ANTI-INFLAMMATORY EFFECTS OF TAURINE DERIVATIVES (TAURINE CHLORAMINE, TAURINE BROMAMINE, AND TAUROLIDINE) ARE MEDIATED BY DIFFERENT MECHANISMS

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1. INTRODUCTION

Taurolidine (TRD) is a synthetic derivative of taurine, which is chemically designed as bis-(1,1-dioxoperhydro-1,2,4-thiadiazinyl-4)methane, originally designed as a chemoterapeutic agent. It is commonly used as adjunctive therapy for various infections (Browne *et al.,* 1976; Traub *et al.,* 1993). Recently, it has been shown that TRD exerts antineoplastic activities and inhibits the growth of a variety of tumor cell lines *in vitro* and *in vivo* (McCourt *et al.,* 2000; Darnowski *et al.,* 2004; Nici *et al.,* 2004). TRD is degraded *in vivo* into three major breakdown products (Scheme 1). Methylol-containing TRD breakdown products, taurultam and taurinamide exert anti-bacterial, anti-endotoxin and anti-adherence activities (Gorman *et al.,* 1987; Willatts *et al.,* 1995). Taurine, the third breakdown product of TRD, does not share those activities. On the other hand, it has been reported that taurine is responsible for TRD immunoregulatory properties, such as inhibition of production of pro-inflammatory cytokines (Bedrosian *et al.,* 1991; Watson *et al.,* 1995). However, the results are controversial and the contribution of taurine in TRD anti-inflammatory activity is not clear.

On the contrary, it is well documented that taurine haloamines, taurine chloramine (TauCl) and taurine bromamine (TauBr) exert immunoregulatory properties (Marcinkiewicz *et al.,* 1995; Schuller-Levis and Park, 2003; Marcinkiewicz *et al.,* 2005). As taurine derivatives, TRD and taurine haloamines, but not taurine itself, can down-regulate inflammation (Marcinkiewicz 1997; Watson *et al.,* 1995), it is reasonable to establish whether myeloperoxidase **(**MPO) halide system may contribute to TRD-

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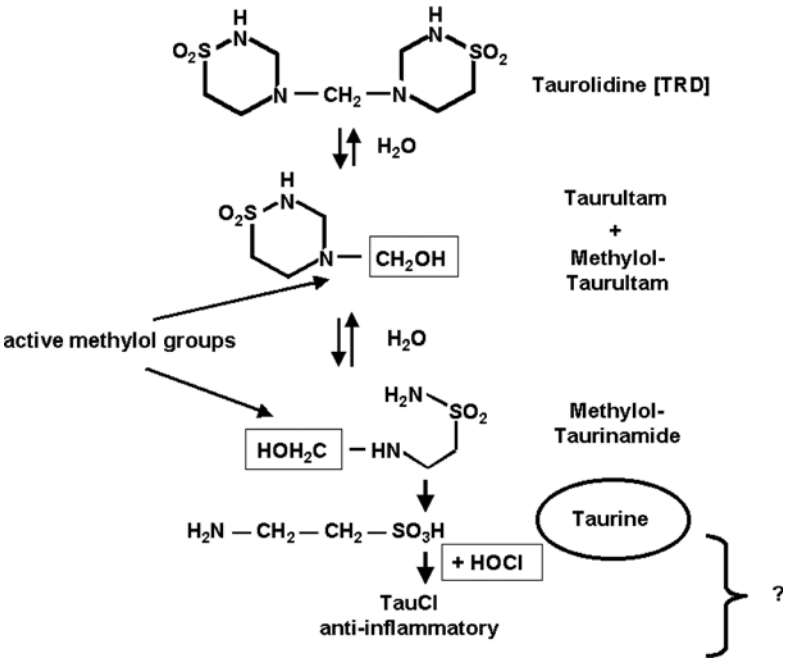
dependent anti-inflammatory activities. Therefore, the formation and biological properties of chlorinated TRD remain to be elucidated.

TauCl and TauBr, the physiological products of reaction between taurine and HOCl or HOBr, are major haloamines generated at a site of inflammation (Klebanoff and Hamon, 1979; Thomas *et al.,* 1995). Both taurine haloamines exert bactericidal and anti-inflammatory properties (Nagl *et al.,* 2000; Marcinkieicz *et al.,* 2005). They decrease the production of pro-inflammatory mediators by various types of activated cells: macrophages, neutrophils, dendritic cells, fibroblast-like synoviocytes and glial cells. TauCl was described to inhibit production of NO, PGE_2, TNF-α, IL-6, IL-8 and ROS (Marcinkiewicz *et al.,* 1995, 1999; Park *et al.,* 1995; Kontny *et al.,* 2000). Studies on the mechanism of its action have revealed that TauCl inhibits the activation of NFκB, a potent signal transducer for inflammatory cytokines, by oxidation of IκBα at methionine[45] (Barua *et al.,* 2001). More recently it has been shown that TauCl and TauBr induce expression of heme oxygenase-1 (HO-1) (Olszanecki and Marcinkiewicz, 2004), a stress-inducible protein with strong anti-inflammatory properties (Vincente *et al.,* 2003).

In our study we have addressed the issue whether TRD down-regulates acute inflammation and whether the MPO-halide system, via the formation of TRD-chlorinated species, may contribute to the anti-inflammatory properties of TRD. To clarify this problem we have tested *in vivo* the effect of local administration of TRD on the development of zymosan-induced peritonitis and *in vitro* the effect of TRD and its



Scheme 1. Structure of taurolidine (TRD) and its major breakdown products taurultam, taurinamid and taurine. Upon breakdown, TRD generates methylol-containing fragments (-CH_2OH) that have been suggested as being responsible for its anti-bacterial, anti-endotoxin, anti-adherence and pro-apoptotic activities.

chlorinated product (TRD-Cl) on cytokine production by macrophages as well as on the expression of HO-1 protein in macrophage cytosol. The effects were compared with those of TauCl. Therefore, the aim of this study was to evaluate the anti-inflammatory activities of TRD and its chlorinated product (TRD-Cl) in comparison to TauCl.

2. MATERIALS AND METHODS

2.1. Reagents

Taurolidine: For *in vivo* study Taurolin® for injections (Boehringer Ingelheim, Germany*)* 2% w/v aqueous isotonic solution of taurolidine (TRD) in 5% polyvinylpyrrolidine (PVP) was used as a source of TRD. PVP (Sigma, St Louis, MO, USA), the pharmaceutical stabilizer of TRD, was used as a control (placebo). For *in vitro* studyTRD, which has a low molecular weight (284), was isolated from Taurolin and separated from PVP (40 000 MW) by centrifugation (2000 x g, 20 min) on Vivaspin membrane 5000 MW (Vivascience, Germany). Chlorinated TRD (TRD-Cl) was prepared by a reaction of TRD with equimolar concentration of HOCl. The reaction was monitored by UV absorption spectra (λ_{max} for chloramines is 252 nm).

2.1.1. Preparation of Taurine Chloramine (TauCl)

TauCl was prepared by a dropwise addition of 5 ml of 20 mM NaOCl (Aldrich, Steinham, Germany) solution in 0.05 M phosphate buffer (pH 7.4) into 5 ml of 24 mM taurine (Tau) (Sigma, St. Louis, MO, USA), with vigorous stirring. Each preparation of TauCl was monitored by UV absorption spectra (λ = 200 to 400 nm) to assure the authenticity of monochloramine (TauCl) (λ_{max} is 252 nm) and the absence of dichloramine ($TauCl_2$) (λ_{max} is 300 nm) and unreacted $HOCl/OCl^-$ (λ_{max} is 292 nm). The concentration of synthesized TauCl was determined using the molar extinction coefficient 429 $M^{-1}cm^{-1}$ at A_{252} (Thomas *et al.,* 1986). The stock solution of TauCl was kept at 4°C for maximum 5 days before use.

2.2. Mice

Inbred Balb/c male mice from the breeding unit, Department of Immunology, Jagiellonian University Medical College, Cracow, Poland, were used between 6 and 8 weeks of age. The authors were granted permission by the Local Ethics Committee to use mice in this study.

2.3. Induction and Evaluation of Acute Peritonitis

The *in vivo* effect of TRD on the development of zymosan-induced peritonitis in Balb/c mice was investigated. Mice received i.p 1.0 mg of zymosan with either Taurolin (TRD 10 mg/kg + PVP) or with placebo (PVP, a dose equivalent to that present in the Taurolin solution). The severity of acute inflammation was determined by measurement of vascular permeability, neutrophil influx into he peritoneal cavity and proinflammatory cytokine production *in vitro* by peritoneal exudate cells.

The influence of TRD on vascular permeability was investigated by determination of Evans blue leakage from the circulation into the peritoneal cavity. Briefly, the mice received i.v 100 μl of 0.5 % solution of Evans blue 30 minutes after induction of peritonitis. After additional 30 min, the mice were sacrificed and the peritoneal cavity was washed with 1.0 ml of phosphate buffer solution (PBS). The concentration of Evans blue at peritoneal exudates was determined by measuring the optical density of cell-free exudates (λ is 630 nm). The content of neutrophils at a site of inflammation was estimated by determination of MPO activity in peritoneal exudate cells. Moreover, the effect of local treatment of TRD on cytokine production by peritoneal exudate cells (PEC) has been tested. PEC were restimulated *in vitro* with IFN-γ for 24 h. The supernatants were collected and tested for TNF-α and IL-6.

2.4. Cells

Peritoneal mouse macrophages (Mϕ) were induced by intraperitoneal injection of 1.0 ml of paraffin oil (Sigma, St.Louis, MO, USA). Cells were collected 48 h later by washing the peritoneal cavity with 5 ml of PBS, containing 5 U/ml heparin (Polfa, Warsaw, Poland). The cells were centrifuged and red blood cells were lysed by osmotic shock using distilled water; and osmolarity was then restored by addition of 2 x concentrated PBS. The presence of macrophages (85-90%) was judged by cytochemical demonstration of non-specific esterase-positive mononuclear cells, using *α*-naphtyl acetate (Sigma, St Louis, MO, US).

2.5. Cell Cultures and Treatment

Mϕ were cultured in 24-well flat-bottom cell culture plates at 5 x 10^5/well in RPMI 1640 medium (JR Scientific Inc., Woodland, CA, USA) supplemented with 5% FCS at 37°C in an atmosphere of 5% CO_2. Cells were activated with either 50 U/ml of IFN-γ (Sigma, Steiham, Germany) or 100 ng/ml of LPS (*E. coli* 0111 B:4, Sigma Steiham, Germany) and cultured in the presence of test agents. After the 24-h culture, the supernatants were collected and frozen at -80°C until used.

2.6. Measurement of Cell Viability

The viability of the cells was routinely monitored by cellular exclusion of trypan blue. In some experiments, cell respiration, an indicator of cell viability, was assessed by mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) to formazan. Cells in 96-well plates were incubated at 37°C with MTT (0.2 mg ml^{-1} for 60 minutes). Then, culture medium was removed by aspiration and the cells were solubilized in dimethylsulfoxide (DMSO, 200 μl). The extent of reduction of MTT to formazan within cells was quantitated by measurement of absorbance at 550 nm.

2.7. Cytokine Determination

The cytokine concentrations in culture supernatants were measured using capture ELISA. Briefly, for IL-6 measurment 96-well plates (Corning, NY, USA) were coated overnight with rat mAb against a mouse cytokine (capture antibody). After blocking the

plates with 4% albumin (2 h), standards and tested supernatants were added and incubated overnight. Finally, the plates were coated with biotinylated antibodies against the same cytokine-detecting antibody for 1 h. ELISA was developed with horseradish peroxidase streptavidin (Vector, Burlingame, CA, USA), followed by *o*-phenylene-diamine and H_2O_2 (both Sigma, Steiham, Germany) as substrates for 30 min. For TNF-α, a peroxidase-conjugated goat anti-rabbit IgG (Sigma, Steiham, Germany) was used to develop the reaction. The reaction was stopped with 3 M H_2SO_4 and the optical density of each well was measured at 492 nm in a plate reader.

IL-6: Rat anti-IL-6 and biotinylated rat anti-IL-6 (both Pharmingen, San Diego, CA, USA) mAbs were used as detecting antibodies. Recombinant mouse IL-6 (PeproTech Rocky Hill, New York, USA) was used as a standard. The detection limit was about 15 pg IL-6/ml.

TNF-α: Rat anti-murine TNF-α and biotinylated rat anti-mouse TNF-α (Pharmingen, San Diego, CA, USA) mAbs were used as detecting antibodies. Recombinant mouse TNF-α (Pharmingen, San Diego, CA, USA) was used as a standard. The limit of detection was 30 pg TNF-α/ml.

2.8. Measurement of Myeloperoxidase Activity

Freshly collected zymosan-induced cells from exudates of the mice treated with either TRD or placebo (PVP, a dose equivalent to that present in the Taurolin solution) were resuspended (~10^6 cells/ml) in 0.5% hexadecyltrimethylammonium (HTAB) (Sigma, Steiham, Germany) in 50 mM potassium phosphate buffer, pH 6.0. The cells were freeze-thawed in three cycles, dispersed by vortexing and the supernatants were collected by centrifugation at 4000 x g at 10 min at 4°C. Twenty µl sample supernatants were then mixed with 180 µl of 50 mM potassium phosphate buffer (pH 6.0) containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide (both Sigma, Steiham, Germany) and placed in the 96-well flat bottom plate. After 20-min incubation at room temperature the absorbance at 460 nm was measured in a plate reader. The MPO activity was calculated from a standard curve prepared the same way as test samples by using the MPO standard (Sigma, Steiham, Germany) and expressed in units per milligrams of protein. One unit of MPO activity was defined as an increase in absorbance of 1.0 per min at room temperature and the concentration of protein in samples was measured at 280 nm calculated from bovine serum albumin (BSA) standard curve. Each sample was measured in duplicate.

2.9. Western Blot Analysis: Evaluation of HO-1 Expression.

Non-stimulated Mϕ were cultured with taurine derivatives and the expression of heme-oxygenase-1 (HO-1) protein in cytosol was determined by a Western blot technique. Twenty four hours after incubation with the agents, the cells were lysed in lysis buffer [1% Triton X-100, 0.1% sodium dodecylsufate (SDS) in PBS containing 1 mM phenylmethylsulphonylofluoride (PMSF), 100 µM leupeptin and 50 µM pepstatin A]. The protein concentrations of lysates were determined using the Bradford method. The samples, containing equal amounts of total protein, were mixed with gel-loading buffer (50 mM Tris, 3% SDS, 10% glycerol, 7% 2-mercaptoethanol and 0.1% bromophenol blue) in a ratio 4:1 (v/v) and boiled (4 min). Then samples (20 µg of total protein per lane) were separated on 10% SDS-polyacrylamide gels (Mini Protean II, Bio-

Rad, USA) using Laemmli buffer system and proteins were semi-dry transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, USA). Non-specific binding sites were blocked overnight at 4°C with 5% 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). Bands were detected with horseradish peroxidase-conjugated secondary antibody (1 h in RT, 1:5000, Amersham Pharmacia Biotech, USA) and developed with ECL reagents (Amersham Pharmacia Biotech, USA). Additionally, membranes were re-probed with monoclonal anti β-actin antibody (Sigma, USA). Rainbow markers (Amersham Pharmacia Biotech, USA) were used for the molecular weight determinations. The protein bands were scanned and analyzed with freeware Scion image (Scion Corporation, USA). The data are normalized to constitutively expressed β-actin protein.

2.10. Statistical Analysis

If not otherwise stated, the statistical significance of differences between groups was analysed using a factorial ANOVA (Microsoft Excel) followed by Student's t test, if appropriate. The results are expressed as mean ± SE. A p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using Statistica PL™ v. 6.0 (StatSoft, Poland).

3. RESULTS

3.1. Effect of *in Vivo* TRD (Taurolin) Administration on Zymosan-Induced Peritonitis

To evaluate the effect of TRD on the development of acute inflammatory response, vascular permeability, MPO activity of peritoneal exudates cells, which linearly correlates with the number of neutrophils, and the release of pro-inflammatory cytokines by peritoneal exudate cells were determined in the mice treated locally with Taurolin (TRD + PVP). The results were compared with control mice (placebo group), which received an equivalent dose of PVP. Local administration of Taurolin, in a dose relevant to a therapeutic dose used in the treatment of peritonitis in humans (10 mg TRD/kg body weight) (Baker *et al.,* 1994), completely inhibited zymosan-induced increased vascular permeability. As shown in Fig. 1A, Evans blue leakage from the circulation into the peritoneal cavity in TRD-treated mice was only slightly above the level of that in the untreated mice (spontaneous leakage). By contrast, taurine, as well as PVP, a stabilizer of TRD in Taurolin solution (Kirsh and Sihn, 1997), did not alter vascular permeability when compared to that of control mice (mice treated with zymosan only) (control OD = 0.360 ± 0.025).

Similarly, the influx of neutrophils into peritoneal cavity in zymosan-induced peritonitis, as measured by MPO activity, was completely blocked by TRD but not by taurine and PVP (Fig. 1B). In addition to beneficial effect of on vascular permeability and on neutrophil influx, TRD significantly attenuated inflammatory activities of peritoneal exudate cells by the reduction of TNF-α and IL-6 production. PVP and taurine did not affect the cytokine production (Fig. 1C).

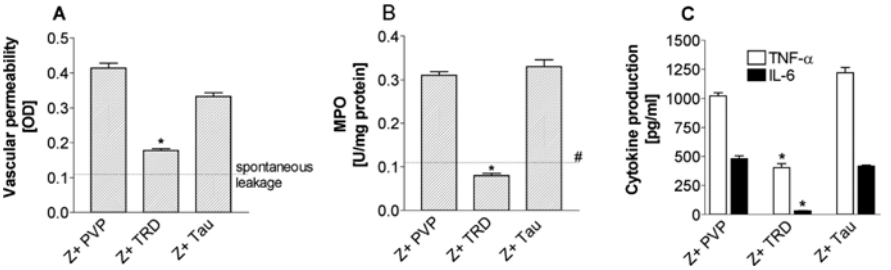


Figure 1. The effect of local TRD administration on the development of zymosan-induced peritonitis. PVP was used as placebo. * TRD *vs*. PVP p< 0.05. (A) Concentration of Evans blue in peritoneal exudates is shown as OD measured at 630 nm. The results represent 6 independent experiments. (B) MPO activity is expressed as units/1 mg of protein (results from 3 exp.) *MPO activity in peritoneal exudates of naïve, untreated mice. (C) TNF-α and IL-6 release from re-stimulated *in vitro* peritoneal exudate cells (results from 3 experiments).

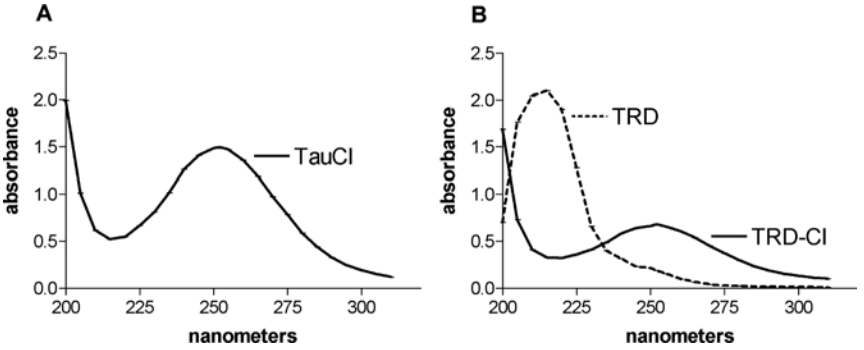


Figure 2. The UV absorption spectra of (A) TauCl (~3 mM), (B) TRD and TRD-Cl (~1.5 mM).

3.2. Effect of *in Vitro* HOCl Reaction With TRD

To determine whether TRD may react with HOCl to form monochloramine, similarly to the reaction between taurine and HOCl, the UV spectra of products of these reactions were analysed. As shown in Fig. 2, TRD and taurine react with HOCl in a similar way to form chloramines, as identified by characteristic UV spectra with λ_{max} for chloramines at 252 nm.

3.3 Effect of Taurine Derivatives on Macrophage Inflammatory Activities

3.3.1 TRD, TRD-Cl and TauCl Inhibit the Production of IL-6 by Activated Macrophages

TauCl, at non-cytotoxic concentrations, inhibited the production of IL-6 in both LPS- and IFN-γ stimulated macrophages in a dose-dependent manner. Taurine did not affect the production of IL-6.

TRD and its chlorinated derivative TRD-Cl inhibited the production of IL-6 by IFN-γ-stimulated macrophages in a similar dose-dependent manner (Fig. 3B). Surprisingly,

chlorination of TRD significantly enhanced its suppressive capacity as measured by the effect on IL-6 production by LPS-stimulated macrophages (Fig. 3A). In our experimental set-up TRD and TRD-CL were significantly stronger than TauCl in reducing IL-6 production.

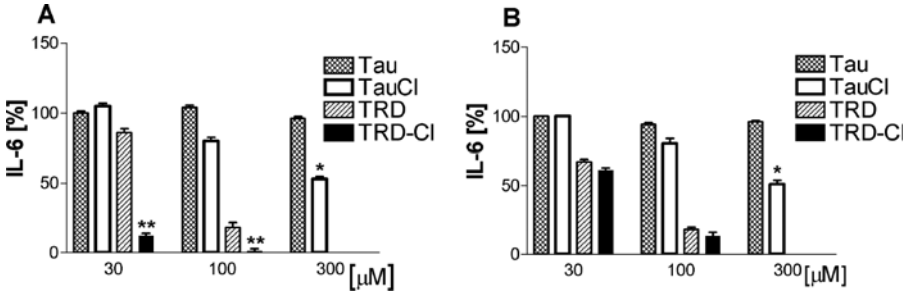


Figure 3. The effect of taurine derivatives on IL-6 release from peritoneal macrophages. Mϕ (5 x 10^5/ml) were stimulated either with LPS (A) or with IFN-γ (B) and cultured in the presence of Tau, TauCl, TRD or TRD-Cl. The agents were used at non-cytotoxic concentrations. Results represent 4 independent experiments. * Tau *vs.* TauCl $p < 0.05$; ** TRD *vs*. TRD-Cl $p < 0.01$.

3.3.2. TRD does Not Induce Expression of HO-1

TauCl induced the expression of HO-1 protein in non-stimulated macrophages in a dose-dependent manner. By contrast, TRD did not induce the expression of HO-1 in our experimental set-up (Fig. 4). Moreover, there was only a slight expression of HO-1 in Mø cytosol after incubation with taurine and with lower (30 μM) concentration of chlorinated TRD in some experiments.

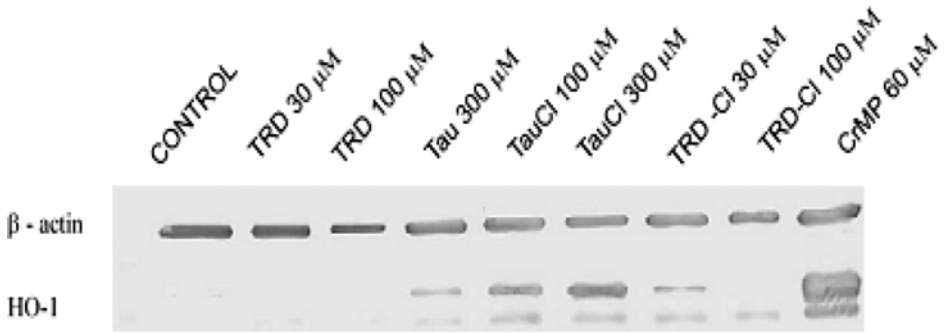


Figure 4. Representative Western blot of HO-1 protein expression in peritoneal macrophages 24 hours after stimulation with Tau, TauCl, TRD and TRD-Cl. Chromium III mesoporhyrin chloride (CrMP) was used as a reference inducer of HO-1 expression.

4. DISCUSSION

Taurolidine due to its anti-endotoxin, anti-bacterial and anti-adherence properties has been administered clinically by peritoneal lavage as a prophylaxis against infections after abdominal surgery and in the treatment of patients with established peritonitis (Browne *et al.,* 1976; Willatts *et al.,* 1995; Staubach, 1997). The bactericidal activity of TRD depends on the generation of active methylol groups. The methylol-containing moieties, taurultam and taurinamide (Scheme 1) appeared to react with bacterial cell wall components resulting in their denaturation (Myers and Allwood, 1980; Torres-Viera *et al.,* 2000). In addition to this direct effect on bacterial cell components, TRD has been reported to reduce the synthesis and activity of pro-inflammatory mediators probably by mechanisms independent of the methylol-containing species (Bedrosian *et al.,* 1991). Taurine, the third breakdown product of TRD, has been suggested to be responsible for its immunoregulatory properties (Watson *et al.,* 1995). Despite the lack of direct evidence, one may speculate that at a site of inflammation TRD may be chlorinated with HOCl to form chloramines, which will exert anti-inflammatory activities, as has been reported for TauCl (Marcinkiewicz, 1997; Schuller-Lewis and Park, 2003). These anti-inflammatory activities along with anti-bacterial and anti-endotoxin activities of TRD fully explain its beneficial therapeutic effect in acute peritonitis.

In our experimental model of acute peritonitis in mice we have shown that TRD exerts strong anti-inflammatory activities. Local administration of Taurolin, a chemioterapeutic, which contains Taurolidine and its stabilizer polyvinylpyrrolidine (PVP), reduced a number of parameters of acute inflammation induced by zymosan. Taurolin (TRD), but not PVP, reduced neutrophil infiltration into the peritoneal cavity. Moreover, plasmatic exudation, the effect of increased vascular permeability, was maintained by TRD at the level of spontaneous leakage. In addition, TRD significantly suppressed the production of TNF-α and IL-6, the two major pro-inflammatory cytokines, closely connected with the development of acute inflammatory states. All these results are in agreement with previous studies in which the effect of TRD on functions of inflammatory cells has been tested (Watson *et al.,* 1995). However, the link between the MPO-halide system and TRD has not been tested yet.

To evaluate the effect of MPO-halide system on the anti-inflammatory properties of TRD, we have tested *in vitro* the influence of TRD and that of chlorinated-TRD on macrophage functions. For the *in vitro* study, purified TRD, instead of Taurolin, was used and compared with TauCl as a reference taurine derivative. We have shown that TRD, as well as taurine, can react with HOCl to form chloramines. Whether, the formation of active chlorinated products of TRD, the most probably TauCl, affects the degradation of TRD into active methylol-moieties remains to be elucidated. Nevertheless our results indicate that *in vitro* at non-cytotoxic concentrations both TRD and TRD-Cl inhibit the production of IL-6 by LPS-stimulated macrophages. The effect of TRD was stronger when compared with that of TauCl which correlates with its stronger cytotoxic and pro-apoptotic activities (Table 1). Moreover, the production of other inflammatory mediators, such as NO and IL-12p40 was inhibited by TRD and TRD-Cl (data not shown). The suppressive effect was not dependent on the anti-endotoxin activity of TRD since the similar reduction of cytokine production was observed in macrophages stimulated with IFN-γ. Interestingly, chlorination of TRD, in contrast to chlorination of taurine, only slightly enhanced the suppressive activity of the parent molecule.

Table 1. Biological activities of TRD and taurine haloamines (TauCl and TauBr)

Activity	TRD	TauCl	TauBr
Cytotoxicity *in vitro* (Mϕ)	>100 μM	>300 μM	>300 μM
Antibacterial	yes	yes	yes
Induction of HO-1 expression	**no**	**yes**	**yes**
Antiendotoxin	yes	no	no
Pro-apoptotic activity	yes	yes	?
Inhibition of cytokine production (IC_{50})	~30 μM	~200 μM	~250 μM

While both native and chlorinated TRD exert anti-inflammatory activities, only taurine haloamines, but not native taurine, down-regulate the activity of inflammatory immune cells *in vitro* (Learn *et al.,* 1990; Marcinkiewicz *et al.,* 1995, 1999, 2005; Barua *et al.,* 2001). Although the precise mechanism of TauCl and TauBr action at a site of inflammation remains unclear, two scenarios, not excluding each other, have been proposed. Haloamines affect target immune cells either directly by inhibiting NFκB signalling, a pathway primarily involved in a cellular inflammatory response (Barua and Quinn, 2001; Kanayama *et al.,* 2002), or indirectly via the heme oxygenase system. Recently, we have described that TauCl and TauBr induce heme oxygenase-1 (HO-1) expression in resting and LPS-stimulated macrophages (Olszanecki and Marcinkiewicz, 2004). HO-1, a cytoprotective enzyme, is a part of the integrated response to oxidative stress. HO-1 catalyses heme degradation to iron, sequestered by ferritin, carbon monoxide (CO) and biliverdin (Abraham *et al.,* 1988; Vicente *et al.,* 2003). Several lines of evidence indicate a link between HO-1 and inflammation (Kushida *et al.,* 2002; Lee and Chau, 2002). We have shown previously that the induction of HO-1 expression by TauCl and TauBr is associated with a concomitant dose-dependent reduction of NO synthesis. As HO-1 products, as well as taurine haloamines, may suppress the production of pro-inflammatory mediators (Kushida *et al.,* 2002; Olszanecki and Marcinkiewicz, 2004), we may speculate that there is a link between taurine-dependent and HO-1-dependent cytoprotective mechanisms. Our present results indicate that TRD, in contrast to TauCl, at concentrations in which the reduction of cytokine production was observed, did not induce the expression of HO-1 protein. Thus, it is unlikely that the anti-inflammatory effect of TRD in zymosan-induced peritonitis is HO-1 dependent.

In conclusion, in this study we have shown TRD as an anti-inflammatory agent by mechanisms distinct from these responsible for immunoregulatory properties of TauCl and TauBr. The major difference was inability of effective dose of TRD to induce expression of HO-1 *in vitro*. Moreover, our results do not confirm the suggestion of Watson *et al.* (1995) that TRD functions primarily through the taurine moiety. However, at a site of inflammation, taurine, as a breakdown product of TRD, may react with HOCl to produce anti-inflammatory agent, TauCl. Further studies are necessary to determine the influence of TRD chlorination on both formation and biological activity of all three TRD breakdown products, taurinamide, taurultam and taurine.

5. SUMMARY

In this study, in an animal model of zymosan-induced peritonitis we have tested anti-inflammatory properties of Taurolidine (TRD), a synthetic derivative of taurine. *In vitro,*

the effect of TRD and HOCl treated TRD on peritoneal macrophages was compared with that of TauCl. We report that locally administered TRD (Taurolin) shows strong anti-inflammatory properties. TRD inhibits vascular permeability increased by inflammatory stimuli; it also significantly attenuates the influx of neutrophils into the peritoneal cavity, as well as the production of pro-inflammatory cytokines (TNF-α, IL-6) by peritoneal exudate cells. Chlorination of TRD resulted in the formation of chloramine (TRD-Cl), as confirmed by characteristic UV spectra. Both TRD and TRD-Cl, more effectively than TauCl, inhibited the production of IL-6 by stimulated macrophages. The effect was not dependent on its well-known anti-endotoxin activity since TRD inhibited cytokine production by macrophages stimulated with either LPS or IFN-γ.

Finally, we report that anti-inflammatory activities of TRD and taurine haloamines are mediated by different mechanisms. TRD, in contrast to TauCl and TauBr, does not induce expression of HO-1, a stress inducible enzyme with strong anti-inflammatory properties.

6. ACKNOWLEDGMENTS

This work was supported in part by Jagiellonian University Medical College (501/P/22/L) and by the "Polish Pharmacy and Medicine Development" (No. 004/2002).

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