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Thiopropanol Induced Changes in Glycogen Breakdown in Alloxan Diabetic Liver

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Abstract: Liver glycogen content and liver glycogen synthesis are lowered in diabetes mellitus due to lack of functioning insulin. Many enzymes of glycogen metabolism as well as glucose metabolism are sulfhydryl in nature and are affected by changes in cellular thiol-disulfide ratio. Certain low molecular weight thiols can influence glucose uptake and utilization in fat cells and in muscle cells. A study was undertaken to establish the effect of thiopropanol(3-mercapto 1-propanol) on glycogen breakdown in isolated alloxan diabetic liver. The results indicate that thiopropanol influences glycogen breakdown, lactic acid production in alloxan diabetic liver which may be attributed to increased activity of hexokinase in thiopropanol-exposed-alloxan diabetic liver.

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I. INTRODUCTION

Glycogen, a stored polysaccharide of liver, is the principal available source of glucose for hepatic as well as other cells in mammalian systems including human beings. It is observed that glycogen synthesis is lowered in liver in diabetes mellitus which may be probably due to lack of insulin as insulin is known to favour liver glycogenesis [1,4,19,21]. This lowered liver glycogenesis in part may also due to decreased cellular thiol concentration which is reciprocal to an elevated reactive oxygen species (ROS), a common phenomenon observed in diabetes mellitus [13,15]. It has been recognized that the stimulatory action of insulin on glucose transport in muscle[5,6] and fat cells[7,12,14] is sensitive to perturbation of cellular sulfhydryl groups. Some earlier workers [23] have shown that certain low molecular weight thiols may mimic some of the actions of the insulin in fat cells. In order to establish the possibility of

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similar effects of thiols in liver, a study was undertaken to assess the effect of thiopropanol (3-mercapto 1-propanol) on glycogen breakdown in isolated alloxan diabetic liver slices.

II. MATERIALS AND METHODS

a) Chemicals:

All the chemicals employed were of analar grade (AR). Alloxan was obtained from Loba chemicals. Thiopropanol was procured from Sigma-Aldrich chemicals Pvt. Ltd. USA.

b) Experimental Animals:

Male albino rats (*Rattus norvegicus*) in the weight range 150-250 g were selected randomly from the stock colony of animal house of Basaveshwara Medical College & Hospital, Chitradurga were employed in the present study. The chosen animals were housed in plastic well aerated cages at normal atmospheric temperature (25 ± 5 °C) and normal 12- hour light/dark cycle. The rats were maintained on standard stock diet (Amruth Rat Feed, manufactured and supplied by Pranav Agro Industries, Pune, India). The feed and the tap water were given *ad libitum*.

c) Induction of Diabetes:

Diabetes was induced into the 12 hours fasted rats with a single intraperitoneal injection of freshly prepared aqueous Alloxan monohydrate (150 mg per kg body weight) [2, 22]. The onset of diabetes was monitored 48 hours after alloxan treatment by using standard Urine Glucose Strips(from Qualigens).The rats, whose urine showing positive for glucose for 3 consecutive days were labeled diabetic and were used in the present work.

d) Experimental Design:

The rats were divided into two groups.

i. Normal group– consisting of 6 male albino rats maintained on stock lab diet and tap water *ad libitum*.

ii. Diabetic group – consisting of 6 male albino alloxan diabetic rats maintained on stock lab diet and tap water *ad libitum*.

The rats of both the groups were anesthetized and sacrificed after 30 days. They were immediately dissected, the liver tissue was procured, washed and refrigerated with PBS (phosphate buffered saline) pH 7.4 has to be added before at 0-2°C till further use. The liver

0.5g each and these slices were employed in the present work. Glycogen levels [9], Lactic acid levels [3] and hexokinase activity [18] were estimated both at zero minute as well as at 60 minutes interval in normal rat liver slices (0.5g), in alloxan diabetic liver slices, as well as in alloxan liver slices exposed to thiopropanol (5mg thiopropanol / 0.5 g)

The glycogen breakdown/depletion per hour was estimated by incubating a known weight (0.5 g) of normal / alloxan liver tissue in isotonic phosphate buffer, pH 7.4, for 1 hour at 37 °C in a thermostatic water bath. The glycogen content was estimated both at 0 minute and at 60 minutes to know the per hour glycogen breakdown/depletion. The experiments were repeated with thiopropanol- exposed - alloxan diabetic liver tissue to know its effect on glycogen breakdown. Lactate production per hour was also estimated in the same way as explained above.

e) *Ethical Considerations:*

The animal experiments were conducted as per the norms of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi and ethical clearance was obtained from IAEC (Institutional Animal Ethical Committee) of Basaveshwara Medical College.

f) *Data management and statistical analysis:*

The data entry was carried out using Microsoft Office Excel worksheet and statistically analyzed. The P value was calculated by student 't' test.

III. RESULTS

The results of the present study are given in table-1. It is evident from the table that the glycogen breakdown, lactate production are significantly lowered ($p < 0.001$) in diabetic liver tissue (group-2) as compared to normal liver tissue (group-1), where as these parameters are significantly elevated ($p < 0.001$) in thiopropanol -exposed -alloxan diabetic liver tissue (group-3) as compared to control diabetic liver tissue (group-2) showing there is a stimulation of glycogen breakdown in alloxan diabetic liver in presence of thiopropanol. It is also evident from the table that liver tissue hexokinase activity is significantly lowered ($p < 0.001$) in group-2 as compared to group-1 but the hexokinase activity is significantly raised ($p < 0.001$) in group-3 as compared to group-2 showing that thiopropanol might have favored liver tissue hexokinase activity.

IV. DISCUSSION

The glycogen stored in liver, in fed state, approximately amounts to 5% of the wet weight of liver tissue. Insulin favors glycogen synthesis in liver by keeping the glycogen synthase, the key enzyme of

glycogenesis, in the active state [1,19]. Glycogenolysis usually occurs to provide glucose when there is a decrease in the available glucose, which promptly mediated by active glycogen phosphorylase. Many enzymes of glycogen breakdown and of glucose catabolism are thiol enzymes and are affected by tissue redox systems as well as by the available free thiols in the tissue [24]. As seen in the table the glycogen content of liver as well as glycogen breakdown after an hour of incubation at 37 °C is significantly decreased in group-2 probably due to lack of insulin as alloxan effectively damages the beta cells of Islets of Langerhans of pancreas [22], hence there is no available insulin thus glycogenesis is lowered and glycogen content is low in alloxan diabetic liver.

Glycogen is broken down to glucose-1-phosphate by glycogen phosphorylase, further converted to lactate via glycolytic pathway. It is evident from the table that lactate produced in group-2 is significantly low ($p < 0.001$) compared to group-1, indicating that in alloxan diabetic rat liver not only the percentage of glycogen breakdown per hour but also the rate of glycolysis is significantly lowered in diabetic liver as compared to normal liver slices, which may be attributed to the lack of insulin as insulin activates the enzymes of glycolytic pathway [20]. The addition of 5 mg thiopropanol/0.5g liver tissue slice significantly increases the glycogen breakdown ($p < 0.001$), lactate production ($p < 0.001$), as well as hexokinase activity ($p < 0.001$) in group-3 as compared to group-2. The key enzymes of glycolytic pathway namely hexokinase, phosphofructokinase and pyruvate kinase are known to be inhibited by smaller disulfides and are reactivated by glutathione and other thiols [10,11,16,17,24] indicating that these enzymes are sulphhydryl in nature. The results obtained in the present study (ref. table-1) indicate that the liver hexokinase activity in group-3 is significantly higher as compared to liver hexokinase activity in group-2. This clearly indicates that thiopropanol, probably similar to GSH (reduced glutathione) might have favored the activity of hexokinase thus promoting the glucose utilization through glycolytic pathway.

A similar favorable action of thiopropanol with respect to glycogen phosphorylase kinase enzyme might have increased the activity of phosphorylase kinase and hence the activity of glycogen phosphorylase thus favoring the glycogen utilization in group-3 (ref. table-1).

In conclusion it can be stated that thiopropanol (3-mercapto-1-propanol) at the concentration employed in the present study may influence glycogen breakdown and lactic acid formation in isolated diabetic liver slices probably favoring glycolytic key enzymes- hexokinase, phosphofructokinase and pyruvate kinase.

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Table -1

Table showing glycogen content, glycogen utilized per hour, percentage glycogen utilized per hour, lactate production per hour and hexokinase activity in normal rat liver slices(Group-1) , alloxan diabetic rat liver Slices(Group-2) as well as in thiopropanol- exposed – alloxan diabetic rat liver slices(Group-3)

GROUP	Glycogen Contents ⁵ mg/g	Glycogen Utilized mg/g/hr	%age glycogen utilized/hr	Lactate Produced μg/g/hr	Hexokinase Activity ⁴ units
Group-1 Normal Liver(6)	38.25 ± 3.02	20.07 ± 1.71	51.64 ± 3.72	684.03 ± 23.40	166.67 ± 2.78
Group-2 Alloxan-Diabetic liver(6)	29.50*** ± 3.22	10.50*** ± 1.27	35.56 *** ± 1.35	341.70 *** ± 12.91	83.43 *** ± 1.43
Group-3 Thiopropanol exposed- alloxan diabetic liver (6)	29.50 ± 3.22	13.80** ± 2.15	46.65*** ± 2.376	552.96 *** ± 7.07	123.80 *** ± 1.42

- Note: 1. Number in parenthesis indicate the number of liver specimen
 2. The values are expressed as their mean ± SD
 3. Statistical evaluation- probability level * p<0.05, ** p< 0.01, *** p< 0.001
 4. Hexokinase: 1 unit = 1mμMol phosphate transferred /hr/mg liver tissue
 5. Glycogen content of group-2 and group-3 is same as the same diabetic liver is employed for these experiments