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Taurine chloramine and taurine bromamine induce heme oxygenase-1 in resting and LPS-stimulated J774.2 macrophages

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Summary. Taurine chloramine (TauCl) and taurine bromamine (TauBr) are products of activated neutrophils and eosinophils, respectively. It has been reported that TauCl, has strong anti-inflammatory properties. In a number of separate studies it has been shown that heme oxygenase-1 (HO-1), a stress inducible protein, exerts similar anti-inflammatory effects. In this study we investigated the influence of HO-1 on TauCl/TauBr mediated suppression of NO generation in J774.2 macrophages. Expression of HO-1 and inducible nitric oxide synthase (NOS-2) in LPS stimulated J774.2 cells provides an opportunity for determining these interactions. TauCl and TauBr, at non-cytotoxic concentrations, in a similar, dose-dependent manner, inhibited the expression of NOS-2, as evidenced by western blotting technique. Surprisingly, TauCl and TauBr induced expression of HO-1 in both non-activated and LPS-activated macrophages. Importantly, the fall in NOS-2 protein level was associated with a concomitant, dose-dependent induction of HO-1. In addition, an inhibitor of HO-1 activity, chromium III mesoporhyrin (CrMP), attenuated the inhibitory activity of TauBr but not that of TauCl, as measured by nitrite accumulation. These results suggest that at a site of inflammation, TauCl and TauBr may provide a link between taurine-dependent and HO-1-dependent cytoprotective mechanisms.

Keywords: Taurine chloramine – N-Chlorotaurine – Taurine bromamine – Heme oxygenase – Macrophages – Nitric oxide

Abbreviations: Tau, taurine; TauCl, taurine chloramine; TauBr, taurine bromamine; HO-1, heme oxygenase type 1; CrMP, chromium(III) mesoporphyrin; NOS-2, nitric oxide synthase type 2; MPO, myeloperoxidase; LPS, lipopolysaccharide

Introduction

Taurine (Tau), the most abundant free amino acid in mammalian tissues, has been shown to be tissue – protective in many models of oxidant – induced injury (Schuller-Levis and Park, 2003). These protective effects of taurine have been attributed to taurine detoxifying properties mainly by trapping hypochlorous (HOCl) and hypobromous (HOBr) acids, highly toxic oxidants formed by neutrophil myeloperoxidase (MPO) and eosinophil peroxidase (EPO), respectively (Thomas et al., 1995; van Dalen and Kettle, 2001). Studies from many laboratories have demonstrated that taurine chloramine (N-chlorotaurine, TauCl), the product of reaction of HOCl with Tau, exerts both bactericidal and anti-inflammatory properties (Park et al., 1995; Nagl et al., 2000). Recently, we have shown that also taurine bromamine (TauBr), the product of reaction of HOBr with Tau, exerts similar biological properties (Marcinkiewicz et al., 2002). Nevertheless, many more studies are referred to TauCl action. TauCl decreased production of pro-inflammatory mediators by activated macrophages (Park et al., 1997; Quinn et al., 2003), neutrophils (Marcinkiewicz et al., 1998), fibroblast-like synoviocytes (Kontny et al., 2000), dendritic cells (Marcinkiewicz et al., 1999), monocytes (Chorazy et al., 2002) and glial cells (Serban et al., 2003). TauCl was described to inhibit production of TNF-α, IL-1β and IL-6 as well as decrease activity of matrix metalloproteinase-9 in lipopolysaccharide (LPS)-stimulated macrophages (Chorazy et al., 2002; Park et al., 2000). It has been shown that pre-treatment with TauCl inhibited LPS-dependent induction of macrophage nitric oxide synthase (NOS-2) and cyclooxygenase-2 (COX-2) (Marcinkiewicz et al., 1995; Park et al., 1997; Barua et al., 2001). Although, the precise mechanism remains uncertain, much interest is now focused on the inhibition by TauCl macrophage NF kB signalling, a pathway primarily involved in cellular inflammatory response (Liu et al., 2002; Kanayama et al., 2002; Barua et al., 2001; Quinn et al., 2003).

Heme oxygenase-1 (HO-1), similarly to taurine, over the past decade has been implicated in the cytoprotective defense response against oxidative injury (Abraham, 2003; Ryter et al., 2002). HO-1 is an inducible enzyme that degrades heme to biliverdin (which is rapidly converted to antioxidant bilirubin), free iron (which leads to the induction of ferritin, an iron binding protein) and carbon monoxide (which is involved in the anti-inflammatory action of HO-1) (Wagener et al., 2003).

It was shown that CO may inhibit the production of pro-inflammatory cytokines in LPS-treated macrophages (Sarady et al., 2002; Otterbein, 2000) and stimulate secretion of anti-inflammatory IL-10 (Otterbein et al., 2000). Moreover, induction of HO-1 by several compounds has been claimed to decrease activation of NF kB signalling and inhibit macrophage NOS-2 induction (Colville-Nash et al., 1998; Kitamura et al., 1999; Lee et al., 2003). Thus, there are striking similarities between influence of TauCl and HO-1 inducers on LPS-dependent NOS-2 induction in macrophages.

The present study is in our knowledge the first report indicating that both TauCl and TauBr can potently induce heme oxygenase (HO-1). This finding rises a question concerning the contribution of HO-1 in TauCl (TauBr) mediated anti-inflammatory effects. Herein, we tested the role of HO-1 in inhibition of nitric oxide generation by TauCl and TauBr in LPS stimulated J774.2 macrophages.

Methods

Reagents

Taurine, lipopolysaccharide (LPS) from Escherichia coli serotype 0127: B8, leupeptin, pepstatine A, PMSF (phenylmethanesulfonyl fluoride) and MTT [3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyltetrazolium bromide] were from Sigma, USA. Chromium III mesoporphyrin IX chloride (CrMP), an inhibitor of heme oxygenase activity was from Frontier Scientific Porphyrin Products, Logan, USA.

Synthesis of taurine chloramines (TauCl) and taurine bromamine (TauBr)

TauCl was prepared according to the method described previously (Marcinkiewicz et al., 1998). Briefly, 5 ml of 20 mM NaOCl (Aldrich, Stenheim, Germany) solution in 0.2 M phosphate buffer (pH 7.4–7.5) was added dropwise to 5 ml of 24 mM taurine (Sigma, USA) with vigorous stirring. Each preparation of TauCl was monitored by ultraviolet absorption spectra (200–400 nm) to ensure the authenticity of TauCl (252 nm) and the absence of dichloramine, NH_2Cl and unreacted $HOCl/OCl^-$. The concentration of synthesized monochloramine was determined after synthesis and before each experiment by the molar extinction coefficient 415 M^{-1} cm^{-1} with absorbance at a wavelength of 252 nm.

TauBr was prepared in a two-step procedure. First, NaOBr was synthesized in reaction between equimolar amounts of NaOCl and NaBr in PBS solution (pH 10–11). In such conditions virtually all the OCl^- present reacts with Br^- to form OBr^- and Cl^-.

The presence and concentration of OBr^- was confirmed by UV spectroscopy (200–400 nm). In the second step, 20 mM NaOBr (5 ml) was added dropwise to equal volume of 100 mM taurine (an excess of taurine is necessary to avoid taurine dibromamine formation). UV absorbtion spectrum was checked to exclude formation of taurine dibromamine or chloramine and to estimate the concentration of TauBr. Using this method, the concentration of synthesized TauBr was usually within range of 4.13 to 6,44 mM. Stock solutions of Taurine, TauCl (10 mM) and TauBr (5 mM) were kept at 4°C for a maximum period of 3 days before use.

Cell culture

The mouse macrophage cell line J774.2 was cultured in T75 flasks in DMEM (Gibco, USA) that contained 10% foetal bovine serum supplemented with streptomycin (100 $\mu g\,ml^{-1}$), penicillin (100 $U\,ml^{-1}$) and fungizone (0.25 $\mu g\,ml^{-1}$). Flasks were kept at 37°C in atmosphere of humidified air containing 5% CO_2. Then, the cells were seeded in 96-well plates and cultured in 200 μl of culture medium until reaching confluence

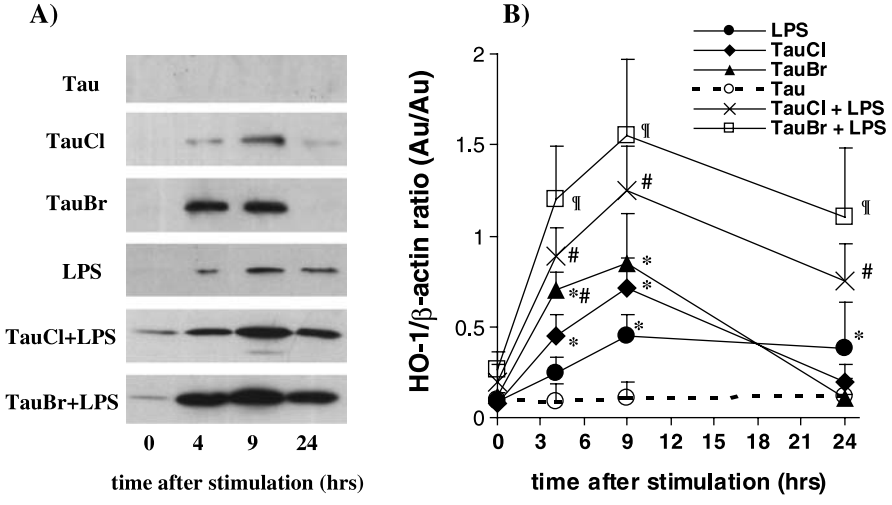

Fig. 1. **A** Representative western blotting of HO-1 protein expression in J774.2 cells in 4, 9 and 24 hours after stimulation with Tau (3 mM), TauCl (300 μM), TauBr (300 μM), LPS (1 μg/ ml), TauCl (300 μM) + LPS (1 μg/ml) and TauBr (300 μM) + LPS (1 μg/ml). **B** Densitometric analysis of bands from n = 3 experiments. Data are normalized to β-actin levels. $^*p < 0.05$ compared to Tau group; #$p < 0.05$ compared to TauCl group; ¶$p < 0.05$ compared to TauBr group

(10^5 cells per well). Additionally, in order to obtain sufficient amount of material for western blot assay, the cells were cultured in 6-well plates (10^6 cells per well) in 2 ml of culture medium. Nitric oxide synthase (NOS-2) in macrophages was induced by lipopolysaccharide from *Escherichia coli* serotype 0127:B8 (LPS, 1 μg ml^{-1}).

The cells were incubated either with TauCl (30–1000 μM), TauBr (30–300 μM) or Tau (100–3000 μM). In some experiments to block the activity of HO-1 the cells were cultured in the presence of chromium III mesoporphyrin chloride (CrMP 3–30 μM). All compounds were added to culture medium 15 minutes prior to LPS (1 μg ml^{-1}).

Cell viability

Cell respiration, an indicator of cell viability, was assessed by mitochondrial-dependent reduction of MTT to formazan (Olszanecki et al., 2002). Cells in 96-well plates were incubated in 37°C with MTT (0,2 mg ml^{-1} for 60 minutes). Then, culture medium was removed by aspiration and cells were solubilized in DMSO (200 μL). The extent of reduction of MTT to formazan within cells was quantitated by measurement of absorbance at 550 nm.

Nitrite measurements

24-hr nitrite accumulation in J774.2 cell culture supernatant was measured, as described previously, using the Griess method (Olszanecki et al. 2002). Briefly, 100 μl of 1% sulphanilamide in 5% phosphotic acid, followed by 100 μl of 0,1% N-(1-naphtyl)-ethylene diamine in 5% phosphoric acid were added to 100 μl of culture medium. After 10 minutes of incubation at 23°C the absorbance at 550 nm was read. Micromolar concentrations of nitrite were calculated from standard curve constructed with sodium nitrite as a reference compound.

Western blot analysis

24 hours after LPS stimulation the cells were lysed in lysis buffer (1% Triton X-100, 0,1% SDS in PBS containing 1 mM PMSF, 100 μM leupeptin, 50 μM pepstatin A). Protein concentrations of lysates were determined using Bradford method. Samples, containing equal amounts of total protein were mixed with gel loading buffer [50 mM Tris, 3% SDS, 10% glycerol, 7% 2-mercaptoethanol, 0.1% bromophenol blue] in a ratio 4:1 (v/v) and boiled (4 min). Then samples (30 μg of total protein per lane) were separated on 7.5% SDS-polyacrylamide gels (Mini Protean II, Bio-Rad, USA) using Laemmli buffer system and proteins were semi-dry transferred to nitrocelulose membranes (Amersham Pharmacia Biotech, USA). Non-specific binding sites were blocked overnight in 4°C with 5% non-fat dried milk and the membranes were then incubated 2 hrs in room temperature (RT) with rabbit polyclonal antibody to NOS-2 (1:1000) (Cayman, USA) or mouse monoclonal antibody to HO-1 (1:2000) (Stressgen, Canada). Bands were detected with horseradish peroxidase-conjugated secondary antibody (1 hr in RT, 1:5000, Amersham Pharmacia Biotech, USA) and developed with ECL reagents (Amersham Pharmacia Biotech, USA). Additionally, membranes were reprobed with monoclonal anti β-actin antibody (Sigma, USA). Rainbow markers (Amersham Pharmacia Biotech, USA) were used for molecular weight determinations. Protein bands were scanned and analyzed with freeware Scion image (Scion Corporation, USA). The data are normalized to constitutively expressed β-actin protein.

Statistics

All values in the figures and text are expressed as mean ± standard deviation (S.D). A one way analysis of variance (ANOVA) followed, if appropriate, by a Bonferroni's test for multiple comparisons was used to compare means between the groups. A P value less than 0.05 was considered to be statistically significant.

Results

TauCl and TauBr induce HO-1 expression in J774.2 macrophages

TauCl and TauBr but not taurine in dose-dependent manner increased the expression of HO-1 protein in J774.2 cells (Fig. 1A and 1B, 4 and 9 hours after challenge, $p<0.05$). TauBr tend to be stronger HO-1 inducer than TauCl (Fig. 1A and 1B), however the difference reached statistical significance only 4 hours after challenge (Fig. 1B, $p<0.05$). Interestingly, both compounds at concentration of 300 μM induced HO-1 much stronger than LPS

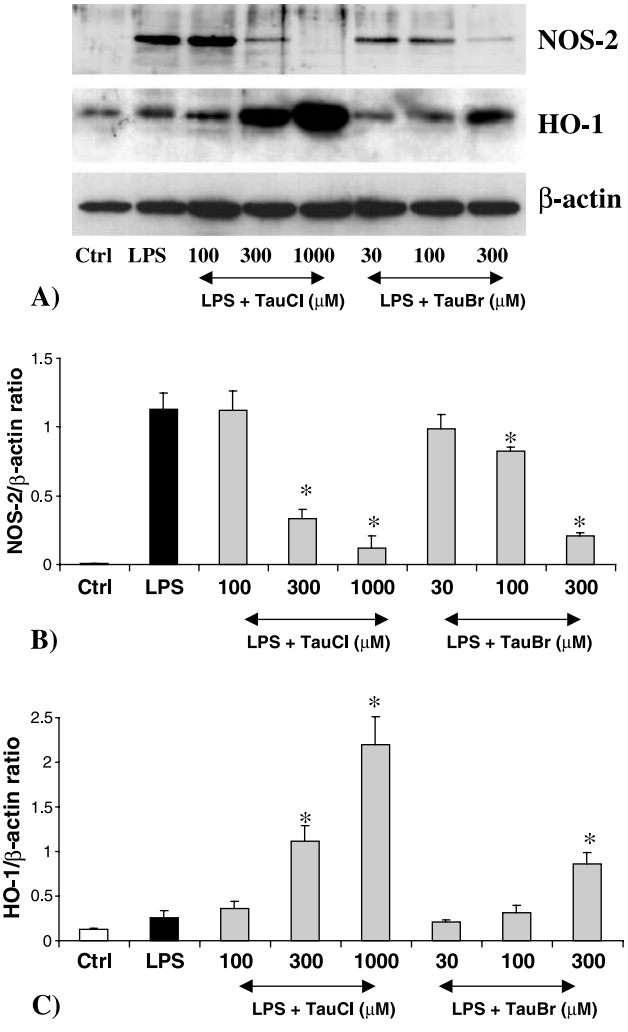

Fig. 2. A Western blotting of NOS-2 and HO-1 proteins expression in J774.2 cells stimulated 24 hrs with LPS (1 μg/ml). TauCl (100–1000 μM) and TauBr (30–300 μM) dose dependently inhibit LPS-dependent NOS-2 induction and potently increase levels of HO-1 protein. β-actin, a constitutively expressed protein was used as control. **B**, **C** Densitometric analyses of bands from n = 3 experiments. Data are normalized to β-actin levels. $^*p<0.05$ compared to LPS group

(1 μg/ml) (Fig. 1A and 1B, 4 and 9 hours after challenge, $p < 0.05$). However, treatment of the cells with LPS (1 μg/ml) resulted in stable increased level of HO-1 for 24 hours. In contrast, the increase of expression of HO-1 induced by TauCl and TauBr was time-dependent (of short duration), with the maximum level at 9 hours. After 24 hours HO-1 expression returned to the basal level. Importantly, there was striking synergy between action of LPS and taurine haloamines (TauCl and TauBr), concerning HO-1 induction in J774.2 cells (Fig. 1A and 1B).

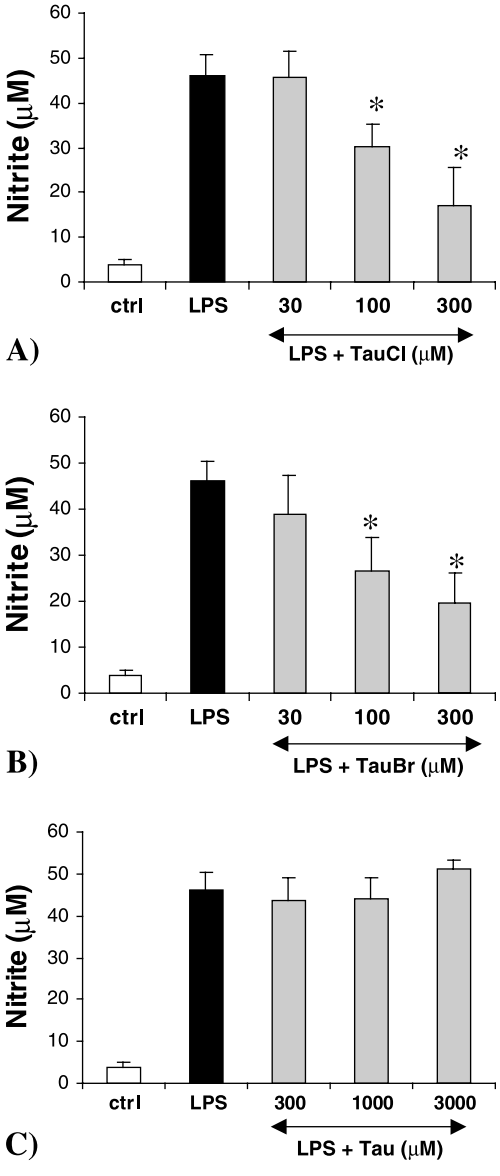

Fig. 3. Influence of TauCl (30–300 μM) (**A**), TauBr (30–300 μM) (**B**) and Tau (300–3000 μM) (**C**), administered 15 minutes prior to LPS (1 μg/ml), on 24-hr accumulation of nitrite in culture medium of J774.2 cells (n = 4 experiments). $^{*}p < 0.05$ compared to LPS group

TauCl and TauBr had no significant effects on cell viability under these conditions.

TauCl/TauBr-dependent expression of HO-1 correlates with the inhibition of NOS-2 expression in LPS-treated macrophages

Stimulation of J774.2 cells with high dose of LPS (1 μg/ml) caused a massive induction of NOS-2 protein in the cells (Fig. 2A and 2B). This was accompanied by significant increase of 24-h nitrite (the stable metabolites of NO) accumulation in culture medium (from 3.9 ± 1.02 to 45.9 ± 4.5 μM) (Fig. 3). Control cells were treated with vehicle but not with LPS.

TauCl (100–1000 μM) and TauBr (30–300 μM) administered 15 minutes prior to LPS (1 μg/ml), dose-dependently inhibited expression of NOS-2 protein in J774.2 cells, as evidenced by western blotting technique (Fig. 2A and 2B). Importantly, inhibition of NOS-2 expression by these compounds was correlated with massive, dose-dependent induction of HO-1 protein in LPS-treated J774.2 macrophages (Fig. 2A and 2C).

Role of HO-1 in TauCl/TauBr-mediated inhibition of nitrite generation by LPS-treated macrophages

Both TauCl (30–300 μM) and TauBr (30–300 μM), added 15 minutes prior to LPS, dose dependently decreased 24-hr nitrite accumulation in culture medium (Fig. 3A and 3B), with half-maximal inhibitory concentrations of 185 and 210 μM, respectively. This effect was not related to their cytotoxic action, as neither TauCl nor TauBr, up to

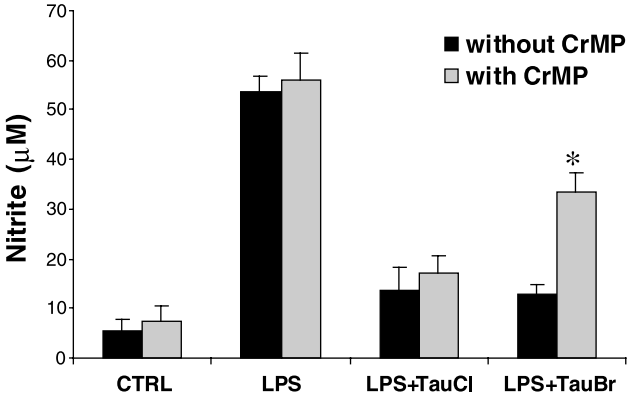

Fig. 4. Influence of chromium (III) mesoporphyrin (CrMP, 30 μM), an inhibitor of HO-1 activity, on 24-h nitrite accumulation in a culture of non-stimulated J774.2 cells and the cells treated with either LPS (1 μg/ml), TauCl (300 μM) + LPS (1μg/ml) or TauBr (300 μM) + LPS (1 μg/ml). In all groups n = 6 experiments. $^{*}p < 0.05$ compared to group without CrMP

300-micromolar concentrations affected viability of J774.2 cells (as determined by the MTT assay). In contrast to its derivatives, taurine (Tau) in concentrations up to 3000 μM did not influence 24-hr nitrite accumulation in culture medium of LPS-treated J774.2 cells (Fig. 3C).

Chromium III mesoporphyrin IX chloride (CrMP, 30 μM), an inhibitor of HO-1 activity, did not affect 24-hr nitrite accumulation by non-activated and LPS-activated J774.2 cells (Fig. 4). Interestingly, CrMP (30 μM) attenuated the inhibitory effect of TauBr (300 μM) but not that of TauCl (300 μM) on LPS-dependent nitrite production by J774.2 cells (Fig. 4).

Discussion

It is generally accepted that taurine protects cells against oxidative injury (Shaffer et al., 2003). Four mechanisms may contribute to taurine-mediated reduction in oxidative stress. First, taurine might up-regulate the anti-oxidant defences. Second, Tau may prevent Ca^{2+} overload, thereby minimizing free radical generation. Third, the major cause of Tau-mediated cytoprotection against certain xenobiotics is the formation of a taurine conjugate that is incapable of generating free radicals. Fourth, TauCl, the major physiological product of reaction of Tau with hypohalous acids, suppresses the activity of phagocytic cells, thereby reducing their ability to generate free radicals (Park et al., 1995; Marcinkiewicz et al., 1995, 1998; Shaffer et al., 2003).

Heme oxygenase-1 (HO-1) is also a part of the integrated response to oxidative stress. Interestingly, HO-1 exerts anti-inflammatory properties similar to TauCl. For example, TauCl and HO-1 inhibit the production of pro-inflammatory cytokines and inhibit the generation of nitric oxide in LPS-activated phagocytes (Park et al., 1997; Colville-Nash et al., 1998). In addition, at a site of inflammation, the same stimuli may activate neutrophils for generation of TauCl and HO-1 (both agents may co-exist in the same cells) (Vicente et al., 2001, 2003; Marcinkiewicz et al., 1997). Thus, it is reasonable to expect that TauCl and HO-1 will cooperate to suppress the generation of free radicals as a part of common response to oxidative stress.

To confirm this hypothesis we investigated the role of HO-1 in the inhibition of NO generation by TauCl incubated with LPS stimulated J774.2 macrophages. In addition, the present study extends the investigations of Tau derivatives anti-inflammatory properties by measuring the inhibitory capacity of TauBr, a product of activated eosinophils.

The model of LPS-treated J774.2 cells is widely used in studies of mechanisms of macrophage NOS-2 induction, which could be easily tracked down by measurement of accumulation of nitrite in culture medium (Wu et al., 1995; Olszanecki et al., 2002). In our experimental set-up, stimulation of macrophages with supra-optimal dose of LPS caused an induction of both enzymes tested, NOS-2 and HO-1, which is in an agreement with previous reports (Hashimoto et al., 2003; Camhi et al., 1995). However, this experimental set-up resulted in nearly maximal induction of NOS-2 and massive production of NO/NO_2^- by J774.2 cells, while the induction of HO-1 protein was not that strong. It may explain why we did not achieve higher concentration of nitrite even in the presence of CrMP, which should abolish an inhibitory effect of HO-1 (Colville-Nash et al., 1998). It also suggests that in J774.2 cells, stimulated with high dose of LPS, an autocrine regulation of NOS-2 production/activation by HO-1 is negligible.

Taurine did not affect the expression of HO-1 and NOS-2 proteins. However, its derivatives, TauCl and TauBr, in a similar, dose dependent manner, significantly enhanced the expression of HO-1 in LPS stimulated macrophages. Importantly, TauCl and TauBr were able to induce HO-1 in non-stimulated J.774.2 cells. Surprisingly, the effect of TauCl and TauBr on HO-1 induction was even stronger than that achieved by administration of high dose of LPS alone. These properties of taurine derivatives seem to be very important for their action *in vivo*. One may speculate that at a site of inflammation TauCl derived from neutrophils or TauBr derived from eosinophils will induce HO-1 in neighbouring non-activated cells (e.g. macrophages) to protect them against oxidative stress.

It was shown previously, that TauCl inhibits LPS-dependent induction of NOS-2 in macrophages (Park et al., 1997; Marcinkiewicz et al., 1995). Here, our results show that TauBr exerts the same effect. TauCl and TauBr, given 15 minutes prior to LPS, dose-dependently inhibited accumulation of nitrite in cell culture supernatant. It means that both compounds decrease nitrite formation due to the inhibition of NOS-2 activity and/or inhibition of induction of enzyme. Noteworthy, inhibitory effects of TauCl and TauBr were much weaker when they were given 10 hours after LPS stimulation – the time of nearly maximum expression of NOS-2 in our model (data not shown). Thus, TauBr, in a way described previously for TauCl (Marcinkiewicz et al., 1995; Quinn et al., 2003; Park et al., 1997) seems to inhibit an induction but not activity of NOS-2. Indeed, as evidenced by western blotting technique, TauCl and TauBr dose-dependently and with almost equal potencies inhibited expression of NOS-2 protein in LPS-stimulated J774.2 cells. To our knowledge, this is the first report about the influence of TauBr on nitric oxide generation by activated macrophages.

Moreover, our results suggest that the inhibitory action of TauCl and TauBr may depend on induction of HO-1, a cytoprotective and anti-inflammatory enzyme. Firstly, TauCl and TauBr dose-dependently potentiate HO-1 induction elicited by LPS. The levels of HO-1 protein were inversely correlated with inhibition of NOS-2 expression elicited by TauCl and TauBr. Secondly, inhibition of HO-1 activity with CrMP, an inhibitor of HO-1 activity, significantly attenuated the inhibitory effect of TauBr on the production of nitrite. On the other hand, CrMP did not affect the production of nitrite in the presence of TauCl in our system. Further studies are necessary to explain these differences by using lower concentrations of both, stimulators (LPS) and inhibitors (TauCl, TauBr) of NOS-2. The results obtained in our experimental set-up do not exclude the possibility that HO-1 may reverse TauBr and TauCl-mediated suppression of other than NO pro-inflammatory mediators. A similar mode of action of HO-1 in IL-10-dependent suppression of TNF-α release was described recently (Lee and Chau, 2002). Moreover, this suggestion is supported by our preliminary experiments in which we have shown that CrMP, in a dose dependent manner, attenuates an inhibitory effect of TauCl and TauBr on TNF-α production by moderately stimulated murine peritoneal macrophages. Nevertheless, it remains to be elucidated whether HO-1 could be solely responsible for anti-inflammatory effect of TauCl/TauBr or alternatively, whether TauCl/TauBr inhibit production of pro-inflammatory mediators in two ways, directly and through the induction of HO-1.

Despite the fact that the mechanisms of anti-inflammatory activity of TauBr have not been studied yet, at this stage of our investigations we can assume that the molecular effects of TauCl/TauBr and HO-1 actions seem to be similar. In LPS-activated macrophages expression of NOS-2 critically depends on the NF κB transcription factor signalling pathway (Xie and Nathan, 1994). Thus far, as much as TauCl action is involved, almost all studies report dampening of LPS-dependent expression of inducible proteins such as NOS-2, COX-2 or cytokines, mainly by interfering with LPS-triggered NF κB signalling, although the precise molecular mechanism remains still controversial. It was proposed that TauCl action might depend on the decrease of NF κB binding to DNA (Liu and Quinn., 2002) as well as on stabilisation of NF κB inhibitor (IκB), through both direct oxidation of methionine 45 in IκB alpha subunit (Kanayama et al., 2002) and inhibition of IκB kinase (Barua et al., 2001).

Others have shown that nitric oxide as well as other inflammatory agents induces HO-1, which in turn inhibits the expression of NOS-2 (Andre and Felley-Bosco, 2003; Hara et al., 1999). For example, induction of HO-1 by PPAR γ agonists, like 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, decreases LPS-dependent production of GM-CSF, TNFα and NO in macrophages (Sarady et al., 2002; Lee et al., 2003), microglial cells (Koppal et al., 2000) and glial cells (Kitamura et al., 1999). Importantly, induction of HO-1 in macrophages by PPAR γ agonists (Colville-Nash et al., 1998) or flavonoids (Lin et al., 2003) is dose-dependently and temporally correlated with inhibition of LPS-dependent NOS-2 induction. Accordingly, strong inducer of HO-1, cobalt protoporphyrin IX (CoPPIX) has been reported to inhibit NO production in LPS-treated RAW264.7 cells (Jozkowicz and Dulak, 2003). Interestingly, several reports point to the role of heme degradation products, of which the most important seems to be carbon monoxide (CO) (Ryter et al., 2002; Dulak and Jozkowicz, 2003; Wagener et al., 2003). HO-1-derived CO was demonstrated to inhibit NF κB pathway in LPS-stimulated macrophages (Sarady et al., 2002; Lee et al., 2003), as well as to stimulate release of anti-inflammatory IL-10 (Otterbein et al., 2000). These data support the hypothesis that HO-1, at least partially, could be responsible for TauCl and TauBr action in our model.

In conclusion, the major finding of this work is that both TauCl and TauBr, produced in inflammation sites by neutrophils and eosinophils, respectively, may act as anti-inflammatory agents in essentially common way, by induction of HO-1. It is tempting to speculate, that this mechanism is relevant to protective action of taurine *in vivo* and represents a natural anti-inflammatory mechanism.

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