

Glibenclamide-pregnenolone derivative has greater hypoglycemic effects and biodistribution than glibenclamide-OH in alloxan-rats

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Aim. The present study was designed to investigate the activity of two glibenclamide derivatives on glucose concentration. An additional aim was to identify the biodistribution of glibenclamide derivatives in different organs in a diabetic animal model.

Methods. The effects of two glibenclamide derivatives on glucose concentration were evaluated in a diabetic animal model. In addition, glibenclamide derivatives were bound to Tc-99m using radioimmunoassay methods. To evaluate the pharmacokinetics of the glibenclamide derivatives over time (15, 30, 45 and 60 min) the Tc-99m-glibenclamide conjugates were used.

Results. The results showed that glibenclamide-pregnenolone had greater hypoglycemic activity than glibenclamide or glibenclamide-OH. The data also showed that the biodistribution of Tc-99m-glibenclamide-OH in all organs was less than that of the Tc-99m-glibenclamide-pregnenolone derivative.

Conclusions. The glibenclamide-pregnenolone derivative had greater hypoglycemic effects and its biodistribution was wider than glibenclamide-OH. The data suggest that the steroid nucleus may be important to the hypoglycemic activity of the glibenclamide-pregnenolone derivative and this could be related to the degree of lipophilicity induced by the steroid nucleus in the chemical structure of glibenclamide-pregnenolone.

Key words: glibenclamide, pregnenolone, biodistribution, glucose

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INTRODUCTION

Sulphonylurea drugs have been used for the treatment of patients with diabetes mellitus for several years¹⁻³. Glibenclamide is a sulphonylurea which has been widely used in the management of non-insulin dependent diabetes mellitus⁴⁻⁶. Nevertheless, there are reports that glibenclamide can induce hypoglycemia, even at low doses, especially in the elderly^{7,8}. In addition, drug interactions and renal dysfunction are suspected to contribute to hypoglycemic episodes but little is known about their effect on glibenclamide pharmacokinetics^{9,10} and information on the characteristics of its dose-response relationship is not clear.

To evaluate several pharmacokinetic and pharmacodynamic aspects of glibenclamide, drugs have been used, for example the HB699 compound (4-[2-(5-chloro-2-methoxybenzamide)-ethyl]-benzoic acid), which showed a mechanism similar to glibenclamide for insulin release *in vitro*¹¹. Other studies showed that treatment with glimepiride (a glibenclamide derivative) had similar pharmacokinetic effects to glibenclamide in diabetic pa-

tients¹². Nevertheless, there are data which suggest that glimepiride is associated with fewer episodes of severe hypoglycemia than glibenclamide in diabetic patients. These data suggest that glimepiride has different pharmacokinetics and pharmacodynamics to glibenclamide¹³.

On the other hand, there are studies¹⁴ comparing the pharmacokinetics activity of glipizide (glibenclamide derivative) with glibenclamide. The results suggest that both drugs are metabolized by liver and kidneys which play roles important in their biotransformation and elimination from plasma. These data suggest that glipizide has similar pharmacokinetics than glibenclamide.

Other data¹⁵ indicate that administration of glipizide and glibenclamide in diabetic patients cause changes in glucose metabolism by sustained stimulation of insulin secretion. The effect was greater with glipizide. All these data suggest that glibenclamide and its derivatives exert an effect on glucose concentration and this phenomenon may be dependent on the functional groups involved in the chemical structure of glibenclamide such as chlorine atom bound to the phenyl ring of this drug. To provide this information, the present study was designed to inves-

tigate the effects of two glibenclamide derivatives on glucose concentration and its distribution in different organs in a diabetic rat model.

MATERIAL AND METHODS

General methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of Universidad Autonoma de Campeche (UAC) and were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) (ref.¹⁶). Female rats (Wisstar; weighing 200-250 g) were obtained from UAC.

Reagents

Glibenclamide derivatives were prepared according to a previously reported method¹⁷. Other reagents were obtained from Sigma-Aldrich Chemical Co.

Experimental induction of diabetes in rats: The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. intraperitoneally¹⁸. After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycemia (*i.e.*, with a blood glucose \geq 200 mg/dl) were used for the experiment.

Experimental design and treatment: In the experiment, a total of 60 rats were used. Diabetes was induced in rats, 2 weeks before starting the experiment. The rats were divided into ten groups after the induction of diabetes. Six rats were used in each group (54 diabetic surviving rats, six normal rats) as follows.

Group 1: Normal rats given 2 ml of normal saline.

Group 2: Diabetic control rats given 2 ml of normal saline.

Group 3: Diabetic rats given aqueous solution of glibenclamide (600 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 4: Diabetic rats given aqueous solution of metformin (350 mg/kg body mass) daily had an intragastric tube for 30 days.

Group 5: Diabetic rats given aqueous solution of glibenclamide-OH derivative (300 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 6: Diabetic rats given aqueous solution of glibenclamide-OH derivative (600 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 7: Diabetic rats given aqueous solution of glibenclamide-OH derivative (1200 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 8: Diabetic rats given aqueous solution of glibenclamide-pregnenolone derivative (300 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 9: Diabetic rats given aqueous solution of glibenclamide-pregnenolone (600 μ g/kg body mass) daily with intragastric tube for 30 days.

Group 10: Diabetic rats given aqueous solution of glib-

enclamide-pregnenolone (1200 μ g/kg body mass) daily with intragastric tube for 30 days.

Biochemical assays

Measured in acute form: Blood glucose was determined from tail blood with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche, U.S.A.) every 48 h.

Radiochemical study

The glibenclamide derivatives were bound to Tc-99m using radioimmunoassay methods^{19,21}. A solution of 20 mg of two glibenclamide derivatives in 1.0ml was adjusted to pH 7.0 with 0.1M NaOH. The solution was then added to another freshly prepared solution (75 ml) of stannous chloride (2mg/ml in 0.1M HCl) and the pH was readjusted to 7.0. In addition, two ml of a Tc-99m pertechnetate solution eluted from a sterile ⁹⁹Mo-99m-Tc shielded generator was added to the mixture solution.

Quality Control

Thin Layer Chromatography was used to quality control²². The labeling efficiencies with Tc-99m were evaluated chromatographically using a Silica-gel 60 F254 plate. To determinate the radiochemical purity of compound studied, a solvent system such as acetonitrile: water (4:1) was used. The plates were counted by images in a gamma camera equipped with a high resolution collimator with a digital computer (VP450). The Rf values were determined using as control Tc-99mpertechnetate and hydrolyzed Tc-99m colloid. The purities of Tc-99m-conjugates were determined by paper electrophoresis. The paper strips were run at a constant voltage of 600 V for 30 min using a buffer solution (0.1M, pH 7.4). The paper strips were counted by images in a gamma camera equipped with a high resolution collimator with a digital computer. Movement was determined relative to Tc-99m pertechnetate and hydrolyzed Tc-99m colloid.

Biodistribution study

Six rats per group were used for each biodistribution study. Each diabetic rat received 0.3 ml (200 μ Ci, 1.3mg) of Tc-99m glibenclamide-OH and Tc-99m glibenclamide-pregnenolone derivative (200 μ Ci, 1.3mg) by tail vein administration. Sequential scintigrams were taken at predetermined intervals (15, 30, 45 and 60 min) with a gamma camera equipped with a high resolution collimator with a digital computer. The animals were sacrificed and the organs were removed and the radioactivity was counted by images in a gamma camera equipped with a high resolution collimator with a digital computer. The percentages of the injected dose per organ were determined by comparison of tissue radioactivity concentration with the total radioactivity. Additionally, the blood (ml) in the heart was collected to evaluate the radioactivity with the same equip.

Statistical analysis

All the experimental data were statistically evaluated and the significance of various treatments was calculated

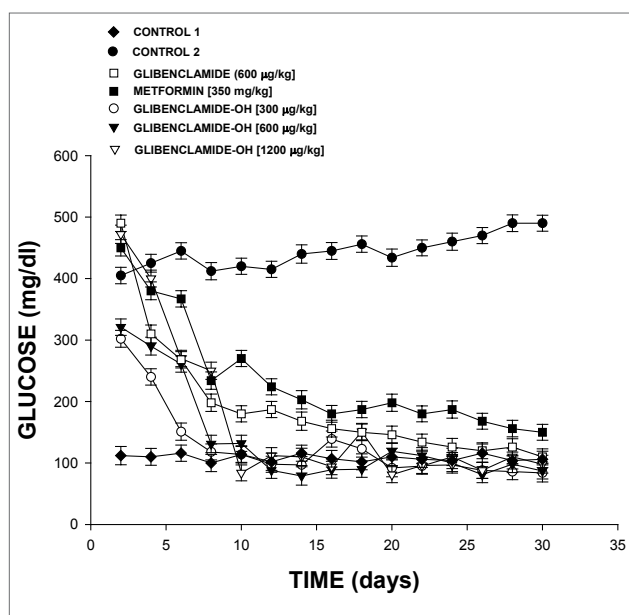


Fig. 1. Activity induced by glibenclamide-OH derivative on glucose concentration in a diabetic rat model. The results showed that glibenclamide-OH significantly reduced ($P=0.05$) the blood glucose concentration at a dose of 300 µg/kg in comparison with glibenclamide and metformin. The effects are expressed as mean \pm S.E. $n = 6$.

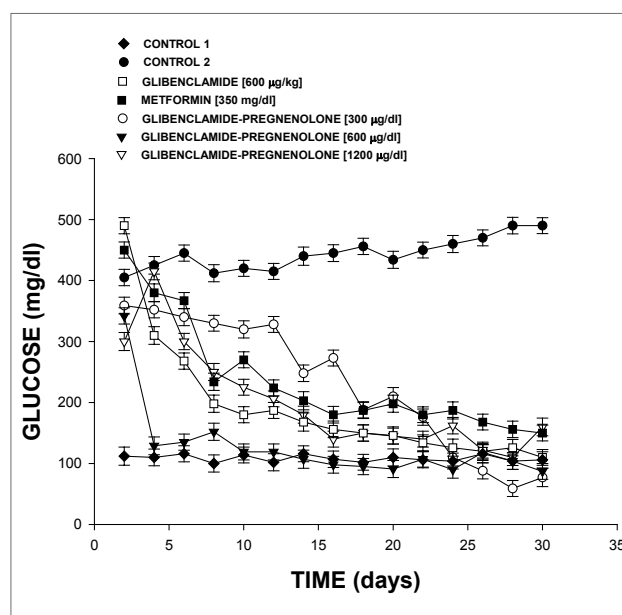


Fig. 2. Effect exerted by glibenclamide-pregnenolone derivative on glucose concentration in a diabetic rat model. The results showed that glibenclamide-pregnenolone significantly reduced ($P=0.05$) the blood glucose concentration at a dose of 600 µg/kg in comparison with glibenclamide and metformin. The effects are expressed as mean \pm S.E. $n = 6$.

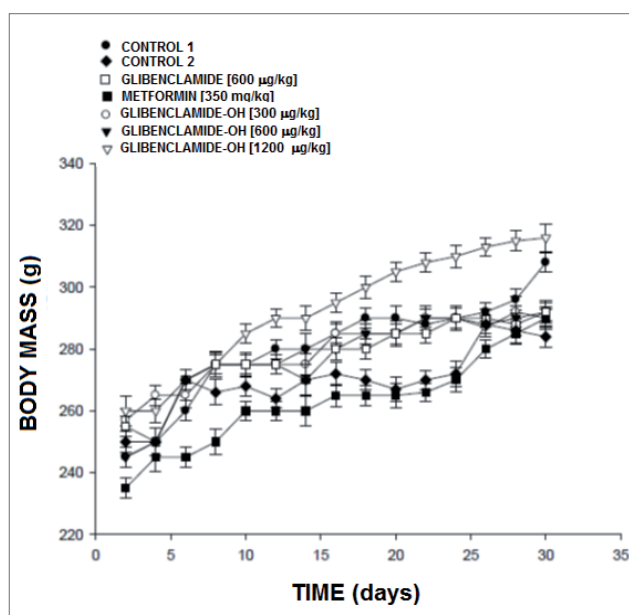


Fig. 3. Activity induced by glibenclamide-OH derivative on the body mass in a diabetic rat model. The results showed that glibenclamide-OH significantly increase ($P=0.05$) the body mass levels at a dose of 1200 µg/kg in comparison with glibenclamide and metformin. The effects are expressed as mean \pm S.E. $n = 6$.

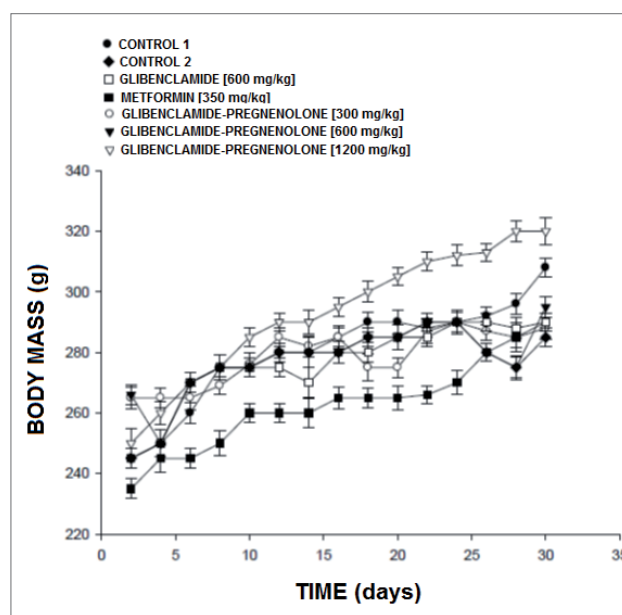


Fig. 4. Effect exerted by glibenclamide-pregnenolone derivative on the body mass in a diabetic rat model. The results showed that glibenclamide-pregnenolone significantly increase ($P=0.05$) the body mass levels at a dose of 1200 µg/kg in comparison with glibenclamide and metformin. The effects are expressed as mean \pm S.E. $n = 6$.

Table 1. Biodistribution of Tc-99m-glibenclamide-OH derivative (μCi). Each value is mean \pm S.E. $n = 6$.

Organ	15 min	30 min	45 min	60 min
Brain	71.75 \pm 2.67	70.33 \pm 2.24	72.47 \pm 2.34	71.80 \pm 2.68
Spleen	10.34 \pm 2.43	10.24 \pm 2.64	9.56 \pm 2.12	10.09 \pm 2.98
Stomach	8.03 \pm 1.68	8.09 \pm 1.53	8.27 \pm 1.87	8.38 \pm 2.78
Intestine	2.94 \pm 2.02	3.21 \pm 1.28	2.87 \pm 1.24	2.39 \pm 1.22
Liver	2.31 \pm 1.87	2.62 \pm 1.42	2.42 \pm 1.76	2.82 \pm 1.56
Kidney	2.04 \pm 1.66	3.46 \pm 1.76	2.82 \pm 1.53	3.19 \pm 1.34
Gonads	4.02 \pm 1.53	3.88 \pm 1.22	3.70 \pm 1.68	3.02 \pm 1.53
Blood	2.64 \pm 1.88	2.02 \pm 1.06	1.56 \pm 1.26	1.44 \pm 1.02

Table 2. Biodistribution of Tc-99m-glibenclamide-pregnenolone conjugate (μCi). Each value is mean \pm S.E. $n = 6$.

Organ	15 min	30 min	45 min	60 min
Brain	188.71 \pm 2.67	178.02 \pm 2.24	170.02 \pm 2.34	171.80 \pm 2.68
Spleen	12.65 \pm 2.43	12.02 \pm 2.64	11.02 \pm 2.12	10.09 \pm 2.98
Stomach	10.82 \pm 1.68	10.24 \pm 1.53	9.27 \pm 1.87	9.02 \pm 2.78
Intestine	5.76 \pm 2.02	6.02 \pm 1.28	5.04 \pm 1.24	4.56 \pm 1.22
Liver	8.30 \pm 1.87	7.70 \pm 1.42	7.02 \pm 1.76	6.90 \pm 1.56
Kidney	3.91 \pm 1.66	4.42 \pm 1.76	3.42 \pm 1.53	3.19 \pm 1.34
Gonads	16.02 \pm 1.22	15.01 \pm 1.87	14.22 \pm 2.64	13.32 \pm 1.68
Blood	3.88 \pm 1.88	3.22 \pm 1.06	2.02 \pm 1.26	1.88 \pm 1.02

using Student's *t*-test. All the results were expressed as mean \pm S.E.

RESULTS

Glucose concentration

The results shown in (Fig. 1) indicate that both glibenclamide (490 to 110 mg/dl; $P=0.05$) and metformin (450 to 150 mg/dl; $P=0.06$) significantly diminished the blood glucose concentration in comparison with diabetic control rats (405–490 mg/dl). In addition, other results show that glibenclamide-OH significantly decreased the blood glucose concentration in doses of 300 $\mu\text{g/kg}$ (499 to 94 mg/dl; $P=0.05$), 600 $\mu\text{g/kg}$ (442 to 97 mg/dl; $P=0.05$) and 1200 $\mu\text{g/kg}$ (450 to 110 mg/dl; $P=0.06$). Other data indicate that glibenclamide-pregnenolone significantly reduced the blood glucose concentration (Fig. 2) in doses of 300 $\mu\text{g/kg}$ (456 to 92 mg/dl; $P=0.06$), 600 $\mu\text{g/kg}$ (452 to 77 mg/dl; $P=0.05$) and 1200 $\mu\text{g/kg}$ (468 to 116 mg/dl; $P=0.06$).

Body mass levels

The results show variations in body mass levels for the group 1 of 245 to 308 g and for group 2 of 245 to 285 g (Fig. 3). In addition, other results showed that glibenclamide-OH derivative at different doses show variations in the body mass levels in 300 $\mu\text{g/kg}$ (257 to 290 g), 600 $\mu\text{g/kg}$ (245 to 292 g) and 1200 $\mu\text{g/kg}$ (260 to 316 g). Other

results indicate that the glibenclamide-pregnenolone derivative (Fig. 4) induces changes in body mass at doses of 300 $\mu\text{g/kg}$ (265 to 288 g), 600 $\mu\text{g/kg}$ (266 to 295 g) and 1200 $\mu\text{g/kg}$ (250 to 320 g).

Radiochemical study

Thin Layer Chromatography method shows that glibenclamide derivatives were bound to Tc-99m ($\cong 90\%$) under the conditions previous described. The purities of Tc-99m-glibenclamide conjugates determined by paper electrophoresis showed a value of R_f of 0.69 for Tc-99m-glibenclamide-OH derivative and a R_f of 0.78 Tc-99m-glibenclamide-pregnenolone derivative.

Pharmacokinetics activity

The biodistribution of Tc-99m-glibenclamide-pregnenolone and Tc-99m-glibenclamide (Fig. 5, Table 1,2) showed the following; 1) the biodistribution of the Tc-99m-glibenclamide derivatives was significantly higher in the brain than in spleen, stomach, intestine liver and kidney; 2) The levels of Tc-99m-glibenclamide-OH in all organs was less than Tc-99m-glibenclamide-pregnenolone.

DISCUSSION

In this study, the activity of two glibenclamide derivatives on glucose concentration in a diabetic animal model was evaluated. Diabetes in the animals studied was in-

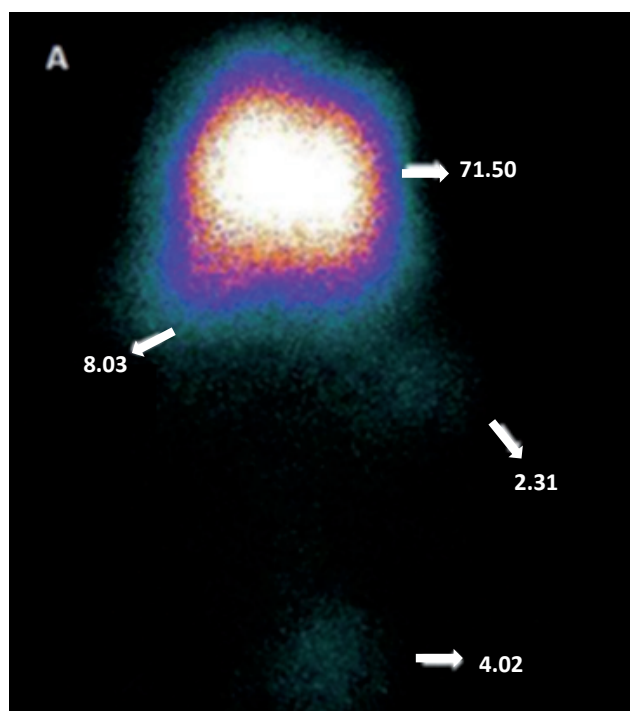


Fig. 5. Scintigrams (μCi) were taken 15 min after the administration of the Tc-99m-glibenclamide-OH derivative. The Tc-99m-glibenclamide-OH derivative (A) show 71.75 μCi different values in brain in comparison with other organs such as stomach (8.03 μCi), liver (2.31 μCi) and gonads (4.02 μCi).

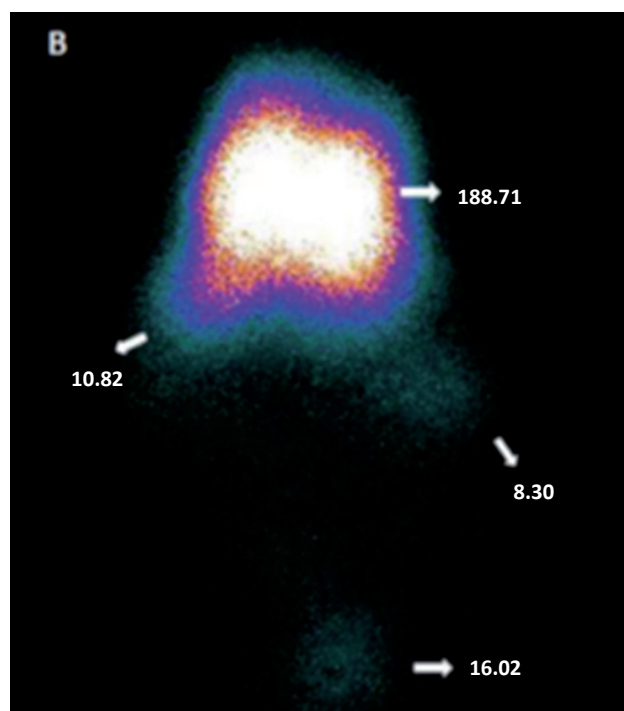


Fig. 6. Scintigrams (μCi) were taken 15 min after the administration of the Tc-99m-glibenclamide-OH derivative. The Tc-99m-glibenclamide-pregnenolone conjugate (B) show 188.71 μCi different values in brain in comparison with other organs such as stomach (10.82 μCi), liver (8.30 μCi) and gonads (16.02 μCi).

duced with alloxan. Alloxan is reported to cause massive reduction in insulin release, through the destruction of β -cells of the islets of Langerhans which consequently causes an indirect increase in the glucose concentration²³. Changes in plasma glucose concentration of different groups studied were then determined (Fig. 1, 2).

Glucose concentration

Glibenclamide-OH effect on glucose concentration was higher than metformin possibly because it involves a different molecular mechanism in terms of hypoglycemic activity. The effect of glibenclamide-OH was similar to that of glibenclamide. These results suggest that the chlorine atom bound to the phenyl group of glibenclamide is not specific for hypoglycemic activity. Analyzing these data, in this study the glibenclamide-pregnenolone derivative was used as a pharmacological tool to evaluate this hypothesis. This compound has as a steroid nucleus in its structural chemical. The results showed that it also affected glucose concentration but showed higher hypoglycemic activity than glibenclamide or glibenclamide-OH in a short time at a dose of 600 $\mu\text{g}/\text{kg}$. These data suggest that the steroid nucleus it is important for the hypoglycemic effects of the glibenclamide-pregnenolone derivative and possibly this is conditioned by degree of lipophilicity induced by steroid nucleus. This premise is supported by other studies²⁴ which indicate that changes in the structural chemical of glibenclamide to form some glibenclamide derivatives such as glimepiride and gli-

zide show variations in degree of lipophilicity and changes in glucose concentration.

Body mass levels

To evaluate whether changes in glucose concentration were related to variations in body mass, this study evaluated the body mass of animals studied. Recurrent hypoglycemia induced by glibenclamide, metformin and the glibenclamide derivatives (300 and 600 $\mu\text{g}/\text{kg}$) had no significant overall effect on weight gain or adiposity. Nevertheless, at a dose of 1200 $\mu\text{g}/\text{kg}$ for both glibenclamide derivatives the body mass was higher than other groups studied. This data suggests that high dose of glibenclamide derivatives could induce changes in body mass, possibly through activation of some molecular mechanism such as occurs in another compounds²⁵.

Pharmacokinetics activity

Analyzing the results previously described, in this study was evaluated the biodistribution of glibenclamide derivatives using radioimmunoassay methods¹⁹⁻²¹. In this technique the glibenclamide derivatives were easily labeled with Tc-99m by the conventional stannous chloride method. The glibenclamide conjugates involved in this study are excellent chelating agents, with both carbonyl and hydroxyl groups binding to Tc-99m.

On the other hand, to evaluate the pharmacokinetics of the glibenclamide derivatives as a consequence of increases in time (15, 30, 45 and 60 min) the Tc-99m

glibenclamide conjugates were used. The results indicate that the biodistribution of the glibenclamide derivatives was significantly higher in brain than in spleen, stomach, intestine liver and kidney although there were differences between the two glibenclamide derivatives in each organ studied. All these data suggest; 1) The glibenclamide derivatives were initially distributed to all tissues, including non-target sites; 2) the concentration of the glibenclamide conjugates in brain was higher than in other organs. This phenomenon may be conditioned by interaction between the glibenclamide derivatives and some endogenous substances involved in the brain; 3) the higher concentration of the glibenclamide-pregnenolone derivative in comparison with glibenclamide-OH may depend on degree of lipophilicity from the glibenclamide-pregnenolone derivative, possibly as a consequence of degree of lipophilicity exerted by the steroid nucleus involved in the chemical structure of this compound.

CONCLUSIONS

Glibenclamide-pregnenolone exerted greater hypoglycemic effects and its biodistribution was greater than glibenclamide-OH. In addition, the biodistribution of glibenclamide-pregnenolone was different from glibenclamide-OH. These data suggest that the steroid nucleus may be important to the hypoglycemic effects of glibenclamide-pregnenolone and this phenomenon could be related with degree of lipophilicity induced by steroid nucleus involved in the chemical structure of glibenclamide-pregnenolone.

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