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Effect of glurenorm on immunohistochemical changes in pancreatic β cells of rats in experimental diabetes

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Immunohistochemical localization of islets of Langerhans of streptozotocin (65 mg/kg, ip) induced diabetic + glurenorm (10 mg/kg, po) treated female albino rats revealed increase in number of β cells and insulin immunoreactivity of β cells. The results suggest that glurenorm can cause the stimulation of β cells of endocrine pancreas in diabetic rats.

Keywords: β cell, Diabetes mellitus, Endocrine pancreas, Glurenorm (gliquidone)

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Diabetic treatment is essential for preventing or at least delaying the onset of diabetic complications. Diabetes mellitus can be treated initially with oral antidiabetic drugs such as sulphonylureas, biguanides[1], alpha-glucosidase inhibitors[2], and plant extracts[3]. Glurenorm (Gliquidone) (1-cyclo-hexyl-3-[[4-[2-(3, 4-dihydro-7-methoxy-4, 4-dimethyl-1,3-dioxo-2(1H)-isochinolyl)ethyl]phenyl]sulphonyl]urea, AR-DF 26 SE) is a second generation blood sugar lowering sulphonylurea oral antidiabetic drug, used as insulinotropic in the treatment of hyperglycemia in patients with diabetes mellitus[4-6]. It is metabolised by hydroxylation and demethylation in the liver into inactive compounds, with a half-life of 1.5 hr and 95% of the metabolites are eliminated in the biliary tract and only 5% via the kidneys[6,7]. Sulphonylureas reduce plasma glucose by stimulating endogenous insulin secretion from β cells after binding to their receptors on plasma membrane and enhancing tissue sensitivity to insulin[6-9]. The cellular mechanism of sulphonylurea-induced insulin secretion is controversial and is supposed to enhance β cell secretion. Other mechanism of action of sulphonylureas is extrapancreatic and is mainly on muscular, adipose tissue and liver[10]. No immunohistochemical study on glurenorm and endocrine pancreas has been reported. The purpose of this study is to understand the mechanism of action of glurenorm by studying immunohistochemical changes in pancreatic β cells of normal and streptozotocin (STZ)-induced diabetic (treated and untreated) rats.

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Materials and Methods

Experimental animals and treatment—Healthy, Swiss albino female, 6 to 6.5 months old rats were fed with chow laboratory pellets and given water *ad libitum*. They were randomly divided into following 4 subgroups; two diabetics and two controls: Group I, healthy group (untreated control animals); group II, control group given glurenorm; group III, untreated diabetic group; and group IV, diabetic group given glurenorm. Group II and IV rats were given 10 mg/kg glurenorm (Gliquidone, Eczacıbaşı, Turkey) by the oral gavage daily from day 0 until the end of the experiment at day 42. At the end of the experiment the rats were sacrificed under ether anaesthesia after 18 hr of fasting.

Induction of diabetes in rats—Diabetes was induced in rats by ip injection of streptozotocin (single dose of 65 mg/kg body weight) dissolved in freshly prepared 0.01 *M* citrate buffer (*p*H 4.5)[11].

Immunohistochemical assay—On the 42nd day, the pancreas tissues taken from animals were fixed with Bouin's fixative and embedded in paraffin. The sections were mounted on histogrip-coated glass slides for immunohistochemical studies. An indirect immunohistochemical method was used by following the procedure with the streptavidin-biotin-peroxidase

kit and insulin primary antibody (Zymed, 95-9943, 08-0067). After washing, the sections were incubated with AEC (3-amino-9-ethyl-carbazole) for 5-10 min. Haematoxylin was used as counterstain. Number of immunoreactive β cells was counted by evaluation of 100 islets of Langerhans for sections of each experimental group. The same observer scored all microscopic slides.

Biochemical assays—In order to correlate immunohistochemical changes with blood glucose, blood samples from rats were collected after 18 hr fasting from the tail vein at 0, 7, and 42 days and fasting blood glucose (FBG) levels were determined using the o-toluidine method[12].

Statistical analyses—All results are shown as mean ± SD. The results were evaluated using an unpaired *t*-test and analysis of variance (ANOVA) using the NCSS statistical computer package[13]. The significance among the groups was evaluated by using 'SPSS for windows 10.0' with Mann Whitney U test for number of β cells.

Results

Immunohistochemical results—There was no statistically significant (P=0.469) difference between the two healthy control groups with and without glurenorm regarding the insulin immunoreactivity in pancreatic β cells (Table 1). A decrease in the number of β cells of islets of Langerhans and non-secretory material in β cells giving positive reaction by immunohistochemical reaction in the diabetic group was observed. This decrease was statistically significant when compared to healthy control group and diabetic group given glurenorm (P-0.0001; Table 1). Insulin immunoreactivity was highly depleted in most of β cells. There was a positive reaction in the insulin immunoreactivity only for a few cells or the immunoreactivity was completely absent in β cells of pancreas of untreated diabetic rats (Fig. 1b). But, the number of β cells of islets of Langerhans and insulin immunoreactivity of β cells (Table 1) in the diabetic group given glurenorm increased in comparison to the untreated diabetic group (Fig. 1c) and it was found statistically significant (P=0.0001). But the number of β cells did not reach the level of control group.

Body weight and blood glucose level—The effect of glurenorm on body weight and blood glucose is already reported. However for giving an idea of diabetic status of the animals in the present experiment, these values are given here. The body weight of unhealthy untreated controls increased from 171 to 179 g, while that of the healthy animals treated with glurenorm decreased from 166 to 153 g. In the diabetic groups, the body wwight of untreated animals decreased from 161 to 139 g, while that of the treated diabetic group decreased from 162 to 131 g. There was no significant difference in the initial FBG levels between four groups on the 0 day (P_{ANOVA}=0.181; Table 1). After STZ injection, there was a significant increase in the FBG levels of untreated diabetic rats from 62±12 mg/dl on day 0 to 205±58 mg/dl on day 7, but came down and stabilized at 141±54 mg/dl on day 42 ($P_{t\text{-test}}$=0.0001). In healthy untreated control group, blood glucose levels did not change significantly between at 0, 7 and 42 days ($P_{t\text{-test}}$=0.233). In diabetic+glurenorm group, the FBG

Table 1—Mean levels of body weights and fasting blood glucose at 0, 7 and 42 days and β cell numbers at 42 days for all groups [Values are mean ± SD. No. of animals are given in parentheses]

	C (23)	C_G (18)	D (25)	D_G (13)	P_{ANOVA}
β cell numbers Day 42	36 ± 13	34 ± 12[a]	2 ± 1[b]	19 ± 7[c]	
Weight (g)					
Day 0	171 ± 21	166 ± 6	161 ± 16	162 ± 10	0.172
Day 7	182 ± 26	145 ± 12	151 ± 16	139 ± 13	0.000
Day 42	179 ± 22	153 ± 16	139 ± 21	131 ± 20	0.000
$P_{(t\text{-test})}$	0.278	0.000	0.000	0.000	
Blood glucose (mg/dl)					
Day 0	67 ± 13	64 ± 11	62 ± 12	72 ± 12	0.181
Day 7	62 ± 16	39 ± 8	205 ± 58	184 ± 29	0.000
Day 42	59 ± 16	43 ± 9	141 ± 54	99 ± 14	0.000
$P_{(t\text{-test})}$	0.233	0.000	0.000	0.000	

C=control; C_G=control + glurenorm; D=diabetic; D_G=diabetic + glurenorm

P values: [a]=0.496 *vs* control group, [b]=0.00 *vs* control group; [c]=0.000 *vs* diabetic group

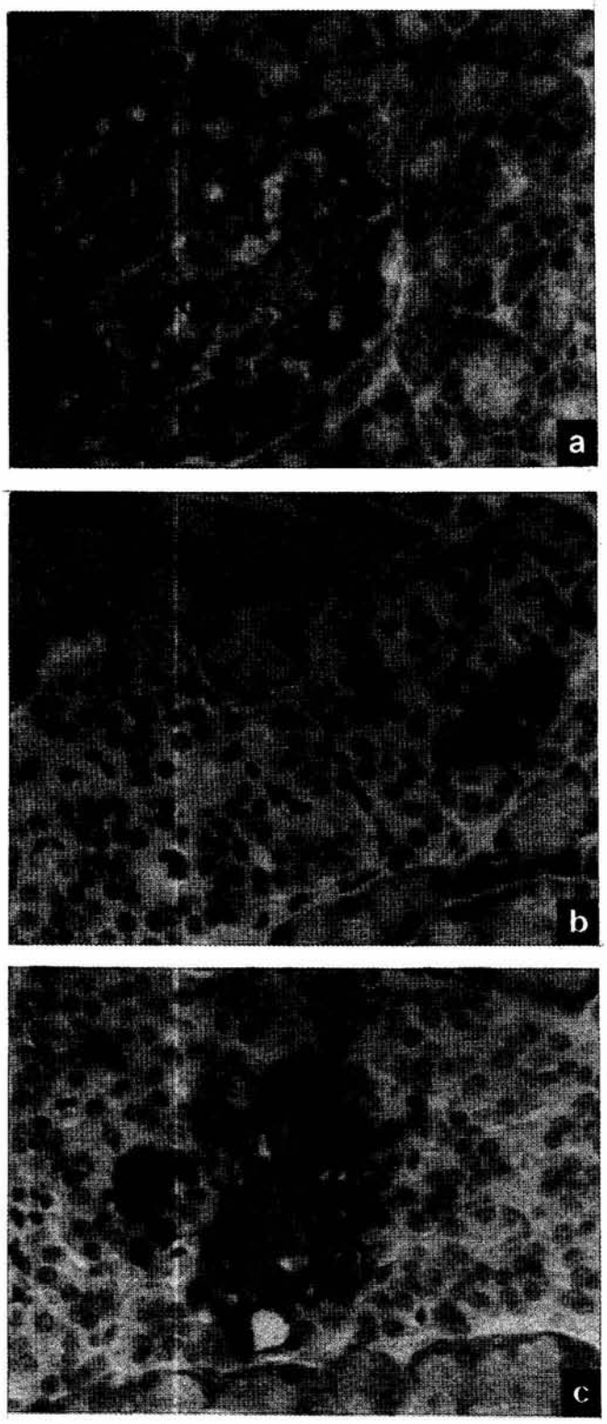

Fig. 1—Insulin immunoreactivity (arrow) in islets of Langerhans in pancreas.[(a) control rat, (b) untreated diabetic group; very few dark stained insulin immunoreactive β cells in islets of Langerhans could be seen, (c) increased insulin immunoreactivity in the diabetic group treated with glurenorm. × 400]

levels increased from 72±12 to 184±29 mg/dl by day 7, whereas in the untreated diabetic group the FBG values increased from 62±12 to 205±58 mg/dl. But by day 42 the FBG values of untreated diabetic group were still very high (141±54 mg/dl), whereas in the treated diabetic group the values came down 99±14 mg/dl which is slightly higher than the 0 day value. In the healthy control animals treated with glurenorm also there was slight fall in FBG from 64±11 to 43±9 mg/dl.

Discussion

The mechanism of action of sulphonylureas is not completely understood. The efficacy of glurenorm (gliquidone) in lowering plasma glucose levels is demonstrated in diabetic patients. This hypoglycemic action of gliquidone seems to occur through either an increased β cell response to glucose stimulus or an enhanced insulin-mediated glucose disposal[14]. Hypoglycemic sulphonylureas stimulate insulin release after binding to receptors on plasma membrane and by facilitating the inflow of Ca^{2+} into the pancreatic β cell[6,15]. The insertion of hypoglycemic sulphonylureas into the phospholipid domain of the β cell membrane could represent a primary event in the mechanism by which the agents stimulate insulin release[15]. It appears to inhibit metabolic disturbances and stimulate regenerating processes in the organs and improve protein-synthetic processes in the pancreas[16].

In present study, the results of light microscopic examination involving diabetic group were in good agreement with the results of other investigators with other drugs[17,18]. In the diabetic group given glurenorm, stimulation of pancreas and increase in the number of β cells of the islets was also indicated.

Glurenorm treated diabetic group of rats showed reduction in FBG levels, which was maximum at day 42. The reduction was about 47%, when compared with spontaneous reduction in FBG of 31% in untreated diabetic group of rats. In the present study, the reduction in the body weight of treated diabetic group of rats is a disadvantage. It appears that glurenorm can cause the stimulation of β cells of pancreas in diabetic rats based, on immunohisto-chemical findings.

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