ORIGINAL ARTICLE

EFFECT OF TAURINE ON BIOMARKER OF OXIDATIVE STRESS IN SERUM OF HIGH FAT DIET-LOW DOSE STREPTOZOTOCIN INJECTED MODEL OF TYPE 2 DIABETIC RATS

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Background: Oxidative stress plays a major role in the development of insulin resistance and pancreatic beta cell dysfunction. Taurine and beta-alanine have been documented to act as antioxidants. The aim of this study was to compare the antioxidant effect of taurine and beta-alanine in type 2 diabetic rats. **Methods:** This Laboratory based experimental study was conducted in Department of Physiology at Army Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad, from July to Sep 2018. Ninety male Sprague-Dawley rats were randomly divided into three groups; diabetic control (DC), diabetic beta-alanine (DBA), and diabetic taurine group (DTau). All ninety rats were fed with taurine free-high fat diet for a period of four weeks, and administered low-dose streptozocin on the 14th day for the induction of type 2 diabetes mellitus. Also, DC rats were supplemented with 0.02% (w/v) taurine, DBA rats with 3% (w/v) beta-alanine, and DTau rats with 3% (w/v) taurine in their respective drinking water, for a period of four weeks. At day 21, plasma glucose levels and insulin resistance were measured to confirm development of type 2 diabetes mellitus in the three groups. At the end of four weeks, terminal intracardiac sampling was done to measure 8-isoprostane levels. **Results:** 8-isoprostane levels were significantly increased in diabetic group. Taurine supplementation ameliorated these effects as compared to beta-alanine. **Conclusion:** Taurine reduces oxidative stress in type 2 diabetes mellitus.

Keywords: High fat diet, Oxidative stress, Taurine supplementation

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INTRODUCTION

Oxidative stress (OS) is a major direct pathway in the development of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) in both rodent and human studies.[1,2] An important consequence of excessive reactive oxygen species and OS, especially linked with IR and diabetes, is lipid peroxidation (LPO). LPO can be measured by its primary (lipid hydroperoxides and conjugated dienes) or secondary (thiobarbituric reactive substances and F_2-isoprostanes) end products. Among these, the F_2-isoprostanes (F_2-IsoPs) have surfaced as a direct and reliable measure of OS. F_2-IsoPs are prostaglandin-like compounds derived non-enzymatically, through free radical, such as superoxide anion (O_2^-)-catalysed peroxidation of arachidonic acid. F_2-IsoPs are first formed in esterified form in tissues, and thereafter enter the circulation in the free form. 8-isoprostane-PGF2α (8-IP), the most abundant F_2-IsoPs, has proved to be highly precise lipid biomarker for *in vivo* OS in both animal and human studies. Elevated levels of 8-IP are reported in biological fluids in T2DM patients.[2,3]

Tau is a methionine and cysteine-derived amino acid found in mammalian tissues. Reduced Tau levels have been reported in diabetes.[4] An inverse correlation exists between plasma Tau levels and diabetic complications, while, dietary Tau supplementation reduced OS and alleviated diabetic complications, implicating Tau as a conditionally essential amino acid in disease states associated with high OS, such as DM and metabolic syndrome.[5]

With regard to its antioxidant property, Tau is responsible for ensuring normal electron transport chain (ETC). In doing so, Tau protects mitochondria from excessive formation of O_2^-.[5] Tau forms a conjugate with uridine on mitochondrial transfer RNA (tRNA) and forms 5-taurinomethyluridine [$\tau m^5(S^2)U$] for adequate formation of mitochondrial encoded proteins (MEPs), which are functional subunits of respiratory chain complexes. Since, the $\tau m^5(S^2)U$ content of tRNA is dependent on Tau levels, Tau deficiency causes a paucity of MEPs (ND5 and ND6) and disruption in activities of complexes I and III of the respiratory chain. This diverts electron to the acceptor oxygen resulting in excessive O_2^- generation and hence, increased OS.[6]

Beta alanine (BA) is another emergent class of therapeutic agent, reported to positively affect metabolic control in various animal models of diabetes, as well as, in human subjects with T2DM, on account of antioxidant properties. In the body, BA combines with L-histidine to form the dipeptide carnosine (β-alanyl-L-histidine).[7–9] Carnosine (CAR) is a non-enzymatic free-radical scavenger. At physiological concentrations, CAR alters the reactivity of O_2^-, by directly reacting with, and forming a charge-transfer complex with O_2^- radical. Being a natural dipeptide, CAR also reduces LPO.[7,9]

Diabetes-associated increased OS in various organs is followed by micro- and macrovascular complications.[1] Therefore, mitigating OS is a major target for preventing, managing, and treating T2DM. This has steered the path for investigation of new compounds having strong antioxidant properties.[5] The current study aimed to compare the antioxidant effect of Tau and BA, in type 2 diabetic Sprague-Dawley rats.

METHODOLOGY

This laboratory-based experimental study was done at Department of Physiology, Army Medical College, Rawalpindi, and National Institute of Health, Islamabad, from July to Sep 2018, after approval of the Ethical Committee of the College.

Preliminary assessment of plasma glucose was done to exclude rats with pre-existing derangement in glucose metabolism. Ninety male Sprague-Dawley rats, aged 60–90 days, and weighing 250±50 grams, were selected for the study. Rats were kept in 2×3 feet steel cages, fitted with clean water bottles, in a well-ventilated room at 20–22 ºC, on 12-hour light/dark cycle.

Rats were divided randomly into 3 groups: diabetic control (DC), diabetic BA (DBA) and diabetic Tau (DTau). All rats were fed for 4 weeks, with Tau free-high fat diet (Table-1). DC rats were supplemented with 0.02% (w/v) Tau which represented Tau content of standard rat chow, DBA rats with 3% (w/v) BA, and DTau rats with 3% (w/v) Tau in their respective drinking water for a period of 4 weeks.

Table-1: Composition of taurine free-high fat diet

Ingredients	Weight (g/Kg)
Animal fat	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-Methionine	3
Yeast powder	1
Sodium chloride	1
Powdered taurine free diet:	365
i. Cornstarch	122.2385
ii. Casein	78.11
iii. Dextrised cornstarch	40.365
iv. Sucrose	36.5
v. Soybean oil	51.1
vi. Cellulose	18.25
vii. Mineral mix	12.775
viii. Vitamin mix	3.65
ix. L-cystine	1.095
x. Choline bitartrate	0.9125

On 14th day of study, a single, low-dose (35 mg/Kg body weight) injection of streptozotocin (STZ) (Calbiochem, USA) was given intraperitoneally, in the lower-right quadrant of the abdomen of all rats, to induce T2DM with insulin resistance. At day 21st, tail vein blood samples were obtained to measure plasma glucose and insulin resistance, to confirm development of T2DM.[10] The same diet and supplementation were continued for another week.

On completion of 4 weeks of study, rats were euthanized by overdose of ether anesthesia. Terminal blood samples were collected by intracardiac sampling, in sodium fluoride tubes for plasma, and in gel separator tubes for serum. The samples were centrifuged and stored at -80 °C until assayed. Plasma glucose was measured by glucose oxidase method (Linear Chemicals, Spain), serum levels of insulin were measured by Sandwich ELISA (Cayman Chemical Company), and HOMA-IR was calculated from these values. Serum 8-IP was measured by enzyme immune assay (Cayman Chemical Company).

Data was entered and analysed on SPSS-21 to calculate Mean±SD of all variables. ANOVA and Post-hoc Tukey test was applied for comparison between groups, and $p<0.05$ was considered significant.

RESULTS

At the completion of 3rd week, T2DM was confirmed according to criteria. At the end of the study, Post hoc Tukey's HSD revealed statistically significant difference in the levels of 8-IP between control and Tau ($p=0.00$), and between BA and Tau ($p<0.001$). Non-significant difference in 8-isoprostane levels was found between control and BA groups ($p=0.07$) (Table-2).

Table-2: Comparison of 8-isoprostane levels among groups of diabetic rats, by Post Hoc Tukey's Test at completion of the study

8-IP (pg/ml)	DC (n=30)	DBA (n=30)	DTau (n=30)
Mean±SD	219.83±64.15	190.79±54.35	141.33±23.26
p	I & II= 0.07	II & III <0.001*	I & III= 0.00*

*Significant

DISCUSSION

In this study the high fat diet-low dose STZ model of T2DM was used because this model closely resembled the human T2DM in its natural history and metabolic profile.[10] The effect of Tau and BA supplementation on oxidative stress related parameter were examined in diabetic rats. Tau significantly ($p=0.00$) ameliorated OS in supplemented rats in comparison to rats supplemented with BA.

Tau has been documented to reduce OS indices in other experimental studies. In one such study, STZ-induced diabetic Wistar dams were orally supplemented with Tau (1 g/Kg bodyweight/day) from 5th till 12th gestation day, and euthanized on the 13th day. The authors found that after the 8 days of treatment, Tau reduced the OS marker, malondialdehyde (MDA) in diabetic dams ($p<0.05$) as well as in mitochondrial and cytosolic fractions of embryonic milieu ($p<0.05$). The authors further found Tau to be embryo-protective in pregnant diabetic dams, and suggested Tau to be used as

a supplemental therapeutic in diabetic pregnancies.[11] In a study by Zhang *et al*, mice were injected once daily with iron, 5 days a week, for a total of 13 weeks, to produce iron overload with consequent hepatic dysfunction and increased OS. Tau treatment elevated hepatic Tau levels (40%), improved hepatic function, and reduced ROS formation, liver LPO, and OS. The authors suggested Tau as a therapeutic agent with potential to reduce iron overload-induced hepatic damage.[12]

In contrast to animal studies, the results of the few clinical trials that have explored the effect of Tau consumption on OS, are contradictory. In one such trial in which type 2 diabetic patients consumed Tau (3,000 mg daily for 4 months), no effect on OS status was reported. It could be because the sample size of the study was small due to the recruitment of only freshly-diagnosed cases of T2DM, and hence, lack of significant alterations in OS marker.[13] On the other hand, Maleki *et al*, conducted a novice controlled clinical trial in type 2 diabetics and found that Tau supplementation (1,000 mg, thrice daily, for 8 weeks) significantly decreased levels of serum MDA (26.33%, p=0.00). The authors attributed these findings to the suppression of superoxide generation in the mitochondria, as well as to stimulatory effect of Tau on the activities of antioxidant enzymes superoxide dismutase (5.1%, p=0.00) and catalase (4.22%, p=0.00), culminating in markedly reduced generation of ROS.[5]

Significant reduction in OS was also noted in Tau supplemented diabetic rats as compared to BA treated rats (p<0.00). The difference in antioxidant capacity of Tau and BA could be because the dosage of BA (3%) was too low or the duration of BA supplementation was too short to cause beneficial effect, as opposed to Tau given in the same dose and for the same duration. Or it could be because 3% BA, being structurally similar to tau, competitively inhibits Tau uptake at transporter site, producing 50% reduction in plasma and tissue Tau levels.[14]

BA also eliminates mitochondrial Tau content by 60%.[15] Taken together, this resulted in increased mitochondrial superoxide generation with resultant increased LPO and OS. Similar to our finding, immersion of isolated neonatal cardiomyocytes of Wistar rats for 2 days, in a 5 mM BA-containing medium produced a 45% decrease in Tau content along with increased mitochondrial OS. Co-administration of 5 mM Tau with BA prevented these effects. BA inhibited the post-transcriptional modification of tRNA by tau, thereby negatively impacting MEP synthesis and assembly of respiratory chain complexes. This ultimately resulted in accumulation of harmful oxidants (Figure-1)[16].

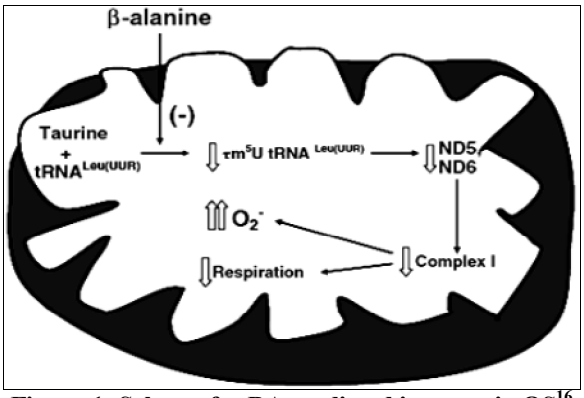

Figure-1: Scheme for BA-mediated increase in OS[16]

In the present study, 8-IP levels of diabetic BA supplemented rats were comparable (p=0.07) with those of control rats. This could be because of insufficient dose of BA used in the present study (3%), or to the absence of nutritional control during supplementation, which may have resulted in insufficient CAR concentration in the body, to be effective as antioxidant and bring serum 8-IP levels down to the value where difference with serum 8-IP levels of control rats became significant. This could also be due to the BA-induced Tau deficiency. It could also be that the duration of supplementation was too short to cause any beneficial effect. In corroboration with our study, Gibbons *et al*, also documented that feeding mice for four weeks with BA supplemented diet had no effect in reducing OS.[17] Kerai *et al* also demonstrated that rats fed on BA (3%) supplemented diet remained susceptible to ethanol-induced hepatic LPO, as a result of Tau depletion.[18]

CONCLUSION & RECOMMENDATIONS

Tau as compared to BA, significantly improved the OS indices in type 2 diabetic rats. Therefore, it can be used as a supplement for the treatment of T2DM. It is suggested that combined supplementation of alanine and taurine should have also been investigated to assess if together these could be more beneficial as an effective therapeutic approach rather than when given independently. Plasma levels of CAR in BA supplemented rats should have been measured to ascertain the amount of CAR generated.

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