Effect of Taurine on Liver Xanthine Oxidase Activity and 3-Nitrotyrosine Level in Endotoxemia

Endotoksemide Taurinin Karaciğer Ksantin Oksidaz Aktivitesi ve 3-Nitrotirozin Düzeylerine Etkisi

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SUMMARY

Objective: Taurine, a semi-essential and sulphur-containing β-amino acid, is not incorporated into proteins. It has been shown in many *in vitro* and *in vivo* studies to have cytoprotective effects, and these actions are often attributed to an antioxidant mechanism. The purpose of this study was to investigate the effect of taurine on 3-nitrotyrosine production and xanthine oxidase activity in hepatocytes during endotoxemia.
Methods: In this study, 40 adult male Dunkin Hartley guinea pig were randomly divided into four groups: control, taurine, endotoxemia and taurine plus endotoxemia (n=10). Animals were administered taurine (300 mg/kg, a single dose), lipopolysaccharide (4 mg/kg), or taurine plus lipopolysaccharide intraperitoneally. After six hours of incubation, when the highest blood levels of taurine and endotoxin were attained, the animals were sacrificed and liver samples were collected. The amount of 3-nitrotyrosine was measured by HPLC and xanthine oxidase enzyme activity was measured spectrophotometrically.
Results: Lipopolysaccharide administration significantly increased protein 3-nitrotyrosine levels and xanthine oxidase activity in hepatic tissue compared with the control group ($p<0.05$). In the taurine plus endotoxemia group, taurine and lipopolysaccharide administration significantly reduced protein 3-nitrotyrosine levels and xanthine oxidase activity compared with the endotoxemia group ($p<0.05$).
Conclusion: It has been concluded that taurine prevented the formation of protein nitration in hepatocytes. Taurine may act as a biological antioxidant not only by decreasing xanthine oxidase activity but also by scavenging peroxynitrite. *(Gazi Med J 2011; 22: 14-7)*

Key Words: Taurine, 3-Nitrotyrosine, xanthine oxidase, liver, endotoxemia

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ÖZET

Amaç: Taurin, protein yapısına katılmayan, semi-esansiyel ve kükürt içeren bir β-amino asittir. Daha önce *in vivo* ve *in vitro* pek çok çalışmada taurinin hücre koruyucu etkilere sahip olduğu gösterilmiştir ve bu etkileri çoğunlukla antioksidan özelliğine bağlanmıştır. Bu çalışmada, taurinin endotoksemi esnasında hepatositlerdeki 3-nitrotirozin üretimi ve ksantin oksidaz aktivitesi üzerindeki etkilerinin araştırılması amaçlanmıştır.
Yöntemler: 40 adet erkek Dunkin Hartley kobay, kontrol, taurin, endotoksemi ve taurin+endotoksemi olmak üzere rastgele 4 gruba ayrılmıştır (n=10). Taurin grubuna 300 mg/kg ve tek doz taurin, endotoksemi grubuna 4 mg/kg lipopolisakkarit, taurin+endotoksemi grubuna ise aynı dozlarda taurin ve lipopolisakkarit birlikte intraperitoneal olarak uygulanmıştır. Uygulamadan 6 saat sonra, taurin ve endotoksin konsantrasyonları kanda en yüksek seviyeye ulaştığında, kobaylar anestezi altında feda edilmiş ve karaciğer dokuları uygun koşullarda alınmıştır. Dokulardaki 3-nitrotirozin düzeyleri HPLC ile, ksantin oksidaz aktivitesi ise spektrofotometrik olarak ölçülmüştür.

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Bulgular: Lipopolisakkarit uygulaması, karaciğerde protein 3-nitrotirozin düzeyini ve ksantin oksidaz aktivitesini kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı şekilde arttırmıştır. Taurin+endotoksemi grubunda ise, 3-nitrotirozin seviyesi ve ksantin oksidaz aktivitesi, endotoksemi grubuna göre belirgin azalma göstermiştir.
Sonuç: Taurin, hepatositlerde proteinleri nitrasyon oluşumuna karşı korumuştur. Hem muhtemel peroksinitrit süpürücü etkisi ile, hem de ksantin oksidaz aktivitesini azaltması yoluyla biyolojik bir antioksidan olarak etki gösterebilir. *(Gazi Med J 2011; 22: 14-7)*

Anahtar Sözcükler: Taurin, 3-Nitrotirozin, ksantin oksidaz, karaciğer, endotoksemi

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INTRODUCTION

Lipopolysaccharide (LPS) is an integral component of the cell wall of gram-negative bacteria. LPS administration has been commonly employed for the experimental induction of inflammation in laboratory animals. The inflammatory response includes activation of free radical-generating enzymes in various types of cells (1, 2). The activated free radical-generating enzymes are xanthine oxidase (XO), inducible nitric oxide synthase (iNOS) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase. XO reduces molecular oxygen, leading to the formation of superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). Another important source of free radical generation by LPS depends on iNOS. This enzyme produces large amounts of nitric oxide (NO) (3-5).

Recent studies have indicated that many of the deleterious effects of NO are mediated by peroxynitrite ($ONOO^{\cdot}$). $ONOO^-$, which is formed *in vivo* by the diffusion-controlled reaction between NO and $O_2^{\cdot-}$, has been receiving increasing attention as a mediator of various human diseases, including neurodegenerative disorders, chronic inflammation, autoimmune diseases, ischaemia-reperfusion injury and septic shock. $ONOO^-$ is a strong oxidating and nitrating agent and reacts with various biomolecules, such as lipids, thiols, amino acids and antioxidants. $ONOO^-$ reaction with proteins can cause nitration and oxidation of residues, leading to changes in protein structure and function. Thus the formation of 3-nitrotyrosine (3-NT) *in vivo* has been considered a possible stable marker for the action of $ONOO^-$ (6, 7). Increased nitration of tyrosine residues was demonstrated in hepatocyte membrane in response to $ONOO^-$-induced oxidative stress by LPS (8). It has been suggested that either NO or $ONOO^-$ could feedback and inhibit XO (5). However, controversy remains regarding the presence and nature of interaction of NO or $ONOO^-$ with XO and the role of this process in regulating oxidant generation.

Taurine (2-aminoethanesulfonic acid), a semi-essential and sulphur-containing β-amino acid, has cytoprotective properties conferred by its antioxidation, detoxification, osmoregulation and membrane-stabilization actions, as well as intracellular calcium flux regulation, due to its molecular structure. It has also been implicated in a wide array of other physiological phenomena including feedback inhibition of macrophage respiratory burst. Several recent studies have reported that taurine may also act as a protective agent able to prevent $ONOO^-$ mediated reactions in *in vitro* assays (9-11).

In the present study, we aimed to investigate the effect of taurine on liver $ONOO^-$ production via 3-NT detection and XO activity during LPS-mediated endotoxemia.

MATERIALS AND METHODS

Chemicals

Sodium phosphate dibasic, potassium phosphate monobasic, hydrochloric acid and trichloroacetic acid used for the study were purchased from Merck (Darmstadt, Germany). Sucrose, 3-nitrotyrosine, xanthine, phosphoric acid, citric acid and methanol (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals and Study Protocol

This study was carried out in accordance with the regulations of the Animal Experimentation Ethics Committee at Gazi University. All experiments were performed with 40 adult male Dunkin Hartley guinea pigs weighing 500-600 g. The animals were randomly divided into four groups. Group I (n=10) served as the control group and was given only saline solution. Group II (n=10) served as the taurine group and taurine was administered intraperitoneally (ip) in a single dose (300mg/kg) (2). Group III (n=10) served as the endotoxemia group and taurine was administered ip LPS (*Escherichia coli* serotype 0111: B4, from Sigma, 4mg/kg) (12). Group IV (n=10) served as the taurine plus endotoxemia group and was administered consecutively in taurine and LPS. After six hours of incubation, when the highest blood levels of taurine and endotoxin were attained, all animals were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) intramuscularly, and sacrificed. The livers were removed and immediately frozen in liquid nitrogen, and then stored at -80°C until assay.

Measurement of 3-NT Levels

Tissue homogenates were prepared according to the method described by Kamisaki et al. (13). Briefly, 0.5 g liver tissue was homogenized in 1.5 ml of 50 mM potassium phosphate buffer (pH 7.2) and then centrifuged for 5 min at 1.000xg. Following the acid hydrolysis of the precipitate, it was evaporated under nitrogen and after adding distilled water, 10 µl sample was applied to HPLC. All samples were analysed by HPLC with an electrochemical detector using the method described by Maruyama et al. (14). For 3-NT detection a Microtech Scientific C_{18} analytical column (50x1mm, 5 µm particle size) was used with 50 mM phosphoric acid/50 mM citric acid/5% methanol (pH 3.1) as the mobile phase. Applied potentials were -850 mV for the reduction and 600 mV for the oxidation/detection cell. Concentrations of 3-NT were calculated from a 3-NT standard curve and expressed as µmol/g wet tissue.

XO Activity Determination

Measurement of XO activity was accomplished spectrophotometrically using the method described by Prajda and Weber (15). Liv-

er tissue samples were homogenized in 0.25 M sucrose solution and then centrifuged at 10.000xg at 4°C for 30 min. The supernatant was incubated for 40 min at 37°C and then added to the reaction mixture containing xanthine (0.17 mM) in phosphate buffer (33 mM, pH 7.5). The reaction was carried out at 37°C and was stopped at 0 and 20 min by addition of 0.1 ml 100% TCA (w/v). The mixture was centrifuged at 10.000xg for 15 min. In the supernatants, the uric acid produced from the substrate, xanthine, was measured by the increase in absorbance at 293 nm. The blank contained the identical reaction mixture without xanthine. The enzyme activity was calculated by using the difference in the reaction rates in samples and blank. Protein concentration was measured by the Lowry method (16).

Statistical Analysis

Results were expressed as mean±standard deviation. Statistical analyses were performed using a software program (SPSS 11.5 for Windows, Chicago, IL, USA). The nonparametric Mann-Whitney U test was used to analyze the significance of the differences between control and experimental groups. $p<0.05$ was considered significant.

RESULTS

Liver 3-NT levels and XO enzyme activities of four group were indicated in Table 1.

In the endotoxemia group, LPS administration increased approximately 2-fold levels of 3-NT in liver tissues compared with the control group ($p=0.007$, $p<0.05$). Levels of 3-NT in the taurine group was lower than both control and endotoxemia groups, and these differences were statistically significant ($p=0.012$ and $p=0.000$, respectively). As for the taurine plus endotoxemia group, taurine and endotoxin administration together significantly reduced tissue levels of 3-NT compared to the endotoxemia group ($p=0.000$). Moreover, the lowest 3-NT level observed was in the taurine plus endotoxemia group (0.64±0.97 µmol/g wet tissue).

As seen in Table 1, liver tissue XO activity was 71.18±14.84 mU/mg protein in the control group and 68.64±11.27 mU/mg protein in the taurine group, and the difference between them was not statistically significant ($p=0.993$). Liver XO activity was significantly elevated in the endotoxemia group compared with the control group ($p=0.002$). In both taurine and taurine plus endotoxemia groups, XO activities were significantly decreased compared with endotoxemia group ($p=0.002$ and $p=0.000$, respectively).

DISCUSSION

Bacterial LPS has been shown to increase xanthine dehydrogenase (XDH)/XO mRNA expression and activity in mouse liver. XO was found to be significantly elevated in patients with sepsis syndrome (8). Bacterial infection, sepsis, endotoxemia induce NO production as well as oxygen free radical and reactive nitrogen anion generation. A critical role in the development of LPS-related oxidative stress has been ascribed to XO activity as it has been identified as a major source of $O_2^{\cdot-}$-driven intracellular damage in these stress situations (3, 17). The present study provides evidence that XO activity is increased in the liver of endotoxemic animals. In addition, we observed enhanced generation of 3-NT, a stable marker of $ONOO^-$ action, after endotoxin administration to guinea pigs. These results are in agreement with a previous study that reported endotoxin-induced reactive oxygen species such as $O_2^{\cdot-}$ generation together with increased NO production in endotoxemia. $ONOO^-$ reacts with tyrosine to form 3-NT *in vivo* (7). It has been shown that $ONOO^-$ modulates XO function and XO-mediated $O_2^{\cdot-}$ generation *in vitro* (5).

Table 1. 3-NT levels and XO activities of experimental groups in liver

	3-Nitrotyrosine (µmol/g wet tissue)	Xanthine oxidase (mU/mg protein)
Control	1.24±0.28	71.18±14.84
Taurine	0.79±0.09[a]	68.64±11.27
Endotoxemia	2.47±0.26[b,c]	174.14±32.56[g,h]
Taurine plus Endotoxemia	0.64±0.97[d,e,f]	67.10±12.88[i,j]

Results were expressed as mean±standard deviation
[a,b,d,g,i]$p<0.05$, compared to control group
[c,e,h]$p<0.05$, compared to taurine group
[f,j]$p<0.05$, compared to endotoxemia group
([a]$p=0.012$, [b]$p=0.007$, [c,f,j]$p=0.000$, [d]$p=0.005$, [e]$p=0.027$, [g,h]$p=0.002$, [i]$p=0.04$'tür)

Taurine has been shown in many *in vitro* and *in vivo* studies to have cytoprotective effects and these actions are often attributed to an antioxidant mechanism (9, 10). Our study demonstrated that taurine reduced XO activity and 3-NT level in the liver during endotoxemia. Previous studies have shown that taurine concentrations are decreased in patients with sepsis, which suggests that taurine may have a role in the management of endotoxemia (2, 18). Taurine has recently been shown to protect hepatocytes against injury mediated by NO (19). In addition, it has beenshown that taurine inhibits iNOS expression and iNOS synthesis in various cell types, including hepatocytes (20). In another *in vitro* study, it has been demonstrated that taurine protects tyrosine from nitration by $ONOO^-$ (7). In an experimental study performed with isolated rat hepatocytes, taurine significantly reduced LPS-mediated hepatocellular damage and this correlated with free radical and $ONOO^-$ inhibition (20).

In conclusion, our findings indicated that taurine may act as a biological antioxidant, not only for its ability to decrease XO activity but also it can act as a scavenger of $ONOO^-$. Thus, the formation of protein 3-NT has been prevented *in vivo*. We suggest that there is a relationship between increased XO activity and $ONOO^-$ generation in endotoxemia.

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Conflict of Interest

No conflict of interest was declared by the authors.

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