Studies on the biochemical effects of glibenclamide on alloxan diabetic rabbits

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Summary. Treatment of alloxan diabetic rabbits with glibenclamide, the most potent of the sulfonylureas, for a period of 2 months, significantly ameliorated the diabetic condition. It produced a decrease in serum and liver lipids, amino acids, serum urea, blood sugar and urine sugar; increase in body weight, serum and liver proteins, liver glycogen, glucose tolerance and serum and liver acid labile phosphates. The possible mechanism of action of this hypoglycemic agent is discussed.

Prolonged use of sulfonylureas has met with several disadvantages compared with the use of insulin, such as hyperlipidemia[1,2], greater incidence of myocardial infarction[3], and unsatisfactory control of postprandial blood sugar[4]. In normal animals also prolonged use of sulfonylureas and phenformin led to hyperlipidemia[5,6]. But a long term detailed study of the effects of a second generation sulfonylurea on diabetic animals has not been conducted so far with a view to analysing the merits and demerits of such effects on carbohydrate, lipid and protein metabolism. Glibenclamide, a member of the second generation sulfonylureas, is 240 times more potent than its classical counterpart tolbutamide[7], and it is very widely used as a hypoglycemic agent in the treatment of diabetes. The present study deals with the effects of glibenclamide treatment on alloxan diabetic rabbits for a period of 2 months.

Materials and methods. Male albino rabbits with an average body weight of 1.4 kg were used for the study. Alloxan diabetes was produced as described previously[8]. After a fortnight rabbits which showed blood sugar levels in the range of 220–260 mg/100 ml were selected for the experiments. They were divided into 2 groups of 6 each, 1 for the control and the other for a glibenclamide test, and their b.wt. recorded. Urine was collected all day from both the groups and its sugar content estimated[9]. Before the drug treatment an initial glucose tolerance test was performed in all the animals as reported earlier[10]. Pure glibenclamide was supplied by Boehringer Knoll Ltd, Bombay. Glibenclamide was administered orally as a fine suspension in water to group 2, the dosage being 0.5 mg/kg b.wt/day. The group 2 animals were fed ad libitum with laboratory diet (Hind lever rabbit feed). After treatment for 2 months the urine sugar and glucose tolerance were again determined in both control and test groups. The animals were weighed, starved over night, sacrificed by cutting the jugular vein and their blood was collected. The livers were quickly removed and transferred to ice cold containers for analysis. Blood sugar was estimated by the method of Asatoor and King[11] using the low alkaline copper reagent of Somogyi[12]. Lipids from the liver and serum were extracted by the method of Entenman[13]. Total cholesterol, phospholipids, triglyceride, and free fatty acids were estimated by different methods[14–17]. Liver glycogen was estimated by the method of Carrol et al.[18]. The inorganic, acid labile and total phosphates of liver and serum were determined by the method of Fiske and Subba Row[19] following the modification suggested by Leloir and Cardini[20]. Total protein in liver and serum were estimated by the biuret method[21]. Serum and liver free amino acids were determined by a combination of the methods described by Sahyun[22] and Frame et al.[23] and serum urea was determined by the method of Natelson[24]. Statistical analyses of the results were made according to the Student's t-test[25].

Results. 1. Body Weights, fasting blood sugar and urine sugar: the results are given in table 1. There was a significant decrease in b.wt. of the control diabetic animals during the 2-month period. Such a decrease in b.wt. was not observed in diabetic rabbits treated with glibenclamide. In contrast, the post-treatment b.wt. was significantly higher than the pretreatment and control weights. The fasting blood sugar and urine sugar significantly increased in the control group compared with the significant decrease in the glibenclamide-treated group. The post-treatment values were also significantly lower than the final control values.

2. Oral glucose tolerance test: treatment with glibenclamide increased the glucose tolerance of diabetic animals. The peak blood sugar value (1.5 h) in the glibenclamide-treated group, 318 ± 13 mg/100 ml, was significantly lower than the corresponding value, 471 ± 12.8 mg/ml, in the control group. However, the 3 h post-glucose blood sugar values did not reach the fasting levels in either group.

3. Glycogen, lipids, proteins, urea, amino acids and phosphates: the values of these parameters, some of which are shown for both serum and liver, are given in table 2. The values of total cholesterol, phospholipids, triglycerides, free fatty acids and free amino acids in serum and liver, and urea in serum of the control diabetic animals, are significantly higher than the normal values. However, the values of glycogen in liver and acid labile phosphates and total proteins in liver and serum of the same group are significantly lower than the normal values (unpublished data). Treatment with glibenclamide ameliorated the diabetic condition and hence significantly altered these parameters compared with those of the control. The values significantly decreased were: cholesterol, triglycerides, free fatty acids and free amino acids in serum and liver and phospholipids in liver and urea in serum. The values significantly increased were: liver glycogen and acid labile

Table 1. Effects of glibenclamide on fasting blood sugar, urine sugar, and body weights of alloxan diabetic animals after 2 months treatment. Values are mean ± S.E. from 6 rabbits of each group

Groups	Control		Glibenclamide-treated		Level of significance between final values
	Initial	Final	Initial	Final	
Fasting blood sugar (mg/100 ml)	239 ± 12	315 ± 11**	244 ± 13	193 ± 13***	$p < 0.001$
Urine sugar (g/24 h)	1.56 ± 0.06	2.55 ± 0.12**	1.6 ± 0.04	0.62 ± 0.03***	$p < 0.001$
Body weights (g)*	1275 ± 4	1100 ± 5***	1293 ± 11	1367 ± 8**	$p < 0.001$

* Body weights decreased on alloxanisation. The weights given are during the period of treatment. ** = increase and *** = decrease, the corresponding levels of significance are shown as follows: ** $p < 0.001$, these values are significantly higher than the initial values. *** $p < 0.001$, these values are significantly lower than the initial values.

phosphates, serum phospholipids and serum and liver proteins.

Discussion. The hypoglycemic action of sulfonylureas has been mainly attributed to their stimulation of the secretion of pancreatic insulin[26] or the release of bound insulin[27,28]. According to some observations sulphonylureas also produce effects independent of insulin action, namely the increased uptake of glucose by the diaphragm[29] and fat pad[30], hypoglycemia without raising plasma insulin levels[31] and amelioration of diabetic symptoms in cases where insulin alone proved inadequate[32]. In maturity-onset diabetes, the common lipid parameters elevated are triglycerides and free fatty acids[33], whereas in alloxan diabetes according to the present study all the lipid parameters are elevated. Although considerable damage to the pancreas occurs in alloxan diabetes, glibenclamide and other sulfonylureas have been found to stimulate insulin secretion in such conditions also[34]. Hence insulin-dependent actions of these drugs are quite possible in alloxan diabetic animals.

Oral glucose tolerance in alloxan diabetic animals improved on treatment with glibenclamide. But the control of either the fasting blood sugar or the post-glucose blood sugar level (3 h) is not satisfactory. It shows that alloxan diabetes cannot be completely controlled even by a very potent hypoglycemic agent like glibenclamide. In the present study the results show that the action of glibenclamide on alloxan diabetes is very similar to that observed in human diabetes[33]. The gain in b.wt., decrease in fasting blood sugar, urine sugar, serum and liver inorganic phosphate, cholesterol, triglycerides and free fatty acid and the increase in liver glycogen, total proteins and acid-labile phosphate and the improvement in glucose tolerance, on treatment of the alloxan diabetic animals with glibenclamide could be attributed to insulin dependent actions of the drug, viz; insulinogenic actions of glibenclamide. Insulin independent actions of the drug as observed in normal animals[6], viz increase in lipids and decrease in liver glycogen and proteins could perhaps not be operative in alloxan diabetes. These differences in the effects of the drug may be due to the entirely different physiological conditions prevalent in alloxan diabetic animals and normal animals. Hence, to establish the steps by which this drug either corrects the abnormalities in the same way as insulin does, or deranges the metabolism by insulin independent or antagonistic actions as observed in normal animals[6], further investigations are warranted.

Table 2. Effects of glibenclamide on serum and liver values of lipids, proteins, amino acids and phosphates and liver glycogen and serum urea of alloxan diabetic rabbits after 2 months treatment. Values are mean ± S.E. from 6 rabbits of each group

Groups	Control	Glibenclamide-treated	Level of significance
Serum	(mg/100 ml)		
Total cholesterol	360±4	229±5*	$p<0.001$
Phospholipids	287±3	303±2**	$p<0.01$
Triglyceride glycerol	21.3±0.6	13±0.3*	$p<0.001$
Free amino acids	3.0±0.11	2.3±0.08*	$p<0.001$
Urea	29.2±1.4	23.7±1.5*	$p<0.05$
	(nEq/ml)		
Free fatty acids	946±34	538.0±32*	$p<0.001$
	(µmoles/ml)		
Inorganic phosphate	1.74±0.10	1.55±0.04	NS
Acid labile phosphate	0.30±0.03	0.41±0.07	NS
Total phosphates	3.5±0.06	3.6±0.12	NS
	(g/100 ml)		
Total protein	5.45±0.1	6.01±0.17**	$p<0.05$
Liver	(mg/100 g wet tissue)		
Total cholesterol	677±11	489±11*	$p<0.001$
Triglyceride glycerol	554±13.5	490±13*	$p<0.05$
Free amino acids	6.52±0.33	4.7±0.31*	$p<0.001$
	(g/100 g)		
Glycogen	0.42±0.02	1.3±0.06**	$p<0.001$
Phospholipids	3.16±0.08	2.90±0.05*	$p<0.001$
Total protein	6.18±0.14	7.04±0.14**	$p<0.01$
	(nmoles/g)		
Free fatty acids	573±19.2	426±12.4*	$p<0.001$
	(µmoles/g)		
Inorganic phosphate	3.59±0.09	3.53±0.07	NS
Acid labile phosphate	0.49±0.07	0.63±0.06**	$p<0.01$
Total phosphate	6.47±0.27	7.03±0.21	NS

* decrease, ** increase, the corresponding levels of significance are shown. NS = not significant.

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