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An experimental study: Diabetic nephropathy and oxidative damage relationship

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Abstract

Aim: The increase of oxidatif stress caused by hyperglycemia in diabetes contributes to diabetic complications such as nephropathy. Experimental Diabetes Mellitus (DM) was induced with streptozotocin. Thioctic acid (TA), which has antioxidant properties, is a vital cofactor of mitochondrial respiration enzymes. This study investigated whether TA administration could reduce oxidative stress to treat diabetic nephropathy.

Materials and Methods: 40 male Wistar albino rats were divided to groups: Control, DM, TA and DM+TA. TA and DM+TA group was administered 100 mg/kg/day TA daily, and blood glucose was assessed for six weeks. The superoxide dismutase, glutathione peroxidase and catalase activities, glutathione levels, malondialdehyde (MDA), protein carbonyl (PC), total antioxidant (TAS), total oxidant status (TOS) and OSI also were evaluated.

Results: MDA and PC were increased, and antioxidant capacity was decreased in the diabetic groups compared to Control ($p < 0.05$). In the DM+TA group, MDA, PC and TOS were decreased and TAS was increased compared to the DM group ($p < 0.05$).

Conclusion: TA exhibited a curative effect on diabetic nephropathy by increasing antioxidant activity and reducing oxidative damage.

Introduction

Diabetes mellitus (DM) is one of the most prevalent illnesses with the world's highest mortality rate. DM is a metabolic and chronic disease during good behaviour with the inability to produce enough insulin due to the inactivation of insulin containing cells. DM is a general lack of insulin action [1]. DM is associated with multiple pathophysiological processes related to insulin deficiency caused by damage to pancreatic β-cells [2, 3]. Diabetes connected kidney disease, also called diabetic nephropathy, is a long term serious microangiopathy that complicates both Type DM1 and DM2 treatment and adversely affects the lives of diabetic patients. Diabetic nephropathy patients account for approximately half of the patients who have recently died as a result of kidney illness worldwide [4, 5].

Reactive oxygen species (ROS) has a primary role in the improvement and etiology of diabetes. High increment of ROS in DM impairs the primary antioxidant defence mechanizm, as observed in the pathogenesis and improvement of diabetic complications like diabetic kidney illness. The increase of oxidative stress mediates tissue damage. At the molecular level, permanent hyperglycemia induced overproduction of ROS, primarily from renal mitochondria, has been shown to play a significant role in developing and worsening diabetic kidney disease. Reactive oxygen radicals and lipid oxidation are increased and the decrease in antioxidant activity may be associated with diabetic tissue damage patients and experimental diabetic models [6-8]. Cells have enzymatic/nonenzymatic endogenous antioxidant defence systems to decrease the efficacy of these harmful radicals. In addition, efforts are being made to reduce the effects of free radicals and antioxidants that can be taken as exogenous. Today, one of the most frequently mentioned antioxidants is a powerful antioxidant against fat and water soluble oxygen radicals [9].

Thioctic acid (TA) is a naturally synthesized fatty acid with a short chain and two thiol groups. TA or α-lipoic acid is water and fat soluble sulphurous organic metabolite, and TA is an essential cofactor of mitochondrial enzymes. It is also available as a supplement with TA foods. TA has been shown in studies to show various bioactivities such as antiaging, antioxidant, immunomodulatory, anticancer, anti-inflammatory antiviral and neuroprotective activities [10, 11]. Its antioxidant property stems from

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the fact that it is reduced to dihydrolipoic acid, clears free radicals and chelates with metal ions [12]. It is also used in treating diabetes, increasing the cell's use of glucose [13]. TA regulates glucose use and increases insulin production, thus curing the damage caused by hyperglycemia. TA, reduce and develop diabetes-related oxidative stress induced disorderliness and autonomic neuropathy, neuropathy and retinopathy due to DM. TA is a drug used in the treatment of polyneuropathy, one of the diabetic complications.

Streptozotocin (STZ), a diabetogenic agent, is synthesized by the soil bacterium Streptomyces achromogenes. STZ is transported to insulin synthesizing β-cells in pancreatic tissue by glucose transporter 2, similar to glucose. This causes damage to cells and impairs insulin synthesis. Thus leading to a diabetic condition such as Type 2 DM [14]. STZ also has oxidant properties like alloxan, and it decreases glutathione levels in β cells and superoxide dismutase activity in erythrocytes. Apart from pancreatic tissue, STZ also damages the kidney and liver [15]. Since it irritates the tissues, it should not be leaked out of the vein during its application.

In this study, diabetic animals were treated with TA to investigate the possible impact of biochemical alterations seen in diabetic nephropathy. Results were obtained to document the significance of TA in preventing diabetic damage in kidney tissue.

Materials and Methods

Animals

Experimental studies were carried out in the experimental animals unit at the Inonu University Experimental Animals Production and Research Center. All administration of the study under the approval and supervision of the local experimental animal ethics committee of Inonu University on Experimental Animal Research Approved the Experimental Animal Protocols and the use of animals in this study (2022/7-7). According to power analysis [16], 40 male Wistar albino rats [17] obtained weighed between 280-350g were randomly separated into four groups (n:10); Control, Diabetes Mellitus (DM), Thioctic acid (TA), Diabetes Mellitus+Thioctic acid (DM+TA). All rats were housed in a cage environment with a 12h/12h light/dark period and a temperature of 21±2°C controlled. All animals were fed with ad libitum access to a standard laboratory chow diet. The animals' experimental procedures and care were carried out by the ARRIVE guidelines [18].

Induction of diabetes

Rats in the diabetic groups were starved for a night before STZ administration. The solution was freshly prepared 50mg/kg STZ (Sigma-Aldrich CAS no: 18883-66-4) with 0.1M sodium citrate buffer (ph 4,5) and injected intraperitonally [19, 20]. To avoid sudden and severe hypoglycemia, glucose solution (10%) was given as drinking water for 24 hours following the STZ injection. Blood glucose levels were evaluated with a glucometer and test strips (On Call, G113-11, Acon Labs USA) blood samples from the tail three days after the STZ injection. The rats whose blood glucose levels of over 250 mg/dl were included in the diabetic groups [21]. With this experimental diabetes model created in our study, type 2 DM in the clinic was tried to be simulated.

TA administration

Animals of TA ve DM+TA groups were treated with TA (CAS No: 1077-28-7, Abcam; ab142952) daily for six weeks. A daily dose of TA as a solution in 1 ml distilled water freshly prepared 100 mg/kg/day was given by oral gavage at the same time every day (10:00-11:00 am) [22].

Completion of experiment and collection of blood and kidney tissues

At the end of the required period (6 weeks), the rats were sacrificed under anaesthesia (80/12 mg/kg ketamine/xylazine). Blood samples were drawn, and kidney tissues separate for biochemical analysis. The tissues were stored at -80 C° under suitable conditions until the day of the biochemical analysis.

Biochemical analysis

Frozen kidney tissues of a hundred milligrams of specimens were homogenized using steal beads (Next Advance BBY24M, USA) in homogenized in PBS buffer (1:9, w/v) for approximately 3 minutes. Remove homogenate for analyzed malondialdehyde (MDA), which is determined as an indicator of lipid structure peroxidation and protein carbonyl (PC), which is determined as an indicator of oxidative damage of protein structures.

The homogenate was centrifuged at 3500g for approximately 45 minutes to remove large debris and supernatant. The supernatant was used for the measurement of other antioxidant enzyme activities, total antioxidant status (TAS) and total oxidant status (TOS) analyses.

Antioxidant enzyme activities were evaluated as described, Superoxide dismutase (SOD) activity by Sun et al. [23], Catalase (CAT) activity by Aebi et al. [24], Glutathione peroxidase (GSH-Px) activity by Valentine and Paglia [25], GSH concentration by Beutler et al. [26], MDA by Cheeseman and Esterbauer [27] and PC by Packers and Reznick [28] protocols.

TAS and TOS were measured using commercial kit sets compatible with animal tissue (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey). Elisa's analyzes were carried out with the Biotek HT Synergy Gen 5 software, an immune plate reader device. Results for TAS analyzed tests were an adjustment, with Trolox solution, the vitamin E analogue, calculated in mmol Trolox Equivalent/mg protein [29, 30]. TOS measurement tests were adjusted with H_2O_2, and the results were expressed as µmol H_2O_2 equivalent/mg protein [31]. Oxidative stress index (OSI), an indicator parameter of oxidative stress, was calculated according to TOS/TAS results.

Statistical analysis

For statistical analysis, version 22.0 of SPSS for Windows statistical package program was used. Kruskal Wallis Analysis in independent group comparisons; Bonferroni corrected Mann Whitney test was used for pairwise

comparison in groups with differences. Spearman correlation analysis was used to examine the relationship between continuous variables. $p<0.05$ was considered statistically significant.

Results

The difference in blood glucose status between the groups is shown in Table 1. The increases in the Control group between the diabetic groups (DM and DM+TA) are statistically significant ($p<0.05$). In contrast, the decrease in blood glucose between the DM+TA and DM groups was meaningless statistically

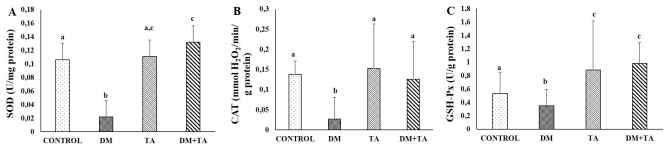

Figure 1. Descriptive statistical criteria for the activities of SOD (A), CAT (B), and GSH-Px (C) parameters in kidney tissues. Data are given as mean ± SD, and a comparison between groups was made with the Kruskal-Wallis test. a, b, c the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

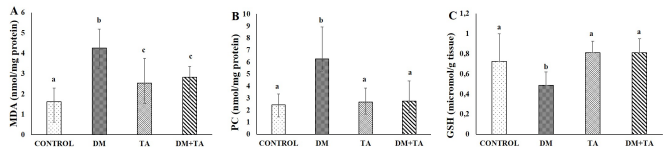

Figure 2. Descriptive statistical criteria for the activities of MDA (A), PC (B), and GSH (C) parameters in kidney tissues. Data are given as mean ± SD, and a comparison between groups was made with the Kruskal-Wallis test. a, b, c the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

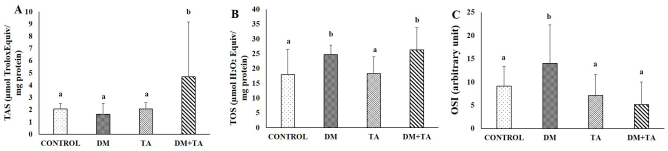

Figure 3. Descriptive statistical criteria for the activities of TAS (A), TOS (B), and OSI (C) parameters in kidney tissues. Data are given as mean ± SD, and a comparison between groups was made with Kruskal Wallis test. a, b the difference between the group with different superscript letters on the same line is statistically significant($p<0.05$) (n=10).

The decrease in SOD activity (Figure 1-A) is statistically significant in kidney in the Diabetic groups contrasted to the Control ($p<0.05$) The increase of SOD values is statistically significant in the DM+TA group contrasted to the control ($p<0.05$). The decrease in CAT activity (Figure 1-B) is statistically significant in kidney tissue in the Diabetic groups contrasted to the Control ($p<0.05$).

The decrease in GSH-Px activity was statistically significant in the DM groups contrasted to the Control ($p<0.05$) (Figure 1-C). The increase in GSH-Px activity values was statistically significant in the TA and DM+TA group contrasted to the Control and DM groups ($p<0.05$).

The results of MDA, PC and GSH levels of kidney are given in Figure 2. The increase in MDA (Figure 2-A) value is statistically significant in the Diabetic groups compared to the Control ($p<0.05$). The decrease in MDA value is statistically significant in the TA and DM+TA groups contrasted to the DM group ($p<0.05$). The increase in PC value (Figure 2-B) is statistically significant in the DM groups compared with the Control ($p<0.05$). The decrease in PC value is statistically significant in the TA and DM+TA groups contrasted to the Diabetic group ($p<0.05$). The decrease in GSH value (Figure 2-C) is statistically significant in the Diabetic groups contrasted with the Control ($p<0.05$). The decrease in GSH value was statistically significant in the TA+DM groups equated to the DM group ($p<0.05$).

Differences in TAS level (Figure 3-A) are meaningless statistically between Control, DM and TA groups. The increase in TAS levels in the DM+TA group is statistically significant contrasted to Control, DM and TA groups ($p<0.05$). The increase in TOS levels (Figure 3-B) in the DM and DM+TA groups is statistically significant contrasted to the Control ($p<0.05$).

The increase in OSI values (Figure 3-C) in the Diabetic group is statistically significant contrasted to the Control and TA ($p<0.05$). The decrease in OSI value was statistically significant in the DM+TA group equated to the Diabetic group ($p<0.05$).

Discussion

Diabetes mellitus has become one of the most metabolic illnesses affecting health in our country and all over the world. In addition, various complications that may impair vital functions may occur in the mid and late periods of diabetes [32]. While DM directly causes many clinical symptoms, complications related to the disease cause serious mortality and morbidity. Retinopathy, dermatitis, and neural, cardiovascular and renal disorders are the most common complications in patients. Diabetic nephropathy is a widespread and considerable diabetic adversity. Diabetic nephropathy is inevitable; by 2050, there will be approximately 786 million diabetic illnesses worldwide, and more than half of the illness will develop diabetic nephropathy. The chronic period, in which clinical symptoms of kidney function begin and histopathological damage is formed, is defined as final-stage renal failure and the patient becomes dialysis dependent. Therefore, it causes

Table 1. Effect of TA on the weekly blood glucose level in control and diabetic rats.

	0	1	2	3	4	5	6 weeks
Control	124±10	121±7	125±11	124±10	121±7	121±13	117±13
DM	392±106[a]	408±133[a]	480±71[a]	395±106[a]	415±105[a]	413±120[a]	452±120[a]
TA	141±19	134±16	135±19	123±6	121±9	125±11	141±19
DM+TA	473±175[a]	451a±107	415±128[a]	322±136[a]	292±169[a]	318±165[a]	300±132[a]

Data are given as mean ± SD and comparison between groups was made with Kruskal Wallis test a; statistically different than control group, $p<0.05$ n=10.

high treatment costs and mortality in DM patients [33]. Because of this, it is essential to discover a new therapeutic agent for diabetic nephropathy and other diabetic complications [34].

Recent studies on diabetes have shown that the onset and course of diabetic nephropathy, one of the microvascular complications of diabetes, can be significantly improved with various interventions. However, these interventions have a significant effect if initiated firstly in the retrogression of diabetic nephropathy. ROS increase the cytokines, formation of transcription and growth factors related to diabetic nephropathy progression. In addition, increases in ROS generally induce lipid peroxidation, leading to the progress of renal fibrosis and renal damage [35]. Excess glucose causes a decrease in antioxidants such as NADPH in cells, leading to increased free radicals in the tissue. STZ induced insulin deficiency and hyperglycemia developing protocols are being studied to establish animal models for experimental type DM2 in rats. Therefore, in this study, we observed that TA has a therapeutic effect, regulating the oxidant/antioxidant balance in nephropathy caused diabetic oxidative stress.

One of the most distinctive features of diabetic complications is the presence of increased ROS and oxidative damage and decreased antioxidant protection mechanism. Due to increased oxidative stress in all phases of DM, extracellular matrix increase in the glomeruli in the renal cortex, tubulointerstitium and vascular structures, an increase in glomerular filtration, yellowing tuberculosis, glomerulosclerosis, myelitis tuberculosis, fibrosis. Today, one of the alternative ways to control the development of diabetic nephropathy and to develop treatment methods is the use of antioxidants. In one of the experimental studies, lipoic acid treatment for 14 days in rats with diabetes mellitus with 90 mg/kg STZ wasinvestigated GSH, GSH-Px, SOD and CAT activities in lung, heart and kidney tissues. As a result, they showed the effect of lipoic acid to avoid oxidative damage by sweeping free radicals and reducing lipid oxidation [36]. In another study, the researcher observed that alpha lipoic acid protects glomerular injury in the early phase of DM even more than high doses of vitamin C and vitamin E [37]. In this study, the therapeutic effect of lipoic acid in rats induced with diabetes by STZ was evaluated. It was observed with immunohistochemical staining that renal damage decreased following the literature. CAT, SOD and GSH-Px are the essantial enzymes that have antioxidant activity in cells and exert their curative effects by scavenging ROS and enhancing the antioxidant defence system [38]. In our study, TA significantly reduced oxidative stress in the kidneys of diabetic groups. Our findings suggest that the observed changes in oxidative stress markers may have contributed to the efficacy of TA on diabetic nephropathy. In the literature, the positive efficacy of the use of antioxidants against active toxic substances is generally mentioned, but the important thing is to maintain the oxidant/antioxidant balance. Although the mechanism has not been fully explained in some studies, there are findings that the use of antioxidants does not always reduce oxidative damage markers and does not increase antioxidant capacity [39]. The use of high doses of antioxidants may trigger the cancerization process by suppressing the programmed death process of the cell. In a cell culture study in which high doses of vitamin C, which is known to have antioxidant activity and widely used in the literature, caused depletion of GSH and NAD, it was reported that it induced the expression of apoptotic genes [40]. In our study, the reason why the antioxidant capacity was higher in the diabetic groups in which we applied TA (DM+TA) than the groups because we applied only TA. This may be because the increased oxidative stress in the DM groups' cells activates the cell's antioxidant defence mechanisms.

To further research the effects and practice of TA on diabetic nephropathy and to find the connections between oxidative stress, inflammation and lipid deposition in ameliorating diabetic nephropathy with TA, the study analyzed major indicators of all three directions with diabetic nephropathy. A recent comprehensive study determined that oxidation markers such as advanced protein carbonyl, oxidation protein products and malondialdehyde increased in the kidney tissues of diabetic rats. Kidney tissues of diabetic rats were examined immunohistochemically, using apoptotic, antiapoptotic, caspase-3 and TUNEL methods and apoptosis was detected in tubular cells and occasionally in glomerular structures [41]. They contain lipid hydroperoxides, oxygen and metal cations formed through certain reactions from various long-chain unsaturated fatty acid precursors. As a result of sequential reactions, highly reactive lipid radicals are formed. In diabetes, individual lipid profiles deteriorate, sensitivity to lipid peroxidation increases and this causes an increase in the incidence of atherosclerosis. As a result, the formation mechanisms of lipid peroxides and their active metabolites gain importance in diabetes studies. However, TA showed a significant effect on lipid backlog in the tissue [38, 42]. The results showed that TA oxidative stress in rats with diabetic nephropathy. In addition, TA significantly reduced the MDA level of the rat with diabetic nephropathy. Studies have shown that chronic lipid deposition reason an improve in the generation of ROS and also leads to oxidative

damage to organism [43]. In addition, oxidative stress in adipocytes induces an immune response that leads to low-grade inflammation [44]. As mentioned before, TA exerts its positive effects on diabetic nephropathy by regulating lipid oxidation.

Another important molecule that free oxygen radicals damage besides lipid structures is protein structures in the cell. ROS can reaction with various amino acids in vitro, thereby converting changed less active enzymes into denatured, nonfunctional proteins. The cleavage of the peptide chain and the aggregation of the cross-linked reaction products cause changes in their electrical charge and increase their susceptibility to proteolysis. As in our study, irreversible damage occurs in collagen, intracellular proteins and DNA in tissues exposed to chronic hyperglycemia and glucose toxicity. Vascular ischemia and increased free radicals in diabetic nephropathy cause inflammation, apoptosis and necrosis. Increasing oxidative stress depending on the severity and duration of diabetes also causes an increase in the value of PC [45]. In our study, the reduce in the amount of PC in the diabetic group. Treatment with TA shows that TA is effective in preventing structural and functional damage and its antioxidant property [16].

Creatinine is formed by the loss of water from creatine in skeletal muscles. Serum Cr level is determined by body muscle mass and muscle breakdown. However, it is only slightly affected by dietary protein content. Therefore, it is more sensitive than the BUN level in assessing renal function. However, this test alone is insufficient to detect mild to moderate kidney damage. Serum creatinine level increases in renal failure, severe congestive heart failure and obstructive uropathies that prevent urine flow. The use of BUN/Cr (not urea) ratio to differentiate renal azotemia from prerenal and postrenal azotemia. It is more valuable than using the test alone. In healthy people, this rate varies between 12/1-20/1 [46]. In our study findings, it is seen that there is a significant increase in kidney damage markers as a result of 6-week diabetes and TA application decreases these values. In light of our findings, using antioxidants will also be effective in regulating kidney functions.

In addition, there are some limitations such as histopathology and inflammatory cytokines measured in this study. It is not clear how TA exert an influence on the kidney in diabetic rat. The effects of TA derivatives apoptotic, autophagic and mitophagy pathways. Furthermore, TA has kidney protection effects for diabetic comlications. TA may reduce the side effects of drugs and DM. The combined action of TA with drugs could be explored to enable the use of TA in clinical practice.

Conclusion

Consequently, previous studies in the literature and our study findings have shown that TA is a potent antioxidant, but it does not have an antidiabetic effect. During our study, glucose level was high in the DM+TA group. It is not possible to talk about its antidiabetic effect. However, thanks to its antioxidant activity, it reduced the damage caused by damage in lipid and protein structures by increased oxidative in diabetes. It may have been effective in reducing the damage in the kidney tissues by acting on apoptotic, autophagic and mitophagy pathways, which we did not evaluate in our study. As our knowledge of the etiopathogenesis of diabetic nephropathy increases, finding an easy way to solve this problem becomes more and more complex. The multifactorial causes in neuropathogenesis indicate that researchers will spend time and effort on the treatment of diabetic nephropathy for a more extended period.

Ethical approval

This study was carried out with the approval of the Ethical Committee of Experimental Animals of the Faculty of Medicine at Inonu University (2022/7-3). The authors have no ethical conflicts to disclose and declare they have no competing interests.

Author contributions

Conceptualization, formal analysis and data curation, K.T.; writing, methodology and software, K.T. and S.S.

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