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Effect of Thiopropanol on Glucose Utilization in Alloxan Diabetic Rat Liver

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Abstract

Cellular thiol-disulfide ratio can be altered by exogenously added, readily absorbable thiols or disulfides. Many sulphydryl enzymes including glycolytic kinases are known to be affected by changes in thiol-disulfide balance. It is known that in diabetes mellitus the tissue total thiol concentration is reduced thereby creating disturbances in various metabolic pathways, especially the pathways of carbohydrate metabolism. Few studies have suggested that the alterations in carbohydrate metabolism can be directly attributed to modifications in tissue thioldisulfide balance. Certain low molecular weight thiols are known to influence glucose utilization in adipocytes probably by replenishing cellular NADP levels hence favoring utility of glucose through HMP pathway. A study was undertaken to assess the effect of Thiopropanol(3- mercapto-1- propanol), a low molecular weight thiol, on glucose utilization in isolated alloxan diabetic liver slices. The results indicate that the thiopropanol at the dosage employed in the present study influences glucose utilization, lactate production, pyruvate production, glucose-6- phosphate dehydrogenase as well as hexokinase activities in isolated alloxan diabetic liver slices, probably by favoring glucose utilization through glycolysis as well as through HMP pathway.

Index terms— : low molecular weight thiol, 3mercapto-1-propanol, thiol-disulfide balance, glucose utilization, diabetes mellitus.

1 INTRODUCTION

n principle, any enzyme or protein having an accessible thiol essential for its activity is capable of yielding itself to cellular changes in thiol-disulfide ratio thus making such enzymes or proteins for easy modulation **??**1]. This cellular thiol -disulfide balance can Authors Author Author be altered by treating animals or isolated tissue with readily absorbable thiols or disulfides [1,2,3]. It is known that many enzymes particularly glycolytic kinases are sulphydryl enzymes and are affected by changes in thiol-disulfide balance [1, [4] **??**5] **??**6] **??**7]. In diabetes mellitus the tissue total-thiol concentration is reduced **??**8] there by creating disturbances in various metabolic pathways especially the pathways of carbohydrate metabolism. There are few studies that suggests that changes in carbohydrate metabolism can be directly attributed to modifications in tissue thiol-disulfide balance [9, 10,11,12]. Certain low molecular weight thiols are known to influence glucose utilization in adipocytes [13,14] which is thought to be probably through replenishing cellular NADP levels hence favoring utilization of glucose through HMP pathway . Hence a study was undertaken to assess the effect of thiopropanol (3-mercapto 1-propanol), a low molecular weight thiol, on the glucose utilization in isolated alloxan diabetic liver slices.

2 II.

3 MATERIALS & METHODS

All the chemicals employed were of analar grade. Alloxan was obtained from Loba chemicals. 3mercapto 1-propanol (Thiopropanol) (TP) was procured from Sigma-Aldrich chemicals Pvt. Ltd. USA. Male albino rats weighing 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 rats 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, supplied by Pranav Agro Industries, Pune, India).

4 I ?

The feed and the tap water were accessible to the animals ad libitum.

A single intraperitoneal injection of freshly prepared aqueous Alloxan monohydrate (150 mg per kg body weight) **??**15,16] was given to 12 hours fasted rats . 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. The rats were divided into two groups.

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

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, The isolated livers of both normal as well as alloxan diabetic rats were cut into small slices of 0.5 g each and were employed in the present studies.©

The glucose [17], lactic acid [18] as well as the glycogen [19] contents of both pre and post incubated liver samples were estimated .Glucose utilization by the isolated normal liver slices, control alloxan diabetic liver slices(control) and TP-exposedalloxan-diabetic liver slices were studied.

5 d)

Procedure :

The zero minute contents of Glucose and lactic acid were estimated as follows. To 0.5g of normal liver tissue slice or control alloxan diabetic liver slice or TPexposed-alloxandiabetic liver slice(Conc. 5mg thiopropanol/0.5g liver tissue slice) 1ml of freshly prepared buffered glucose solution (0.1g % glucose in phosphate buffer, pH 7.4) was added and immediately 3.5ml of 10% TCA(trichloro acetic acid) was added and allowed to stand at room temperature for 15 minutes for protein precipitation. The contents were thoroughly homogenized using Potter Elvehjam Homogenizer and centrifuged at 3000rpm for 5minutes. The supernatant obtained was employed for both Glucose and Lactic acid estimations. Like wise, for the 60 minutes (post incubation) levels of glucose and lactic acid, 0.5g normal liver slice or control alloxan diabetic liver slice or TP-exposed-alloxan-diabetic liver slice was added with 1ml buffered glucose solution and the tubes were incubated at 37 0 C in a thermostatically regulated water bath for 60 minutes. Then processed to get the protein free supernatant as described above. The glucose formed by the liver glycogen breakdown during this period was also taken into account by estimating glycogen content in the beginning (at zero minute) and at the end of incubation period(at 60 minutes). This glycogen-glucose value was taken into consideration during glucose utilization calculations.

6 Glucose utilization was calculated as follows:

Lactate Production was calculated by subtracting zero min lactate from 60 minutes lactate.

Glucose -6 -phosphatedehydrogenase (G6PD) {EC:1.1.1.49} and Hexokinase (HK){EC:2.7.11} activities were estimated in isolated normal liver slices , in control alloxan diabetic liver slices as well as in TP-exposedalloxan-diabetic liver slices(5mg thiopropanol/0.5g liver tissue).

7 e) :

Procedure :

Four test tubes were taken and marked as B(reagent blank), S(standard), T(test), C(test control). Then 0.2 ml of buffered substrate(L-Alanine [200mMol'L] , Oxo-2 -Glutarate [2mMol/L] prepared in Phosphate buffer, pH 7.4) was taken in all 4 test tubes. The tubes were kept at 37 0 C in a thermostatically controlled water bath for 5 minutes. Then 0.02 ml of glass distilled water, 0.02 ml standard pyruvate solution (2mMol/L) and 0.02ml supernatant were added into tubes B, S and T respectively and the contents were mixed well. All the tubes were incubated for 30 min. at 37 0 C in a water bath. At the end of the incubation, 0.2ml of DNPH (1mMol/L) was added to all the tubes. Then 0.02 ml of supernatant was added to the tube 'C' and all the tubes were allowed to stand at room temperature for 20minutes. Later 2ml of 0.4N NaOH was finally added into all the tubes, the contents were mixed and the tubes were allowed to stand for 5 min. at room temperature. The optical density (OD) was read at 540nm in Spectrophotometer against glass distilled water. The test-control OD

gives the pyruvate content in the beginning ie, at zero minute and the test OD gives the pyruvate content at the end of 30minutes. Pyruvate produced was calculated by subtracting T from C.

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The statistical analysis of the data obtained was done using Microsoft Office Excel worksheet and the P washed and refrigerated with cold PBS(Phosphate buffer saline , pH 7.4) at 0-2 0 C till further use. Procedure : 0.5g of normal liver slice or control alloxan diabetic liver slice or TP-exposed-alloxan-diabetic liver slice was taken in a test tube containing 1ml of phosphate end of the from the water bath and 3.5ml of was added to all the tubes. and centrifuged for 5 employed for the estimation of G6PD [20, ??1,22] and HK [23].

at 37 0 C in The pyruvate content in isolated normal liver slices , in control alloxan diabetic liver slices as well as in TP -exposed -alloxan -diabetic liver slices (5mg thiopropanol/0.5g liver tissue) was estimated using Dinitro phenyl hydrazine (DNPH) [24] reaction. The same supernatant which was used for the enzyme assays as described above was employed for pyruvate estimation also.

buffer (pH 7.4) and the contents were incubated for 60 minutes a thermostatically regulated water bath. At the incubation period , the tubes were removed Phosphate buffer, (pH7.4) Then contents were homogenized min at 3000rpm.The supernatant was Glucose utilization/hr/g liver tissue ={zero min. glucose + (zero min. glycogen -60 min. glycogen). -60 min.glucose} III. Where as the same parameters are significantly increased ($p<0.001$) in TP-exposed-alloxan diabetic liver slices as compared to control alloxan diabetic liver slices.

9 RESULTS

Graph 1, 2 and 3 gives the comparative results of glucose utilization, pyruvate production, lactate production, HK activity as well as G6PD activity in isolated normal liver slices, control alloxan diabetic liver slices and in TP-exposed-alloxan diabetic liver slices. It is evident from these graphs that these parameters are significantly lowered in control alloxan diabetic liver slices as compared to normal liver slices while the same parameters are statistically improved upon exposure of alloxan diabetic liver slices to thiopropanol(5mg/0.5g liver).

IV.

10 DISCUSSION

Alloxan is known to induce diabetes by selectively damaging beta-cells of pancreas[15] thereby affecting insulin production and insulin release. This decreased or non-availability of insulin results in lowered glucose uptake and utilization by alloxan diabetic liver slices. The decreased glucose utilization in control alloxan diabetic liver as compared to normal liver observed in the present study may be due to decreased insulin levels in alloxan diabetic rats. There are few earlier studies regarding influence of thiols on glucose utilization [25][26][27][28][29] suggesting that thiols stimulate utilization of glucose through pentose cycle as well as favor incorporation of glucose-carbon into fatty acids which are more similar to insulin action. Many enzymes of glycolytic pathway, including hexokinase, phosphofructokinase and pyruvate kinase are thiol enzymes[1, [4] ??5] ??6] ??7] and are expected to be altered by cellular thiol concentrations. The data of the present study given in table -1 as well as in graphs 1, 2 and 3 are 5mg/0.5g) might have improved the cellular thiol levels hence keeping the enzymes in their thiol nature thus favoring their activities resulting in increased glucose utilization as evidenced by increased lactate and pyruvate production as well as raise in HK activity in TP-diabetic liver slices, observed in the present study agrees with our previous report [30].Further it is known that certain low molecular weight thiols mimics the actions of insulin probably by acting as substrates for NADPH oxidase (NOX) system [31] thus, may show certain actions of insulin, hence may favor glucose utilization. This action of low molecular weight thiols through NOX system may increase the cellular NADP levels and may facilitate glucose utilization through HMP pathway. Our results shown in table-1 as well as in graphs 1, 2 and 3 agrees with this as there is an increase in glucose utilization with a parallel raise in the G6PD activities in TP-exposed-alloxan -diabetic rat liver slices(test) as compared to control alloxan diabetic rat liver slices

It may be concluded from the present study that thiopropanol at the concentration of 5mg/0.5 g liver tissue slice increases glucose utilization by the alloxan diabetic liver slices probably by favoring glucoseutilization through glycolysis as well as HMP pathway.

11 Graph-2

Graph-1 [1] [2]

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Figure 1: 2011 26 Volume

propanol),
a low
molecular

low molecular weight thiol, 3mercapto-1-
propanol, thiol-disulfide balance, glucose utilization,
diabetes mellitus.

a)
Induction
of Diabetes
Mellitus :

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Figure 2:

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Figure 3: Table - 1

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Figure 4: Table 1 :

.1 Graph-3

Graph showing HK activity and G6PD activity in normal liver, control-alloxan diabetic liver and in TP-exposed alloxan diabetic liver

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