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The effect of taurine on polymorphonuclear leukocyte functions in endotoxemia

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Summary. The aim of the present study was to measure MPO activity in PMN leukocytes after endotoxin administration, and to compare the levels of NO_2^- competing with taurine for reaction with HOCl. Furthermore we aimed to determine TauCl levels, a product of MPO–H_2O_2–Halide system, and to evaluate anti-inflammatory properties of PMN in endotoxemia. In addition, our second objective was to investigate the effect of taurine, an antioxidant amino acid, on anti-bactericidal and anti-inflammatory functions of PMN after administration of endotoxin together with taurine.

All experiments were performed with four groups (control, taurine, endotoxemia, and taurine plus endotoxin) of ten guinea pigs. After endotoxin administration (4 mg/kg), MPO activities increased and taurine levels decreased. Therefore levels of TauCl, $NO_2^{\bullet-}$ increased. We observed the effects of taurine as conflicting. When taurine was administrated alone (300 mg/kg), all of these parameters decreased.

Consequently, we suggested that taurine is influential in infected subjects but not on healthy ones as an antioxidative amino acid. In addition, we believe that in vivo effects of taurine may differ from those in vitro depending on its dosage.

Keywords: Polymorphonuclear leukocyte – Taurine – Myeloperoxidase – Nitric oxide

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; iNOS, inducible nitric oxide synthase; ip, intraperitoneally; LPS, Lipopolysaccharide; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; $O_2^{\bullet-}$, superoxide; $ONOO^-$, peroxynitrite; PMN, polymorphonuclear; ROS, reactive oxygen species; XO, xanthine oxidase

Introduction

PMN, the major cells of acute inflammation, during phagocytosis generate a variety of microbicidal agents, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hypochlorous acid (HOCl), a highly toxic product of the myeloperoxidase-hydogen peroxide-halide system (Ding et al., 1988; Klebanoff and Hamon, 1972; Marcinkiewicz et al., 1999).

RNS may also react with a number of ROS to form novel biologically active species. Recently, it has been suggested that nitrite (NO_2^-) converts HOCl into the more potent chlorinating species – nitryl chloride (NO_2Cl) that is capable of nitrating, chlorinating and dimerizing phenolic compounds including tyrosine (Babior, 2000; Marcinkiewicz et al., 2000).

On the other hand, HOCl reacts rapidly with a variety of different molecules to produce less toxic and more long-lived chloramines. Because of extremely high concentration of taurine in neutrophils, taurine chloramine (TauCl) is the predominant long-lived chloramines produced in this way (Chorazy et al., 2002; Marcinkiewicz et al., 2000).

Taurine (2 amino-ethane sulfonic acid) is a sulphur-containing β-amino acid and the most abundant free amino acid in leukocytes particularly PMN. Taurine can act as a direct antioxidant by scavenging ROS and/or as indirect antioxidant by preventing changes in membrane permeability due to oxidant injury. Many of the biological functions of taurine rely upon the intracellular concentration of itself (Kim et al., 1998; Schuller-Levis and Park, 2004; Tappaz, 2004).

In addition, it has been suggested the production of RNS in neutrophils is more controversial (Evans et al., 1996). Despite intense interest in the role of RNS in phagocytic activity of PMN, the in vivo systems remain poorly understood.

The aim of the present study was to compare NO_2^- and taurine in their ability to affect the antimicrobial/cytotoxic properties of HOCl after endotoxin adminis-

tration in PMN of guinea pigs. In addition, we aimed to investigate the effect of taurine on the antimicrobial and cytotoxic functions of PMN after administration of endotoxin together with taurine. Since many of the biological functions of taurine rely upon the intracellular concentration of itself, we want to evaluate the level of taurine in PMN of all the groups.

Materials and methods

Reagents

NaOH, Na_2CO_3, KCl, $CuSO_4$, KCN, Na_2HPO_4, KH_2PO_4, glucose, HCl, boric acid and $MgCl_2$ used for the study were purchased from Merck Chemical Co (Darmstadt, Germany). Taurine (99%), fluroscamine (98%), disodium tetraborate, 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), and O-dianisidine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and tetrahydrofuran were of HPLC grade and purchased from Labscan (Dublin, Ireland).

Animals and study protocol

All experiments were performed with 40 adult male Dunkin Hartley guinea pigs weighing 500–600 g. The animals were randomized into four groups. Group I ($n = 10$) served as control group and was given only saline solution. Group II ($n = 10$) served as taurine group and was administered intraperitoneally (ip) in a single dose (300 mg/kg) (Egan et al., 2001). Group III ($n = 10$) served as endotoxemia group and was administered ip LPS (*Escherichia coli* LPS serotype 0111:B4, from Sigma, 4 mg/kg) (Duffy et al., 2000). Group IV ($n = 10$) served as taurine plus endotoxin group and was administered consecutively taurine and LPS through the same way. At the end of the 6th hour at which both taurine and endotoxin rise up to blood level, all animals were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) intramuscularly, and blood samples were collected via intracardiac puncture using heparin as an anticoagulant.

PMN isolation

PMN were isolated from 10-ml heparinized blood sample according to the method as described by Cutts (1970). Briefly, for this experiment, blood was collected in a tube containing 4 ml 6.5% dextran and incubated at 4 °C for 45 min. The supernatant was then gently removed and taken into another tube and 20 ml Hanks' solution was added and centrifuged at 4 °C, 2000 rpm for 5 min. The supernatant was removed and sediment mixed with 15 ml distilled water for 30 sec and then 5 ml 3.5% NaCl was added. The mixture was centrifuged at 4 °C, 2000 rpm for 5 min, thereby removing the erythrocytes. The supernatant was removed and the sediment was washed in 20 ml Hanks' solution. The mixture was centrifuged at 4 °C, 2000 rpm for 5 min. The supernatant was removed and 3 ml 0.16 M KCl was added to the sediment and then sonicated at 50 watt, for 30 sec. The mixture was stored at −80 °C until use.

Measurement of NO$^{\bullet}$ levels

NO levels in leukocyte suspension were determined spectrophotometrically, based on the reduction of NO_3^- to NO_2^- by $VaCl_3$ (Miranda et al., 2001). NO levels were measured by the Griess reaction. Sodium nitrite and nitrate solutions (1, 10, 50, 100 μM) were used as standards. Leukocyte suspension samples were deproteinized prior to assay. Samples were added to 96% cold ethanol (1/2 v/v) and then vortexed for 5 min. After incubation for 30 min at +4 °C, the mixture was centrifuged at 14,000 rpm for 5 min and the supernatants were used for the Griess assay (Miranda et al., 2001).

MPO activity determination

The MPO activity was assayed by measuring the H_2O_2-dependent oxidation of O-dianisidine. In its oxidized form O-dianisidine has a brown color. This was measured spectrophotometrically at 410 nm. The results are given as U/mg-protein. One unit of MPO activity was defined as the amount of enzyme caused absorbance change in 1 min at 410 nm and 37 °C (Glowick, 1955).

Measurement of taurine levels

Measurement of taurine was accomplished by HPLC, using the method described by McMahon et al. (1996). In brief, leukocyte suspension samples (10 μl) were treated with 150 μl of acetonitrile and centrifuged at 5800 *g* for 10 min and 50 μl of 10 mM borate buffer (pH 9.2) were added to the supernatant solution. This was followed by the addition of a 50 μl aliquot of 5 mM fluorescamine in acetonitrile and immediate vortex mixing. A 20 μl sample was on to a reversed-phase HPLC system using Bondclone C-18 10 μm analytical column ($300 \times 3.9\ mm^2$). The mobile phase was tetrahydrofuran-acetonitrile-phosphate buffer (15 mM, pH 3.5) (4:24:72, v/v/v). The taurine derivative was detected by measuring the UV absorbance of 385 nm.

Measurement of TauCl levels

The TauCl assay is based on the reaction of HOCl with taurine to produce TauCl, which is measured by reacting it with 5-thio-2-nitrobenzoic acid (TNB). Yellow TNB is oxidized to colorless DTNB. A 1 mM solution of TNB is prepared by dissolving 2 mM DTNB in 50 mM phosphate buffer (pH 7.4). The solution of DTNB is titrated to pH 12 with NaOH to promote its hydrolysis, and after 5 min the pH is brought back to 7.4 with HCl. This assay was carried out in a 96-well format. Changes in absorbance were determined at 405 nm (Kettle and Winterbourn, 1994).

Protein measurement

Protein level of leukocyte suspension was assessed by the Lowry method (Lowry et al., 1951).

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. For tests of significance a p value of less than 0.05 was considered to be significant.

Results

The leukocyte suspension MPO activity, and levels of NO_2^-, taurine and TauCl of the four groups are indicated in Tables 1 and 2.

MPO activity

Endotoxin administration significantly increased MPO activity compared with the control group ($p < 0.05$). Also taurine administration increased MPO activity but this elevation was not statically significant ($p > 0.05$). Endotoxin plus taurine administration significantly reduced

Table 1. $NO_2^{\bullet-}$ levels and MPO activities in PMN leukocytes of experimental groups

	$NO_2^{\bullet-}$ (μmol/l)	MPO (U/mg protein)
Control group	33.28 ± 13.21	149.50 ± 44.56
Taurine group	$53.33 \pm 12.94^{a,f}$	$212.20 \pm 76.37^{b,e}$
Endotoxemia group	$60.11 \pm 18.96^{a,d,e}$	$245.90 \pm 97.71^{a,d,e}$
Taurine plus endotoxin group	$44.07 \pm 9.37^{b,d}$	$76.50 \pm 36.91^{a,c}$

Results were expressed as mean ± standard deviation
[a] $p < 0.05$, compared to the corresponding value of control group
[b] $p > 0.05$, compared to the corresponding value of control group
[c] $p < 0.05$, compared to the corresponding value of taurine group
[d] $p > 0.05$, compared to the corresponding value of taurine group
[e] $p < 0.05$, compared to the corresponding value of taurine plus endotoxine group
[f] $p > 0.05$, compared to the corresponding value of taurine plus endotoxine group
$NO_2^{\bullet-}$ Nitrite, *MPO* myeloperoxidase, *PMN* polymorphonuclear

Table 2. The levels of taurine and taurochloramine in PMN leukocytes of experimental groups

	Taurine (μg/ml)	Taurochlorine (μg/ml)
Control group	11.62 ± 3.39	326 ± 0.39
Taurine group	$1.92 \pm 0.23^{a,e}$	$1.68 \pm 0.54^{a,f}$
Endotoxemia group	$3.37 \pm 1.12^{a,c,e}$	$4.27 \pm 0.59^{a,c,e}$
Taurine plus endotoxin group	$5.61 \pm 1.12^{a,c}$	$2.19 \pm 0.63^{a,d}$

Results were expressed as mean ± standard deviation
[a] $p < 0.05$, compared to the corresponding value of control group
[b] $p > 0.05$, compared to the corresponding value of control group
[c] $p < 0.05$, compared to the corresponding value of taurine group
[d] $p > 0.05$, compared to the corresponding value of taurine group
[e] $p < 0.05$, compared to the corresponding value of taurine plus endotoxine group
[f] $p > 0.05$, compared to the corresponding value of taurine plus endotoxine group
PMN Polymorphonuclear

MPO activity compared with the control group ($p < 0.05$). The maximal MPO activity was seen in the endotoxemia group.

Leukocyte suspension levels of NO_2^-

Both endotoxin and taurine administration significantly increased levels of NO_2^- compared with the control group (for both $p < 0.05$). The mean level of NO_2^- of endotoxeia group was higher than that of taurine group and this difference was not statistically significant ($p > 0.05$). Endotoxin plus taurine administration reduced leukocyte suspension level of NO_2^- and the difference between control and endotoxin plus taurine group was not statistically significant ($p > 0.05$).

Leukocyte suspension levels of taurine

Endotoxin, taurine, and endotoxin plus taurine administration significantly reduced levels of taurine (for all $p < 0.001$). The maximal mean of taurine level was seen in the control group, even though the minimal mean of taurine level was seen in the taurine group. The mean level of taurine of endotoxin plus taurine group was higher than that of endotoxemia group and this difference was statistically significant ($p < 0.001$).

Leukocyte suspension levels of TauCl

Endotoxin administration significantly increased levels of TauCl compared with the control group ($p < 0.001$). Taurine and endotoxin plus taurine administration significantly reduced levels of TauCl ($p < 0.001$ and $p = 0.001$, respectively). The mean levels of TauCl of endotoxin plus taurine group was higher than that of taurine group and this difference was not statistically significant ($p > 0.05$). The maximal mean of TauCl level was seen in the endotoxemia group.

Discussion

Monocytes and PMN utilize the MPO–H_2O_2–halide system to generate HOCl and chlorinated oxidants in inflammation. Nitrating oxidants are implicated in host defenses mechanism and the pathogenesis of many diseases. NO_2^-, a stable end product of NO metabolism, converts HOCl into the more potent chlorinating species, such as NO_2Cl (Gaut et al., 2002; Van Dalen et al., 2000). In addition, NO_2^- competes with taurine for reaction with HOCl released by PMN. TauCl is less toxic than HOCl and contributes to PMN dependent modulating of the inflammatory reaction (Marcinkiewicz et al., 1999, 2000). In the present study, NO_2^-, TauCl level and MPO activity were studied after endotoxemia (4 mg/kg) at 6 h. After 6 h following the endotoxin injection, we obtained PMN at the 6th hour. Since iNOS is maximally induced by endotoxin at 6 h after treatment (Cimen et al., 2004). Together with endotoxin, taurine (300 mg/kg) was administrated in order to investigate the effect of taurine on NO_2^- and TauCl level and MPO activity.

To demonstrate which concentration was effective on these parameters, we measured the taurine level of PMN by HPLC method in all groups. In this study, after endotoxin treatment, there was marked increase in PMN MPO activity, NO_2^- and TauCl levels compared with the control group. On the contrary, taurine level reduced after

endotoxemia in PMN. Our results were consistent with previous reports (Evans et al., 1996; Gaut et al., 2002; Gebska et al., 2005).

It has been reported that MPO and iNOS are colocalized in the primary granules of leukocytes. During phagocytic activity, such as during ingestion of bacteria, activated MPO and iNOS generate HOCl and NO respectively. As a result of this, the MPO-dependent oxidation of NO_2^- increases forming a reactive RNS (Gebska et al., 2005; Podrez et al., 2000).

Gaut et al. (2002) demonstrated that the levels of NO_2^- were 20 fold higher in infected mice than in control mice. Their observations strongly support that MPO uses NO_2^- to produce inflammatory nitrating oxidant in vivo (Gaut et al., 2002; Van Dalen et al., 2000).

On the other hand, in our experimental conditions, we observed the increased levels of TauCl, but taurine level was lower than that of control group after endotoxemia. As shown in our study, taurine was used for producing of TauCl after administration of endotoxin. Our study confirmed with previous reports demonstrating that HOCl reacts with taurine to form stable TauCl which inhibits the generation of PMN inflammatory mediators such as $NO^\bullet$, TNF-α in inflammation conditions (Chorazy et al., 2002).

An important aspect of this experiment was to find out if treatment with taurine would have effects on the endotoxin-induced MPO–H_2O_2–NO_2^- pathway or MPO–H_2O_2–taurine pathway. Taurine is a sulphur containing amino acid and naturally occurring antioxidant. One of its main roles is to protect tissues against attack by chlorinated oxidant, particularly HOCl (Egan et al., 2001; Marcinkiewicz et al., 1999, 2000). Based on this fact, we used the taurine intraperitoneally alone and with endotoxin. In our study, taurine treatment decreased MPO activity and NO_2^- level compared to endotoxemia group. But taurine alone had no effects on MPO activity and NO_2^- level. Our results were in accordance with previous studies. Several studies demonstrated that exogenous taurine was shown to possess significant anti-inflammatory properties in various in vivo and in vitro models of inflammation (Egan et al., 2001; Marcinkiewicz et al., 1999). It was reported that chronic nicotine treatment increased MPO activity in aorta and heart tissues samples compared to the control, taurine supplementation reversed this effect (Sener et al., 2005).

On the other hand, Egan et al. (2001) observed that there was a significant reduction in pulmonary MPO activity in those animals treated with taurine (300 mg/kg) before given endotoxin. In addition taurine can also quench and detoxify $NO^\bullet$, H_2O_2, and $OH^\bullet$ (Egan et al., 2001). But as seen in our results, the effect of taurine was different whether alone and together with endotoxin.

In addition, the effects of taurine and TauCl levels of PMN were investigated in the present study. Taurine and TauCl levels were lower when taurine was injected alone and together with endotoxin compared to the control group. Interestingly when taurine was administrated alone, it decreased the taurine and TauCl levels significantly compared to the corresponding endotoxin plus taurine group. It was reported previously that taurine transport capacity of cells was inversely related to altered dietary intake of taurine. Taurine transport appeared thus enhanced following low taurine diet and conversely decreased following high taurine diet (Tappaz, 2004). Tomi et al. (2006) demonstrated that taurine level of cells decreased following pretreatment with excess taurine under hypertonic conditions.

In conclusion, while taurine administration may be useful in endotoxemia because of its relative antioxidative effect, it has additional effects on MPO–H_2O_2–$NO_2^{\bullet-}$ system as an endotoxin in healthy subjects.

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