by 5%), but had no effect on mixed plasma proteins. Dietary restrictions also decreased the plasma concentrations of urea (by 63%), triglycerides (by 56%), creatinine (by 15%) and glucose (by 25%). Similar effects of dietary restrictions were obtained for urea, glucose and triglyceride concentrations in the aminoguanidine-treated rats. However, in aminoguanidine-treated rats plasma albumin and creatinine concentrations were unaltered by dietary restriction.

Aminoguanidine treatment caused significant decreases (13%) in plasma creatinine concentrations in free-fed rats, which probably explains why concomitant dietary deprivation did not cause any significant change in plasma creatinine levels. Furthermore, in dietary-restricted rats, aminoguanidine treatment caused total plasma proteins and albumin to decrease by 5%, and urea to significantly increase by 26%.

Effect on haematological parameters (Table 4)

Dietary restriction in control rats caused an apparent increase in red cell count (by 15%), but red cell volume was significantly reduced by 5%. Haematocrit was also increased (10%) as was haemoglobin content (16%). White blood cells were relatively unaltered by dietary restriction. Similar results due to dietary restrictions were obtained for aminoguanidine-treated rats.

Aminoguanidine treatment in free-fed rats, and in dietary-restricted rats had no effect on any haematological variable.

Effect on aminoguanidine in-vitro (Table 5)

The addition of aminoguanidine to plasma in-vitro caused the apparent concentration of creatinine to decrease. At a concentration of 0·1 mg mL^{-1}, the creatinine concentration was significantly reduced by 7%. Much greater reductions in creatinine were observed (i.e. to over 80% inhibition) with increasing concentrations of aminoguanidine. At concentrations of 0·1 to 10 mg mL^{-1}, aminoguanidine had no effect on urea determinations.

Table 3. The effect of aminoguanidine treatment and dietary-restriction on plasma proteins and metabolites.

	Free-fed rats	
Metabolite	Control	+Aminoguanidine
Total plasma protein (g L^{-1})	58·3±2·0	57·0±1·0
Plasma albumin (g L^{-1})	32·7±0·4	32·7±0·4
Urea (mmol L^{-1})	6·3±0·2	6·3±0·2
Triglycerides (mmol L^{-1})	1·80±0·12	2·04±0·25
Creatinine (μmol L^{-1})	46·2±1·5	40·4±2·3#
Glucose (mmol L^{-1})	9·83±0·21	9·56±0·37
	Dietary-restricted rats	
Metabolite	Control	+Aminoguanidine
Total plasma protein (g L^{-1})	59·2±0·7	56·2±0·6##
Plasma albumin (g L^{-1})	34·3±0·6*	32·5±0·6##
Urea (mmol L^{-1})	2·3±0·2***	2·9±0·2#
Triglycerides (mmol L^{-1})	0·79±0·09**	0·85±0·10
Creatinine (μmol L^{-1})	39·2±1·6**	35·5±1·6
Glucose (mmol L^{-1})	7·32±0·27***	7·33±0·17

All data are means±s.e.m. of 5–6 observations. Differences between free-fed controls and dietary-restricted controls $*P<0·05$; $**P<0·01$; $***P<0·001$. Differences between free-fed controls and free-fed aminoguanidine-treated rats or dietary-restricted controls and dietary-restricted aminoguanidine-treated rats #$P<0·05$; ##$P<0·025$.

Table 4. The effects of aminoguanidine treatment and dietary restriction on whole-blood haematology.

	Free-fed rats	
	Control	+Aminoguanidine
Red blood cell count (× 10^8 mL^{-1})	54·6±1·4	53·6±0·5
Red cell volume (fL)	65·2±0·8	66·8±0·6
Red cell haematocrit (%)	40·5±0·7	40·7±0·5
Haemoglobin (g/100 mL)	12·8±0·2	12·9±0·1
White cell count (× 10^6 mL^{-1})	9·26±0·86	8·16±0·84
White cell volume (fL)	65·5±2·5	66·2±2·8
	Dietary-restricted rats	
	Control	+Aminoguanidine
Red blood cell count (× 10^8 mL^{-1})	62·8±1·5**	62·8±1·2
Red cell volume (fL)	61·9±0·3*	62·0±0·8
Red cell haematocrit (%)	44·7±1·3*	44·6±0·8
Haemoglobin (g/100 mL)	14·9±0·3**	14·4±0·2
White cell count (× 10^6 mL^{-1})	7·18±0·58	7·69±0·49
White cell volume (fL)	62·6±2·5	60·6±2·1

All data are means±s.e.m. of 5–6 observations. Differences between free-fed controls and dietary-restricted controls $*P<0·01$, $**P<0·001$. Differences between free-fed controls and free-fed aminoguanidine-treated rats or dietary-restricted controls and dietary-restricted aminoguanidine-treated rats were not significant.

Table 5. The effects of adding aminoguanidine to plasma on the assay of plasma creatinine and urea.

	Plasma analyte	
	Creatinine (μmol L^{-1})	Urea (mmol L^{-1})
Aminoguanidine concn (mg mL^{-1})		
0	52·5±2·0	5·83±0·24
0·10	49·0±1·5*	5·88±0·23
1·00	27·3±0·9**	5·78±0·23
10	<10±0***	5·80±0·26

All data are means±s.e.m. of 4 observations. Differences between controls (without addition of aminoguanidine) and groups with 0·1, 1, or 10 mg aminoguanidine mL^{-1}: $*P<0·05$, $**P<0·01$, $***P<0·001$.

Discussion

The basis of these experiments was the original observations by Baylin et al (1975), who showed that the diamine oxidase

inhibitor aminoguanidine reduces nitrogen excretion in cancer cachexia and promoted tissue growth in laboratory rats. Studies by various other groups have also shown that aminoguanidine has other novel pharmacological effects (Moulinoux et al 1977; Dabrowski & Szczepanowska 1984; Khatami et al 1988; Tamura et al 1989; Nicholls & Mandel 1989). However, there is a paucity of data on its possible effects on plasma analytes. An experimental protocol was therefore devised in which the pharmacotoxicological effects on blood and plasma were investigated in normal free-fed rats. An additional group of dietary-restricted rats was also investigated which represented a model in which nitrogen availability for tissue protein accretion was limiting for optimum growth.

The results of the present investigation have indicated that dietary restriction itself caused various alterations in plasma biochemistry and blood haematology. These included increases in sodium, albumin, erythrocyte count, haematocrit, and haemoglobin. There were also reductions in calcium, phosphate, alkaline phosphatase, urea, triglycerides, creatinine, glucose, and red cell volumes. These presumably occurred as a normal adaptive response, and/or as a result of organ dysfunction. Changes in hydration may also explain increases in individual variables such as erythrocyte count, haemoglobin and haematocrit. This appeared to be supported by the slightly increased albumin concentration as a consequence of dietary restriction: however, water intake relative to body weights was not reduced in the dietary-restricted group.

The only effect attributable to aminoguanidine treatment in free-fed rats was a reduction in plasma creatinine concentration. However, we showed that aminoguanidine interfered with the assay of this substrate. Aminoguanidine concentrations as low as 0·1 mg mL^{-1} reduced the apparent creatinine values by 7% and much greater inhibitions were obtained when the concentration of aminoguanidine was increased. These observations suggest that the aminoguanidine-induced reductions in plasma creatinine concentrations in fed rats may have been an anomaly. Consideration must be given to the question of whether the in-vivo aminoguanidine concentrations attain values as high as 0·1 mg mL^{-1}. Baylin et al (1975) reported that the serum aminoguanidine concentrations were approx. 10 $\mu g\ mL^{-1}$ after rats were fed aminoguanidine for 34 days at 50 mg $kg^{-1}\ day^{-1}$. There do not appear to be other published data on normal plasma levels of aminoguanidine during normal episodic feeding and drinking patterns. However, a factor contributing to plasma aminoguanidine concentrations would be the amount of drinking water consumed by the rat as this will undoubtedly influence peak levels.

In dietary-restricted rats, aminoguanidine reduced plasma sodium and total plasma proteins (largely as a consequence of a fall in albumin), and also increased urea concentrations. We believe the data for urea to be irrefutable as the assay method for this plasma analyte was unaltered by the addition of very high levels of aminoguanidine in-vitro. The contrasting effects in free-fed and dietary-restricted rats suggests that the effects of aminoguanidine on blood biochemistry was dependent upon the prior nutritional state of the animal.

It is possible to ascribe some of the above effects of aminoguanidine treatment to alterations in the biochemistry of individual tissues, as changes in the plasma may reflect specific pathological processes. Increases in plasma urea may occur as a consequence of impaired renal function or as a result of enhanced hepatic ureagenesis. The reduction in plasma albumin (which is synthesized exclusively in the liver) supports the contention that aminoguanidine had additional effects on the liver. In contrast, the activities of those marker enzymes which reflect overt liver damage were not altered by aminoguanidine treatment. Although aminoguanidine was shown to alter sodium concentrations, the changes were very small, i.e. 2%, and it is doubtful if this would be of physiological importance.

Conclusion

The results indicated that nutritional deprivation induced variable changes in blood biochemistry. In general, relatively chronic aminoguanidine treatment did not abate these alterations, and indeed there was tentative evidence to suggest that aminoguanidine treatment itself may have caused mild perturbations in hepatic or kidney biochemistry. The nature of these changes appears to be dependent on a prior nutritional state. There was no evidence of any overt or gross toxicological effects of aminoguanidine.

Acknowledgements

We wish to thank Dr M. A. Yamin for the gift of aminoguanidine. We also wish to acknowledge the unfailing support and encouragement of Professor T. J. Peters. Part of this work was carried out at the Rayne Institute, London SE5, UK.

References

Baylin, S., Horakova, Z., Beaven, M. A. (1975) Increase in food consumption and growth after treatment with aminoguanidine. Experientia 31: 562–564

Bergmeyer, H. U., Horder, M. (1980) IFCC method for alanine aminotransferase. Clin. Chim. Acta 105: 147–154

Bowers, G. N., McComb, R. B. (1975) Measurement of total alkaline phosphatase activity in human serum. Clin. Chem. 21: 1988–1995

Cooper, G. R. (1973) Methods for determining the amount of glucose in blood. CRC Crit. Rev. Lab. Sci. 4: 101–145

Dabrowski, R., Szczepanowska, A. (1984) Alterations in histamine and collagen induced in chick embryos by aminoguanidine and 48/80. Agents Actions 14: 458–460

Daly, J. A., Ertingshausen, G. (1972) Direct methods for determining inorganic phosphate in serum with the "Centrifi Chem". Clin. Chem. 18: 263–265

Forbes, A., Brown, I. R. F., McBain, A. (1985) Mg^{2+} determined with a commercial kit and the Technicon RA1000 analyser. Ibid. 31: 1570

Fossati, P., Prencipe, L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Ibid. 28: 2077–2088

Gitelman, H. J. (1967) An improved procedure for the determination of calcium in biochemical specimens. Anal. Biochem. 18: 521–531

Gornall, A. G., Bardawill, F. J., David, M. W. (1949) Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751–766

Gowenlock, A. H., McMurray, J. R., McLauchlan, D. M., Varley, H. (1988) Varley's Practical Biochemistry. 6th edn, Heinemann Medical Books, London, pp 476–549

Khatami, M., Suldan, Z., David, I., Li, W., Rockey, J. H. (1988) Inhibitory effects of pyridoxal phosphate, ascorbate and aminoguanidine on non-enzymatic glycosylation. Life Sci. 43: 1725–1731

Lustgarten, J. A., Wenk, R. F. (1972) Simple, rapid kinetic method for serum creatinine measurement. Clin. Chem. 18: 1419–1422

Moulinoux, J. P., Fontaine, N., Chambon, Y. (1977) Effects of aminoguanidine sulphate on hepatic and blood levels of histamine after partial hepatectomy in rats. C.R. Soc. Biol. (Paris) 171: 1288–1292

Nicholls, K., Mandel, T. E. (1989) Advanced glycosylation end-products in experimental murine diabetic nephropathy: effect of islet isografting and of aminoguanidine. Lab. Invest. 60: 486–491

Rao, J., Pelavin, M. H., Morgenstern, S. (1972) SMAC: high-speed, continuous-flow, ion-selective electrodes for sodium and potassium; theory and design. In: Advances in Automated Analysis. Technicon International Congress. Vol I, Clinical Chemistry Methods. Tarrytown, New York, pp 33–36

Talke, H., Schubert, G. E. (1965) Enzymatishe harnstoffbestimmung in blut und serum in optischen test nach Warburg. Klin Wochemschr 43: 174–185

Tamura, H., Horiike, K., Fukuda, H., Watanabe, T. (1989) Kinetic studies on the inhibition mechanism of diamine oxidase from porcine kidney by aminoguanidine. J. Biochem. (Tokyo) 105: 299–306

Webster, D. (1977) The immediate reaction between bromcresol green and serum as a measure of albumin content. Clin. Chem. 23: 663–665