Inflammatory Cytokine Regulation by Taurine-chloramine

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INTRODUCTION

Taurine chloramine (Tau-Cl) is a chlorinated form of taurine, which is an exceptionally abundant free amino acid in the cytosol of inflammatory cell, by the halide-dependent myeloperoxidase system. Taurine protects tissue from damage resulting from overt inflammatory reactions as demonstrated in various in vivo and in vitro models of inflammation (Davies *et al.*, 1993; McLoughlin *et al.*, 1991; Schuller-Levis *et al.*, 1994). Tissue inflammatory damage is mediated by reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI), and proinflammatory cytokines such as tumor necrosis factor (TNF) (Moncada *et al.*, 1993; Fantone *et al.*, 1982). Polymorphonuclear cells and macrophages respond to a variety of stimulants to produce ROI, RNI, and TNF (Tachibana *et al.*, 1992; Pendino *et al.*, 1993; Xing *et al.*, 1993). The reactive oxygen species produced during the respiratory burst of phagocytic cells, along with NO and TNF play a major role in host defense against bacterial or parasite infections and tumors (Rossi, 1986; Klebanoff, 1985; Nathan *et al.*, 1981; Stuehr *et al.*, 1989). However, when these inflammatory mediators are produced in greater quantities, they mediate the tissue damage associated with inflammation and ischemic injury (Kroencke *et al.*, 1991; Grisham, 1994).

High concentrations of taurine are present in the cytosol of human leukocytes (Green *et al.*, 1991; Learn *et al.*, 1990). Under physiological conditions, Tau-Cl is thought to be produced from hypochlorous acid (HOCl/ OCl^-), a product of the oxidative burst from PMN, in a reaction catalyzed by the halide-H_2O_2-myeloperoxidase system (Weiss *et al.*, 1982; Witko *et al.*, 1992). Formation of Tau-Cl may also be catalyzed directly by the halide-dependent myeloperoxidase associated with PMN (Marquez and Dunford, 1994). Although Tau-Cl inherently possesses oxidative potential, it is more stable and less toxic than HOCl.

In this context, the effects of Tau-Cl and its precursor, taurine, on the production of O_2^- by murine peritoneal PMN were determined. In addition, activated murine peritoneal macrophages and RAW 264.7 cells were used to determine the effects of Tau-Cl on production of NO and TNF-α.

Effects on Superoxide Anion Production

BALB/c peritoneal PMN were tested for their capacity to generate O_2^- during 45 min of stimulation by PMA in the presence of Tau-Cl and taurine. Since RAW 264.7 cells do not produce O_2^- and ROI are potent mediators of tissue damage, murine peritoneal PMN were used to examine the effect of Tau-Cl on O_2^- production. Tau-Cl was an effective and potent inhibitor of O_2^- production (Fig. 1). Production of O_2^-

was inhibited in a concentration dependent manner and was inhibited 50% by 0.5 mM Tau-Cl. Although taurine was much less effective and less potent than Tau-Cl, significant ($p \leq 0.05$) inhibition of O_2^- production was also observed. Production of O_2^- was inhibited 34% by 0.5 mM taurine, with no further inhibition by 1 mM taurine. It is likely that the halide-dependent MPO that is associated with PMN converted taurine to Tau-Cl in vitro (Weiss *et al.*, 1982; Witko *et al.*, 1992; Marquez and Dunford, 1994) and that Tau-Cl rather than taurine caused the inhibition.

To determine the duration of Tau-Cl effects, PMN were pretreated for 2 hr with various concentrations (0-1 mM) of Tau-Cl. PMN were then washed, stimulated with PMA in the presence or absence of Tau-Cl, and assayed for production of O_2^-. Pretreatment of PMN with Tau-Cl, followed by removal of Tau-Cl, had no effect on the capacity of the cells to generate O_2^- (Fig. 2). The effects of Tau-Cl on PMN appears to be short-lived and to result from processes that occur during stimulation of PMN because Tau-Cl had no direct effect on ferricytochrome c reduction in the absence or presence of SOD.

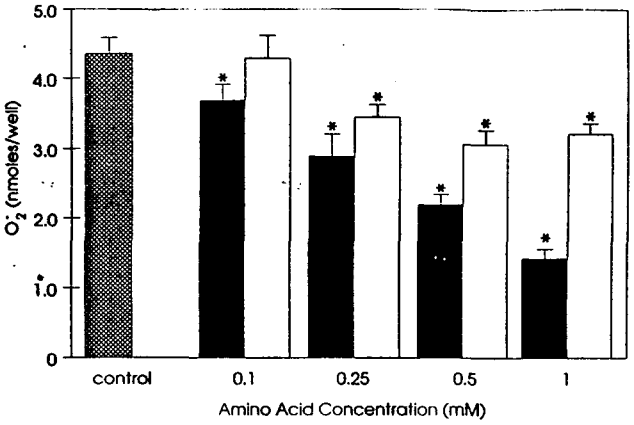

Fig. 1. Effect of Tau-Cl and taurine on superoxide anion production by PMN. PMN were stimulated by PMA in the absence (control) or presence of various concentrations of taurine (open bars) or Tau-Cl (dark bars) and O_2^- production was measured. Values are expressed as mean ± SD for triplicate samples. Similar results were obtained in five independent experiments. *, significantly different from control by ANOVA, $p<0.05$.

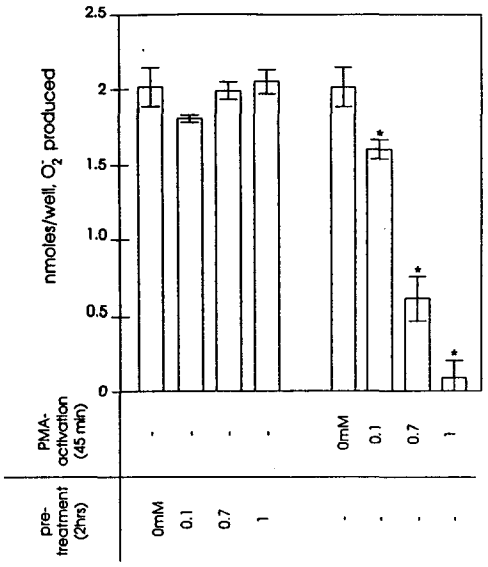

Fig. 2. Effect of Tau-Cl pretreatment on superoxide anion production by PMN. PMN were exposed to various concentrations (0, 0.1, 0.7, 1 mM) of Tau-Cl for 2 hr before activation or during the 45 min PMA activation period. PMN were washed before PMA activation and O_2^- production was measured. Values are expressed as mean ± S.D. for triplicate samples. Similar results were obtained in three independent experiments. *, significantly different from control (PMA activated PMN) by ANOVA, $p<0.05$.

Time Course of NO_2^- and TNF-α Production

Murine peritoneal macrophages were activated with LPS (10 ug/ml) and rINF-γ (50 U/ml) for various times before NO_2^- and TNF-α production were measured. Only trace amounts of NO_2^- and TNF-α were determined in the absence of stimulant or in the

presence of rINF-γ without LPS. Small amounts of NO_2^- were produced 10 hr after stimulation and increased linearly over the following 38 hr (Fig. 3). After 48 hr of incubation, the accumulation of NO_2^- were increased only slightly. TNF-α was detected as early as 3 hr after activation and continued to accumulate linearly over the following 7 hr (10 hr incubation time). The amount of TNF-α in the media did not increase any further after the first 10 hr of activation (Fig. 3).

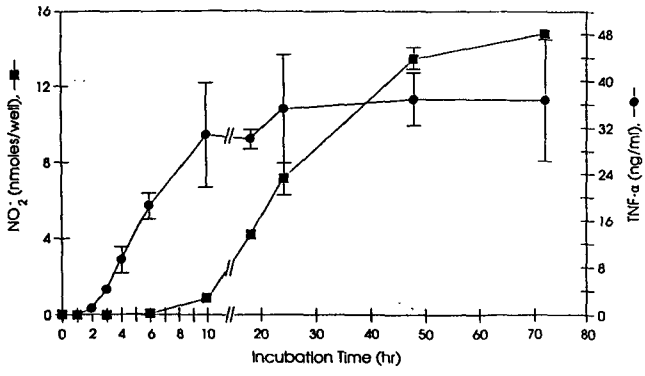

Fig. 3. Time course of NO_2^- (-■-) and TNF-α (-●-) production by murine peritoneal macrophages. Cultured adherent macrophages were activated by addition of LPS (10 μg/ml) and rIFN-γ (50 U/ml). Conditioned media samples were collected at various times post-activation and analyzed for nitrite and TNF-α content. The data are the mean ± SD for triplicate samples. Similar results were obtained in four independent experiments.

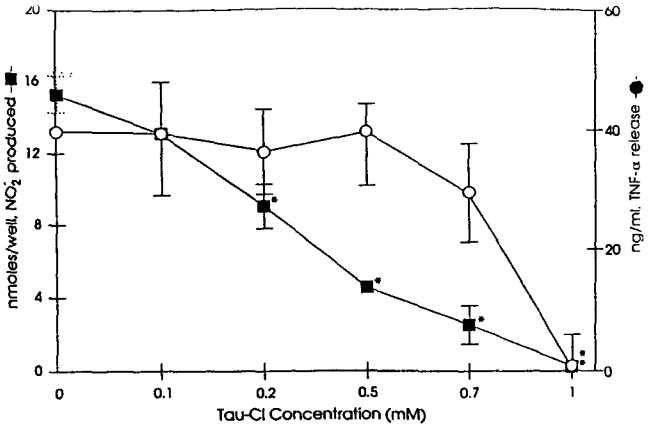

Fig. 4. Inhibition of NO_2^- (-■-) and TNF-α (-●-) production by Tau-Cl. Murine peritoneal macrophages were cultured with LPS and rIFN-γ in the presence of different concentrations of Tau-Cl. Supernatants were removed after 48 hr and assayed for nitrite and TNF-α. The data are the mean ± SD for triplicate samples. Similar results were obtained in 10 independent experiments. *, significantly different compared to control (LPS and rIFN-γ only) by ANOVA, $p<0.05$.

Inhibition of NO_2^- and TNF-α Production

The effects of Tau-Cl and taurine on NO_2^- and TNF-α production by mouse peritoneal macrophages activated with LPS (10 ug/ml) and rINF-γ (50 U/ml) were determined. Either Tau-Cl or taurine was added simultaneously with activators and accumulation of NO_2^- and TNF was measured 48 hr later (Fig. 4). Tau-Cl inhibited production of NO_2^- and TNF with equal effectiveness but exerted a more potent inhibition of NO_2^- secretion. For instance, 0.5 mM Tau-Cl inhibited NO_2^- production 72% without affecting TNF-α while accumulation of both NO_2^- and TNF-α was completely inhibited when cells were activated in the presence of 1.0 mM Tau-Cl. Taurine was without effect on these parameters, as measured in parallel cultures (not shown). The viability of Tau-Cl treated cells was evaluated by trypane blue dye exclusion and was similar to that of untreated cells over the range concentrations used in these studies.

To gain insight into possible mechanisms of action, Tau-Cl (1.0 mM) was added to the cell culture media at different times following the addition of the activators. Media content of NO_2^- and TNF-α was measured 48 hr after activation. The greatest inhibition of NO_2^- production was achieved when Tau-Cl (1.0 mM) was added

within the first 6 hr of activation (Fig. 5). The production of TNF-α was significantly inhibited when Tau-Cl was added within 3 hr of activation, but was unaffected if Tau-Cl was added thereafter. Values were statistically compared to those for TNF-α (36±5 ng/ml) and NO_2^- (13±1 nmol/well) obtained after 48 hr activation in the absence of Tau-Cl (Fig. 3). In contrast to TNF-α, NO_2^- production was inhibited to various extents if Tau-Cl was added to the media within the first 24 hr of activation. Tau-Cl inhibited NO_2^- production 38% even when added 24 hr after activation (Fig. 5). The degree of Tau-Cl inhibition of NO_2^- production was graded, depending on the post-activation time of Tau-Cl addition.

The similar effects of Tau-Cl on NO and TNF-α production were observed in activated RAW 264.7 cells..

Inhibition of iNOS

The direct effect of Tau-Cl on NOS activity was determined by using a cell-free cytosolic preparation from activated RAW 264.7 cells. NOS activity was linear over 4 hr of incubation and omission of the cofactors NADPH, BH_4, and FAD, resulted in 56% inhibition of NOS activity. Addition of a competitive substrate inhibitor, monomethylarginine, or 1 mM Tau-Cl in the assay mixture inhibited NOS activity by 90% and 59%, respectively. To determine whether Tau-Cl inhibited NOS activity by interacting with enzyme or by inactivating cofactors, we pretreated the cytosolic preparation with 1 mM Tau-Cl, removed unreacted Tau-Cl, and then measured NOS activity. NOS was irreversibly inhibited, thus demonstrating a direct effect of Tau-Cl on NOS rather than on cofactors.

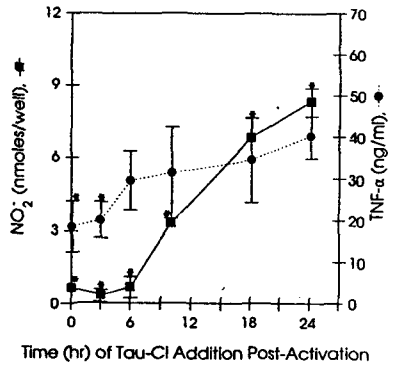

Fig. 5. Effects of Tau-Cl added at various times post-activation on NO_2^- (-■-) and TNF-α (-●-) production. Murine peritoneal macrophages were activated (LPS and rIFN-γ) and NO and TNF-α were measured 48 hr later. Tau-Cl (1 mM) was added at various times post-activation during the 48 hr incubation period. In the absence of Tau-Cl, 10.7 ± 0.3 nmoles/well of NO_2^- and 39.1 ± 0.5 ng/ml of TNF-α were produced by activated cells during the same 48 hr incubation period. The data are the mean ± SD for triplicate samples. Similar results were obtained in four independent experiments. *, significantly different from control (48 hr production without Tau-Cl) by ANOVA, $p<0.01$.

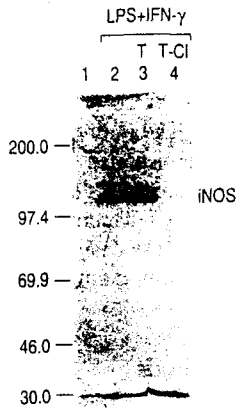

Figure 6. Western blot analysis of iNOS expression in RAW 264.7 cell lysates; lysates (50 ug protein/lane) from unactivated cells (lane 1), activated (LPS and IFN-γ) cells (lane 2), and from cells activated in the presence of either 0.8 mM taurine (lane 3) or 0.8 mM Tau-Cl (lane 4) are shown.

To determine whether Tau-Cl inhibited iNOS activity by a direct interaction with the enzyme of by affecting the expression of iNOS protein, cell lysates from the same bulk-cultured cells were analyzed by SDS-PAGE and Western blot (Fig.6). Lysates from activated RAW 264.7 cells were positive for iNOS, which appeared as a 130 kDa band. This band was absent from lysates prepared from cells activated in the presence of Tau-Cl (0.8 mM), similar to that observed with naive, inactivated cells. Expression of iNOS in lysates from cells activated in the presence of taurine (0.8 mM) was unaffected. The results in murine macrophages are consistent with that in RAW 264.7 cells.

DISCUSSION

Both Tau-Cl and taurine inhibited O_2^- production. However, Tau-Cl demonstrated greater inhibition of O_2^- than taurine. Several studies have shown that the formation of Tau-Cl is catalyzed by a reaction of PMN derived MPO and $HOCl/OCl^-$ with taurine and addition of exogenous taurine strongly enhances chloramine formation (Weiss *et al.*, 1982; Witko *et al.*, 1992). Thus, the inhibition of O_2^- production by taurine is likely to be from the in vitro formation of Tau-Cl in this culture system.

Inhibition by Tau-Cl of O_2^- production required Tau-Cl to be present during the activation process and was reversible in the sense that inhibition of O_2^- was not observed when Tau-Cl was removed from previously exposed PMN. Since NADPH oxidase is assembled as a holoenzyme only after activation (Chanock *et al.*, 1994), Tau-Cl must either inhibit the assembly of the subunit components of NADPH oxidase or interact with the assembled multiprotein complex. These results cannot be accounted for by a direct effect of Tau-Cl on the O_2^- assay because Tau-Cl added to supernatant after PMA stimulation and prior to spectrophotometric analysis did not effect the amount of SOD inhibitable O_2^- detected relative to the same samples not containing exogenous Tau-Cl (Data not shown).

Similar to RAW 264.7 cells, the most prominent inhibition of NO in macrophages is at or near activation and prior to induction of iNOS (6 hr). However, there is still considerable inhibition of NO in macrophages after 6 hr of activation indicating possible direct inhibition of iNOS by Tau-Cl. NO synthase activity of cytosolic preparations from activated RAW 264.7 cells is irreversibly inhibited Tau-Cl. Inhibition of TNF-α is seen only when Tau-Cl is added within 3 hr of activation because expression of TNF-α mRNA peaks within 4 hr of activation (Park *et al.*, 1995).

In RAW 264.7 cells, Tau-Cl inhibits production of TNF-α and NO by different mechanisms, Tau-Cl inhibited transcription of the iNOS gene, or some earlier event in the signal transduction pathway, because iNOS protein and iNOS mRNA were undetected in lysates of cells activated in the continuous presence of Tau-Cl. In contrast, steady-state levels of TNF-α mRNA increased in the presence of Tau-Cl to at least the same extent as that in untreated activated cells and persisted for a longer period time (Park *et al.*, 1995). The data in murine macrophages are consistent with the mechanisms that are operative in RAW 264.7 cells.

In the present study, we demonstrate that Tau-Cl, which is actively transported into RAW 264.7 cells (Park *et al.*, 1993) and peritoneal macrophages (data not shown), inhibits the production of O_2^-, NO_2^-, and TNF-α by cultured peritoneal leukocytes in a dose-dependent manner. These data suggest that Tau-Cl decreases production of inflammatory mediators and thus may play an important role in inflammation.

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