INSULIN- LIKE ACTIONS OF THIOPROPANOL DISULFIDE IN ISOLATED ALLOXAN DIABETIC RAT LIVER

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ABSTRACT

BACKGROUND

Major actions of insulin include glucose uptake, utilization and storage as well as protein and fat synthesis. Diabetes mellitus (DM) is a metabolic disease mainly results due to insulin deficiency or failure in insulin action. Plant extracts have been used as medicine for DM since ancient times. The allium plants' active principles are found to be mainly disulfides and sulfoxides and their hypoglycaemic values are well established. Our earlier studies with a low molecular weight thiol, 3-mercapto-1-propanol (thiopropanol), have shown insulin-like actions in isolated liver tissues of alloxan diabetic rats. A study was undertaken to assess the insulin-like actions of thiopropanol disulfide (TPDS) prepared from thiopropanol.

MATERIALS AND METHODS

Male Swiss albino rats weighing 200-250 g were employed. Alloxan monohydrate was used to induce diabetes. Optimum dosage of TPDS was determined. Six normal and six diabetic rats were sacrificed on the 31st day of the study. Extracted liver tissues were divided into three groups namely- normal, control alloxan diabetic and TPDS-exposed alloxan diabetic groups. Levels of liver tissue glucose, glycogen, lactate, pyruvate, total thiol groups, alanine, total amino acid nitrogen (AAN) as well as activities of hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), alanine transaminase (ALT) and aspartate transaminase (AST) were estimated. Per hour glucose utilization, lactate production, pyruvate production and amino acid utilization were calculated.

RESULTS

The results showed significant increase ($p<0.001$) in glucose utilization, lactate production, pyruvate production, total thiol groups, AAN, alanine, HK activity and G6PD activity, and significant decrease ($p<0.001$) in activities of ALT and AST in TPDS-exposed alloxan diabetic liver slices as compared to control alloxan diabetic liver slices.

CONCLUSION

TPDS undergoes sulfhydryl cleavage like any other disulfide to produce its corresponding thiol component which is responsible for the increased glucose utilization.

KEYWORDS

Thiopropanol Disulfide, Insulin-Like Action, Alloxan Diabetes, Glucose Utilization.

HOW TO CITE THIS ARTICLE: Dattaprasad D, Vickram, Thirumalarao KR. Insulin- like actions of thiopropanol disulfide in isolated alloxan diabetic rat liver. J. Evid. Based Med. Healthc. 2019; 6(8), 585-589. DOI: 10.18410/jebmh/2019/121

BACKGROUND

Insulin, a peptide hormone synthesised and secreted by beta cells of islets of Langerhans of pancreas, is the only hypoglycaemic hormone and regarded as the paramount anabolic hormone present in the body.[1] Its actions include glucose uptake, utilization and storage as well as protein and fat synthesis.[2] Diabetes mellitus is a metabolic disease mainly results due to insulin deficiency or failure in insulin action (insulin resistance).[3] Many herbal extracts have been used since the ancient times as medicine for diabetes.[4,5] Various studies on the hypoglycaemic values of extracts of allium group of plants- garlic and onion, in animal models, have revealed that the active principles of these plants and their extracts are disulfides and sulfoxides.[6,7,8,9,10] Joseph PK and Kashinath RT[11] have shown that a modified disulfide, diacetodibutyl disulfide, exhibit significant hypoglycaemic effects in alloxan diabetic rats. It is thought that these disulfides may have insulin sparing action by substituting for insulin in disulfide bond reduction by thiol groups. Our earlier studies.[12,13] with a low molecular weight thiol, 3-mercapto-1-propanol (thiopropanol), has shown insulin-like effects in isolated liver tissues of alloxan diabetic rats. In the present study we have prepared a disulfide from thiopropanol, we called it thiopropanol disulfide (TPDS) and studied its insulin mimicking actions.

Financial or Other, Competing Interest: None.
Submission 06-02-2019, Peer Review 08-02-2019,
Acceptance 22-02-2019, Published 25-02-2019.
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DOI: 10.18410/jebmh/2019/121

MATERIALS AND METHODS

Chemicals

3-mercapto-1-propanol (thiopropanol) was purchased from Sigma-Aldrich Company, USA. Alloxan was purchased from

Loba chemicals, India. All chemicals used in the present study were of A.R. grade.

Study Animals and Induction of Diabetes

Healthy male albino rats weighing 150-250 g were randomly selected from the stock colony of animal house of B M C H, Chitradurga (1284/ac/09/CPCSEA). These rats were maintained on a standard lab feed supplied by Amruth feeds, Bangalore, and kept in well aerated plastic cages at room temperature with food and water ad libitum. For the induction of diabetes, single intraperitoneal shots of freshly prepared aqueous alloxan monohydrate[14] (150 mg/kg body weight) were given to six 12 hours fasted rats. Diabetes was confirmed by checking urine glucose, using standard urine glucose strips, which came positive three days after alloxan treatment and for the next three consecutive days.

Ethics

Animal maintenance and procedures were performed as per the guidelines of CPCSEA, New Delhi, and ethical clearance was taken from IAEC of B M C H, Chitradurga.

Preparation of TPDS

Procedure- We followed the procedure described by Joseph PK and Kashinath RT[11] for the crude preparation of TPDS. An aliquot of thiopropanol is titrated with 1N iodine solution (Iodine in KI) till a faint iodine colour retained. The mixture was extracted with diethyl ether. Later the ether was evaporated and the left over crude disulfide is considered as thiopropanol disulfide and was employed in the present studies.

Studies to Find out the Optimum Dosage of TPDS

Four alloxan diabetic rats were sacrificed on the 31st day (after 30 days of onset of diabetes) and liver tissues were extracted. Concentrations of TPDS (2.0, 5.0, 10.0 and 20.0 mg / 0.5 g liver slice) have been exposed to alloxan diabetic liver slices and the glucose utilization through glycolysis has been studied by estimating liver lactate production. The preparation of varying concentrations of TPDS was formulated such that the final volume exposed to 0.5 g liver slice was 10 µl which contains the desired concentration. The necessary dilutions were made using normal saline solution. Our earlier article[12] documents the details of tissue processing and estimation of pre and post incubation liver lactate levels. Lactate production per hour was calculated by subtracting post incubation (60 min) lactate levels with pre-incubation (zero min) lactate levels.

Experimental Design:

Animal Groups

The rats were divided into-

1. Normal rats (n=6) - healthy male albino rats maintained on stock lab diet and water ad libitum.
2. Diabetic Rats (n=6) – alloxan diabetic rats maintained on stock lab diet and water ad libitum.

Liver Tissue Grouping, Processing and Parameter Procedures

On the 31st day of the study period both the rat groups were anesthetized and sacrificed. The liver tissues were extracted and refrigerated with cold phosphate buffer solution in separate beakers at 0-2^0 C till further use. The liver slices (0.5 g each) of normal rats served as Group-I. The liver slices of alloxan diabetic rats served both as control alloxan diabetic liver slices (Group-II) and TPDS (5.0 mg/0.5 g liver)-exposed alloxan diabetic liver slices (Group-III).

Studies to Assess TPDS Effect on Hepatic Glucose Metabolism

(a) To study the glucose-utilization-promotional influence of optimum dosage of TPDS, levels of glucose,[15] glycogen,[16] lactate,[17] pyruvate,[18] total thiol groups[19] and activities of hexokinase (HK)[20] and glucose-6-phosphate dehydrogenase (G6PD)[21] were analysed in both pre-incubated (zero min) and post incubated (60 min) liver slices of group-I, group-II as well as group-III. Later, glucose utilization, lactate production and pyruvate production were calculated as described below.

(b) To assess the effects of TPDS on liver gluconeogenesis, levels of total amino acid nitrogen (AAN),[22] alanine,[23] and activities of aspartate transaminase (AST)[18] and alanine transaminase (ALT)[18] were analysed in all three groups as mentioned above.

The aliquots of liver slices (0.5 g each) were taken in glass test tubes and were suspended in freshly prepared phosphate buffer, pH 7.4. To this, 10 µl normal saline for group-I and II labelled tubes or with 10 µl (5.0 mg) TPDS for group-III labelled tubes were added. The tubes were incubated for 1 hour at 37^0 C and after, treated with trichloroacetic acid for the estimation of glucose and lactate, with sodium tungstate-sulphuric acid for total AAN and alanine estimations, and with cold phosphate buffer for pyruvate, total thiol groups estimations and for analysing the activities of HK, G6PD, ALT and AST. All the parameters were analysed in supernatants after homogenization and centrifugation. The detailed procedures for tissue processing and their analyses were documented in our previous studies.[12,13]

Glucose formed from liver glycogen breakdown during this one-hour period was also taken into account by estimating glycogen content in the beginning (zero min.) and at the end of incubation (60 min.). This glycogen-glucose content was taken into consideration for glucose utilization calculations.

Glucose Utilization[12] is Calculated as Follows-

Glucose utilization/hr/g liver = {zero min. glucose + (zero min. glycogen – 60 min. glycogen) – 60 min. glucose}

Per hour liver lactate production was calculated by subtracting post incubation (60 min) lactate levels with pre-incubation (zero min) lactate levels. Similarly, pyruvate production was also estimated. Assessment of amino acid utilization for gluconeogenesis was explained in our earlier article.[13]

RESULTS

Chart-1 shows increase in lactate production in alloxan diabetic liver slices exposed to different concentrations of TPDS as compared to control alloxan diabetic liver slices. The lactate production is slightly more in alloxan diabetic liver slices exposed to 5.0 mg TPDS as compared to 2.0 mg, 10.0 mg and 20.0 mg TPDS exposure. Results in table-1 depict a significant decrease ($p<0.001$) in glucose utilization, lactate production, pyruvate production, total thiol groups and in activities of HK and G6PD in group-II as compared to group-I whereas the same parameters show a significant increase ($p<0.001$) in group-III as compared to group-II. Results in table-2 show significant decrease ($p<0.001$) in levels of total AAN and alanine content, and significant increase ($p<0.001$) in activities of ALT and AST in group-II as compared to group-I whereas there is a significant raise ($p<0.001$) in levels of total AAN and alanine content, and significant fall ($p<0.001$) in ALT and AST activities in group-III as compared to group-II. The results signify insulin-like glucose utilization-promotional effects by TPDS (5.0 mg/0.5 g liver slice).

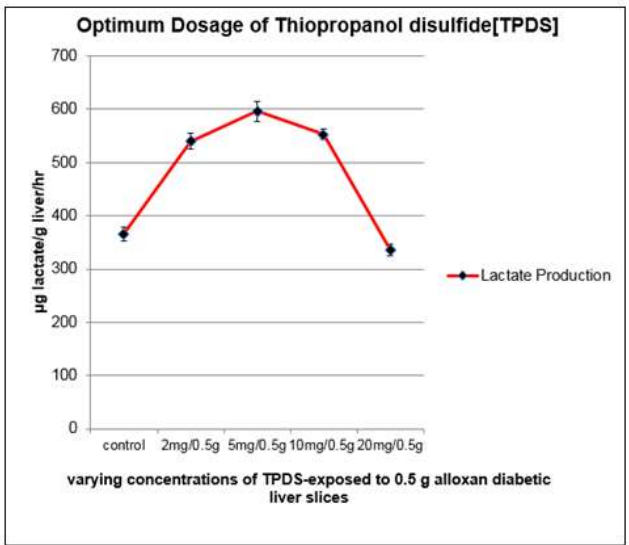

Chart 1. Showing the Effect of Varying Dosages of TPDS on Lactate Production in Alloxan Diabetic Liver Slices

Groups (n=6)	Glucose Utilization mg/g/hr	Lactate Production µg/g/hr	Pyruvate Production mg/g/hr	HK Activity U	G6PD Activity U	Total Thiol (-SH) Groups mg/g
I. Normal Rat Liver	9.01±0.40	692.41±29.16	13.74±0.69	169.08±3.46	77.17±1.44	2.06±0.10
II. Control Alloxan Diabetic Rat Liver	5.03***±0.46	352.30***±13.93	10.45***±0.34	84.01***±1.07	17.10***±1.10	1.36***±0.10
III. TPDS Exposed-Alloxan Diabetic Rat Liver	7.64***±0.54	583.05***±32.41	12.11***±0.44	103.95***±1.94	36.55***±1.63	1.59**±0.13

Table 1. Showing Glucose Utilization Per Hour, Lactate Production Per Hour, Pyruvate Production Per Hour, Total Thiol Group Levels as Well as G6PD & HK Activities in Group-I, Group-II and In Group-III Liver Slices

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. G6PD 1 unit = amount of NADPH produced/min/g liver tissue.
5. HK 1 unit = 1mµMol phosphate transferred /hr/mg liver tissue.
6. TPDS exposed dosage: 5.0 mg/0.5 g liver slice.

Groups (n=6)	Total AAN µg/g	Alanine Content mg/g	ALT Activity U/mg	AST Activity U/mg
I. Normal Rat Liver	455.33±12.02	2.72±0.06	71.02±1.11	73.69±1.15
II. Control Alloxan Diabetic Rat Liver	222.67***±10.28	1.36***±0.06	78.20***±1.32	81.01***±0.94
III. TPDS Exposed-Alloxan Diabetic Rat Liver	280.16***±14.82	1.70***±0.09	75.04***±1.22	76.60***±0.97

Table 2. Showing Total AAN, Alanine Content and AST & ALT Activities in Group-I, Group-II and in Group-III Liver Slices

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. TPDS exposed dosage: 5.0 mg/0.5 g liver slice

DISCUSSION

The decrease or non-availability of insulin results in poor glucose uptake and utilization by alloxan diabetic liver as alloxan is known to induce diabetes by selectively destroying the beta cells of Islets of Langerhans of pancreas.[24,25] Diminished glucose utilization observed in group-II as compared to group-I may be due to the absence of insulin. In diabetes mellitus, cellular thiol concentration decreases[26] which create disturbances in many metabolic pathways especially the pathways of carbohydrate metabolism. The cellular total thiol groups concentration is significantly decreased in group-II in comparison with group-I as a result of alloxan induced diabetes. The cellular perturbation by readily absorbable thiols or disulfides in thiol-disulfide balance has been documented.[27] Most of the enzymes specifically glycolytic kinases are thiol enzymes and are easily influenced by changes in thiol-disulfide balance.[27] TPDS, a low molecular weight disulfide prepared from thiopropanol exhibited glucose-utilization-promotional effects in group-III as compared to group-II probably by undergoing sulfhydryl cleavage to yield its thiol counter parts and these thiols might have mimicked similar actions of thiopropanol[12] as evidenced by increase in glucose utilization, lactate production, pyruvate production, as well as raise in the activities of HK and G6PD in group-III as compared to group-II (table-1). This action of TPDS through formation of its thiol component is further evidenced by increase in concentration of total thiol groups in group-III as compared to group-II. Glucose from gluconeogenesis becomes crucial in fasting state and in diabetic conditions. Glucogenic amino acids mainly alanine and glutamic acid are converted to their corresponding alpha keto acids by transaminases, ALT and AST, and are utilised for the synthesis of glucose via gluconeogenic pathway.[28] The present study with respect to amino acid utilization for gluconeogenesis in all three groups (table-2) shows that the levels of total AAN and alanine are significantly decreased ($p<0.001$) in group-II as compared to group-I. This may be because of increased gluconeogenesis due to lack of insulin as insulin is known to suppress gluconeogenic pathway. This decrease in part may be due to increased activity of transaminases resulting from diabetes induced hepatic dysfunction[29,30] as evidenced by significant increase ($p<0.001$) in ALT and AST levels in group-II as compared to group-I. Further, exposure of alloxan diabetic liver to TPDS (5.0 mg/ 0.5 g liver) shows a significant increase ($p<0.001$) in levels of total AAN, alanine and significant decrease ($p<0.001$) in activities of ALT and AST in group-III as compared to group-II which suggests that TPDS, like insulin,[31,32] might have lowered the activities of these transaminases as well as might have suppressed gluconeogenesis.

CONCLUSION

TPDS at the dosage employed shows insulin-like actions in isolated alloxan diabetic liver slices by undergoing sulfhydryl cleavage like any other disulfides to its corresponding thiol component, which mimicked some actions of insulin by increasing glucose utilization mainly via glycolysis, partly via HMP pathway and decreasing amino acid utilization via gluconeogenesis by suppressing the transaminases. Though we have not studied the exact mechanism of action of TPDS, there is a definite positive influence on hepatic glucose utilization by altering cellular thiol/disulfide ratio. Further, molecular level studies with the purified and characterized disulfide would provide a larger insight about its mechanism of action.

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