Amino Acids (2008) 35: 359–364
DOI 10.1007/s00726-007-0609-x
Printed in The Netherlands

Amino Acids

# The role of heme oxygenase-1 in down regulation of $PGE_2$ production by taurine chloramine and taurine bromamine in J774.2 macrophages

**R. Olszanecki**[2], **M. Kurnyta**[1], **R. Biedroń**[1], **P. Chorobik**[1], **M. Bereta**[1], and **J. Marcinkiewicz**[1]

[1] Chair of Immunology, Jagiellonian University Medical College, Kraków, Poland
[2] Chair of Pharmacology, Jagiellonian University Medical College, Kraków, Poland

Received July 16, 2007
Accepted October 1, 2007
Published online December 21, 2007; 

**Summary.** Taurine chloramine (TauCl) and taurine bromamine (TauBr), products of myeloperoxidase halide system, exert anti-inflammatory properties. TauCl was demonstrated to inhibit the production of a variety of pro-inflammatory mediators including cyclooxygenase-2 (COX-2) dependent production of prostaglandin $E_2$ ($PGE_2$). Recently we have demonstrated that both major leukocyte haloamines, TauCl and TauBr, induced expression of HO-1 in non-activated and LPS-activated J774.2 macrophages. In this study, we have shown that TauCl and TauBr, at non-cytotoxic concentrations, inhibited the production of ($PGE_2$) without altering the expression of COX-2 protein, in LPS/IFN-$\gamma$ stimulated J774.2 cells. The inhibitory effect of TauCl and TauBr was reversed by chromium III mesoporhyrin (CrMP), an inhibitor of HO-1 activity. Our data suggest that HO-1 might participate in anti-inflammatory effects of TauCl/TauBr possibly by inhibition of COX-2 activity and decrease of $PGE_2$ production.

**Keywords:** Inflammation – Macrophages – Heme oxygenase-1 – Taurine bromamine – Taurine chloramine – Prostaglandin $E_2$ – Cyclooxygenase-2

**Abbreviations:** COX-2, Cyclooxygenase-2; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; FCS, fetal calf serum; HO-1, heme oxygenase-1; IFN$\gamma$, interferon $\gamma$; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12, interleukin-12; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; PBS, phosphate buffered saline; PD 98059, 2′-amino-3′-methoxyflavone; $PGE_2$, prostaglandin $E_2$; $PGJ_2$, prostaglandin $J_2$; ROS, reactive oxygen species; RT, room temperature; SB 203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole-; SDS, dodecyl sodium sulfate; TauBr, taurine bromamine; TauCl, taurine chloramine; TGF-$\beta$, tumor growth factor-$\beta$; TNF-$\alpha$, tumor necrosis factor

## Introduction

Heme oxygenase-1 (HO-1), the rate-limiting enzyme responsible for heme catabolism, is induced by various inflammatory and oxidative agents (Wagener et al., 2003). Degradation of free heme by HO-1 leads to the generation of biliverdin (an anti-oxidant), free iron (rapidly exhausted by ferritin) and carbon monoxide (an anti-inflammatory agent) (Ryter et al., 2002; Abraham, 2003). It has been reported that some well known anti-inflammatory and cytoprotective mediators like interleukin-10 (IL-10), tumor growth factor-$\beta$ (TGF-$\beta$) and prostaglandin $J_2$ ($PGJ_2$) act *via* activation of the HO-1 pathway. Only recently we have demonstrated that HO-1 can be induced in macrophages by taurine chloramine (TauCl) and taurine bromamine (TauBr) (Olszanecki and Marcinkiewicz, 2004). TauCl and TauBr, the physiological products of reaction between taurine and HOCl or HOBr, are major haloamines generated by leukocytes at a site of inflammation (Thomas et al., 1995) and both exert bactericidal and anti-inflammatory actions (Marcinkiewicz et al., 2000, 2005; Nagl et al., 2000). Compared to TauBr, the anti-inflammatory potencies of TauCl are much more elucidated. It has been shown to inhibit production of reactive oxygen species (ROS), nitric oxide (NO), chemokines and proinflammatory cytokines [tumor necrosis factor (TNF-$\alpha$), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12)] as well as to decrease production of COX-derived proinflammatory eicosanoids (especially $PGE_2$) in various inflammatory cells in vitro (Marcinkiewicz et al., 1995, 1998; Park et al., 1997; Kontny et al., 2000). Yet it should be noted that the molecular mechanisms of haloamine actions of are still unclear and a part of them might well depend on the induction of HO-1. For example, HO-1 controls the availability of heme for synthesis of enzymatic heme proteins (e.g. COX), and generates CO, which binds the heme moiety of heme proteins thus

affecting their enzymatic activity (Wagener et al., 2003). This is why HO-1 could be responsible for TauCl/TauBr-mediated suppression of production of COX-derived eicosanoids in inflammatory cells. In this study we address the issue of HO-1 contribution in the regulation of $PGE_2$ production by TauCl and TauBr in macrophages stimulated with LPS and IFNγ.

## Materials and methods

*Reagents*

Taurine (Tau), interferon γ (IFN-γ), lipopolysaccharide (LPS) from *Escherichia coli* serotype 0127:B8, protease inhibitor cocktail and MTT [3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyltetrazolium bromide] were from Sigma-Aldrich, Germany. Chromium III mesoporphyrin IX chloride (CrMP), an inhibitor of heme oxygenase activity was from Frontier Scientific Porphyrin Products, Logan, U.S.A. Inhibitors of MAP kinases: SB 203580 [4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole] – the specific p38 MAPK inhibitor and PD 98059 [2′-amino-3′-methoxyflavone] – the specific ERK inhibitor, were from Calbiochem, Germany.

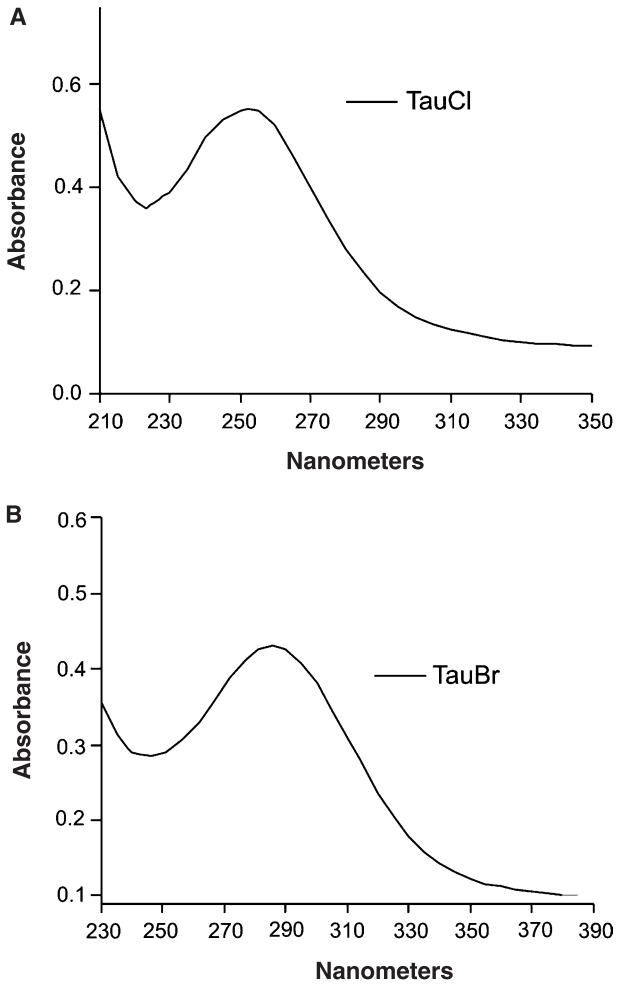


**Fig. 1.** The UV absorption spectra of (**A**) TauCl $\lambda_{max} = 252$ nm; (**B**) TauBr $\lambda_{max} = 286$ nm

*Synthesis of taurine chloramine (TauCl) and taurine bromamine (TauBr)*

TauCl was prepared by dropwise addition of 5 ml of 20 mM NaOCl (Sigma-Aldrich, Germany) solution in phosphate buffer (pH 7.4), with vigorous stirring, to 5 ml of 100 mM taurine (Tau) (Fluka, Japan). Each preparation of TauCl was monitored by UV absorption spectra ($\lambda =$ 200–400 nm) to assure the authenticity of TauCl ($\lambda = 252$ nm) and the absence of dichloramine ($TauCl_2$) ($\lambda = 300$ nm) and free $HOCl/OCl^-$ ($\lambda = 292$ nm) (Fig. 1A). The concentration of synthesized TauCl was determined using the molar extinction coefficient w 429 $M^{-1}$ $cm^{-1}$ at $A_{252}$.

TauBr was prepared in a two-step procedure. First, NaOBr was synthesized in reaction between equimolar amounts of NaOCl and NaBr (POCH, Poland) in phosphate buffered saline (PBS) solution (pH 10). 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 spectra ($\lambda = 200$–400 nm, molar extinction coefficient was 332 $M^{-1}$ $cm^{-1}$ at $A_{329}$). In the second step, 5 ml of 20 mM NaOBr was added dropwise to equal volume of 400 mM taurine. UV absorption spectrum was checked to exclude formation of taurine dibromamide or chloramines and to estimate the concentration of TauBr (molar extinction coefficient 430 $M^{-1}$ $cm^{-1}$ at $A_{288}$) (Fig. 1B). Stock solution of TauCl and TauBr was kept at 4 °C for a maximum period of 3 days before use.

*Cell culture*

The mouse macrophages cell line J774.2 was cultured in T 75 flasks in RPMI 1640 medium (JR Scientific Inc., U.S.A.) supplemented with 10% fetal calf serum (FCS), streptomycin (100 μg $ml^{-1}$) and amphotericin B (Fungizone® (0.25 μg $ml^{-1}$). Flasks were kept at 37 °C in an atmosphere of humidified air containing 5% $CO_2$. In order to obtain sufficient amount of material for Western blot assay and ELISA the cells were cultured in 24-well plates ($10^6$ cell per well) in 2 ml of culture medium (RPMI with 5–10% FCS). The cells were activated with 100 ng/ml of LPS and IFNγ (50 U/ml) for 24 h, if not stated otherwise.

*Protocol of experiments*

The cells were incubated either with TauCl (30–1000 μM), TauBr (30–300 μM) or Tau (100–10000 μM). In some experiments in order 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 min prior to LPS (100 ng/ml). Addition of TauCl and TauBr slightly preceded (1–2 min) addition of CrMP. The cells were incubated with compounds for 24 h. In experiments with inhibition of MAP kinases, SB 203580 and PD 98059 were added 30 min prior to TauCl or TauBr.

*Cell viability*

Viability of the cells was routinely monitored by cellular exclusion of trypan blue. In some experiments the effect of treatment on cell viability was assessed by the CellTiter 96 $AQ_{ueous}$ one solution cell proliferation assay (Promega, U.S.A.), according to manufacturers' instructions. In some experiments 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 at 37 °C with MTT (0.2 mg $ml^{-1}$ for 60 min) (Promega, U.S.A.). Then, the culture medium was removed by aspiration and cells were solubilized in dimethyl sulfoxide (DMSO) (200 μl). The

extent of reduction of MTT to formazan within cells was quantitated by measurement of absorbance at 550 nm.

*Western blot analysis (HO-1, COX-2 and iNOS expression)*

24 h after stimulation of J774.2 cells with LPS/IFN-γ or incubation with TauCl/TauBr the expression of HO-1, COX-2 and inducible nitric oxide synthase (iNOS) proteins in cytosol of the cells was determined by western blot technique. Briefly, after the incubation time, the cells were lysed in lysis buffer (1% Triton X-100, 0.1% dodecyl sodium sulfate (SDS) in PBS) containing protease inhibitor cocktail, (Sigma-Aldrich, Germany). Protein concentrations in lysates were determined using bicinchoninic acid protein assay kit (Sigma-Aldrich, Germany). Samples, containing equal amounts of total protein were mixed with gel loading buffer [0.125 M Tris, 4% SDS, 20% glycerol, 0.2 M DTT (dithiothreitol), 0.02% bromophenol blue] in a ratio 2:1 (v/v) and boiled (4 min). Then samples (20 µg of total protein per lane) were separated on 10% SDS-polyacrylamide gels (Mighty Small II, Amersham Biosciences, U.S.A.) using Laemmli buffer system and proteins were transferred to nitrocelulose membranes (Bio-Rad, U.S.A.). Non-specific binding sites were blocked overnight at 4 °C with 3% non-fat, dried milk, and the membranes were then incubated for 2 h at room temperature (RT) with mouse monoclonal antibody to HO-1 (1:2000) (Stressgen, Canada) or rabbit polyclonal antibody to COX-2 (1:1000) (Cayman, U.S.A.) or rabbit polyclonal antibody to iNOS (1:2000) (Stressgen, Canada). Bands were detected with alkaline phosphatase-conjugated secondary antibody (1 h at RT, 1:3000, Sigma-Aldrich, Germany) and developed with BCIP/NBT alkaline phosphatase substrate (Sigma-Aldrich, Germany). Additionally, membranes were reprobed with monoclonal anti β-actin antibody (1 h at RT, 1:3000, Sigma-Aldrich, Germany). Prestained SDS-PAGE Standards, Low Range, (Bio-Rad, U.S.A.) were used for molecular weight determinations. Protein bands were scanned and analyzed with freeware Scion image (Scion Corporation, U.S.A.). The data were normalized to constitutively expressed β-actin protein.

*$PGE_2$ immunoassay*

$PGE_2$ concentration in supernatants was determined by using a monoclonal antibody/enzyme immunoassay kit from Cayman Chemical Co., according to the manufacturer's instruction.

*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

### *Involvement of MAPK pathways in the induction of HO-1 protein by TauCl/TauBr*

Both taurine haloamines, TauCl (300 µM) and TauBr (300 µM), induced HO-1 protein in resting J774.2 cells (Fig. 2). Induction of HO-1 by TauCl was inhibited by SB 203580, an inhibitor of p38 MAPK, but not by PD 98059, a specific inhibitor of ERK (Fig. 2). Neither SB 203580 nor PD 98059 influenced induction of HO-1 by TauBr (Fig. 2).

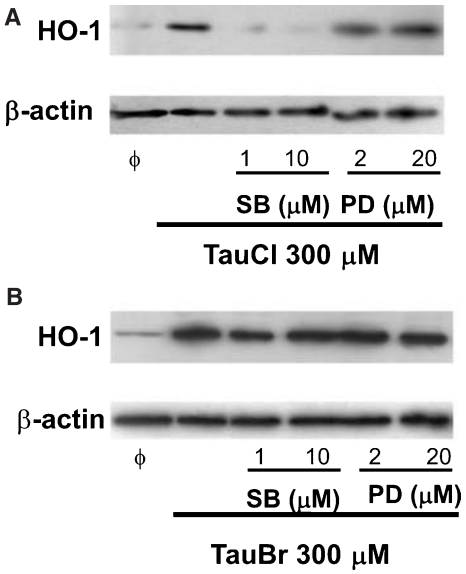


**Fig. 2.** Western blotting of HO-1 protein in resting J774.2 cells incubated with TauCl (**A**) and TauBr (**B**) in the presence of SB 203580 (p38 MAP kinase inhibitor) (1 and 10 µM) or PD 98059 (ERK inhibitor) (2 and 20 µM), *ϕ control groups of non stimulated cells

### *Effects of TauCl/TauBr on the induction of HO-1, COX-2 and iNOS in stimulated macrophages*

Stimulation of J774.2 macrophages with LPS and IFN-γ resulted in an increase of expression of HO-1 and COX-2 proteins (Fig. 3) as well as in a significant increase of $PGE_2$ accumulation in culture medium (from ~500 to ~7000 pg/ml). Moreover, the cells stimulated with LPS/IFN-γ expressed high levels of iNOS (Fig. 4).

Both haloamines, at concentrations of 100 and 300 µM, enhanced the expression of HO-1 in LPS/IFN-γ-stimulated macrophages (Fig. 3). However, clear dose-dependence effect was observed only in the case of TauCl action; TauBr at a concentration of 300 µM induced HO-1 less efficiently than at a concentration of 100 µM (Fig. 2). Neither TauCl nor TauBr influenced LPS/IFN-γ-mediated induction of COX-2 in J774.2 cells (Fig. 3). Importantly, despite of the lack of effect on COX-2 expression, both compounds decreased $PGE_2$ accumulation in culture medium of LPS/IFN-γ- treated cells, in a dose dependent manner, (Fig. 5). In contrast to COX-2, the expression of iNOS was inhibited by both taurine haloamines in a dose dependent manner, in LPS/IFN-γ-stimulated J774.2 cells (Fig. 4).

Taurine (Tau 100–10000 µM) did not affect either HO-1, iNOS and COX-2 induction as well as $PGE_2$ production in LPS/IFN-γ-stimulated J774.2 cells. Neither TauCl nor TauBr had any significant effects on cell viability in the experimental conditions (data not shown).

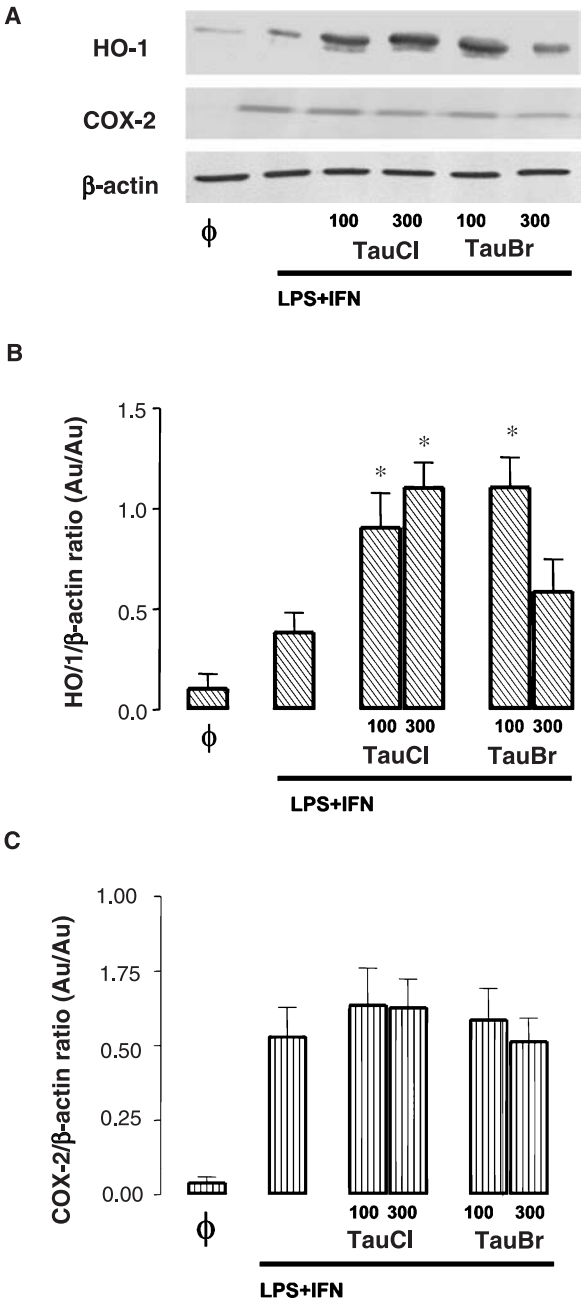


**Fig. 3.** (**A**) Western blotting of HO-1 and COX-2 proteins in J774.2 cells; (**B**, **C**) densitometric analyses of bands from $n = 8$ experiments. Data are normalized to β-actin levels. $^{*}p < 0.05$ vs. LPS/IFN-γ-stimulated cells, $^{*}\phi$ control groups of non stimulated cells

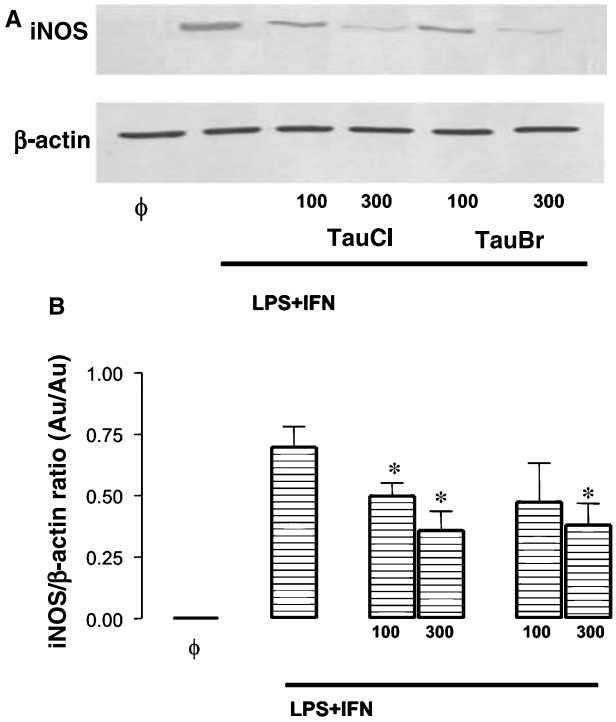


**Fig. 4.** (**A**) Western blotting of iNOS protein in J774.2 cells; (**B**) densitometric analyses of bands from $n = 6$ experiments. Data are normalized to β-actin levels. $^{*}p < 0.05$ vs. LPS/IFN-γ-stimulated cells, $^{*}\phi$ control groups of non stimulated cells

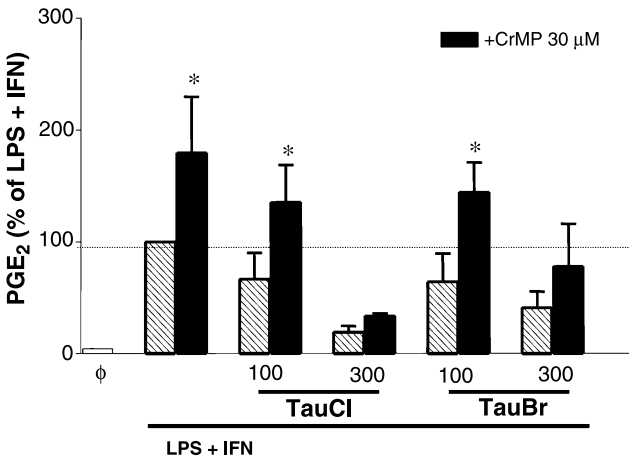


**Fig. 5.** The effect of CrMP, an inhibitor of HO-1 activity on the inhibition of $PGE_2$ synthesis by TauCl and TauBr in LPS/IFN-γ-stimulated macrophages. $^{*}p < 0.05$ CrMP vs. control groups, $^{*}\phi$ control groups of non stimulated cells

### *Effects of TauCl/TauBr and the inhibitor of HO-1 on $PGE_2$ production*

Chromium III mesoporphyrin IX chloride (CrMP 30 μM), an inhibitor of HO activity, augmented $PGE_2$ release by LPS/IFN-γ-stimulated J774.2 cells and abolished inhibitory effect of both haloamines on macrophage $PGE_2$ production (Fig. 5).

## Discussion

At a site of inflammation, the increased synthesis of the HO-1 protein occurs as a general response to oxidative stress. In vitro, in cell culture models, HO-1 is induced by a broad spectrum of agents such as hemin, reactive oxy-

gen and nitrogen species, heavy metals, growth factors and cytokines (Ryter et al., 2006). Our previous reports have shown up-regulation of HO-1 protein expression in TauCl and TauBr treated macrophages (Olszanecki and Marcinkiewicz, 2004; Marcinkiewicz et al., 2006). This study shows that induction of HO-1 plays a major role in inhibition of $PGE_2$ production by both taurine haloamines in activated macrophages.

COX-2-derived $PGE_2$ has been shown to be a major eicosanoid released from LPS/IFN-γ-stimulated J774.2 macrophages (Stenson et al., 1981; Swierkosz et al., 1995). Importantly, in our hands, taurine haloamines did not alter expression of COX-2 in LPS/IFN-γ-stimulated J774.2 cells. This finding was surprising for us for two reasons: (i) previously, we have shown that TauCl is able to inhibit the induction of COX-2 in rheumatoid arthritis synoviocytes (Kontny et al., 2003), (ii) both haloamines inhibited induction of iNOS in LPS/IFN-γ-stimulated J774.2 cells. Apparently, COX-2 induction pathway in various cell types responds to TauCl differently. Moreover, COX-2, but not iNOS induction pathway is somehow refractory to the action of haloamines in J774.2 cells. Thus, the model of LPS/IFN-γ-treated J774.2 cells revealed inhibitory activity of TauCl and TauBr on COX pathway. The question arises about the physiological chemistry and mechanism of action of haloamines (despite possibility of transhalogenation in culture medium a significant part of action still depend on TauCl/TauBr itself, because of its relatively high stability).

It has been reported that TauCl (with an $IC_{50}$ of 0.4 mM) inhibited $PGE_2$ production in RAW 264 macrophages by post-transcriptional effects on inducible COX-2 (Quinn et al., 1996). This, however, does not seem to be a case in our setting: (i) the effect of TauCl/Tau Br was observed at lower concentrations, (ii) it was essentially dependent on HO activity and (iii) it was reversed by the use of CrMP, an inhibitor of HO activity.

Functional link between HO and COX-2 pathways has been described (Vairano et al., 2001). HO-1 controls the availability of heme for synthesis of enzymatic heme proteins, and generates CO, which binds the heme moiety of heme proteins thus affecting their enzymatic activity (Wagener et al., 2003). The increase of HO-1 by non-heme inducers was shown to be associated with a decrease in COX-2 activity in microvessel endothelial cells. On the contrary, HO-1 up-regulation by heme increases prostaglandin production (Haider et al., 2002). Therefore, it is possible that down regulation of $PGE_2$ production by taurine haloamines in J774.2 cells is due to a reduction in catalytically active COX-2 as a result of cellular heme being decreased to suboptimal levels. Alternatively/concomitantly, it may involve the direct action of CO on COX-2. Moreover, the influence of TauCl/TauBr on expression/activity of PGE synthase may be affected. Clearly, further studies are necessary to determine more selectively the effect of HO-1 – TauCl system on the production of eicosanoids in macrophages.

Another question is which intracellular signaling pathways are responsible for HO-1 induction by TauCl and TauBr. The MAP kinases were shown to be involved in HO-1 induction by various stimuli (Gong et al., 2003; Wijayanti et al., 2005; Chen et al., 2006). Interestingly, use of pharmacological inhibitors of MAP kinases revealed the differences between TauCl and TauBr actions in terms of HO-1 induction. TauCl did not induce HO-1 in the presence of p38 MAPK inhibitor. On the other hand, neither inhibitor of p38 nor ERK inhibitor affected the capacity of TauBr to induce HO-1. These results may suggest that TauCl activates p38- but not ERK- dependent signaling pathway for the induction of HO-1. This observation is not in full agreement with some reports. For example, Midwinter et al. (2004) have shown that TauCl activates ERK via epidermal growth factor receptor. However, the experimental conditions used in these studies differ markedly from ours. Their TauCl concentrations were about 10-fold lower; incubation times were much shorter, and most importantly, they used a different cell type, namely human endothelial cells (HUVEC). On the other hand, preliminary experiments have shown that TauCl inhibits phosphorylation of ERK but not of p38, which supports the present results. Further studies are necessary to dismantle the role of MAPK in the mechanisms of cell activation by taurine haloamines.

We conclude that in activated macrophages in vitro, TauCl and TauBr decrease production of $PGE_2$ due to the induction of HO-1, with subsequent inhibition of activity of COX-2 pathway. However, the exact nature of HO-1/COX-2 interaction as well as physiological significance of this mechanism require further investigation.

## Acknowledgement

This work was supported by a grant from the State Committee for Scientific Research of Poland (No 2 PO5A 091 29).

## References

Abraham NG (2003) Therapeutic applications of human heme oxygenase gene transfer and gene therapy. Curr Pharm Des 9: 2513–2524

Chen IC, Huang KC, Lin WW (2006) HMG-CoA reductase inhibitors upregulate heme oxygenase-1 expression in murine RAW264.7 macro-

phages via ERK, P38 MAPK and protein kinase G pathways. Cell Signal 18: 32–39

Gong P, Cederbaum AI, Nieto N (2003) Increased expression of cytochrome P450 2E1 induces heme oxygenase-1 through ERK MAPK pathway. J Biol Chem 278: 29693–29700

Haider A, Olszanecki R, Gryglewski R, Schwartzman ML, Lianos E, Kappas A, Nasjletti A, Abraham NG (2002) Regulation of cyclooxygenase by the heme–heme oxygenase system in microvessel endothelial cells. J Pharmacol Exp Ther 300: 188–194

Kontny E, Rudnicka W, Kowalczewski J, Marcinkiewicz J, Maslinski W (2003) Selective inhibition of cyclooxygenase 2-generated prostaglandin E2 synthesis in rheumatoid arthritis synoviocytes by taurine chloramine. Arthritis Rheum 48: 1551–1555

Kontny E, Szczepanska K, Kowalczewski J, Kurowska M, Janicka I, Marcinkiewicz J, Maslinski W (2000) The mechanism of taurine chloramine inhibition of cytokine (interleukin-6, interleukin-8) production by rheumatoid arthritis fibroblast-like synoviocytes. Arthritis Rheum 43: 2169–2177

Marcinkiewicz J, Grabowska A, Bereta J, Stelmaszynska T (1995) Taurine chloramine, a product of activated neutrophils, inhibits in vitro the generation of nitric oxide and other macrophage inflammatory mediators. J Leukoc Biol 58: 667–674

Marcinkiewicz J, Chain B, Nowak B, Grabowska A, Bryniarski K, Baran J (2000) Antimicrobial and cytotoxic activity of hypochlorous acid: interactions with taurine and nitrite. Inflamm Res 49: 280–289

Marcinkiewicz J, Mak M, Bobek M, Biedron R, Bialecka A, Koprowski M, Kontny E, Maslinski W (2005) Is there a role of taurine bromamine in inflammation? Interactive effects with nitrite and hydrogen peroxide. Inflamm Res 54: 42–49

Marcinkiewicz J, Kurnyta M, Biedron R, Bobek M, Kontny E, Maslinski W (2006) Anti-inflammatory effects of taurine derivatives (taurine chloramine, taurine bromamine, and taurolidine) are mediated by different mechanisms. Adv Exp Med Biol 583: 481–492

Midwinter RG, Peskin AV, Vissers MC, Winterbourn CC (2004) Extracellular oxidation by taurine chloramine activates ERK via the epidermal growth factor receptor. J Biol Chem 30; 279: 32205–32211

Nagl M, Hess MW, Pfaller K, Hengster P, Gottardi W (2000) Bactericidal activity of micromolar N-chlorotaurine: evidence for its antimicrobial function in the human defense system. Antimicrob Agents Chemother 44: 2507–2513

Olszanecki R, Gebska A, Kozlovski VI, Gryglewski RJ (2002) Flavonoids and nitric oxide synthase. J Physiol Pharmacol 53: 571–584

Olszanecki R, Marcinkiewicz J (2004) Taurine chloramine and taurine bromamine induce heme oxygenase-1 in resting and LPS-stimulated J774.2 macrophages. Amino Acids 27: 29–35

Park E, Schuller-Levis G, Jia JH, Quinn MR (1997) Preactivation exposure of RAW 264.7 cells to taurine chloramine attenuates subsequent production of nitric oxide and expression of iNOS mRNA. J Leukoc Biol 61: 161–166

Quinn MR, Park E, Schuller-Levis G (1996) Taurine chloramine inhibits prostaglandin E2 production in activated RAW 264.7 cells by post-transcriptional effects on inducible cyclooxygenase expression. Immunol Lett 50: 185–188

Ryter SW, Otterbein LE, Morse D, Choi AM (2002) Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. Mol Cell Biochem 234–235: 249–263

Ryter SW, Alam J, Choi AM (2006) Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. Physiol Rev 86: 583–650

Swierkosz TA, Mitchell JA, Warner TD, Botting RM, Vane JR (1995) Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. Br J Pharmacol 114: 1335–1342

Stenson WF, Nickells MW, Atkinson JP (1981) Metabolism of exogenous arachidonic acid by murine macrophage-like tumor cell lines. Prostaglandins 21: 675–689

Thomas EL, Bozeman PM, Jefferson MM, King CC (1995) Oxidation of bromide by the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. Formation of bromamines. J Biol Chem 270: 2906–2913

Vairano M, Dello Russo C, Pozzoli G, Tringali G, Preziosi P, Navarra P (2001) A functional link between heme oxygenase and cyclo-oxygenase activities in cortical rat astrocytes. Biochem Pharmacol 61: 437–441

Wagener FA, Volk HD, Willis D, Abraham NG, Soares MP, Adema GJ, Figdor CG (2003) Different faces of the heme-heme oxygenase system in inflammation. Pharmacol Rev 55: 551–571

Wijayanti N, Kietzman T, Immenschuh S (2005) Heme oxygenase-1 gene activation by the NAD(P)H oxidase inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride via protein kinase B, p38-dependent signalling pathway in monocytes. J Biol Chem 280: 21820–21829

**Authors' address:** Janusz Marcinkiewicz, Department of Immunology, Jagiellonian University Medical College, 18 Czysta St, 31-121 Kraków, Poland,

Fax: +48 12 633 94 31, E-mail: mmmarcin@cyf-kr.edu.pl