

EFFECT OF ACETAZOLAMIDE ON INSULIN SENSITIVITY IN DOGS WITH ALLOXAN DIABETES

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Summary: The effect of acetazolamide on the sensitivity to exogenous insulin in the alloxanised diabetic dogs was studied. The administration of acetazolamide caused acidosis and insulin resistance. The liver insulinase activity of diabetic dogs after acetazolamide administration was also studied to evaluate the role of this enzyme for the destruction of exogenous insulin. It was observed that insulinase did not play role for the development of insulin resistance after acetazolamide administration.

Key words: acetazolamide insulin resistance
 insulinase alloxanised diabetic dog

INTRODUCTION

There are many reports suggesting that insulin fails to bring the desired hypoglycaemic effect in ketoacidotic condition. Reports are available to show that acidosis *per se* blunts the action of insulin. Mackler *et al.* (3,4) produced severe acidosis in dogs with ammonium chloride and these animals were refractory to exogenous insulin and there was deterioration in glucose tolerance. At the same time Baird (1) could not detect any alteration in carbohydrate metabolism in non-diabetic humans where acidosis was induced by ammonium chloride and acetazolamide. Similar type of observations has been made in normal rats by Frost and Reaven (2).

Thus there is a lack of consensus of opinion with regard to the glucose tolerance and the activity of exogenous insulin in metabolic acidotic subjects. In the present study, an attempt was made to investigate whether acetazolamide acidosis without other complex factors of ketonaemic acidosis could produce insulin resistance in diabetic dogs. The second object of the study was to evaluate the activity of insulinase enzyme in the livers of acetazolamide acidotic diabetic dogs.

MATERIALS AND METHODS

Twelve dogs (4 to 7 kg) of both sexes and free from diabetes mellitus were used in this study. They were housed in separate cages and were offered standard laboratory diet for 7 days. These animals were injected through radial vein with alloxan monohydrate (Kochlite)

at the dose of 115 mg/kg as 10% aqueous solution. The diabetes mellitus was established after 24 hr of alloxan injection which was confirmed by glycosuria and hyperglycaemia. Then the animals were paired according to their nearest body weight. One group served as control; the animals of experimental group were fasted for 48 hr (except water). During the same period 250 mg acetazolamide (DIAMOX-Lederle) was administered orally at 6 hr interval for eight times to produce acidosis. For the determination of blood pH the syringe and needle were moistened with heparin and dead space filled. The needle was inserted into the radial vein and blood was allowed to flow into the syringe and no suction was applied. The needle and syringe were removed from vein; the needle was detached from syringe and blood was discharged under liquid paraffin in a container and blood pH was determined immediately with Systronic pH meter type 322 (± 0.05 pH units). Immediately after blood pH determination, the glucose-insulin tolerance test was conducted according to Varley (9) in control and experimental group.

Glucose was administered orally through stomach tube at the dose of 0.8 g/kg as 8% aqueous solution. Immediately after glucose administration, plain insulin (Boots Co.) at the dose of 0.1 U/kg was injected through the radial vein. After insulin administration blood was drawn from the radial vein at 30, 60, 90 and 120 min and blood glucose was estimated according to Nelson (6) and Somogyi (7). The index of sensitivity of exogenous insulin was studied by observing blood sugar lowering effect at 30, 60, 90 and 120 min after glucose-insulin tolerance test.

The liver insulinase activity of six alloxan diabetic dogs with acetazolamide administration and six alloxan diabetic dogs without acetazolamide administration was studied. These animals were subjected to similar experimental conditions as during the study of the effect of acetazolamide and insulin sensitivity. The following procedure was adopted for the study of insulinase activity according to Mirsky and Broh-Khan (5). The animals were sedated with trifluromazine hydrochloride (SIQUIL Sarabhai) which was injected through the radial vein at the dose 4 mg/kg. Then both jugular veins were severed for complete bleeding. The abdomen was opened and liver pieces free from adjacent tissues were rapidly removed. The liver tissues weighing 0.5 g from each dog were taken and blended immediately in a Waring blender with 5 ml of ice water exactly for 2 min. The homogenates were centrifuged in a cold room for 5 min and supernatant fluid decanted through gauze. The extract so obtained was adjusted to pH 7.5. 1 ml of the extract was mixed with 0.1 ml (4 U) of plain insulin (Boots Co.) and incubated for 30 min at 37°C. Then the mixtures were injected subcutaneously into fasted rabbits. The destruction of insulin in each mixture was estimated by the determination of blood sugar lowering effect in rabbits of insulin at 60 and 120 min after injection.

RESULTS

Table I shows that acetazolamide administration altered the sensitivity to exogenous insulin. The decreases in blood sugar of acetazolamide treated group were 7.42%, 9.44%,

3.43% and 9.7% at 30, 60, 90 and 120 min respectively after glucose-insulin tolerance test, while those in the control group (without acetazolamide), they were 20.80%, 17.88%, 20.49% and 17.79% at 30, 60, 90 and 120 min respectively. The blood pH value of acetazolamide treated group ranged from 7.1 to 7.2 with a mean value of 7.17 ± 0.2 and the blood pH value of control group (without acetazolamide) ranged from 7.3 to 7.4 with a mean value of 7.36 ± 0.02 . Analysis of variance of blood sugar levels at 30, 60, 90 and 120 min between acetazolamide-treated group and control group (without acetazolamide) gave "F" values (18.85, 13.19, 12.46, 24.85 respectively) that were highly significant at 1% level.

TABLE I: Blood glucose level after glucose-insulin tolerance test in alloxan diabetic dogs with acetazolamide treatment and in alloxan diabetic dogs without acetazolamide treatment.

Period in min	Group without acetazolamide	Group with acetazolamide
	Glucose mg% (mean \pm S.E.M.)	
Initial	550.83 \pm 9.83	577.5 \pm 19.88
After 30 min of glucose-insulin tolerance test	436.25 \pm 17.81 (-20.80%)	534.6 \pm 17.31 (-7.42%)
After 60 min of glucose-insulin tolerance test	358.25 \pm 22.13 (-17.88%)	484.1 \pm 33.38 (-9.44%)
After 90 min of glucose-insulin tolerance test	284.83 \pm 30.87 (-20.49%)	467.5 \pm 26.39 (-3.43%)
After 120 min of glucose-insulin tolerance test	234.16 \pm 30.95 (-17.79%)	422.08 \pm 27.30 (-9.7%)

Minus values in parentheses depict decreases in relation to immediate preceding values.

The insulin destroying effect (insulinase) of liver tissues of alloxan diabetic dogs with and without acetazolamide was studied. The decreases in blood sugar in fasted rabbits from fasting level injected with 4 U of insulin and pre-treated with liver extracts of alloxan diabetic dogs without acetazolamide treatment were $41.87 \pm 1.45\%$ and $45.56 \pm 1.25\%$ at 60 and 120 min respectively, while those in rabbits injected with 4 U of insulin and pre-treated with liver extracts of alloxan diabetic dogs treated with acetazolamide were $40.20 \pm 1.58\%$ and $43.47 \pm 1.44\%$ at 60 and 120 min respectively.

DISCUSSION

The retardation of fall in blood sugar after the acetazolamide administration in alloxan diabetic dogs has been recorded in this investigation. The anti-insulin activity of acetazolamide was not mediated through the insulin-destroying enzyme insulinase. One reason for this anti-insulin activity of acetazolamide may be due to the alteration of blood pH towards the acidosis. The present findings are in agreement with that of Mackler *et al.*(3) who observed a retardation of fall in blood sugar in dogs with ammonium chloride acidosis. The findings of the present

investigations do not corroborate with that of Baird (1) who did not observe any deterioration in glucose tolerance in acidotic non-diabetic humans. The negative findings of Baird (1) might be because he used non-diabetic subjects. Besides, the animals were kept in complete fasting in the present study. It is possible that the effect of acetazolamide and fasting may be responsible for the development of insulin resistance.

Acetazolamide is a carbonic anhydrase inhibitor and it also causes acidosis. The condition which is established in alloxan diabetic dogs after acetazolamide treatment may not simulate the conditions which are encountered in human diabetic acidosis. The findings obtained in the present study may not be similar with the situation occurring in human diabetic acidosis where there is a marked rise of keto-acids and the condition is now successfully managed by a small dose of insulin by intravenous drip.

The acidotic condition is definitely a stress condition. In acidosis high level of cortisol can be expected and may mediate anti-insulin activity. It has indeed been demonstrated that cortisol secretion is directly correlated with the degree of acidosis (8).

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