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Influence of Diallyldisulphide on Glycogen Breakdown in Alloxan Diabetic Liver

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Abstract: *Diabetes mellitus is chronic metabolic disorder of glucose metabolism in particular due to deficiency of insulin. The glycogen formation as well as glucose utilization are profoundly affected in diabetes mellitus. Diallyldisulphide (DADS), an important disulphide of garlic oil, is known to improve glycemic conditions in diabetic animals, but its specific action on glycogen metabolism is not well understood. Hence a study was undertaken to assess the effects of DADS on liver glycogenolysis in alloxan diabetic rats. The alloxan diabetic liver slices were incubated with DADS(4mg/g liver tissue) and the glycogen breakdown was studied. The results indicate that glycogen formation as well as glycogen breakdown in alloxan diabetic liver is significantly decreased and DADS shows a stimulatory effect on glycogen breakdown in alloxan diabetic liver.*
Keywords: *Alloxan, Diabetes Mellitus, DADS, Glycogen breakdown.*

I. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and glucosuria due to deficiency or subnormal functioning of insulin [1,2]. Insulin, a peptide hormone, influences the glucose utilization by favoring the action of various enzymes involved in glucose metabolism including glycogen metabolism [3,4]. Many herbal preparations specifically extract of garlic (Allium Sativum) have been claimed useful in controlling glycemic conditions in DM [5-8]. The important sulfur compound of garlic oil, DADS has been claimed to be beneficial in DM [9]. However, the role of DADS in regulating glucose and glycogen metabolism in DM is obscure though it is known DADS, may enhance insulin action as well as may favor insulin half life [10-12]. Hence a study was undertaken to assess the effect of DADS on glycogen breakdown in isolated alloxan diabetic rat liver slices.

II. Materials And Methods

2.1 Chemicals

DADS and Alloxan monohydrate were obtained from Sigma Aldrich Chemicals (St. Louis, U.S.A.) and the remaining chemicals used were of Analytical Reagent (AR) grade.

2.2 Experimental Animals

Healthy male Wistar rats in the weight range 150-200g were randomly selected from the Institutional Animal House and housed in polycarbonate cages under normal 12 hours day-night cycle at temperature of 22 ± 2^0c. They were maintained on commercial rat feed (Amruth Rat Feed supplied by M/s. Pranav Agro Industries, Pune, India) and water was provided *ad libitum*. The experiments were conducted as per stipulations of CPCSEA under the supervision of Institutional Animal Ethics Committee.

2.3 Induction of Diabetes Mellitus

DM was induced by administering a single intra-peritoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg b.w.) in normal saline to the overnight fasted rats[13-15]. The onset of diabetes was monitored 48 hours after alloxan injection by using urine strips (Mission Urine strips) and the animals that tested positive for urine glucose for 3 consecutive days were considered as diabetic rats and were employed in the present study.

2.4 Experimental design

Groups: For the present study the rats were divided into two groups.
Normal group : Cosisting 6 rats and were maintained on stock lab diet and water *ad libitum*.
Diabetic group : Cosisting 6 Alloxan diabetic rats and were maintained on stock lab diet and water *ad libitum*
The rats of both the groups after the stipulated period of 30 days were anesthetized and sacrificed. Blood samples were collected in Heparinised tubes. The animals were dissected and liver tissue was procured and put

into pre-cooled beaker containing phosphate buffer saline (pH 7.4). The blood samples were centrifuged at 3000 rpm for 8 minutes and the separated plasma was employed for Glucose estimation [16]

A 0.5g of liver slice from each liver of both normal and control alloxan diabetic group were employed for glycogen estimation [17,18]. The liver slices of alloxan diabetic group served both as control liver samples as well as for exposure to DADS.

2.5 Glycogen Breakdown Studies

For estimation of pre-incubation glycogen levels 0.5g each of normal liver slice in one tube and control alloxan diabetic liver slices separately in another two tubes were taken, to all the tubes 1ml isotonic phosphate buffer (pH 7.4) was added. To one of the test tube containing control alloxan diabetic liver slice 10µl DADS solution (2mgDADS) in warm normal saline was added. To all the tubes 4.5 ml of Trichloro acetic acid (10% TCA) was added and allowed to stand for 15mins at room temperature. Then the tubes were thoroughly homogenized using Remi homogenizer for 6 mins and clear supernatant was used for glycogen estimation [18]. This value was taken as pre-incubation glycogen content.

For estimation of post-incubation glycogen levels 0.5g each of normal liver slice in one tube and control alloxan diabetic liver slices separately in another two tubes were taken, to all the tubes 1ml isotonic phosphate buffer (pH 7.4) was added. To one of the test tube containing control alloxan diabetic liver slice 10µl DADS solution (2mgDADS) in warm normal saline was added. The test tubes were incubated at 37^0C in thermostatically regulated water bath for 60 mins. Later the test tubes were cooled and 4.5 ml 10% TCA was added and were processed as stated above. The clear supernatant was employed for for glycogen estimation[18]. The value obtained was taken as post-incubation glycogen content.

III. Results

The results obtained are narrated inTable-1. The Table shows the glycogen content(in mg/g liver tissue) and glycogen breakdown (in mg/ g liver tissue/hour) in isolated normal liver (Group-1), in control alloxan diabetic liver (Group-2) and in alloxan diabetic liver exposed to DADS(4mg/g liver tissue)(Group-3). It is evident from the table that the liver glycogen content as well as glycogen breakdown is significantly ($p<0.001$) lowered in Group-2 compared to Group-1, where as an increase($p<0.001$) in liver glycogen breakdown was observed in Group-3 as compared to Group-2, indicating DADS may favor glycogen breakdown.

IV. Discussion

Glycogen, a stored homopolysacharide, stored in liver to the extent of 5% wet weight of liver. This is synthesized starting from glucose through glycogenesis and and converted back to glucose when require by glycogenolysis. Both these pathways of glycogen metabolism are well regulated through the hormones, principally insulin and glucagon by influencing the key enzymes of pathway, i.e, glycogen synthase and glycogen phosphorylase[19]. The end regulation of these pathways are achieved by protein tyrosine phosphatases (PTPs). Which are thiol enzymes and are modulated by thiol disulphide interaction[20].

The results of the present studies indicate significant decrease ($p<0.001$) in glycogen content in alloxan diabetic liver(refer table)which is due to lack of insulin as alloxan is known to damage β-cells [14]. Further the glycogen breakdown by alloxan diabetic liver (Group-2) is significantly lowered ($p<0.001$)as compared to normal liver (Group-1) where as a significant raise ($p<0.001$) in glycogen breakdown is observed in DADS exposed alloxan diabetic liver (Group-3) as compared to control alloxan diabetic group(Group-2). This increased glycogen breakdown in DADS exposed alloxan diabetic liver may be due to modulating effect of DADS on PTPs. It is known that disulfides do undergo sulfhydryl exchange reaction with cellular thiol protein and enzymes as depicted bellow[21,22].

$$RSSR + Enzyme\text{-}SH \longrightarrow RSH + enzyme\text{-}S\text{-}S\text{-}R$$

Such a possible interaction of DADS with PTPs involved may inactivate glycogen synthase, may prolong the actions of glycogen phosphorylase, hence facilitating glycogenolysis. Thus such an action of DADS increases glycogenolytic breakdon in alloxan diabetic liver exposed to DADS(Group-3), which is evident from the result observed in the present study.

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VI. Conclusion

It can be concluded by the results of the present study with respect to effect DADS on glycogen breakdown in DADS exposed alloxan diabetic liver, That DADS at the dosage employed may facilitate glycogenolysis by prolonging the activities of glycogen phosphorylase through a disulphide exchange regulation of PTPs involved in regulation of glycogen phosphorylase.

References

[1]. American Diabetes Association. "Diagnosis and classification of diabetes mellitus." Diabetes care 29.1 (2006): S43.
[2]. Altan, V. Melih. "The pharmacology of diabetic complications." Current medicinal chemistry 10.15 (2003): 1317-1327.
[3]. Gold, Alvin H. "The effect of diabetes and insulin on liver glycogen synthetase activation." Journal of Biological Chemistry 245.4 (1970): 903-905.
[4]. Ortmeyer, HEIDI K., N. L. Bodkin, and BARBARA C. Hansen. "Insulin regulates liver glycogen synthase and glycogen phosphorylase activity reciprocally in rhesus monkeys." American Journal of Physiology-Endocrinology And Metabolism 272.1 (1997): E133-E138.
[5]. Augusti, K. T. "Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.)." Indian journal of experimental biology 34.7 (1996): 634-640.
[6]. Augusti, K. Thomas. "Therapeutic and medicinal values of onions and garlic."Onions and allied crops 3 (1990): 93-108.
[7]. Thomson, Martha, et al. "Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats." Int J Diabetes & Metabolism 15 (2007): 108-15.
[8]. Jalal, Razieh, et al. "Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance." Journal of Clinical Biochemistry and Nutrition 41.3 (2007): 218-223.
[9]. Liu, Cheng-Tzu, et al. "Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats." European journal of pharmacology 516.2 (2005): 165-173.
[10]. Mathew, P. T., and K. T. Augusti. "Studies on the effect of allicin (diallyl disulphide-oxide) on alloxan diabetes. I. Hypoglycaemic action and enhancement of serum insulin effect and glycogen synthesis." (1973).
[11]. Adoga, Godwin I. "The mechanism of the hypolipidemic effect of garlic oil extract in rats fed on high sucrose and alcohol diets." Biochemical and biophysical research communications 142.3 (1987): 1046-1052.
[12]. Augusti, K. T. "Studies on the effects of a hypoglycemic principle from Allium Cepa Linn." The Indian journal of medical research 61.7 (1973): 1066-1071.
[13]. Etuk, E. U. "Animals models for studying diabetes mellitus." Agric Biol JN Am 1.2 (2010): 130-134.
[14]. Szkudelski, T. "The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas." Physiological research 50.6 (2001): 537-546.
[15]. Chougale, Ashok D., et al. "Optimization of alloxan dose is essential to induce stable diabetes for prolonged period." Asian Journal of Biochemistry2.6 (2007): 402-408.
[16]. Richard J.Henry, Donald,C.Connan and James Winkelman. Clinical Chemistry ;II Edition , Harper row Publishers NY,Chapter -25 Carbohydrates, 1974: 1285-1289.
[17]. Vickram, Divya D., and V. Vijay. "Thiopropanol Induced Changes in Glycogen Breakdown in Alloxan Diabetic Liver." Global Journal of Medical Research11.1 (2011).
[18]. David T. Plummer: An introduction to Practical Biochemistry,III-Edition,Chapte9,Carbohydrates. The isolation & assay of glycogen from the liver & skeletal muscle of rats. pp: 182-184, McGraw-Hill publishing Company Ltd.
[19]. Bishop, Jonathan S., and Joseph Larner. "Rapid activation-inactivation of liver uridine diphosphate glucose-glycogen transferase and phosphorylase by insulin and glucagon in vivo." Journal of Biological Chemistry 242.6 (1967): 1354-1356.
[20]. Ziegler, D. M. "Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation." Annual review of biochemistry 54.1 (1985): 305-329.
[21]. Adamu, I., P. K. Joseph, and K. T. Augusti. "Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two-month period."Cellular and Molecular Life Sciences 38.8 (1982): 899-901.
[22]. Adoga, Godwin I. "Effect of garlic oil extract on glutathione reductase levels in rats fed on high sucrose and alcohol diets: a possible mechanism of the activity of the oil." Bioscience reports 6.10 (1986): 909-912.

Table-1

Table showing the glycogen breakdown in isolated normal liver (Group-1), in control alloxan diabetic liver (Group-2) and in alloxan diabetic liver exposed to DADS(4mg/g liver tissue)(Group-3).

Groups	Pre-incubation glycogen content(mg/g of liver tissue)	Post-incubation glycogen content (mg/g of liver tissue)	Glycogen breakdown (mg/g of liver tissue/h)	% of glycogen breakdown
Normal liver (Group-1) (6)	93.50 ± 6.50	10.85 ± 3.43	82.31 ± 7.99	88.23 ± 3.95
Control alloxan diabetic liver (Group-2) (6)	15.81*** ± 0.91	13.26 ± 0.69	2.55*** ± 0.53	16.0*** ± 2.82
DADS exposed alloxan diabetic liver(Group-3) (6)	17.35** ± 1.06	13.25 ± 0.56	4.1** ± 1.03	23.40*** ± 4.85

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* $p< 0.05$, ** $p<0.01$, *** $p< 0.001$.