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RESEARCH ARTICLE

Effect of Taurine on Hydrogen Peroxide Production in Lipopolisaccharide Induced RAW 264.7 Macrophage Cells

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Summary

Taurine, one of the most abundant aminoacids in mammalian tissue, regulates various cellular functions, including membrane stabilization, antioxidation, calcium homeostasis maintenance and immunity. It is known that taurine has no significant effect on lipopolisaccharide (LPS) induced nitric oxide production in RAW 264.7 cells. In this study, we investigated the effect of taurine on hydrogen peroxide (H_2O_2) production in LPS induced RAW 264.7 macrophage cells. Nitrite and H_2O_2 levels in cell supernatants were determined spectrophotometrically. Our results show that taurine significantly decreases H_2O_2 levels without affecting nitrite production in LPS activated RAW 264.7 macrophages.

Key Words: *Taurine, macrophage, hydrogen peroxide, LPS*

Lipopolisakkarit ile Uyarılan RAW 264.7 Makrofaj Hücrelerinde Taurinin Hidrojen Peroksit Üretimi Üzerindeki Etkisi

Özet

Memeli dokusunda yüksek düzeyde bulunan aminoasitlerden biri olan taurin, membran stabilizasyonu, antioksidasyon, kalsiyum dengesinin sağlanması ve immünite gibi çeşitli hücresel fonksiyonları düzenler. Taurinin RAW 264.7 makrofaj hücrelerinde LPS ile indüklenen nitrik oksit oluşumu üzerinde anlamlı bir etkisinin olmadığı bilinmektedir. Bu çalışmada taurinin, LPS ile uyarılan RAW 264.7 makrofaj hücrelerinde hidrojen peroksit üretimi üzerindeki etkisi araştırılmıştır. Hücre süpernatanlarındaki nitrit ve H_2O_2 düzeyleri spektrofotometrik olarak belirlenmiştir. Sonuçlarımız, taurinin LPS ile aktive edilmiş RAW 264.7 makrofajlarda nitrit üretimini etkilemeksizin H_2O_2 düzeylerini düşürdüğünü göstermektedir.

Anahtar Kelimeler: *Taurin, makrofaj, hidrojen peroksit, LPS*

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INTRODUCTION

Taurine is a free aminoacid found at high millimolar concentrations in mammalian tissue (1). The various roles attributed to taurine in mammalian system include membrane stabilization (2), bile salt formation (3), antioxidation (4), calcium homeostasis maintenance, immunity (5), osmoregulation (6) and vision (7). Oliveira et al.(2010) reported that taurine exhibited in vitro scavenging activity of reactive oxygen (peroxyl radical and anion superoxide) and nitrogen (nitric oxide and peroxinitrite) species

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without reacting with H_2O_2 (8). In neutrophils, taurine decreases the toxicity of hypochlorous acid (HOCl) produced by the myeloperoxidase (MPO) system and exerts protective effects against inflammatory injury (9). Taurine reacts with HOCl to form the more stable and less toxic taurine chloramine (TauCl) which is mainly responsible for the acclaimed anti-inflammatory effects (10,11). Taurine was found to be ineffective as an inhibitor of nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages but its chloramine form inhibits LPS-induced inducible nitric oxide synthase (iNOS) expression and nuclear factor kappa B (NFkB) activation (12). It was reported that TauCl protected RAW 264.7 macrophages from H_2O_2 induced cell death and activated cytoprotective antioxidant enzymes (13). Taurine bromamine (TauBr), despite the existance of very low concentration of brom (Br) in body fluids, may support TauCl and HOCl in the regulation of inflammatory response and in killing of bacteria by neutrophils dependent on the presence of H_2O_2 (14).

Bacterial lipopolysaccharide (LPS) is one of the major constituents of the outer membrane of Gram-negative bacteria, which triggers gene induction of proinflammatory cytokines and nitric oxide biosynthesis upon release into the circulation (15,16). LPS activates multiple signaling pathways and induces the production of several inflammatory mediators, such as nitric oxide, tumor necrosis factor alfa (TNF α), and interleukins in macrophages (17).. CD 14 is the receptor for LPS and plays an essential role in pro-inflammatory responses in monocytes and macrophages via activation of the NFkB pathway (18).

NFkB takes charge in regulation of genes which are involved in apoptosis, tumorigenesis, various autoimmune diseases, and inflammation (19). Enhanced expression of iNOS gene is mediated by NFkB activation in LPS stimulated mouse macrophages (20-22). LPS-induced stimulation of NFkB activity via phosphorylation and degradation of inhibitor kappa B (IkB) is the major mechanism of increased synthesis of NO in a variety of immune cells (23,24). In unstimulated cells IkB-NFkB complex is inactive in the cytosol. After activation with stimulators such as potent exogenous inducer (LPS) and endogenous inducers (cytokines, interferon-γ and TNFα), IkB-NFkB complex is phosphorylated by IkB kinase (IKK) and free NFkB translocates from cytosol to the nucleus and induces iNOS gene expression (25-27). Reactive oxygen species (ROS) such as H_2O_2 activate NFkB with enhanced iNOS expression and this activation is suppressed by antioxidant compounds and enzymes. Thus, H_2O_2 production by LPS in macrophages participates in the upregulation of iNOS. H_2O_2 increases degradation of IkB and activation of NFkB pathway (28,29).

H_2O_2 is a toxic product of aerobic metabolism which is generated abundantly in inflammatory tissues. High concentrations of H_2O_2 leads to host cell damage (30,31). H_2O_2 which is not a radical itself diffuses readily across cellular membranes and leads to a highly reactive and toxic hydroxyl radical generation. Hydroxyl radical can damage lipids, nucleic acids, carbohydrates and aminoacids. Cells respond to H_2O_2-induced oxidative stress in part by increasing the expression of cytoprotective anti-oxidant enzymes (32,33).

Concerning the effect of H_2O_2 production on iNOS upregulation in LPS activated macrophages, this study may help to gain a better insight into taurine's effect on NO production when considered with its effect on H_2O_2.

MATERIALS AND METHODS

Materials

LPS (from Escherichia coli, serotype O55:B5), MTT (3-[4,5- dimethyltiazol - 2 - yl] - 2,5 - diphenynl-tetrazoliumbromide), xylenol orange were purchased from Sigma Chemical (Turkey). RPMI 1640, fetal bovine serum, L-glutamine and antibiotics (1,000,000 U/mL penicilin and 1gr/mL streptomycin) were obtained from PAA.

Cell Culture

The mouse macrophage cell line RAW 264.7 were cultured in 75 cm^2 plastic flasks with RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum, antibiotic (1,000,000 U/mL penicilin and 1 g/mL streptomycin) and 2 mM glutamine at 37°C

in a humidified 5% CO_2 - 95 % air incubator under standard conditions.

Incubation with chemicals

Cells were incubated with 0.5-20 mM taurine for 1 h and then stimulated with LPS for 20 or 24 h. This treatment period was choosen because preliminary studies revealed that nitric oxide production in LPS stimulated RAW 264.7 cells peaks between 16 and 24 h, and after 24 h of exposure to LPS, nitric oxide dependent cell deaths might occur. Similar considerations were made to choose LPS concentration. We achieved optimum stimulation with 1 µg/mL LPS without cytotoxicity. It is important to treat cells with taurine 1 h before stimulation with LPS because of the probable effect of taurine on antioxidant status and defense capacity of the cell before LPS stimulation.

Cell Viability

Cytotoxicity was assessed by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay of Mossman (34) with some modifications. Firstly, supernatants were removed from the plate for nitrite determination, then cells were incubated at 37°C with 0.5 mg/ml MTT for 1 h. The medium was aspirated and the insoluble formazan product was dissolved in dimethyl sulfoxide (DMSO). The extent of MTT reduction was quantified by measuring the absorbance at 550 and 690 nm. Survival (%) was calculated relative to control.

Nitrite Assay

Cells were seeded in 96 well-plates, 6 well-plates and 75 cm^2 flasks, cultured for one night and then incubated with 1µg/ml LPS. The nitrite concentration in the supernatant was assessed by Griess reaction (35). Griess reagent (0.1% N-(1-naphtyl)ethylenediamide dihydrochloride, 1% sulphanilamide in 5% phosphoric acid) and equal volume of RAW 264.7 cell supernatants were mixed and the absorbance at 550 nm was measured.

Hydrogen Peroxide Measurement

Cells were seeded in 6 well-plates, cultured for one night and then incubated with 1µg/ml LPS. Hydrogen peroxide concentration in the supernatant was assessed by colorimetric method of Jiang (36) with some modifications. One hundred µl cell culture supernatant were mixed with reaction reagent including 100 pM xylenol orange, 250 µM ammonium ferrous sulfate, 100 mM sorbitol, 25 mM H_2SO_4 and incubated for 45 min at room temperature. The absorbance was read at 560 nm.

Statistical Analysis

Differences among groups were evaluated by One Way Anova analysis. P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

To determine the cytotoxic effect of taurine, cells were incubated with 0.5-20 mM taurine for 1 h and then stimulated with LPS for 24 h. We found that 0.5-20 mM taurine is not cytotoxic to cells (Figure 1).

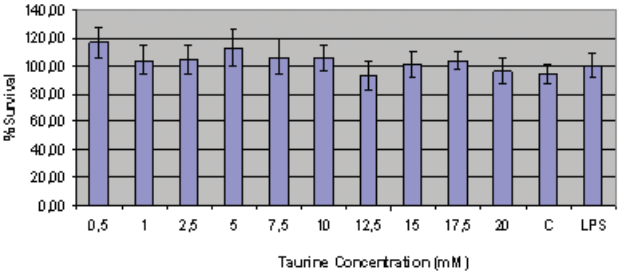

Figure 1: Effect of taurine (0.5-20 mM) on cell survival. Differences among groups are not significant (p>0.05). (C: No stimulation LPS: LPS stimulated cells).

To determine the effects of taurine on NO production, cells were incubated with different concentrations of taurine (1-20 mM) for 1 h before stimulation with LPS. When LPS (1 µg/mL) was added to RAW 264.7 cells, nitric oxide production, measured as nitrite, increased after 20 and 24 h (Figure 2). 20 h after stimulation, nitrite levels were significantly different ($p< 0.001$) between unstimulated cells and cells stimulated with LPS (2.74± 0.11 µM and 8.67± 0.08 µM respectively). There was no significant difference between taurine treated and LPS treated cells. After 24 h of stimulation, nitrite levels of LPS treated and unstimulated cells were significantly higher than

their measured levels at 20 h ($p<0.001$). 24 h after stimulation, nitrite levels were significantly higher ($p<0.001$) in LPS stimulated cells than unstimulated cells (11.72± 0.40 µM and 4.89± 0.13 µM, respectively). Taurine treatment did not have significant effect on nitrite levels at 24 h.

To determine the effects of taurine on H_2O_2 production, cells were incubated with different concentrations of taurine (1- 20mM) for 1 h before stimulation with LPS. When LPS (1µg/mL) was added to RAW 264.7 cells, H_2O_2 increased after 20 and 24 h (Figure 2). After 20 h, H_2O_2 level of LPS stimulated cells was significantly ($p<0.001$) higher than unstimulated cells (100.5 ± 5.65 ng/mL and 150.3±4.60 ng/mL). H_2O_2 level of LPS stimulated cells decreased with all concentrations of taurine (1-20mM) significantly ($p<0.001$). After 24 h, hydrogen peroxide level of LPS stimulated cells was significantly ($p<0.001$) higher than unstimulated cells (121.5± 4 ng/mL and 166.25±8.53 ng/mL respectively) H_2O_2 level of LPS stimulated cells decreased with all concentrations of taurine (1-20 mM) significantly ($p<0.001$) (Figure 3).

In LPS stimulated macrophages, upregulated iNOS can lead to excessive NO production. Upon reaction with superoxide anions, NO forms peroxynitrite, the reactive nitrogen species considered responsible for tissue and molecular damage. A variety of extracellular signals such as LPS activates the enzyme IkB kinase (IKK) which phosphorylates the IkBα protein. Phosphorylated IkBα dissociates from NFkB and translocates to the nucleus and turns on the expression of specific genes (37,38,39).

Reactive molecules such as H_2O_2 are produced in macrophages following exposure to LPS and involved in cellular signalling for gene expression. H_2O_2 is involved in the intracellular signaling for NFkB activation of LPS-stimulated macrophages and may regulate iNOS expression and/or activity in immune-activated macrophages (40). Various pathways can regulate NFkB and most of them lead to IkB phosphorylation by IKK-containing signal cascade (41). It has been suggested that H_2O_2 acts as the central second messenger to NFkB activation (42). H_2O_2 leads to IkB degradation, which is responsible for the activation of NFkB pathway. Our results show that taurine treatment does not effect nitrite levels but decreases H_2O_2 levels in LPS activated macrophages. The decrease in H_2O_2 levels mediated by taurine may possibly decrease IkB degredation and NFkB activation. However, this decrease does not seem to inhibit NFkB activation potently.

CONCLUSION

Upon stimulation with LPS for 20 and 24 h, taurine did not effect nitrite production in macrophages; however, we found that hydrogen peroxide concentrations significantly decreased with taurine treatment. These findings may suggest a better understanding of the effect of taurine which is known as an antioxidant aminoacid on LPS activated macrophages.

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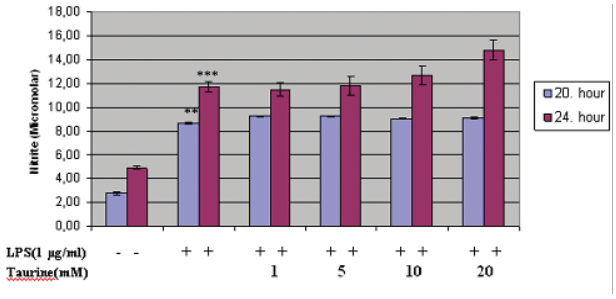

Figure 2: Effect of Taurine on nitrite concentration. Cells were treated with taurine in the presence of LPS and nitrite production was measured. (*): $p<0.001$, versus LPS at 20 h. (**): $p<0.001$, versus LPS at 24 h.

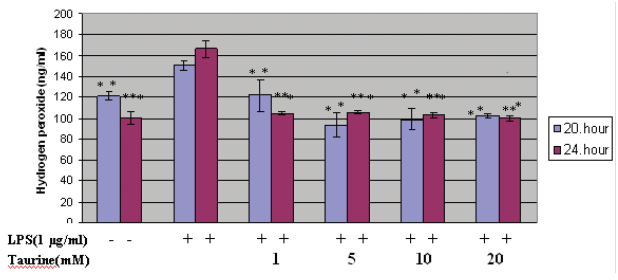

Figure 3 Effect of Taurine on hydrogen peroxide concentration. Cells were treated with taurine in the presence of LPS and hydrogen peroxide production was measured. (*): $p<0.001$, versus LPS at 20 h. (**): $p<0.001$, versus LPS at 24 h.

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