Experimental Research

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Effects of Diaceto-Dipropyl-Disulphide on Plasma Sialic Acid and Renal Tissue Thiol Levels in Alloxan Diabetic Rats

Biochemistry Section

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ABSTRACT

Introduction: Plasma sialic acid levels are elevated in Diabetes Mellitus (DM) patients with proteinuria. Renal damage is mainly caused by free radicals that are excessively generated in DM. Thiols play an important role in the cellular antioxidative defence mechanisms mainly through thiol-disulphide exchange reaction. Diallyl disulphide, a garlic oil principle component, is known for its anti-diabetic properties. Its structural analogue, Diaceto-Dipropyl Disulphide (DADPDS), is a less toxic and more palatable disulphide and possesses similar anti-diabetic actions.

Aim: This study was undertaken to assess the usefulness of DADPDS in prevention of de-sialation of Glomerular Basement Membrane (GBM) in alloxan diabetic rats and to assess effect of DADPDS on renal tissue thiol levels.

Materials and Methods: Rats were divided into Normal, Diabetic and DADPDS treated diabetic groups. Diabetes was induced by intraperitoneal injection (IP) of alloxan. DADPDS was fed by gastric intubation. Plasma Sialic acid was determined by Ehrlich's method and renal tissue thiol levels by Nitroprusside reaction method.

Results: This study showed a significant decrease ($p<0.001$) in plasma sialic acid, plasma glucose and renal tissue TBARS levels along with significant increase ($p<0.001$) in renal tissue thiol levels in DADPDS treated alloxan diabetic rats when compared to diabetic control rats.

Conclusion: Hence it may be concluded that DADPDS helps in preventing de-sialation of GBM in alloxan diabetic rats and improves renal tissue antioxidant defence mechanisms, may be through thiol-disulphide exchange reaction and thereby exhibits a possible clinical use in prevention of renal complications like diabetic nephropathy.

Keywords: Diabetic nephropathy, Thiol-disulphide exchange reaction, Desialation, Sialidase

INTRODUCTION

Diabetes Nephropathy (DN)

DN is major life threatening microvascular complication of Diabetes Mellitus (DM) and is the commonest cause of end stage renal disease, in India [1,2]. Prevalence of DN among DM patients varies from 13% to 70.8% in India [3].

Sialic Acid (SA)

SA, also known as N-acetyl neuraminic acid, is an essential component of glycoproteins and glycolipids present on cell membrane [4]. SA is a nine-carbon sugar derived from mannosamine and pyruvate [5]. It acts as a cofactor of many cell surface receptors (e.g., insulin receptor) [6]. The unique structural feature of SA, which includes a negative charge owing to a carboxyl group, enable it to play an important role in cellular functions, such as cell-to-cell repulsion, recognition, transportation of positively charged compounds and tumour cell metastasis. These sialic acid molecules apparently enter the circulation by either shedding or cell lysis [7].

SA contributes to the maintenance of the negative charge of the renal Glomerular Basement Membrane (GBM) [8]. Plasma SA level is elevated in DM patients with microalbuminuria and clinical proteinuria i.e., DN, which is a well-established diabetic complication accompanied by an increased risk of coronary artery disease [9].

ROS and Thiols in Diabetes

Cellular functional variations as well as cellular protein alterations are commonly observed in DM which are due to increased production of Reactive Oxygen Species (ROS). This diabetes induced ROS elevation may result in decreased total cellular / tissue thiol levels [10].

Disulphides of Garlic, (*Allium sativum Linn*)

Disulphides of garlic, (*Allium sativum Linn*) are known for their anti-diabetic, anti-hyperlipidaemic, anti-atherogenic, antioxidant as well as anti-carcinogenic actions [11]. Diallyl Disulphide (DADS), the principle disulphide compound of garlic oil is responsible for the above-mentioned beneficial properties of garlic. In our previous studies we have documented the anti-glycation effect of DADS on GBM and its beneficial effect in reducing de-sialation of GBM [12,13]. But DADS is very pungent oil and often it is difficult to feed rats. Also, few studies have shown toxic effects of DADS on kidneys [14,15]. But its structural analogue, Diaceto- Dipropyl Disulphide (DADPDS) is pleasant smelling, more palatable, less toxic and possesses similar anti-diabetic properties [15].

AIM

This study was undertaken to assess the effects of DADPDS on GBM sialic acid and renal tissue antioxidant levels in alloxan diabetic rats.

MATERIALS AND METHODS

Materials

Alloxan and thiopropanol were procured from Sigma Chemicals. All other chemicals used in the present study were of analytical grade. DADPDS was synthesized from thiopropanol as explained by Veena GR [15].

Maintenance of Animals

Male albino rats, weighing 200-250g were randomly selected and used for the present study. The animals were maintained on a standard rat feed supplied from Amrut rat feeds, Bengaluru. The rats were maintained at room temperature of 25^{O}C with 24 hours water and food supply.

Ethics

The experiments were conducted according to the norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee (IAEC) guidelines.

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single IP injection of freshly prepared alloxan (150mg/kg body wt.) [11,13] in sterile normal saline. The animals were considered diabetic if their blood glucose were above 250mg/dl and urine showed consistent glucosuria. The treatment was started on 5th day after alloxan injection and was considered as first day of treatment.

Grouping of Animals

The rats were divided into three groups comprising six rats in each group as follows:

Group I: Normal rats – which were fed on 30 ml of normal saline per kg body weight, through gastric intubation, for a period of 60 days.

Group II: Diabetic Control rats – which were fed on normal saline 30ml/kg body weight, through gastric intubation, for a period of 60 days.

Group III: Diaceto- Dipropyl Disulphide (DADPDS) treated Diabetic rats – which were fed on DADPDS (100mg/ kg body weight) prepared in normal saline, given as 30ml/kg body weight suspension, through gastric intubation, for a period of 60 days.

Procedure

On completion of 60 days, the rats were anaesthetised and then sacrificed. Blood was collected in heparinised test tubes. Kidneys were dissected and their net weight was noted. Immediately the kidneys were processed as follows. One part of kidney was homogenised with 9 parts of cold Phosphate buffer (pH 7.4) and the extract was used for estimation of tissue total thiols [16] and total proteins [17]. A second part of the kidney was homogenised with 9 parts of trichloro acetic acid (10%) and the extract was used for the estimation of thiobarbituric acid reactive substances (TBARS) levels by Nitroprusside reaction method [18]. A part of whole blood was centrifuged at 3500 rpm for 6-8mins and the plasma was used for the estimation of glucose by glucose oxidase-peroxidase method [19] and sialic acid levels by Ehrlich's method [20].

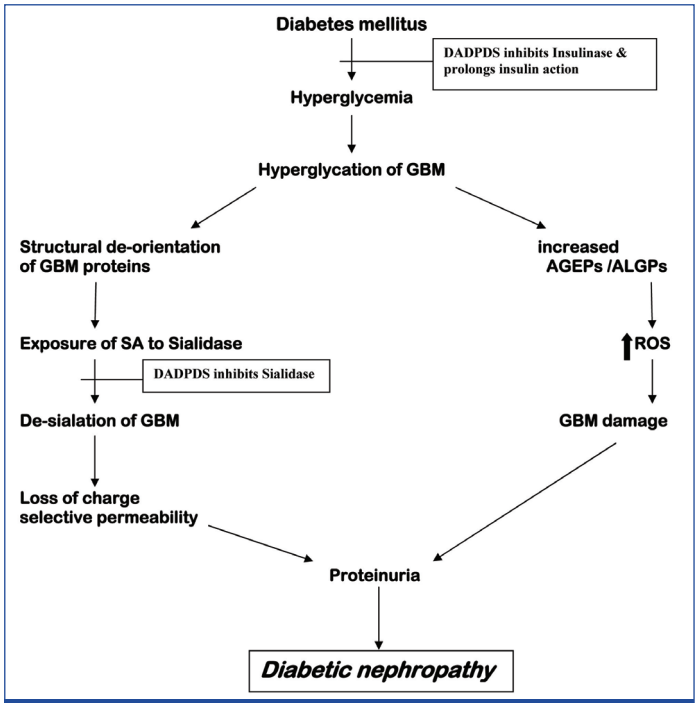
STATISTICAL ANALYSIS

The obtained results were statistically analysed by Student's t-test.

RESULTS

Results obtained in the present study have been presented in [Table/Fig-1]. The plasma glucose levels, plasma creatinine levels, plasma sialic acid levels along with renal glycated proteins, renal thiol levels and renal TBARS levels of normal rats (group I), diabetic control rats (group II) and DADPDS treated diabetic rats (group III) have been incorporated.

There was a significant increase in plasma glucose ($p<0.001$), plasma sialic acid levels ($p<0.001$) and renal tissue TBARS levels ($p<0.001$); significant decrease ($p<0.001$) in renal tissue thiol levels in group II rats as compared to group I rats. However, there was no significant change in plasma creatinine levels between these two groups. Further, it was observed that there was a significant decrease in plasma sialic acid levels ($p<0.001$) and renal tissue TBARS levels ($p<0.001$) as well as significant increase ($p<0.001$) in renal tissue thiol levels in group III rats as compared to group II rats. However, there was no appreciable observed in plasma glucose and plasma creatinine levels between these two groups.



[Table/Fig-2]: Possible actions of DADPDS in preventing diabetic nephropathy in alloxan diabetic rats. (* Advanced glycated end products/advanced lipid glycation products).

DISCUSSION

SA residues are important constituents of the carbohydrate moiety of the GBM. Along with glycosaminoglycans, SA contributes to the polyanionic components and the charge-selective permeability / barrier of the GBM. Sialic acid content of GBM has been found to be decreased in diabetic subjects where the decrease seems to correlate with duration of diabetes. Similar reductions in sialo-glycoproteins were found in alloxan diabetic rats. These findings were due in part to the raised activity of the degradative enzyme, sialidase, in the diabetic kidney cortex [21]. Studies have suggested possible role of hyperglycation of GBM proteins in DM, altering its structural orientation and exposing sialic acid to sialidase. This may lead to removal of sialic acid which in part may account for the increased plasma sialic acid levels [Table/Fig-1]. The observed elevated sialic acid levels in alloxan diabetic rats is in agreement with earlier studies in diabetic nephropathy [8,11,13,22,23]. In our previous study we have documented that this de-sialation of GBM proteins may alter their ionic nature; a decrease in negative charges leading to percolation of little albumin which probably results in microalbuminuria, a predisposing factor observed prior to frank nephropathy [12,13]. A significant decrease in plasma

	Plasma glucose (mg/dl)	Plasma Sialic acid (mg/dl)	Plasma Creatinine (mg/dl)	Renal thiols (mg of Cysteine equivalent/g)	Renal TBARS (µ mol/g)
Normal rats (n=6)	122.61 ± 20.1	71.08 ± 8.35	0.67 ± 0.08	1.77 ± 0.44	7.96 ± 1.81
Diabetic control rats (n=6)	421.66*** ± 102.08 (p< 0.0001)	98.81*** ± 17.33 (p= 0.0011)	0.72 ± 0.04 (p= 0.1362)	0.88*** ± 0.16 (p <0.0001)	21.01*** ± 4.23 (p <0.0001)
DADPDS treated rats (n=6)	264.18*** ± 87.41 (p= 0.0051)	73.97*** ± 11.49 (p= 0.0045)	0.70 ± 0.03 (p= 0.2769)	1.08*** ± 0.06 (p= 0.0052)	10.68*** ± 2.29 (p< 0.0001)

[Table/Fig-1]: Plasma glucose, sialic acid, renal tissue thiols and TBARS levels in all the rat groups.
1. Number in parentheses indicate the number of animals in each group.
2. The values are expressed as their mean ± SD
3. Significance level * p < 0.05; ** p < 0.01; *** p < 0.001.

sialic acid levels ($p<0.001$) as well as a significant increase in tissue thiol levels ($p<0.001$) in DADPDS treated alloxan diabetic rats (group II rats) decisively indicates that DADS has a significant role in inhibiting de-sialation of GBM in alloxan diabetic rats.

DADPDS a disulphide, may be involved in sulphydryl exchange reactions with proteins or enzymes [24,25] similar to any other disulphide as follows:

R1- S-S-R1 + R2 –SH ---------- > R1-S-S-R2 + R1- SH

Sialidase being a sulphydryl enzyme [26], may be involved in sulphydryl exchange reaction with DADPDS. Such a sulphydryl exchange reaction with sialidase, may alter the activity of the enzyme resulting in retention of sialic acid residues on the GBM and thus maintaining its shape as well as negative nature of GBM proteins. Such an action of DADPDS should result in lower activity of sialidase enzyme and hence the decreased plasma sialic acid levels observed in the present study [Table/Fig-1,2].

Total thiol pool contributes to the majority of antioxidant activity of the body. Disturbances of thiol-related mechanisms have been observed in diabetes. Major contribution to total thiol pool is from reduced glutathione and free thiol group present in the amino acid cysteine, which can be found in many cellular peptides or proteins [27]. For instance, plasma levels of protein-bound thiols were found to be lower in type 2 diabetics compared with their controls [28]. Thiol groups can be oxidised to form disulphide. The oxidation of the thiol groups in several peptides and proteins including GBM proteins are specifically coupled with the reduction of other molecules. These thiol antioxidative peptides or proteins, such as glutathione, thioredoxin etc., play an important role in the cellular antioxidative defence as well as the regulation of cellular functions involving the thiol-disulphide exchange [25,29]. Such a thiol-disulphide exchange reaction with DADPDS should result in increased thiol content in the renal tissue and decreased TBARS levels which has been observed in the present study [Table/Fig-1].

LIMITATION

The study was conducted in short term (2 months) diabetic rats. Histopathological changes of the renal tissue in these rats were not done.

CONCLUSION

Hence, it may be concluded that DADPDS helps in preventing de-sialation of GBM in alloxan diabetic rats and improves renal tissue antioxidant defence mechanisms, possibly through thiol-disulphide exchange reaction.

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