Inhibition of Septic Arthritis by Local Administration of Taurine Chloramine, a Product of Activated Neutrophils

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ABSTRACT. ***Objective.*** Taurine is an amino acid able to react with hypochlorous acid, produced endogenously by neutrophils, resulting in the more stable and less toxic taurine chloramine (Tau-Cl). Since Tau-Cl has been shown to down-regulate the production of proinflammatory mediators and to exert anti-bacterial properties, we investigated the efficacy of Tau-Cl treatment for infectious arthritis.

Methods. The murine model of hematogenous septic arthritis involved intravenous injection of a single dose of *Staphylococcus aureus*. Tau-Cl was administered by daily intraperitoneal injections. In another experiment *S. aureus* and Tau-Cl were injected intra-articularly. Evaluation of arthritis was performed clinically and histologically. The effect of Tau-Cl on bacterial growth *in vitro* was also assessed.

Results. Growth of staphylococci, including the methicillin-resistant strain 67-0, was inhibited by Tau-Cl. Mice injected with bacteria and Tau-Cl locally in the joint exhibited significantly fewer arthritic lesions. In contrast, there were no obvious differences between Tau-Cl-treated animals and controls with regard to clinical or histological signs of arthritis when bacteria and Tau-Cl were administered systemically.

Conclusion. Our results show that Tau-Cl exerts an inhibitory effect on the development of bone and cartilage damage in the infected joint when administered intra-articularly. (J Rheumatol 2005; 32:1513–7)

Key Indexing Terms:
TAURINE CHLORAMINE STAPHYLOCOCCUS AUREUS ARTHRITIS

Taurine is the most abundant free intracellular amino acid in the cytosol of leukocytes, particularly in neutrophils. It acts as a scavenger for hypochlorous acid (HOCl), which is produced by the myeloperoxidase-hydrogen peroxide-chloride system of activated neutrophils and monocytes[1]. Taurine is the major target for chlorination by HOCl, which leads to the formation of taurine chloramine (Tau-Cl)[2]. Tau-Cl, in contrast to other chloramines, is relatively stable and non-cytotoxic at physiological concentrations[3]. Recent studies have shown that Tau-Cl itself has a strong antiinflammatory activity, inhibiting the production of tissue damaging inflammatory mediators like nitrous oxide (NO), prostaglandin E_2, tumor necrosis factor (TNF)-α, and interleukin (IL)-6[4,5]. In addition, Tau-Cl displays significant bactericidal effects and has been used in clinical studies as an antimicrobial agent[6,7].

We have shown that in the initial stage of *Staphylococcus aureus* infection, phagocytosis by recruited neutrophils is critical in disease outcome[8]. Neutrophils are not only an effective defense against bacterial pathogens but also act as mediators of tissue-destructive events in many inflammatory diseases[9]. A massive infiltration of granulocytes in the joint may mediate cartilage and bone destruction by a series of well-defined mechanisms[10]. Since Tau-Cl has been shown to down-regulate release of inflammatory mediators generated by activated phagocytes, we assessed the role of Tau-Cl in septic arthritis.

MATERIALS AND METHODS

Mice. Six to 8-week-old NMRI female mice were obtained from B&K Universal AB (Stockholm, Sweden). They were housed in the animal facility of the Department of Rheumatology and Inflammation Research, University of Göteborg under standard light and temperature, and were fed standard laboratory chow and water *ad libitum*. Experiments were performed with approval of the Ethical Committee of Göteborg University.

Preparation of Tau-Cl and bacteria. NaOCl and taurine were purchased from Sigma Chemical Co. (St. Louis, MO). Tau-Cl was freshly synthesized on the day of use as described[11,12]. Briefly, Tau-Cl was synthesized by adding equimolar amounts of NaOCl slowly to taurine and vortexing at

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room temperature. Tau-Cl and NaOCl were diluted in Hanks' Balanced Salt Solution (HBSS, phenol red-free), resulting in a stock solution of 100 mM Tau-Cl, pH 8.3. Each preparation was measured in the UV absorption spectra (190-350 nm) to assure monochloramine formation. The extinction coefficient of Tau-Cl is 429 at the absorption peak 252 nm.

S. aureus strain LS-1 was originally isolated from a swollen joint of a spontaneously arthritic NZB/W mouse[13]. Bacteria were kept frozen at –20˚C in phosphate buffered saline (PBS; 0.13 M NaCl, 10 mM sodium phosphate, pH 7.4) containing 5% bovine serum albumin and 10% dimethylsulfoxide (C$_2$H$_6$OS) until used. Before use, the bacterial suspension was thawed and washed in PBS. Viable count was used to check the number of bacteria in each bacterial suspension.

Treatment of mice with Tau-Cl and injection of bacteria. To determine the impact of locally administered Tau-Cl on septic arthritis, mice were injected intraarticularly (IA) into one knee joint with a total volume of 20 µl of solution consisting of equal volumes of Tau-Cl and bacteria, mixed shortly prior to injection. In the first experiment the amount of injected bacteria was 3×10^3 colony forming units (CFU) per knee joint, and in the second experiment, 3×10^4 CFU. Concentrations of Tau-Cl were 0.1, 1, 10, or 100 mM. Controls received vehicle solution consisting of HBSS, PBS, and bacteria. To confirm that the antibacterial action of Tau-Cl had not occurred *in vitro* (after mixing and before injection), we performed viable counts of bacteria mixed with Tau-Cl (10 mM) in leftover solutions after 30 minutes. Viable counts of bacteria were decreased by only 9% following this time period. This finding precluded the possibility of significant *in vitro* killing of staphylococci by Tau-Cl prior to IA injection. After 3 days, knee joints were removed for histological analysis.

In another experiment, bacterial load in the knee joint was determined 3 days after IA injection with 10 µl of 5×10^3 CFU of *S. aureus* and 10 µl of 10 mM of Tau-Cl. Control mice received vehicle solution and bacteria. A joint harboring more than 20 CFU of *S. aureus* was considered positive. To evaluate the impact of Tau-Cl on systemic septic arthritis, mice were injected intraperitoneally every day with 200 µl of 1 mM or 100 mM Tau-Cl, starting 2 h prior to injection in the tail vein with 0.2 ml of bacteria and then every day until the end of the experiment. Two experiments were performed in which mice received 1.8×10^7 or 8×10^6 CFU of *S. aureus*, respectively. The first experiment included 7 mice per group (controls and Tau-Cl-treated) the second, 12 mice per group. To examine the impact of Tau-Cl-treatment alone, the first experiment also included 4 mice treated with 200 µl of 100 mM of Tau-Cl without injection of bacteria. All mice were monitored individually. Their limbs were inspected visually every day during the experiment. Arthritis was defined as visible joint erythema and/or swelling of at least one joint. To evaluate the intensity of arthritis, a clinical scoring system of 0 to 3 points for each limb was used (1 point, mild swelling and/or erythema; 2 points, moderate swelling and erythema; 3 points, marked swelling and erythema)[14]. The arthritic index was constructed by adding the scores from all 4 limbs for each animal. The overall condition of each mouse was evaluated by assessing its weight, general appearance, alertness, and skin abnormalities. Mice were sacrificed after 6 or 8 days. Paws were removed for histological analysis and blood and kidneys for assessment of bacterial load.

Histological examination. Histological examination was performed after routine fixation, decalcification, paraffin embedding, and staining with hematoxylin and eosin[15]. All slides were coded and joints were studied with regard to synovial hypertrophy (defined as a synovial membrane thickness of more than 2 cell layers) and cartilage and bone destruction (loss of tissue integrity with resulting ingrowth of fibrotic tissue). The severity of synovial hypertrophy and cartilage/bone destruction was scored from 0 (intact synovial, cartilage/bone tissue) to 3 (intense synovitis with total destruction of cartilage and/or bone).

Determination of staphylococcal load in sera and kidneys. Eight days after *S. aureus* inoculation the experiment was terminated, blood samples were obtained, and kidneys were aseptically removed. Appropriate dilutions were made and 0.1 ml of tissue suspensions and whole blood were plated on agar plates containing 5% horse blood. After incubation for 48 h at 37˚C, bacterial colonies were counted and tested for catalase and coagulase activity.

In vitro stimulation of spleen mononuclear cells for proliferation and cytokine production. To analyze the impact of Tau-Cl on naïve lymphocytes, spleens were obtained from healthy NMRI mice. Preparation of spleen mononuclear cells was performed as described[16]. Cells were seeded into a 24-well plate (Nunc, Roskilde, Denmark) at α concentration of 1×10^5 per well. The cells were maintained in continous culture using Iscove's medium supplemented with 10% fetal calf serum, L-glutamine, and gentamicin. Tau-Cl in concentrations ranging from 10 to 500 µM was added together with stimuli to splenocyte cultures. The cells were stimulated with 0.6 g/ml of concanavalin A (Con A; ICN Biochemicals, Cleveland, OH), 3 µg/ml of toxic shock syndrome toxin-1 (TSST-1; Toxin Technology, Sarasota, FL), and 2×10^7 CFU of formalin-killed staphylococci. After 24 h of incubation at 37˚C and 5% CO$_2$, the supernatants were collected and stored at –20˚C until analysis.

Proliferation assay was performed in the same way in 96-well microtiterplates (Nunc) and cultured for 72 h. During the last 12 h one µCi ^{3}H-thymidine/well (Radiochemical Centre, Amersham, UK) was added.

To measure lactate dehydrogenase (LDH) release in supernatant from Con A and TSST-1-stimulated cells treated with Tau-Cl, the cytotoxicity detection kit (LDH) (Roche Diagnostics GmbH, Mannheim, Germany) was used.

In vitro impact of Tau-Cl on bacterial growth. *S. aureus* LS-1 or methicillin-resistant strain 67-0 were cultured in Ex-broth, which consists of meat-extract "Lab-Lemco" Powder Oxoid L29, Peptone Lab MC24, physiological saline, and distilled water. Tau-Cl was added at concentrations ranging from 0 to 1000 µM to tubes containing bacterial cells. Control cultures were incubated in vehicle solution, i.e. HBSS and PBS. Bacteria were incubated on a rotary shaker (150 rpm) at 37˚C. After 5 h, bacterial growth was monitored by viable counts.

Statistical analysis. Statistical comparisons were made by the Mann-Whitney U test and chi-square test with Yates correction. All values are reported as the mean ± standard error of the mean (SEM).

RESULTS

IA injection of Tau-Cl ameliorates septic arthritis. Tau-Cl at a concentration of 1 mM significantly reduced arthritic lesions in mice inoculated IA with *S. aureus* (Table 1). In addition, the frequency of mice developing arthritis was reduced in mice receiving Tau-Cl in this concentration. Tau-Cl at low (0.1 mM) or high (100 mM) concentration had no ameliorating effect on the disease (Table 1).

Bacteria were recovered from 2 out of 14 mice (14%) injected IA with 10 mM of Tau-Cl and *S. aureus* in comparison with 6 out of 14 control animals (43%) injected with bacteria and vehicle solution (HBSS and PBS). The difference is not statistically significant.

Impact of systemic treatment with Tau-Cl on septic arthritis. Two experiments were performed. In the first experiment there was a tendency toward less severe and less frequent arthritis in the animals treated with 1 mM of Tau-Cl. Thus, 4 days after bacterial inoculation, 2 out of 7 mice in the Tau-Cl-treated group displayed clinical signs of arthritis compared to 5 of 7 mice in the control group. The index for arthritis severity was 0.4 versus 1.0, respectively. Tau-Cl-treated mice decreased in weight significantly less (p < 0.05,

Table 1. Local intraarticular treatment with Tau-Cl significantly reduced arthritic lesions in mice inoculated with *S. aureus*. Mice received local intraarticular injection in one knee joint with 3×10^3 or 3×10^4 CFU of *S. aureus* and Tau-Cl at concentrations 0, 0.1, 10, or 100 mM. Data are the pooled results from 2 experiments with similar outcome.

Treatment	Incidence (Arthritic Mice/Total)	Mean Arthritic Scores*	Mice with Cartilage or Bone Destruction/Total
Controls	11/16	1.3	9/16
Tau-Cl (mM)			
0.1	5/6	1.4	2/6
1	5/16	0.5[b]	2/16[b]
10	5/16	0.6	3/16
100	8/16	1.2	3/16

* Arthritic scores were determined on a scale from 1 to 3, as described in Methods. [b] $p < 0.05$ versus controls.

0.05, and 0.01, respectively) as compared to controls on day 3, 5, and 7 (termination of experiment) following bacterial inoculation. No mice died in either of these groups. In the second experiment, mice administered 100 mM Tau-Cl and infected with *S. aureus* displayed signs of sepsis, and 3 days after bacterial inoculation 4 out of 7 animals had died. In contrast, mice that received only the 100 mM Tau-Cl but no bacteria were all healthy, indicating that systemic Tau-Cl at this concentration diminished the inflammatory response to bacteria to a degree detrimental to the host.

In the second experiment there were no differences in arthritis outcome between mice treated with Tau-Cl and controls. In contrast, 8 days after inoculation with bacteria, 6 out of 13 mice in the Tau-Cl-treated group had died compared to none in the control group. Animals in both groups decreased equally in weight but bacterial load in the kidneys was significantly higher in mice treated with Tau-Cl than in control animals (114 ± 29 versus 56 ± 10 CFU; $p = 0.04$).

In both experiments histological examination displayed no differences in arthritic lesions between Tau-Cl-treated mice and controls.

In vitro down-regulation of IL-6. Production of IL-6 by activated murine spleen cells was inhibited by Tau-Cl at the 200 μM concentration (Figure 1). In addition, Tau-Cl decreased the proliferative response to TSST-1 and formalin-killed bacteria but not to Con A (Figure 2). Presence of Tau-Cl in concentrations higher than 200 μM turned out to be toxic to murine mononuclear spleen-cells, as measured by LDH-release (Figure 3) and trypan blue exclusion.

Tau-Cl inhibits in vitro growth of S. aureus including the methicillin-resistant (MRSA) strain. Two experiments were performed to assess bacteriolytic properties of Tau-Cl. The results were similar: Tau-Cl at a concentration of 500 μM and above inhibited the growth of *S. aureus*, including MRSA 67-0 (Table 2).

DISCUSSION
Our results show a significantly ameliorating effect of Tau-Cl on the arthritic lesions in joints injected with *S. aureus*.
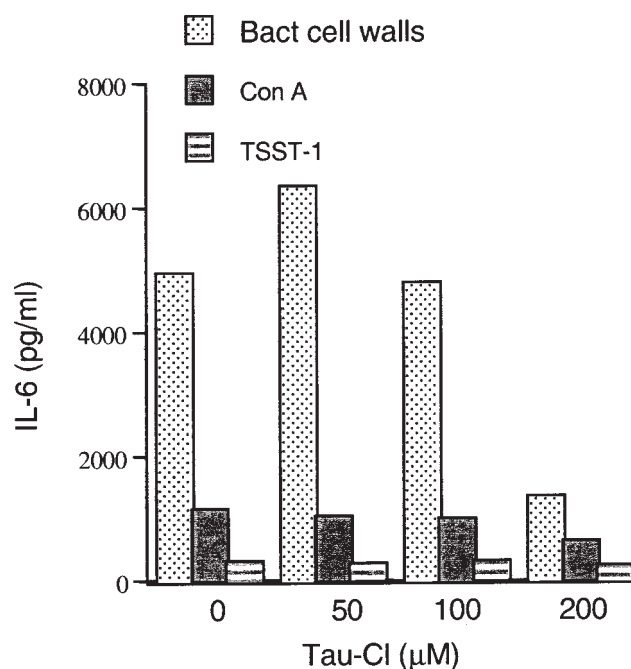

Figure 1. Production of IL-6 by activated murine spleen mononuclear cells. Cells were incubated for 24 h with Tau-Cl and stimulated with Con A, TSST-1, and formalin-killed *S. aureus* LS-1. The results are the mean of 2 experiments with similar outcome.

Studies by Nagl and coworkers also found a significant bactericidal function of Tau-Cl during infectious peritonitis[17]. We also found a bactericidal effect of Tau-Cl on MRSA strains. This finding might be of importance considering the scarcity of antibiotic approaches in a MRSA setting. When injected locally in the joint, Tau-Cl significantly reduced lesions caused by staphylococci. The mechanism is most probably due to its antiinflammatory rather than to its antibacterial properties since (1) bacteria and Tau-Cl solutions were mixed shortly prior to injection into the knee-joint avoiding *in vitro* killing of bacteria and (2) use of concentrations of Tau-Cl higher than 10 mM did not further ameliorate the arthritic lesions, indicating that the joint inflammation was minimally affected by killing of bacteria.
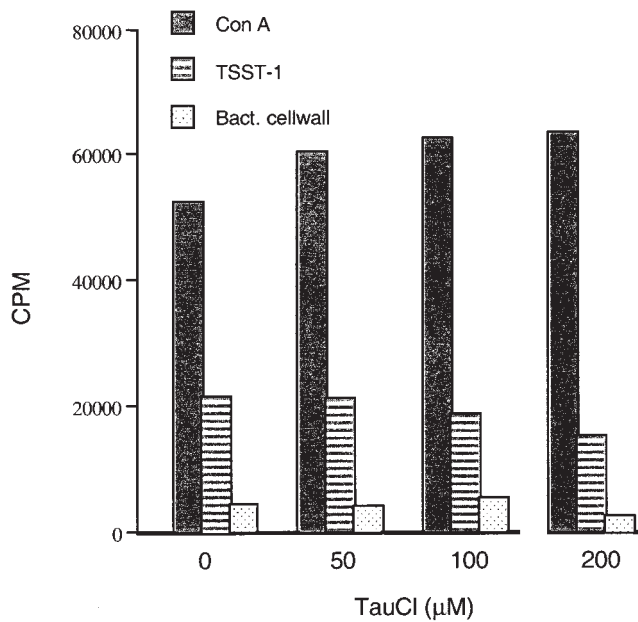
Figure 2. Proliferative responses of mouse spleen mononuclear cells incubated with different concentrations of Tau-Cl and stimulated with Con A, TSST-1, and formalin killed staphylococci, respectively. The results are the mean of 2 experiments with similar outcome.
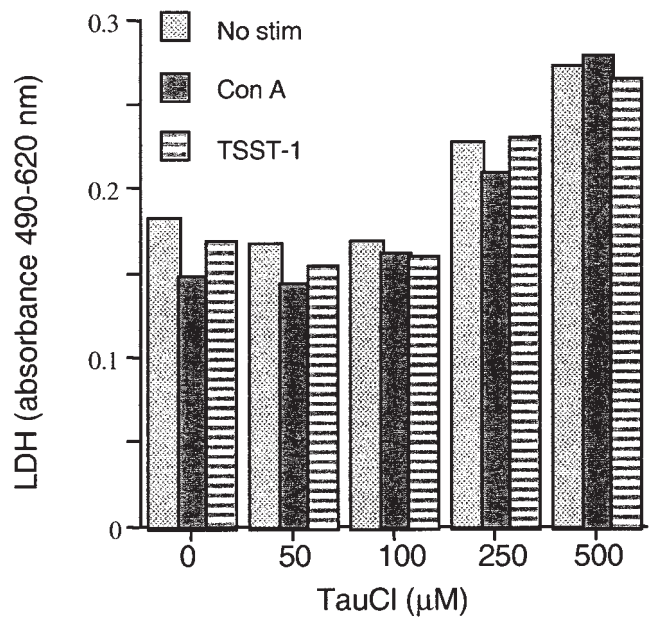
Figure 3. Release of lactate dehydrogenase (LDH) in supernatants of mouse mononuclear spleen cells. Cells were stimulated with Con A and TSST-1, respectively, and treated with different concentrations of Tau-Cl. The results are the mean of 2 experiments with similar outcome.

Table 2. Tau-Cl at the concentration of 500 µM inhibits the growth of *S. aureus*, including the methicillin resistant strain 67–0. Thawed bacteria, $1-8 \times 10^5$ ml, were added to tubes containing broth and various concentrations of Tau-Cl. After 5 h incubation at 37°C bacterial counts were performed. The results are expressed as the mean of 2 experiments.

Bacterium	Tau-Cl (µM)				
	0	10	100	500	1000
S. aureus LS-1 (CFU; $\times 10^5$)	100	68	75	6	0
S. aureus 67-0 (CFU; $\times 10^4$)	17	17	10	3	0

Indeed, the concentrations of Tau-Cl used corresponded to levels of Tau-Cl endogenously produced in infected sites. Thus, in leukocytes capable of generating HOCl from hydrogen peroxide and chloride via the myeloperoxidase pathway, intracellular concentrations of taurine may be 20-50 mM[18].

Neutrophils are the host immune defense cells that are the earliest to migrate into tissues in response to invading pathogens. They can be seen in synovial joint tissue within 24 h after hematogenous spread of *S. aureus*, and they predominate in early joint lesions[13]. One of their principal roles in inflammatory and immune response is thought to be the phagocytosis and killing of bacteria via generation of reactive oxygen intermediates and release of lytic enzymes stored in granules[19,20]. During phagocytosis a variety of microbicidal and proinflammatory agents are generated. These agents are responsible for intracellular killing of pathogens but paradoxically they are also involved in damage to host tissue[9]. In inflammatory sites, taurine can suppress inflammation by acting as a scavenger for HOCl and by formation of Tau-Cl which is much less toxic than HOCl. However, the biological function of taurine in the neutrophil is not only reduction of the cytotoxicity of HOCl but also amelioration of excessive inflammatory reactions[21]. In addition, Tau-Cl exerts antiinflammatory properties by inhibiting generation of proinflammatory mediators[5,22-24].

Thus, reduced production of proinflammatory cytokines and chemokines like IL-6 and IL-8 could have contributed to the reduction of *S. aureus*-induced lesions. In this respect it has been shown that Tau-Cl inhibited the production of IL-6 from fibroblast-like synoviocytes isolated from patients with rheumatoid arthritis[25]. Also the proliferation of these cells was inhibited by Tau-Cl[26]. IL-6 is a pleiotropic cytokine, produced in high amounts in *S. aureus*-induced arthritis[13]. Indeed, when stimulated with formalin killed staphylococci or TSST-1 and treated with Tau-Cl, murine mononuclear cells displayed reduced production of IL-6 as well as reduced proliferative response.

When infected mice were treated systemically with IP Tau-Cl, no amelioration in the development of arthritis was seen. This could be ascribed to the massive inflammatory reaction in response to bacteria and their debris. In fact, when mice were treated with high concentration (100 mM) of Tau-Cl and inoculated with bacteria they developed septicemia and died, while naïve Tau-Cl-treated mice remained healthy. These results indicate that Tau-Cl is unsuitable for systemic use in case of sepsis.

In contrast, local IA injection with Tau-Cl significantly reduced the damage caused by *S. aureus*. Since Tau-Cl is a non-toxic, endogenous substance, it may be considered for local treatment of septic arthritis in man.

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