Amino Acids (2002) 23: 419–426
DOI 10.1007/s00726-002-0207-x

Amino
Acids
Printed in Austria

Effect of taurine chloramine, the product of activated neutrophils, on the development of collagen-induced arthritis in DBA 1/J mice

B. Kwaśny-Krochin[1], **M. Bobek**[2], **E. Kontny**[3], **P. Gluszko**[1], **R. Biedroń**[2], **B.M. Chain**[4], **W. Maśliński**[3], and **J. Marcinkiewicz**[2]

[1] Department of Rheumatology, Jagiellonian University School of Medicine, Cracow, Poland
[2] Department of Immunology, Jagiellonian University School of Medicine, Cracow, Poland
[3] Department of Pathophysiology and Immunology, Institute of Rheumatology, Warsaw, Poland
[4] Department of Immunology, University College London, UK

Received November 30, 2001
Accepted January 25, 2002
Published online August 20, 2002;

Summary. Taurine chloramine (TauCl), a product of neutrophil myeloperoxidase – halide system, formed by a reaction of taurine with HOCl, is known as an anti-microbial and anti-inflammatory long-lived oxidant. We previously reported that TauCl inhibits *in vitro* the production of proinflammatory cytokines (IL-6, IL-8) by RA synoviocytes. Therefore we performed this study to investigate the effect of TauCl treatment on the development of collagen-induced arthritis (CIA) in DBA1/J mice. Early administration of TauCl (after primary immunization) resulted in the delay of the onset of CIA, but had no effect on severity of arthritis. TauCl, given daily for 21 days after booster immunization, did not reduce the symptoms of arthritis in those mice, which already developed CIA, but significantly diminished incidence of the disease (55% vs. 90% of placebo mice). The mechanism of this effect is unknown. This is the first *in vivo* study suggesting that TauCl may be used for immune intervention in chronic inflammatory diseases.

Keywords: Taurine chloramine – Neutrophils – Collagen induced arthritis – DBA 1/J mice

Introduction

The animal model of collagen-induced arthritis (CIA) has been extensively used to elucidate pathogenic mechanisms relevant to human rheumatoid arthritis (RA) and to identify potential targets for therapeutic intervention (Trentham et al., 1977). CIA can be induced in genetically susceptible mice by immunization with native type II collagen (CII) in adjuvant (Trentham, 1982). The disease is characterized by severe swelling and erythema in one or more paws associated with an early massive, predominantly neutrophil cell (PMN) infiltration into the synovium (Weissman, 1982; Edwards et al., 1997). The immune response to CII involves cellular and humoral mechanisms (Klareskog et al., 1983). In RA, the normally acellular joint is heavily infiltrated with a variety of leukocytes. A pannus is infiltrated with endothelial cells, synovial cells (fibroblast-like and macrophage-like), B cells and T cells. On the contrary, synovial fluid from patients with active RA is predominantly infiltrated with neutrophils. All these cells are involved in the pathogenesis of RA (Firestein, 1997). However, the neutrophils have the greatest potential to inflict damage. Neutrophils play an active role in cartilage destruction and erosion secreting enzymes like collagenase, cathepsin, gelatinase, elastase and phospholipase (Edwards et al., 1997). In addition they may generate nitric oxide (NO), a series of reactive oxygen intermediates, including HOCl, and pro-inflammatory cytokines (TNF-α, IL-6, IL-8). All these mediators can act synergistically to accelerate inflammation and a joint damage (Davies et al., 1993; Edwards et al., 1997).

In the present study, we focused on taurine chloramine (TauCl), a physiologic factor generated in activated neutrophils from taurine (Tau) and hypochlorous acid (HOCl), a product of the myeloperoxidase (MPO) – hydrogen peroxide – halide system (Zgliczyński et al., 1971; Thomas et al., 1986; Marcinkiewicz, 1997). The reaction of taurine with HOCl forms the long-lived oxidant TauCl and reduces

HOCl toxicity (Weiss et al., 1982; Grisham et al., 1984; Marcinkiewicz, 1997). TauCl, but not Tau alone, shows *in vitro* various anti-inflammatory properties. For example, TauCl reduces production of NO, prostaglandin E_2, IL-6, TNF-α (Marcinkiewicz et al., 1995, 1998; Park et al., 1995; Kim et al., 1996).

Moreover, we have shown recently that TauCl inhibits *in vitro* the production of proinflammatory cytokines (IL-6 and IL-8) by RA fibroblast-like synoviocytes (FLS), as well as proliferation of these cells. Thus, TauCl may act as a physiological modulator of FLS functions related to their pathogenic role in RA (Kontny et al., 1999, 2000).

Therefore, it was reasonable to ask whether *in vivo* administration of TauCl may affect the development of CIA in mice, an experimental animal model relevant to RA.

Materials and methods

Synthesis of taurine chloramine

TauCl was prepared by dropwise addition of 5 ml of 20 mM NaOCl (Aldrich, Steinham, Germany) solution in 0,05 M phosphate buffer (pH 7,4), with vigorous stirring, to 5 ml of 24 mM taurine (Tau) (Sigma, St. Louis MO) (Marcinkiewicz et al., 1998). Each preparation of TauCl was monitored by ultraviolet absorption spectra (200–400 nm) to confirm the presence of monochloramine (TauCl) (λ_{max} = 252 nm) and the absence of dichloramine ($TauCl_2$) (λ_{max} = 300 nm) and unreacted $HOCl/OCl^-$. The concentration of taurine monochloramine was determined by absorption at 252 nm (ε_M = 415) (Thomas et al., 1986). Stock solution of TauCl (10 mM) was kept at 4°C for a maximum 7 days before use.

Mice

Inbred DBA1/J male mice from the breeding unit, Department of Immunology, Jagiellonian University Medical College, Krakow, Poland, were used between 8 to 10 weeks of age.

Induction of CIA and evaluation of arthritis

Male DBA/1 mice were immunized with 200 μg of chicken collagen II emulsified in complete Freund's adjuvant (CFA) (Sigma) by intradermal injection at the base of the tail (primary immunization). On day 21 after the first immunization the mice were immunized subcutaneously with 100 μg of collagen II in CFA (booster immunization) (Trentham, 1982). In some experiments mice received only primary immunization.

Mice were examined visually for the appearance of arthritis in the peripheral joints for 42 days after the primary immunization. Mice were considered to have arthritis when swelling and erythema, in at least 1 digit or paw, were observed on 2 consecutive observation dates. The severity of arthritis in each affected paw was graded according to an established scoring system, as follows: 0 = normal, 1 = visible inflammation of interphalangeal joints or mild footpad swelling 2 = pronounced foodpad swelling associated with erythema and edema, 3 = join deformity, 4 = ankylosis. The cumulative score of all 4 paws of each mouse was used as the "arthritis index" (maximum score of 16 per mouse) to represent overall disease severity and progression in an animal (Trentham et al., 1977).

TauCl treatment

TauCl treatment was performed as follows: the mice received daily subcutanous injections of 0.5 ml of 2 mM TauCl on 21 consecutive days. TauCl treatment was initiated either 1 day after primary immunization with collagen ("protocol **A**" – treatment from day 1 to 21), or 1 day after booster immunization ("protocol **B**" – treatment from day 22 to 42). Animals not treated with TauCl received D'PBS as a placebo.

Measurement of serum anti-collagen antibody titers

Mice were anesthetized and bled by a retroorbital puncture on days 21 and 42. Serum level of antibody against type II collagen was measured using a standard ELISA assay.

Briefly, individual serum samples were stored at −80°C until they were used for the ELISA. Microtitter plates (Corning, NY) were coated overnight with 5μg/ml of collagen type II (acid soluble) (Sigma, Steinham, Germany) in phosphate buffered saline (PBS) (Sigma) at 4°C. Non specific binding was blocked with 4% bovine serum albumin (BSA) in PBS at room temperature for 1 hour. Diluted serum samples (in 1% BSA in PBS) were added and incubated for 1 hour at room temperature. The plates were then incubated with biotinylated goat anti-mouse IgG antibody (Sigma) for 45 minutes at room temperature. Horseradish peroxidase (HRP) conjugated streptavidin diluted 1 : 1000 in 1% BSA/PBS was added and plates were incubated for 45 minutes at room temperature. Then OPD (o-phenylenediamine dihydrochloride) (Sigma) was used as a substrate (5 mg of. OPD in 10 ml of phosphate-citrate buffer, pH = 5,0) and incubated with 40 μl of 30% H_2O_2 for 30 min at room temperature. The reaction was stopped with 3 M H_2SO_4. Optical density was measured at 492 nm.

Measurement of myeloperoxidase activity

On day 42 of experiment MPO activity was measured in periarticular tissue as described by Bradley et al. (1982). Briefly, periarticular tissue was homogenized in ice-cold 0,5% hexadecyltrimethylammonium (HTAB) (Sigma) in 50 mM potassium phosphate buffer, pH 6,0. The tissue was freeze-thawed three times and dispersed by vortexing. Suspensions were centrifuged at 4000× g for 15 min at 4°C. Aliquots (0,1 ml) of the supernatant were mixed with 2,9 ml of 50 mM phosphate buffer, pH 6,0 containing 0,167 mg/ml o-dianisidine dihydrochloride (Sigma) and 0,0005% hydrogen peroxide. 200 μl of mixture were placed in 96-well flat bottom plate and incubated for 20 min in a room temperature. The absorbency was measured at 460 nm using a Sumal-PE2 spectrophotometer. The activity of MPO was calculated from a MPO (Calbiochem, San Diego, CA) standard curve and expressed in units. One unit of MPO activity was defined as that degrading 1 μmol of hydrogen peroxide per minute at a room temperature. Each sample was measured in duplicate.

Measurement of pro-inflammatory mediators production by peritoneal macrophages

On day 42 of experiment mice were sacrificed and residual peritoneal macrophages were collected by washing out the peritoneal cavity with 5 ml of phosphate buffer solution (PBS) containing 5 U heparin/ml (Polfa, Warsaw, Poland). Cells were centrifuged and red blood cells were removed by osmotic shock using distilled water. The collected cells were at least 90–95% macrophages as judged by

microscopic examination of cytospin preparations stained with May Grunwald/Giemsa (Merck, UK) and by chemical demonstration of non-specific esterase positive mononuclear cells, using α-naphtyl acetate (Sigma, St. Louis, MO).

Macrophages were cultured in 24-well flat bottom cell culture plates at 5×10^5/well in RMPI 1640 medium (JR Scientific, Woodland, CA) supplemented with 5% fetal calf serum (FCS) at 37°C in an atmosphere of 5% CO_2. Cells were activated with LPS 100 ng/ml (Escherichia coli 0111 B:4, Sigma) in the presence of IFN-γ 30 U/ml (Sigma). After 24 hours of culture supernatants were collected and frozen at −20°C for IL-6, TNF-α and NO_2 assays.

Nitrite (NO_2^-) determination

Nitric oxide quantified by the accumulation of nitrite as a stable end product was determined by a microplate assay (Ding et al., 1988). Briefly, 100 μl samples were removed from supernatants and incubated with an equal volume of Griess reagent (1% sulphanilamide/0.1%, N-1-naphtylenediamine dihydrochloride/2,5% H_3PO_4) at room temperature for 10 min. The absorbency at 550 nm was measured with a microplate reader. Nitrite concentration was calculated from a sodium nitrite standard curve.

IL-6 and TNF-α immunoassays

Interlekin-6 was measured using a capture ELISA. Microtiter plates (Corning NY) were coated with the rat monoclonal antibody against murine IL-6 (Pharmingen San Diego, CA), and the biotinylated rat monoclonal antibody against murine IL-6 (Pharmingen, San Diego CA) and horseradish peroxidase streptavidin (Vector Laboratories, Burlingame, CA) and o-phenylenediamine and H_2O_2 (both Sigma) as substrates (Starnes et al). The reaction was stopped with 3 M H_2SO_4 and plates were read at 492 nm in a 96-well plate reader. Recombinant mouse IL-6 (Pepro Tech, Rocky Hill NY) was used as a standard.

TNF-α was measured using a capture ELISA, with microtiter plates coated with the hamster monoclonal antibody against murine TNF-α (Genzyme) and rabbit polyclonal serum against murine TNF-α (Genzyme) and rabbit polyclonal serum against murine TNF-α (Genzyme), peroxidase-conjugated anti-rabit IgG (Sigma) and with o-phenylenediamine and H_2O_2 (both Sigma) as substrates. The reaction was stopped with 3 M H_2SO_4 and plates were read at 492 nm in 96-well plate reader. Recombinant murine TNF-α (Genzyme) was used as a standard.

Statistical analysis

The non-parametric Mann-Whitney U test was applied to examine differences in amounts of cytokine production, antibody synthesis and in an onset of CIA. Differences in cumulative incidence of arthritis at various time points were analyzed by the χ^2 analysis.

Results are expressed as mean ± SD. Probability values less than 0.05 were considered to be statistically significant.

Results

Effect of TauCl treatment on CIA development

To determine the effect of TauCl *in vivo* administration on the development of CIA we used two protocols. The mice received daily subcutanous injection of TauCl on 21 consecutive days initiated either 1

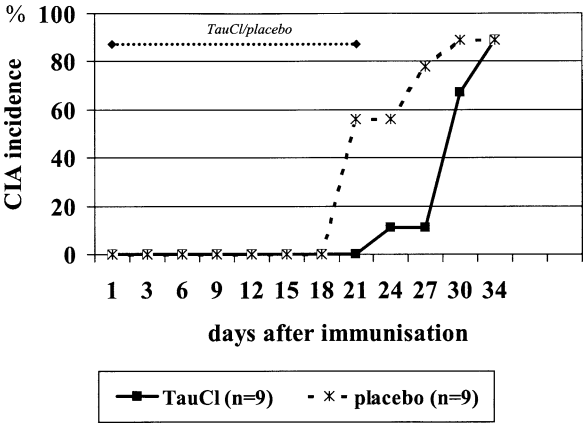

Fig. 1. Effect of TauCl early administration on the development of CIA in DBA1/J mice. Animals were immunized with a single dose of CII in CFA, as described in Methods. TauCl was injected daily for 21 days, starting 1 day after the immunization ("protocol A"). Control mice received D'PBS as placebo. CIA incidence (the percentages of arthritic animals) at the indicated times are given. Difference in the onset of CIA, between the experimental groups, was determined by Mann – Whitney U test ($p = 0.002$)

day after primary immunization with CII in CFA ("protocol A"), or 1 day after booster immunization ("protocol B"). The patterns of expression of CIA were reproducible. However, the mean day of onset of disease, in control (placebo) mice, varied from experiment to experiment (from day 21 to 29). In the majority of experiments, arthritis was fully expressed by the day 35 after primary immunization.

Figure 1 shows the effect of TauCl administration performed according to the "protocol A" (TauCl treatment from day 1 to 21). We observed statistically significant ($p = 0.002$) delay of the appearance of arthritic symptoms in mice receiving TauCl. Seven days after the last injection of TauCl (27 days after immunization) the incidence of arthritis was 10% in TauCl treated animals and 76% in control mice (Fig. 1). However, the symptoms of arthritis flared in TauCl treated mice upon cessation of TauCl injections. On the day 34 of experiment the incidence and severity of arthritis were similar in both groups of animals.

In the other group of experiments in which mice were treated with TauCl according to the "protocol B" (TauCl treatment from day 22 to 42), we observed statistically significant reduction of the incidence of arthritis ($p = 0{,}004$ in χ^2 test). In those experiments the incidence of CIA, evaluated on the day 42, in mice receiving TauCl or placebo was 52% and 89%, respectively (Fig. 2). In contrast to the effect, which was achieved using "protocol A", in these experiments

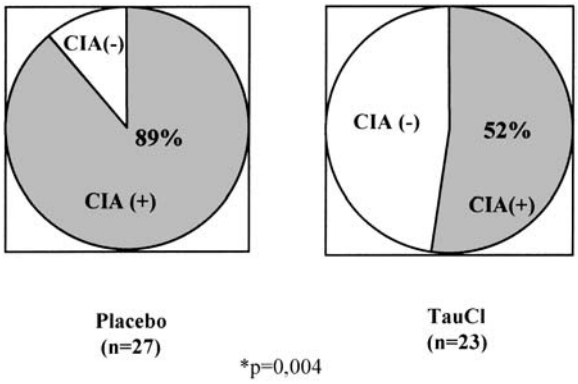

Fig. 2. Effect of TauCl late administration on the development of CIA in DBA1/J mice. Animals were immunized with CII in CFA twice (the primary immunization was followed after 21 days by the booster immunization), as described in Methods. TauCl was injected daily for 21 days, starting 1 day after the booster immunization ("protocol B"). Control mice received D'PBS as placebo. Results (*CIA* incidence) are expressed in the percentages of arthritic mice estimated at the last day of experiment (day 42), and represent five independent experiments

TauCl treatment had no effect on the onset of arthritis. There was no statistic difference in the time of appearance of arthritic symptoms in mice given TauCl and mice given DPBS (25 to 27 days after primary immunization, data not shown). Moreover, administration of TauCl did not ameliorate joint disease in those mice, which did develop CIA. Surprisingly, arthritic index was even statistic significantly higher in the group of mice given TauCl (mean arthritic index = 7,08 ± 3,96) when compared to the mice receiving D'PBS (4,35 ± 2,39) (p = 0,039).

Effect of TauCl treatment on humoral anti-collagen II response

In naive DBA1/J mice the titer of CII specific IgG antibodies was below 1 : 200. To determine the effect of TauCl treatment on CII specific antibody synthesis, mice were bled 21 days after primary and 21 days after booster immunization (day 42 of experiment). In control mice, bled on day 21, the serum titer of IgG anti-CII was at a range 1 : 25,600–1 : 51,200. The booster immunization increased the level of antibodies 4–8 times in animals with CIA. We observed only slight increase of antibodies synthesis measured 21 days after the booster immunization in those mice, which did not develop arthritis. The difference in final IgG titers, specific to collagen II, was statistically significant between CIA-positive and CIA-negative

Table 1. Humoral response to type II collagen*

Mice treatment**	CII antibody titer***		
	Day 21	Day 42	
		CIA (+)	CIA (−)
Placebo	0,227 ± 0,090	0,706 ± 0,195	0,253 ± 0,188
TauCl	0,268 ± 0,164	0,560 ± 0,008	0,312 ± 0,105

* Male DBA mice were immunized twice with collagen II in CFA as described in Materials and methods.
** TauCl was injected daily from day 21 till day 42 of experiment (n = 22). Control mice received DPBS as placebo (n = 19)
*** Mice were bled at day 21 and day 42 and IgG anti-CII levels were determined by ELISA and expressed as mean optical density at 1 : 25600 dilution

mice, whether they received TauCl (p = 0.013) or placebo (p = 0.025). TauCl treatment had no effect on humoral response. The results are shown in Table 1.

MPO activity in periarticular tissue

To determine the effect of TauCl treatment on neutrophil influx into arthritic joints we measured the activity of MPO, the neutrophil specific constitutive enzyme, in periarticular tissue of paws taken from collagen immunized animals. The MPO activity in tissue taken from naive animals was negligible. We found statistically significant difference in MPO activity between mice with and without arthritis in both groups of animals receiving either TauCl or DPBS (Fig. 3). There was no statistically significant difference in the MPO activity between mice given TauCl and placebo.

Effect of TauCl treatment on synthesis of proinflammatory mediators by peritoneal macrophages taken from CII immunized mice

To determine whether *in vivo* TauCl administration inhibits the production of pro-inflammatory mediators, we tested *in vitro* the ability of resident peritoneal macrophages, taken from experimental mice, for synthesis of nitric oxide (NO), IL-6 and TNF-α. There was no statistically significant difference in the production of all tested mediators by peritoneal macrophages taken from mice given TauCl or placebo (Fig. 4).

Interestingly, macrophages taken from those animals, which did develop CIA, produced more proinflammatory mediators than macrophages taken from CIA negative or naive mice. The results were repro-

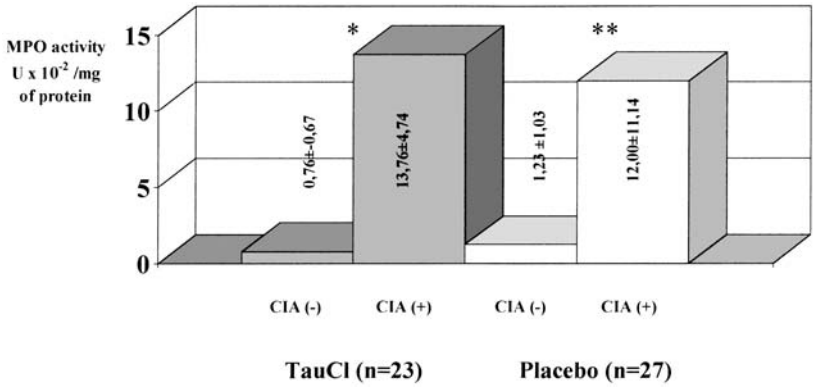

Fig. 3. Effect of TauCl treatment on myeloperoxidase activity in periarticular tissues. One day after the booster immunization mice were treated daily with TauCl (grey bars) or placebo (empty bars) for 21 consecutive days. On day 42 of experiment mice were sacrificed and MPO activity was measured in homogenized paws. The results are expressed as mean of MPO activity ± SD of samples from five independent experiments. * TauCl CIA (+) vs. CIA (−) p = 0.003; ** Placebo CIA (+) vs. CIA (−) p = 0.013

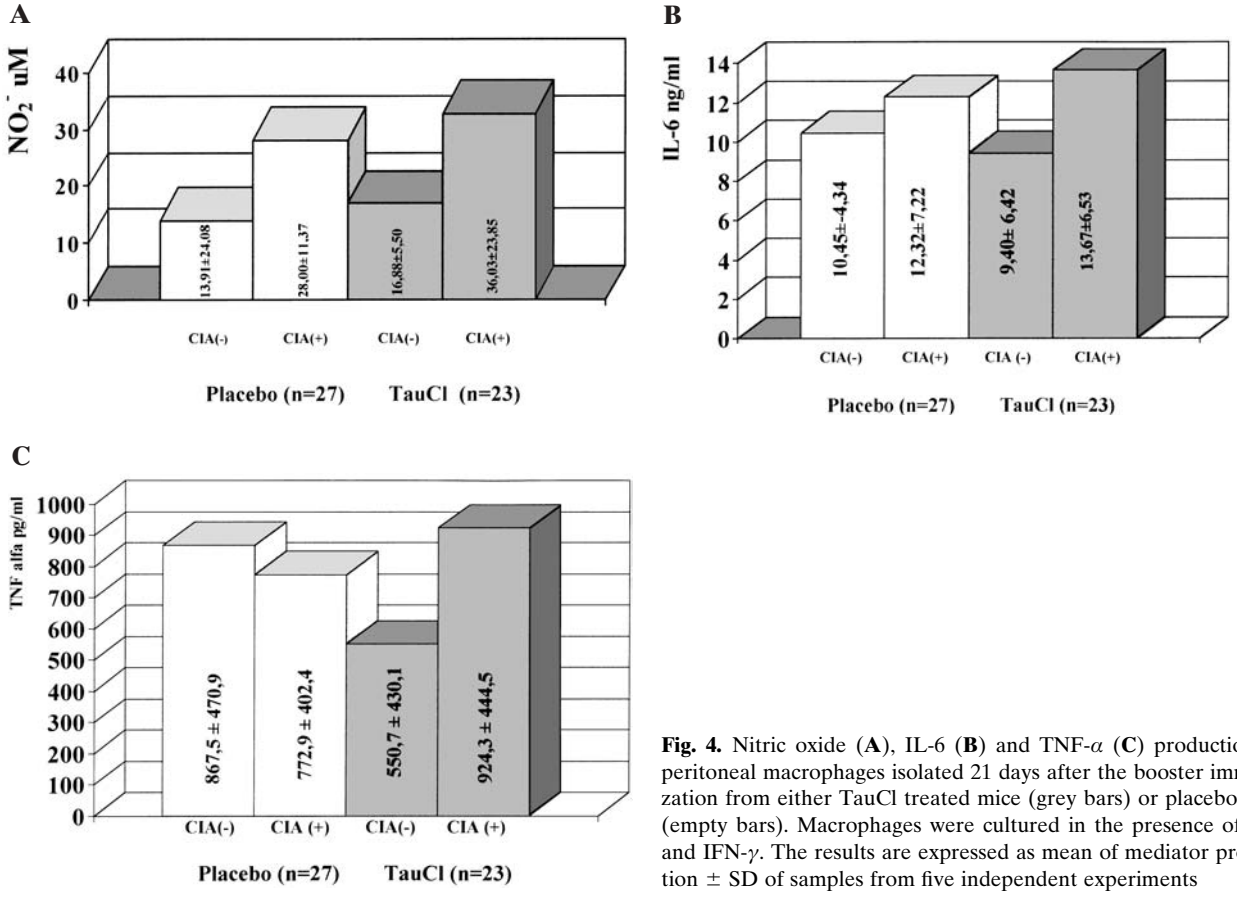

Fig. 4. Nitric oxide (**A**), IL-6 (**B**) and TNF-α (**C**) production by peritoneal macrophages isolated 21 days after the booster immunization from either TauCl treated mice (grey bars) or placebo mice (empty bars). Macrophages were cultured in the presence of LPS and IFN-γ. The results are expressed as mean of mediator production ± SD of samples from five independent experiments

ducible and consistent between experiments, however, the differences between groups were not statistically significant.

Discussion

Taurine chloramine (TauCl), a product of the neutrophil/monocyte myeloperoxidase – halide system, formed by a reaction of taurine with hypochlorous acid, is known to play an important role in the human defense system as an anti-microbial and anti-inflammatory long-lived oxidant (Marcinkiewicz, 1997; Nagl et al., 2000). TauCl shows significant bactericidal and fungicidal properties along with relatively low toxicity, as compared with other oxidants (Nagl et al., 1999; Marcinkiewicz et al., 2000). This optimal compromise between microbicidal activity and low toxicity suggests TauCl as a useful anti-inflammatory

and desinfectant agent for a special application in human medicine. A recent clinical study showed that TauCl could be considered as a therapeutic option in some infectious diseases (Nagl et al., 1998). Much less is known about the ability of exogenous TauCl to inhibit inflammatory response *in vivo*. On the other hand, there is evidence that taurine (Tau), either directly or indirectly (via TauCl), exerts anti-inflammatory effects *in vivo* (Cantin, 1994). For example, oral administration of Tau was reported to prevent or attenuate lung inflammation in animals (Gould et al., 1986).

The present study shows, for the first time, the effect of *in vivo* TauCl administration on the development of an inflammatory disease. To determine the effect of *in vivo* TauCl administration we have used murine type II collagen-induced arthritis (CIA), an animal model for rheumatoid arthritis (RA) (Trentham et al., 1977). The CIA model has been used to test most of the currently accepted therapies for RA (Hess et al., 1996). Many therapeutic approaches for immune intervention in RA have been proposed, including anticytokine strategies (directed towards TNF-α, IL-1β, IL-6), application of anti-inflammatory cytokines such as IL-4 and IL-10, or approaches that interfere with the functions of synoviocytes (Moreland et al., 1997; Firestein, 1998). The treatment of CIA with TauCl is based on the results coming from *in vitro* studies. These studies show that TauCl may modulate the immune reactions, which contribute to the pathogenesis of CIA and RA.

Firstly, it was shown that TauCl inhibits neutrophil functions, which are implicated in tissue damage and inflammation. TauCl down-regulates the production of TNF-α, PGE_2, NO and the generation of other oxygen free radicals (Kim et al., 1996; Marcinkiewicz et al., 1998). Secondly, TauCl competes with HOCl for target molecules neutralizing HOCl toxic effect. It is well documented that HOCl, the product of neutrophil MPO-halide system, is involved in pathogenesis of RA (Edwards and Hallett, 1997). Tissue inhibitors of metalloproteinases (TIMPs) prevent uncontrolled connective tissue destruction by limiting the activity of matrix metalloproteinases. HOCl, but not TauCl, can inactivate TIMPs at concentrations achieved at sites of inflammation (Shabani et al., 1998). HOCl also contributes to cartilage destruction mediated by neutrophil/macrophage enzyme, collagenase – a major putative candidate for collagen breakdown in synovitis. Collagenase has been shown to undergo conversion from the inactive procollagenase state to the fully active collagenase form when oxidized by HOCl. In contrast to HOCl, TauCl has been reported to be essentially unreactive with collagenase, and it has been suggested that taurine may act as a "sink" for the oxidizing potential of HOCl to minimize joint damage (Davies et al., 1993).

Finally, TauCl is able to modulate induction of a specific immune response at several independent points of the overall antigen-presenting pathway. We have shown previously that TauCl inhibits the release of IL-2, a Th1 type cytokine, from CD4+ T cells and modulates the functions of antigen-presenting cells (Marcinkiewicz et al., 1998, 1999). The development of CIA is known to be dependent on CD4+ T cell activation, however, the relative importance of Th1 and Th2 cells in CIA is not clear (Mauri et al., 1996).

The role of endogenous Tau/TauCl in pathogenesis of RA is still unknown, however, some observations have suggested a disturbance in the metabolism of Tau in this disease. These observations include: i. Neutrophils within RA synovial fluid are activated and secrete granule enzymes, including myeloperoxidase (Edwards et al., 1997); ii. Elevated levels of Tau have been detected in plasma; iii. Hypertaurinuria has been noted in RA patients (Trang et al., 1985); iiii, TauCl inhibits, *in vitro*, production of pro-inflammatory cytokines, as well as proliferation of fibroblast-like synoviocytes isolated from rheumatoid arthritis patients (Kontny et al., 1999, 2000).

All these data suggest that application of TauCl may affect the development of arthritis. In a situation of hypertaurinuria in RA, application of exogenous taurine in treatment of CIA does not seem to be appropriate. Indeed, our preliminary experiments showed that Tau administration had no effect on disease outcome (data not shown). On the contrary, administration of TauCl had a strong effect on the development of CIA. TauCl treatment prior to disease onset, simultaneously with primary immunization, significantly delayed the appearance of arthritic symptoms. However, the symptoms flared up one week after the last injection of TauCl (day 21). On the last day of experiment (day 36), incidence and severity of arthritis was similar in mice treated with TauCl and placebo mice. These results suggest that the effect of TauCl was short-term and reversible. TauCl has been described as a long-lived oxidant, undergoing less than 5% decomposition/h at pH 7 and 37°C, towards inactive sulphoacetaldehyde (Cunningham et al., 1998), although this

process has been reported to be accelerated at lower pH, a factor that would be relevant during phagocytosis. A different effect was observed when mice were treated with TauCl during the time of manifestation of arthritis, but starting before the onset of disease. Surprisingly, TauCl reduced the incidence, but had no effect on severity of arthritis. TauCl, in those mice which did develop CIA, did not reduce clinical symptoms of arthritis, the humoral anti-collagen II immune response and the production of proinflammatory mediators by peritoneal macrophages. In our opinion, TauCl, injected subcutaneously, could not suppress the inflammation in arthritic joints because the local concentration of exogenous TauCl was to low. Moreover, TauCl is not able to inhibit the production of mediators by already activated cells. This is in agreement with our previous study showing that the anti-inflammatory effect of TauCl *in vitro*, was observed only when TauCl was added either before or simultaneously with neutrophil/macrophage activating agents (LPS, IFN-γ, bacteria) (Marcinkiewicz et al., 1995, 1998).

In conclusion, it is apparent that systemic TauCl administration *in vivo* could not alleviate the symptoms of arthritis, which already took its course, but surprisingly, it may prevent the development of CIA. The mechanism of this phenomenon is unclear, but this observation strongly indicates that TauCl may be used for immune intervention *in vivo*. Further investigation is necessary to elucidate this problem. At the moment, we can only speculate that TauCl may affect the induction of inflammatory response e.g. by modulating the balance between Th1 and Th2 cytokines. Finally, to improve the effectiveness of TauCl therapy, the future investigations should include local, intraarticular administration of TauCl and evaluation of potential combination therapies.

Acknowledgments

We express our gratitude to K. Szewczyk for skillful technical assistance. This work was supported by grants from the Committee of Scientific Research (Warsaw, Poland) Grant No. 4PO5B01018, and partially by Grant No.: PO 5A 10419.

References

Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cuteneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78: 206–209

Cantin AM (1994) Taurine modulation of hypochlorous acid-induced lung epithelial cell injury *in vitro*. J Clin Invest 93: 606–614

Cunningham C, Tipton KF, Dixon HB (1998) Conversion of taurine into N-chlorotaurine (taurine chloramine) and sulphoacetalaldehyde in response to oxidative stress. Biochem J 330: 939–945

Davies JM, Horowitz DA, Davies KJ (1993) Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Rad Biol Med 15: 637–643

Ding AH, Nathan CF, Stuehr DJ (1988) Release of reactive nitrogen intermediates from mouse peritoneal macrophages: comparison of activating cytokines and evidence for independent production. J Immunol 141: 2407–2412

Edwards SW, Hallet MB (1997) Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. Immunol Today 18: 320–324

Firestein GS (1997) Etiology and pathogenesis of rheumatoid arthritis. In: Kelley WN, Ruddy S, Harris ED, Sledge CB (eds) Textbook of rheumatology, 5th edn. Saunders, Philadelphia, pp 851–897

Firestein GS (1998) Novel therapeutic strategies involving animals, arthritis and apoptosis. Curr Opin Rheumatol 10: 236–241

Gould RE, Shaked AA, Solano DF (1996) Taurine protects hamsters' bronchiols from acute NO_2 – induced alteration. Am J Pathol 125: 585–600

Grisham MB, Jefferson MM, Melton DF, Thomas LE (1984) Chlorination of endogenous amins by isolated neutrophils: ammoniadependent bactericidal cytotoxic and cytolitic activities of chloramines. J Biol Chem 259: 10404–10413

Hess H, Gately M, Rude E, Schmitt E, Szeliga J, Germann T (1996) High doses of interleukin-12 inhibit the development of joint disease in DBA/1 mice immunized with type II collagen in complete Freund's adjuvant. Eur J Immunol 26: 187–191

Kim C, Park E, Quinn MR, Schuller-Levis G (1996) The production of superoxide anion and nitric oxide by cultured murine leukocytes and the accumulation of TNF-α in the conditoned media is inhibited by taurine chloramine. Immunopharmacology 34: 89–95

Klareskog L, Homdahl R, Larrson E, Wigzell H (1983) Role of T lymphocytes in collagen II induced arthritis in rats. Clin Exp Immunol 51: 117–125

Kontny E, Grabowska A, Kowalczewski J, Kurowska M, Janicka I, Marcinkiewicz J, Maśliński W (1999) Taurine chloramine inhibition of cell proliferation and cytokine prooduction by rheumatoid arthritis fibroblast-like synoviocytes. Arthritis Rheum 42: 2552–2560

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

Marcinkiewicz J (1997) Neutrophil chloramines: missing links between innate and aquired immunity. Immunol Today 18: 577–580

Marcinkiewicz J, Grabowska A, Bereta J, Stelmaszyńska 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, Grabowska A, Bereta J, Bryniarski K, Nowak B (1998) Taurine chloramine down-regulates the generation of murine neutrophil inflammatory mediators. Immunopharmacology 40: 27–38

Marcinkiewicz J, Nowak B, Grabowska A, Bobek M, Petrovska L, Chain B (1999) Regulation of murine dendritic cell functions in vitro by taurine chloramine, a major product of the neutrophil myeloperoxidase-halide system. Immunology 98: 371–378

Marcinkiewicz J, Chain B, Nowak B, Grabowska A, Bryniarski K, Baran J (2000) Antimicrobial and cytotoxic activity of hypochlo-

rous acid: interactions with taurine and nitrite. Inflamm Res 49: 280–289

Mauri C, Williams OR, Walmsley M, Feldmann M (1996) Relationship between Th1/Th2 cytokine patterns and the arthritogenic response in collagen-induced arthritis. Eur J Immunol 26: 1511–1518

Moreland LW, Heck LW Jr, Koopman WJ (1997) Biologic agents for treating rheumatoid arthritis: concept and progress. Arthritis Rheum 40: 397–409

Nagl M, Gottardi W (1998) Rapid killing of Mycobacterium terrae by N-chlorotaurine in the presence of ammonium is caused by the reaction product monochloramine. J Pharm Pharmacol 50: 1317–1320

Nagl M, Hengster P, Semenitz E, Gottardi W (1999) The post-antibiotic effect of N-chlorotaurine on *Staphylococcus aureus*. Application in the mouse peritonitis model. J Antimicrob Chemother 43: 805–809

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

Park E, Schuller-Levis G, Quinn MR (1995) Taurine chloramine inhibits production of nitric oxide and TNF-α in activated RAW 264.7 cells by mechanisms that involve transcriptional and translational events. J Immunol 154: 4778–4789

Shabani F, McNeil J, Tippett L (1998) The oxidative inactivation of tissue inhibitor of metalloproteinase-1 (TIMP-1) by hypochlorous acid (HOCl) is suppressed by anti-rheumatic drugs. Free Radic Res 28: 115–123

Starnes H, Pearce M, Tewari A, Yim J, Zou JC, Abrams J (1990) Anti-Il-6 monoclonal antibodies protec against lethal *Escherichia coli* infection and lethal tumor necrosing factor-alfa challenge in mice. J Immunol 145: 4185–4191

Thomas EL, Grisham MB, Jefferson MM (1986) Preparation and characterisation of chloramines. Methods Enzymol 132: 569–571

Trang LE, Furst P, Odeback AC, Lovgren O (1985) Plasma amino acids in rheumatoid arthritis. Scand J Rheumatol 14: 393–402

Trentham DE (1982) Collagen arthritis as a relevant model for rheumatoid arthritis. Arthritis Rheum 25: 911–916

Trentham DE, Townes AS, Kang AH (1977) Autoimmunity to type II collagen: an experimental model of arthritis. Exp Med 146: 857–868

Weiss SJ, Klein R, Slivka A, Wei M (1982) Chlorination of taurine by human neutrophils: evidence for hypochlorous acid generation. J Clin Invest 70: 598–603

Weissmann G (1982) Activation of neutrophils and the lesions of rheumatoid arthritis. J Lab Clin Med 100: 333

Zgliczyński MJ, Stelmaszyńska T, Domański J, Ostrowski W (1971) Chloramines as intermediates of oxidation reaction of amino acids by myeloperoxidase. Biochim Biophys Acta 253: 419–424

Authors' address: Janusz Marcinkiewicz, Ph.D., M.D., Department of Immunology, Jagiellonian University Medical College, 18 Czysta St., 31-121 Kraków, Poland, E-mail: mmmarcin@cyf-kr.edu.pl