

The effect of taurine and its metabolites on the pathogenic functions of rheumatoid arthritis fibroblast-like synoviocytes

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Abstract

Fibroblast-like synoviocytes (FLS) are thought to contribute to rheumatoid arthritis (RA) pathogenesis via several mechanisms, e.g. by secreting numerous pro-inflammatory cytokines or by COX-2-generated synthesis of prostaglandin E₂ (PGE₂), another pleiotropic mediator of inflammation. Moreover, synovial hyperplasia, the most characteristic feature of RA, is believed to be a consequence of intractable proliferation and partial resistance of FLS to apoptosis. We have previously reported taurine chloramine (Tau-Cl) to represent potent inhibitory compound counteracting in vitro these pathogenic function of RA FLS. Tau-Cl is a chlorinated derivative of a dominant free amino acid taurine (Tau), originates from activated neutrophils, and is further converted into sulphoacetaldehyde (SA). The effect of SA on RA FLS functions has not been estimated yet. Therefore, the aim of present study was to compare simultaneously the effect of Tau, Tau-Cl and SA on select cellular responses of FLS, using FLS isolated from the same RA patients. We have found that among tested compounds only Tau-Cl, but neither Tau nor SA, exerts inhibitory effect on RA FLS proliferation as well as on the synthesis of IL-6 and PGE₂ by these cells.

Key words: rheumatoid arthritis synoviocytes, taurine, taurine chloramine, sulphoacetaldehyde, inflammation

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by hyperplasia of synovial membrane, chronic synovitis and progressive destruction of joint cartilage and bone. Fibroblast-like synoviocytes (FLS), the cells of mesenchymal origin located in the joint intimal lining, are believed to contribute to all of these pathological processes resulting in the development of RA symptoms [1]. Therefore, the limitation of an excessive growth of these cells as well as the reduction of synthesis of pro-inflammatory mediators and tissue destructive factors originated from FLS are considered to be a promising therapeutic approach for the treatment of RA patients. We have recently reported that taurine chloramine (Tau-Cl) inhibits in vitro several

pathogenic functions of RA FLS [for review see 2], and found this compound to give some protection against development of collagen-induced arthritis in mice, although the latter in vivo effect was short-term and reversible [3]. Taurine chloramine [for review see 4 and 5] is formed in the reaction between hypochlorous acid (HOCl), that represent one of the major product of the respiratory burst, and a dominant free amino acid taurine (Tau). Activated neutrophils are the major source of Tau-Cl. This physiologic compound has been reported to possess oxidative, bactericidal and immunomodulatory properties. Although Tau-Cl is rather stable oxidant, it is reduced to Tau and Cl⁻ while oxidizing its targets [6] and it was also shown to be slowly decomposed to sulphoacetaldehyde (SA) [7]. Thus,

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Tau, Tau-Cl and SA may co-exist at a site of inflammation. It has been well documented that Tau, in contrast to Tau-Cl, despite its cytoprotective and antioxidant properties does not affect the functions of RA FLS tested *in vitro* [2, 9-11]. On the other hand, it is completely obscure whether SA influences any functions of cells implicated in the inflammatory response. Therefore, in the present study we have focused on the comparison of the effects of Tau, Tau-Cl and SA on RA FLS proliferation and synthesis of pro-inflammatory agents (IL-6, PGE₂) by these cells.

Materials and methods

Synovial samples and synoviocyte cultures

Synovial tissues were obtained from knee joints at the time of total joint surgery or synovectomy, performed as a normal part of clinical care, from 27 female patients who fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for the diagnosis of RA [12]. All patients had stage III or IV disease [13]. The mean \pm SEM age of the patients was 55.2 ± 2.8 years, and the mean \pm SEM disease duration was 11.16 ± 2.7 years. FLS were isolated and cultured *in vitro* as described previously [9]. Cells were treated with medium alone (control) or were stimulated as described below. Either taurine (Tau) (Sigma, St. Louis, MO) or taurine chloramine (Tau-Cl) or sulphoacetaldehyde (SA) were added at physiologically relevant (50-500 μ M) concentrations together with the stimuli. Tau-Cl was prepared by chlorination of taurine [9]. Sulphoacetaldehyde was synthesized according to the method described previously [14]. To make the results more credible, the effect of all tested compounds on cell responses was estimated using FLS isolated from the same patients. The viability of cells was controlled by measurement of lactate dehydrogenase activity, using a lactate dehydrogenase assay kit (Takara Shuzo, Otsu, Japan).

Cell proliferation assay

Proliferation of the cells ($5 \times 10^3/0.2$ ml/well) was determined by an assessment of DNA synthesis in FLS, based on the incorporation of tritiated thymidine, according to the method described previously [9]. Recombinant human basic fibroblast growth factor (bFGF) or recombinant human tumor necrosis factor- α (TNF- α) (both from R&D Systems, Abingdon, UK) were used as the stimuli at 1 ng/ml or 10 ng/ml concentrations, respectively.

Measurement of interleukin-6 (IL-6) production

To trigger IL-6 production FLS (4×10^4 /ml/well) were stimulated with 1 ng/ml of recombinant human interleukin-1 β (R&D Systems). The concentration of IL-6 was determined in both culture supernatants (secreted form) and

in cell lysates (cell-associated form) collected 24 hours after stimulation. The IL-6 specific enzyme-linked immunosorbent assay (ELISA) was applied as described in detail before [9, 15]. Briefly, goat polyclonal neutralizing antibody specific for human IL-6 (R&D Systems) was used to capture IL-6 from samples, while IL-6-specific rabbit polyclonal antibody (Sigma) was applied to detect the cytokine, followed by horseradish peroxidase-conjugated goat anti-rabbit immunoglobulins and 0-phenylenediamine dihydrochloride (OPD) (both from Sigma) as a substrate. Optical density was measured at 492 nm using an automatic ELISA reader (LP-400; Diagnostic Pasteur, Marnes-La-Coquette, France). The detection limit for IL-6 was 39 pg/ml.

Measurement of prostaglandin E₂ (PGE₂) synthesis

The concentration of PGE₂ was determined in culture supernatants collected 24 hours after stimulation of the cells (4×10^4 /ml/well) with 1 ng/ml rhIL-1 β . The competitive acetylcholinesterase enzyme immunoassay (PGE₂ EIA kit; Cayman Chemical, Ann Arbor, MI) was applied according to the manufacturer's protocol.

Statistical analysis

Repeated-measures analysis of variance, followed by Tukey's test, was applied to evaluate the effects of the stimuli and tested compounds. Results are expressed as the mean \pm SEM. *P* values less than 0.05 were considered significant.

Results

Spontaneous proliferation of the cells cultured in medium alone (4081 ± 758 cpm) was significantly raised in the presence of bFGF (6906 ± 1196 cpm; $P=0.007$) or TNF- α (6337 ± 1266 ; $P=0.035$). Tau-Cl inhibited proliferation of TNF- α stimulated cells in a dose dependent manner, while Tau did not affect the cell response (Fig. 1.). These results correspond to our previous observation showing inhibitory effect of Tau-Cl, but not Tau, on both spontaneous and bFGF-triggered RA FLS proliferation [9]. Similarly to Tau, SA had no significant effect on either spontaneous or bFGF- or TNF- α -triggered cell proliferation (Fig. 1.). Under these experimental conditions, neither Tau (≤ 500 μ M) nor SA (≤ 500 μ M) nor Tau-Cl (≤ 400 μ M) was cytotoxic. However, at a concentration of 500 μ M Tau-Cl the cells were progressively damaged (mean \pm SEM cytotoxicity was 8.7 ± 3.6 % and 41.8 ± 6.5 % after 24 and 72 hours of treatment, respectively). Similar results were obtained in cell cultures treated with tested compounds alone or together with stimuli.

FLS produced IL-6 spontaneously (934.3 ± 213 pg/ml of secreted and 144.5 ± 31.2 pg/ml of cell-associated forms, respectively). In the presence of IL-1 β production of IL-6 was significantly ($P=0.001 - 0.0001$) elevated ($8222.3 \pm$

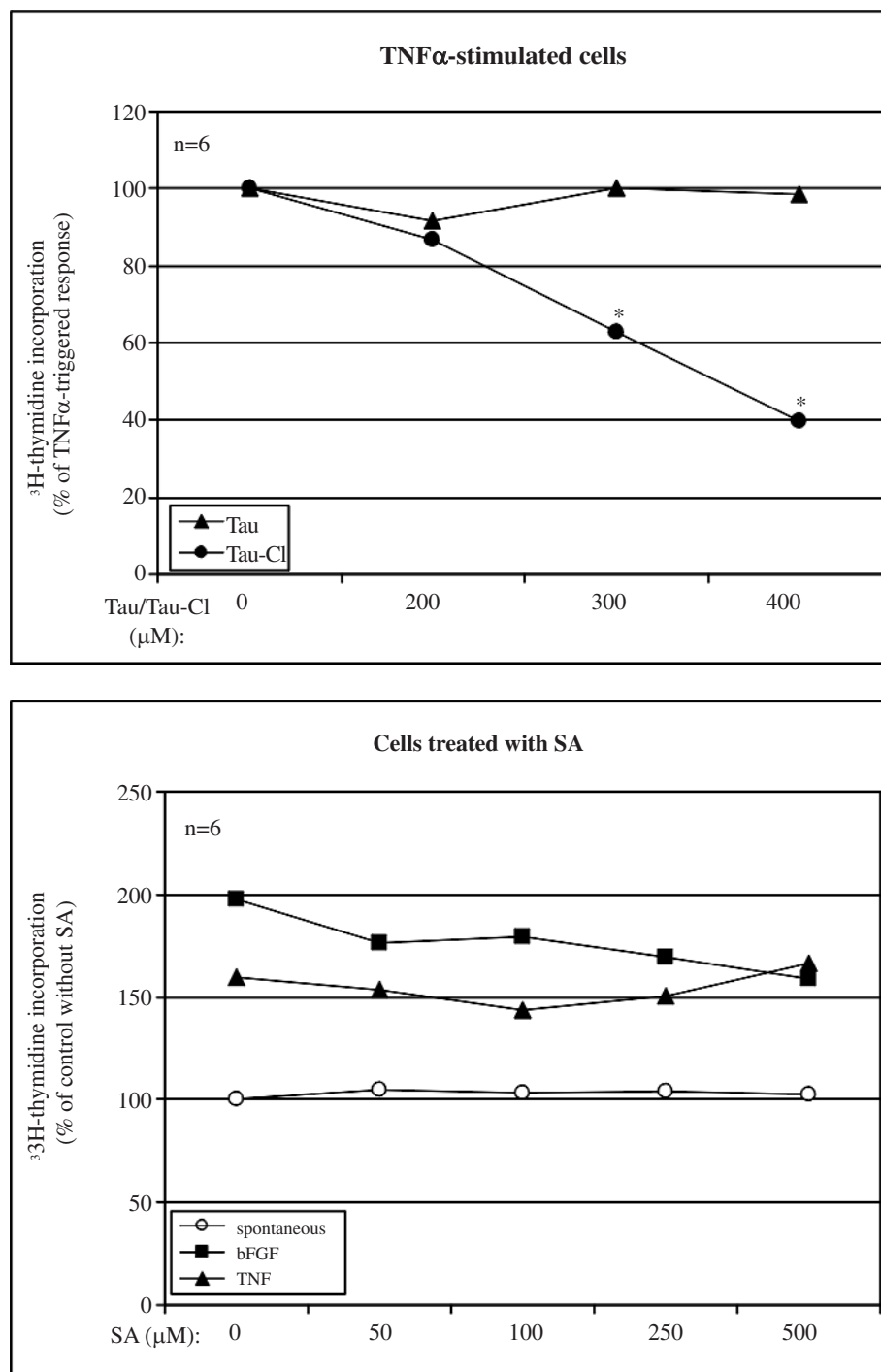


Fig. 1. Effect of taurine (Tau), taurine chloramine (Tau-Cl) and sulphoacetaldehyde (SA) on the proliferation of fibroblast-like synoviocytes. Cells were stimulated for 72 hours with TNF- α (10 ng/ml), bFGF (1 ng/ml) or cultured without stimuli (spontaneous proliferation). Tau, Tau-Cl and SA were added at indicated concentrations together with stimuli and for entire period of cell culture. Incorporation of ^3H -thymidine was measured as described in Materials and methods. Results are expressed as a percentage of the responses noted in cell cultures without Tau, Tau-Cl and SA. Values are the mean and SEM of 6 experiments in which FLS from 6 RA patients were used. * = $P=0.05-0.01$ for TNF- α -stimulated versus TNF- α + Tau-Cl-treated cell cultures. The other differences were statistically insignificant

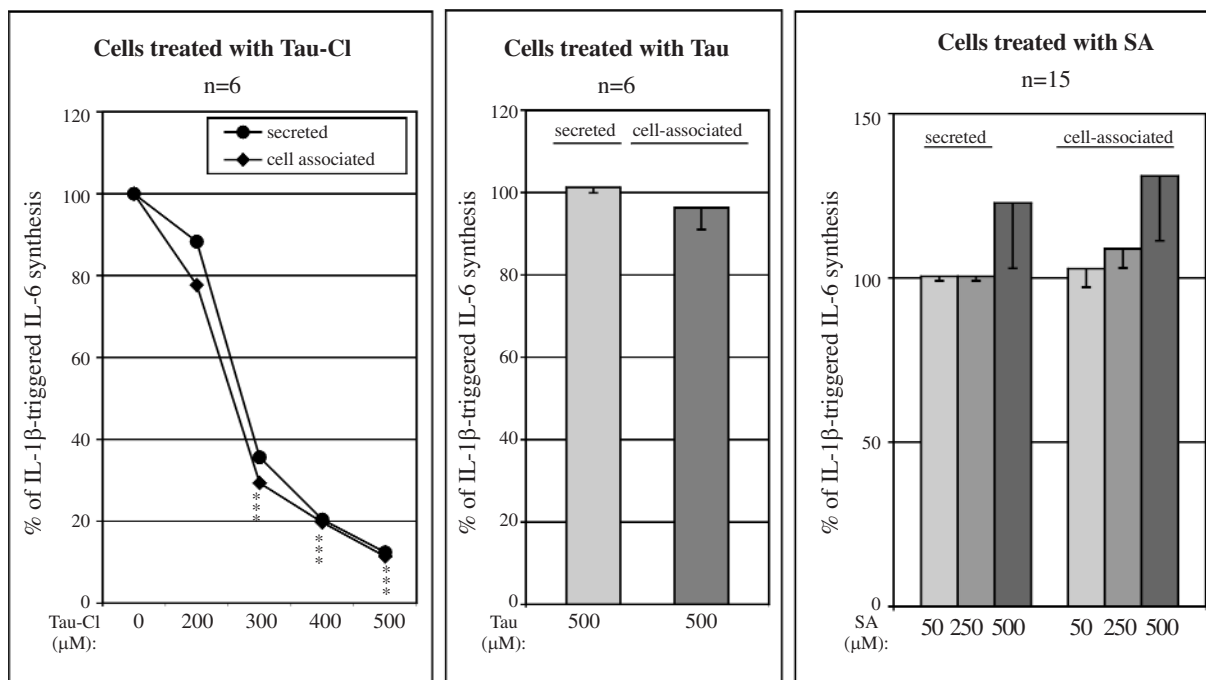


Fig. 2. Effect of taurine (Tau), taurine chloramine (Tau-Cl) and sulphoacetaldehyde (SA) on IL-6 synthesis. Fibroblast-like synoviocytes (FLS) were cultured for 24 hours in the presence of either IL-1 β (1 ng/ml) alone or together with indicated concentrations of Tau, Tau-Cl or SA. Concentration of IL-6 was measured in culture supernatants (secreted form) and cell-lysates (cell-associated form) by ELISA (see Materials and methods for details). Results are expressed as a percentage of IL-1 β -triggered responses and represent IL-6 production by FLS isolated from the synovial tissues of 6 RA patients (cells treated with Tau or Tau-Cl) and 15 RA patients (cells treated with SA). Values are the mean \pm SEM. *** = $P = 0.001-0.0001$ for IL-1 β -stimulated versus IL-1 β + Tau-Cl-treated cell cultures. The other differences were statistically insignificant

992 pg/ml of secreted form and 1638 ± 351 pg/ml of cell-associated form; $n=15$). Consistently with previous results [9,10] Tau-Cl significantly and in a dose-dependent way inhibited IL-1 β -triggered production of both secreted and cell-associated forms of IL-6. By contrast, neither Tau nor SA affected IL-6 production (Fig. 2). The effect of all tested compounds on spontaneous IL-6 production was similar to that stated in IL-1 β -stimulated cells (not shown).

Untreated control cells secreted little amount of PGE₂ (95 ± 40 pg/ml), while in IL-1 β stimulated cell cultures PGE₂ concentration (2081 ± 827 pg/ml) was significantly ($P=0.036$) higher. Similarly to previously published results (5) Tau had no effect, while TauCl inhibited IL-1 β -triggered PGE₂ synthesis (Fig. 3.) in a dose-dependent manner. Interestingly, SA failed to influence this cell response (Fig. 3.).

Discussion

We have confirmed our previous observations [2, 9-11] that Tau-Cl inhibits in vitro several pathogenic functions of RA FLS: (i) cell proliferation, (ii) synthesis of IL-6, and (iii) generation of PGE₂. These inhibitory effects of Tau-Cl were

statistically significant at non-cytotoxic (300-400 μ M) concentrations of this compound. Present results clearly show that neither Tau nor SA possess such inhibitory properties. The inability of SA to influence RA FLS functions is our new original finding.

Consistently with the opinion of others [16] we propose that during inflammatory response generation of Tau-Cl may represent negative regulatory loop to damp inflammation. Consequently, low Tau-Cl concentration at the inflammatory site may favour chronicity of the response. Interestingly, our recent data suggest impaired ability of RA synovial fluid neutrophils to generate Tau-Cl [17]. Thus, it is likely that in RA Tau-Cl-mediated downregulation of inflammatory response is not effective enough to prevent transition from the acute to the chronic phase. Compensation of this defect seems to be another therapeutic approach to treat RA patients. In this connexion our finding that SA neither damage nor affect activity of RA FLS is informative.

The mechanism of anti-inflammatory action of Tau-Cl is not fully understood. This compound was reported to affect either transcription or translation of genes encoding crucial mediators of the inflammatory response [for review

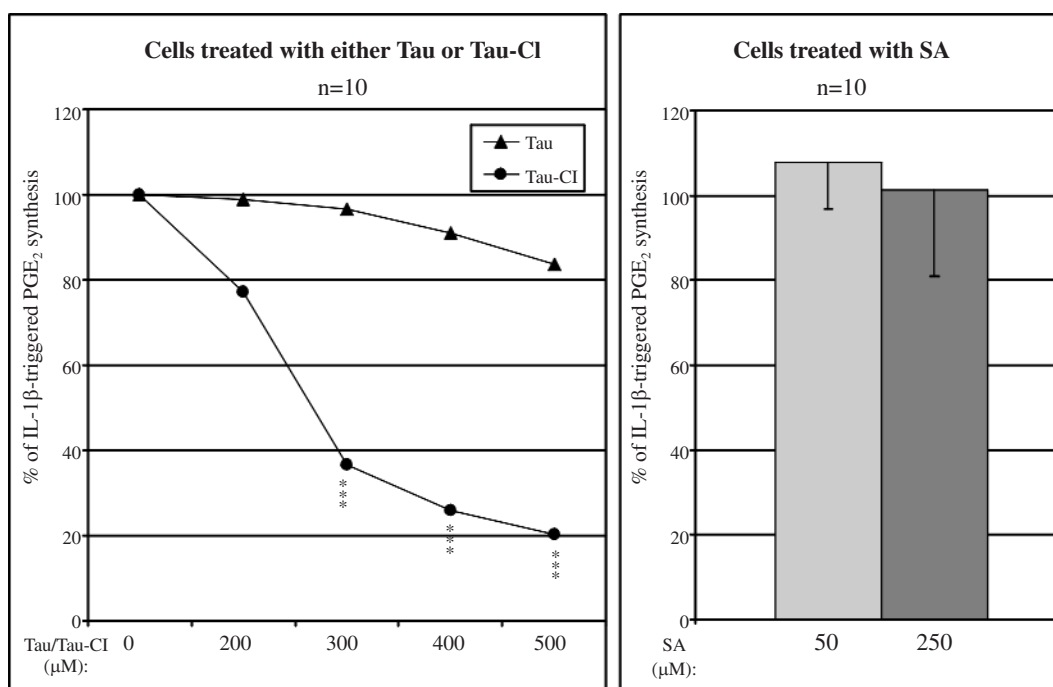


Fig. 3. Effect of taurine (Tau), taurine chloramine (Tau-Cl) and sulphoacetaldehyde (SA) on interleukin-1 β (IL-1 β)-triggered prostaglandin E₂ (PGE₂) synthesis. Cells were treated as in Fig. 2. Concentration of PGE₂ was determined in culture supernatants by competitive acetylcholinesterase enzyme immunoassay as described in Materials and methods. Results are expressed as a percentage of the responses noted in the IL-1 β -stimulated cell cultures that were treated with neither Tau nor Tau-Cl nor SA. Values are the mean \pm SEM of 10 experiments in which FLS from 10 RA patients were used. *** P = 0.001-0.0001 for IL-1 β -stimulated versus IL-1 β + Tau-Cl-treated cell cultures. The other differences were statistically insignificant

see 2 and 5]. Importantly, Tau-Cl diminishes DNA-binding activity of AP-1 and NF κ B transcription factors [10, 16, 18], known to be the key regulators of a broad range of genes implicated in the inflammatory response. It seems that oxidative properties of Tau-Cl may account for this, because it has recently been revealed that oxidation but not chlorination of I κ B α inhibitor by Tau-Cl stabilizes the inhibitor and results in NF κ B inactivation [19].

Because of the myriad of Tau actions, one can not exclude possibility that in vivo also this amino acid may importantly regulate the inflammatory response. Taurine was shown to protect cells against oxidative injury not only by scavenging highly toxic HOCl and formation of anti-inflammatory Tau-Cl, but also by e.g. limiting the availability of biological membrane lipids for lipid peroxidation or by acting as an important organic osmolyte and regulator of cellular Ca⁺² homeostasis, and thus preventing cells from necrotic or apoptotic death [for review see 8]. Despite this, we have failed to observe Tau protection against RA FLS apoptosis triggered in vitro by anti-Fas antibody (data not shown). It is well documented that in vivo Tau treatment protects against tissue injury in a variety

of animal models that share inflammation as a common pathogenic feature. However, this beneficial effect of Tau seems to be mediated mostly via generation of anti-inflammatory Tau-Cl [20]. Recent data have shown that also in animal model of allergic asthma Tau treatment produces anti-hyperreactivity and anti-inflammatory effects [21]. Although the precise mechanism of this protection needs further explanation, authors propose that it seems to be mediated via Tau-Cl, at least partially. Interesting new findings reveal Tau to be an important regulator of bone metabolism, both in vivo and in vitro [for review see 22]. Due to its ability to stimulate bone formation (e.g. via activation of osteoblasts) and inhibition of bone loss (e.g. via inhibition of osteoclasts formation) Tau is proposed to be introduced as an alternative to bisphosphonate therapy in osteoporosis and to prevent inflammatory bone resorption in periodontal disease.

Based on these data it is rational to consider in the future treatment of inflammatory diseases either by application of Tau (if the ability of neutrophils to generate Tau-Cl is retained) or Tau-Cl (in patients with a disturbed neutrophil

myeloperoxidase-H₂O₂-halide system). Present results give further support to this proposal.

Acknowledgements

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