EFFECT OF THIOPROPANOL ON AMINO ACID TURNOVER AND REDOX STATUS IN ALLOXAN DIABETIC RAT LIVER

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ABSTRACT

BACKGROUND

Decreased cellular thiol levels seen in diabetes mellitus (DM) may be in part attributed to increased free radical generation. The free radical mediated oxidative stress has been implicated in the pathogenesis of DM and its complications. The relative deficiency or non-availability of insulin in DM affects the metabolism of biomolecules, specifically the carbohydrate metabolism. The insulin-mimicking actions of various thiols have been studied. In our previous study, we have documented that 3-mercapto-1-propanol (Thiopropanol), a low molecular weight thiol, at the dosage employed has increased glucose utilisation in alloxan-diabetic rat liver tissue probably by favouring utilisation of glucose through glycolysis and HMP pathway. It is known that insulin inhibits gluconeogenesis by inhibiting the key enzymes of the same and by controlling the channelling of amino acids for the glucose biosynthesis through gluconeogenic pathway. A study was undertaken to assess the effects of thiopropanol (TP) on amino acid turnover and the redox status in alloxan diabetic rat liver.

METHODS

Male albino rats weighing 150-250 g were used. Diabetes was induced using alloxan monohydrate. Rats were divided into normal and diabetic groups. Levels of amino acid nitrogen (AAN), alanine, total thiol (-SH) groups, TBARS (Thiobarbituric acid reactive substances), and activities of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated in liver specimens of normal, control-alloxan diabetic and TP-exposed-alloxan-diabetic rats.

RESULTS

The results showed a significant increase ($p<0.001$) in AAN levels, alanine levels, and total -SH groups concentration; and a significant decrease ($p<0.001$) in TBARS levels, ALT and AST activities in TP-exposed-alloxan diabetic liver slices as compared to control-alloxan diabetic liver slices.

CONCLUSIONS

Hence, it may be concluded that TP, at the concentration employed, inhibits gluconeogenesis from amino acids probably by lowering transaminases activity and suppresses free radical production probably by maintaining cellular reduced glutathione (G-SH) levels.

KEYWORDS

Thiopropanol, Low-molecular Weight Thiol, Alloxan Diabetes, Amino Acid Turnover, Redox Status, Gluconeogenesis.

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INTRODUCTION: Diabetes mellitus (DM) is a group of metabolic diseases characterised by hyperglycaemia resulting from defects either in insulin secretion or in its action or both[1]. Free radicals and associated stress have been implicated in eliciting pathological changes of diabetes[2]. In diabetes, the tissue total -thiol levels are reduced. Decreased cellular thiol levels may be in part attributed to the increased free radical generation seen in DM[3] leading to disturbances in various metabolic pathways, importantly the pathways of carbohydrate metabolism. Insulin promotes glycolysis and HMP pathway by activating the key enzymes of the respective pathways[3]. On the other hand, insulin has a negative effect on gluconeogenesis. The glucogenic amino acids are one of the most important non-carbohydrate precursors for gluconeogenesis[4]. Increased protein breakdown results in increased mobilisation of amino acids which acts as substrates for gluconeogenesis, a scenario commonly observed in DM[5]. The insulin mimicking actions of various low molecular weight thiol compounds have been studied[6-8]. In our previous study, we have documented the glucose-utilisation-promotional effects of a low molecular weight synthetic thiol, 3-mercapto-1-propanol (Thiopropanol) in alloxan diabetic rat liver[9] focusing mainly on the pathways of glucose oxidation - glycolysis and pentose phosphate pathway. There was a need to understand the influence of thiopropanol (TP) on amino acid turnover and its possible role in gluconeogenesis.

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AIM: The present study was undertaken to assess the influence of thiopropanol (TP) on amino acid turnover as well as on the redox status in isolated alloxan diabetic rat liver slices.

MATERIALS AND METHODS:

Chemicals & Animals: 3-mercapto-1-propanol (Thiopropanol) was procured from Sigma-Aldrich chemicals Ltd. USA. All other chemicals used were of Analar grade. Randomly selected male albino rats in the weight range of 150-250 g from the stock colony of the Institutional animal house were used in the present study. The rats were maintained at room temperature with 12-hour light/dark cycle on standard stock diet (Amruth Rat Feed, manufactured and supplied by Pranav Agro Industries, Pune, India). The food and water were available 24 hours throughout the study.

Ethics: The animal experiments were performed as per the regulations of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi) and IAEC (Institutional Animal Ethics Committee), Basaveshwara Medical College & Hospital, Chitradurga.

Induction of Diabetes: Diabetes was induced into the 12 hours fasted rats with a single intra-peritoneal injection of freshly prepared aqueous alloxan monohydrate (150 mg per kg body weight)[10]. The onset of diabetes was monitored for the next 48 hours after alloxan treatment by using standard Urine Glucose Strips (from Qualigens). The rats, whose urine showed positive for glucose for three consecutive days and a final confirmation by Accu-Chek Glucometer readings of >250 mg/dL were labelled diabetic.

Animal Groups: The rats were divided into two groups: (1) Normal group - consisting of 6 normal male albino rats, and (2) Diabetic group - consisting of 6 male albino alloxan diabetic rats.

Tissue Processing and Parameter Procedures: After 30 days of maintenance, the rats of both the groups were anaesthetised and sacrificed. The liver tissues were procured from the dissected rats and preserved immediately by washing and refrigerating with ice cold phosphate buffered saline(PBS) at 0- 2 ^{0}C till further use.

Parameters Analysed: Amino acid nitrogen(AAN)[11] concentration, alanine[12] content, total thiol (-SH) groups[13] concentration, activities of transaminases - alanine transaminase (ALT)[14] and aspartate transaminase (AST)[14], and TBARS (thiobarbituric acid reactive substances)[15] level were estimated in normal liver slices, in control-alloxan diabetic liver slices and in TP-exposed-alloxan-diabetic liver slices.

Common incubation procedure: In separate tubes, 0.5 g normal liver slice, 0.5 g control-alloxan diabetic liver slice and 0.5 g alloxan-diabetic liver slice + 5.0 mg TP (TP-exposed-alloxan-diabetic liver slice) were taken. To these, 1.0 mL Phosphate buffer, pH 7.4, was added and incubated for 60 minutes at 37^{0}C in a thermostatically controlled water bath. Multiple batches of tubes were incubated. These were called as "60 minutes incubated tubes". After the incubation period, tissue processing employed were as follows:

(a) 1.0 mL 10% sodium tungstate + 1.0 mL 2/3 N sulphuric acid + 6.5 mL glass distilled water were added to a batch of 60 minutes incubated tubes. The contents were mixed well and allowed to stand for 15 minutes for proteins to precipitate. Then the contents were homogenised for 3 min. using Potter-Elvehjem tissue homogeniser and centrifuged at 3000 rpm for 5 minutes. The supernatants obtained were employed for the estimation of AAN and Alanine.

(b) 3.5 mL phosphate buffer, pH 7.4, were added to a batch of 60 minutes incubated tubes and homogenised for 5 minutes and centrifuged for 5 minutes at 3000 rpm. The supernatants were employed for the estimation of total -SH groups, and AST and ALT activities.

(c) 3.5 mL 10% trichloroacetic acid were added to a batch of 60 minutes incubated tubes, mixed well and allowed to stand for 10 minutes. Later the contents were thoroughly homogenised and centrifuged and supernatants were employed for the estimation of TBARS levels.

STATISTICS: The results obtained were statistically analysed using Student's t-test.

RESULTS: The results in table-1 shows a significant decrease ($p<0.001$) in total AAN as well as alanine content in control-alloxan diabetic liver slices as compared to normal liver slices. However, exposure of alloxan diabetic liver to TP have shown a significant raise ($p<0.001$) in total AAN as well as alanine content as compared to control-alloxan diabetic liver slices. Also there is a significant increase ($p<0.001$) in ALT and AST activities in control-alloxan diabetic liver as compared to normal liver slices whereas same are significantly decreased ($p<0.001$) in TP exposed-alloxan diabetic liver slices as compared to control-alloxan diabetic liver slices. The results in table-2 depicts a significant raise ($p<0.001$) in TBARS levels and a significant decrease ($p<0.001$) in total -SH groups in control-alloxan diabetic liver slices as compared to normal liver slices. However, exposure of alloxan diabetic liver to TP has shown a significant raise ($p<0.001$) in total -SH groups and a significant fall ($p<0.001$) in TBARS levels when compared to control-alloxan diabetic liver slices.

Groups	Total Amino acid nitrogen µg/g	Alanine Content mg/g	ALT activity U/mg	AST activity U/mg
Normal liver (6)	446.16 ± 14.16	2.71 ± 0.09	70.83 ± 1.21	73.97 ± 1.41
Control-alloxan diabetic liver (6)	219.66*** ± 11.96	1.34*** ± 0.07	78.04*** ± 1.55	80.77*** ± 0.99
TP-exposed-alloxan diabetic liver (6)	266.00*** ± 18.85	1.62*** ± 0.12	74.25*** ± 2.13	76.42*** ± 1.91

Table 1

Note:
1. Number in parentheses indicate the number of liver specimen.
2. The values are expressed as their mean ± SD.
3. Statistical evaluation- probability level * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Groups	Total -SH groups mg/g	TBARS µmol/g/hr
Normal liver (6)	2.04 ± 0.12	3.56 ± 0.49
Control-alloxan diabetic liver (6)	1.32*** ± 0.13	7.64*** ± 0.75
TP-exposed-alloxan diabetic liver (6)	1.76*** ± 0.19	5.65*** ± 0.28

Table 2

Note:
1. Number in parentheses indicate the number of liver specimen.
2. The values are expressed as their mean ± SD.
3. Statistical evaluation- probability level * $p< 0.05$, ** $p<0.01$, *** $p< 0.001$.

DISCUSSION: The gluconeogenic supply of glucose becomes prominent in fasting state and in diabetic conditions. The principle gluconeogenic precursors include glucogenic amino acids mainly alanine and glutamic acid, alanine being more significant. These amino acids are converted to their corresponding α-keto acids through respective transamination reactions and the keto acids produced are channelled through gluconeogenic pathway to produce glucose. The present study with respect to amino acid turnover in normal liver slices, control-alloxan diabetic liver slices and TP-exposed-alloxan diabetic liver slices indicates that decreased concentrations of total AAN and alanine in control-alloxan diabetic liver slices as compared to normal liver slices may be due to increased gluconeogenesis because of lack of insulin as insulin is known to suppress gluconeogenesis[3]. This decrease is partly may be due to increased activities of transaminases which may be due to diabetes induced hepatic dysfunction[16] and this is in agreement with the reports of El-Demerdash FM et al[17]. It is known that insulin decreases transaminase activity[18,19]; hence a decrease in insulin may favour transaminases activities and increases the flow of amino acids towards keto acid formation. Thus, liver tissue amino acid levels might have been lowered. Further, exposure of alloxan diabetic liver slices to TP (5.0 mg/ 0.5 g liver) shows a significant raise in total liver tissue AAN, liver tissue alanine levels and a significant fall in liver tissue AST and ALT activities as compared to control-alloxan diabetic liver slices, which suggests that TP mimics the actions of insulin. Thus, like insulin, TP might have suppressed liver tissue transaminase activity as well as might have suppressed gluconeogenic activity thereby function as hypoglycaemic factor through suppressing gluconeogenesis partially. Alloxan, a diabetogenic substance, causes diabetes mellitus by damaging β-cells of Langerhans of pancreas[20] thereby decreases the levels of available insulin thus results in hyperglycaemia. This β-cell damage by alloxan may be due to free radical production from alloxan. Alloxan is known to produce hydroxyl radicals[20] which are responsible for cytotoxic effects of alloxan and thus hydroxyl radical production from alloxan requires NADPH[21]. A significant increase in TBARS levels in alloxan diabetic liver slices suggests that alloxan induced free radical generation through the above explained mechanisms. Further, due to lack of insulin in alloxan diabetic rats, glucose oxidation is suppressed and fat oxidation is favoured, resulting in a possible increase in cellular NADPH levels which might have increased the production of hydroxyl radicals from alloxan. This is further evidenced by a significant fall in liver tissue total –SH groups concentration in alloxan diabetic liver suggesting that this decrease in total thiols may be the result of increased free radical generation. Exposure of alloxan diabetic liver slices to TP (5.0 mg/0.5 g liver) significantly decreases the liver TBARS levels and significantly increases the total –SH groups concentration suggesting that TP might have increased the total cellular thiol concentration, either

directly or indirectly, favouring maintenance of cellular reduced glutathione levels which is important in suppressing free radical generation.

CONCLUSIONS: From the present study, it may be concluded that TP at the concentration of 5.0 mg/0.5 g liver slice increases the AAN and alanine concentrations in alloxan diabetic rat liver slices probably by lowering the activities of transaminases thereby inhibiting gluconeogenesis from amino acids suggesting insulin-like actions of TP on amino acid turnover. Decrease in tissue TBARS and increase in total -SH groups in alloxan diabetic liver exposed to TP indicates that TP probably by maintaining cellular reduced glutathione levels, may act as free radical scavenger.

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