Formation of leukotriene C_4 by human leukocytes exposed to monosodium urate crystals

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Monosodium urate (MSU) crystals stimulate the metabolism of arachidonic acid in mixed populations of human leukocytes. Leukocytes exposed to MSU crystals released leukotriene C_4. Leukotriene C_4 (LTC_4) was characterized and detected by high-performance liquid chromatography (HPLC), UV absorption, bioassay with guinea pig ileum, and radioimmunoassay. Results indicate that MSU crystals stimulate the transformation of arachidonic acid and the formation of leukotriene C_4 in human leukocytes; an effect inhibited by colchicine. Moreover, they suggest that LTC_4 may serve as a mediator of inflammation in crystal-associated diseases.

Leukotriene C_4 *Monosodium urate crystal* *Radioimmunoassay* *Human leukocyte*

1. INTRODUCTION

In acute gouty arthritis crystalline monosodium urate (MSU) deposits in joint tissues. When injected into the joints of experimental animals, MSU crystals provoke an acute inflammatory response suggesting that MSU crystals play a major role in the pathophysiology of gouty arthritis [1,2]. Studies [1] indicate that polymorphonuclear leukocytes are required for crystal-induced arthritis and suggest that interactions between these cells and crystals are essential for initiating the inflammatory response. Yet results of other in vivo studies, utilizing continuous recording techniques suggest that mediators derived from platelets, macrophages and possibly synovial lining cells may initiate MSU-induced inflammation [2].

Although the cellular events responsible for crystal-induced inflammation are not completely clear, results of several studies indicate that uncoated MSU crystals activate various cell types in vitro. For example, human neutrophils exposed to MSU release lysosomal enzymes and generate active oxygen species [3] and human platelets exposed to MSU release their dense body constituents [4,5] before lysis of the cells. The nature of MSU crystal-induced activation and secretion in these cells is unclear. Recent studies indicate however, that crystal-induced secretion is not solely caused by the well-appreciated membranolytic effect of the crystal [7], but that interactions between crystals and membrane proteins may lead to activation of inflammatory cells before lysis [7].

Recently, we demonstrated that MSU crystals stimulate the production of arachidonic acid metabolites by human neutrophils and platelets in the absence of demonstrable release of the cytoplasmic enzyme (LDH) [8]. Briefly, neutrophils exposed to uncoated MSU crystals generate leukotriene B_4 (LTB_4), its non-enzymatic isomers, and the double dioxygenation product 5*S*,12*S*-DHETE from endogenous sources of arachidonate. In addition to these products both 5-HETE and the ω-oxidation products of LTB_4 and 5*S*,12*S*-DHETE were formed. Human platelets exposed to MSU released free arachi-

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donate and products of both the cyclooxygenase and lipoxygenase pathways including thromboxane and 12-HETE [8]. Authors in [9] found leukotriene B_4 to be elevated in gouty effusions but not in synovial fluids of patients with either osteoarthritis or rheumatoid arthritis. Since arachidonate metabolites may serve as mediators of inflammation (review [10]) these studies suggest that metabolites of arachidonate may serve as mediators in crystal-associated diseases. This report describes the identification of leukotriene C_4 in suspensions of human leukocytes exposed to crystals of MSU.

2. MATERIALS AND METHODS

Arachidonic acid was purchased from Nu-Check, MN, Dextran T-500 was obtained from Pharmacia (Uppsala) and Lymphoprep from Nyegaard (Oslo). Uric acid was from Merck (Darmstadt). Ionophore A23187 and colchicine were from Sigma (St. Louis, MO) each were dissolved in ethanol, stored at −20°C and diluted in buffer immediately before use. HPLC equipment was from Waters Associates (Milford, MA − pump 6000A, injector U6K) and from LDC, Laboratory Data Control (Riviera Beach, FL − UV-detector LDC-III). All solvents were of HPLC grade obtained from Rathburn Chemicals (Walkerburne). FPL 55712 was a gift from Fisons Pharmaceutical Division (Loughborough) and synthetic LTC_4 was generously supplied by Dr F. Fitzpatrick of the Upjohn Company (Kalamazoo, MI).

2.1. *Preparation of monosodium urate crystals*

Crystals of monosodium urate were prepared as in [11]. Fresh crystals (approx. 10–15 μm in length) were prepared before incubations with leukocytes and each preparation was examined with polarising microscopy for the presence of strongly negative birefringent crystals. The native crystals were not coated with proteins.

2.2. *Cell preparations and incubations*

Suspensions of human leukocytes (e.g., neutrophils, eosinophils, basophils) were prepared from the peripheral blood of healthy donors collected at the Karolinska Hospital. Leukocyte suspensions − 100×10^6 cells/ml of phosphate-buffered saline containing 0.6 mM $CaCl_2$ and 1.0 mM $MgCl_2$ (pH 7.45) − were prepared as in [12]. Leukocyte suspensions (50 ml) were warmed to 37°C in a water bath with slow continuous shaking (~24 rpm) for 5 min. Crystals of MSU were suspended in buffer and then added to the incubations at final concentrations of 1–4 mg/ml. Incubations were stopped after 15 min by centrifugation and addition of 80% ethanol to the supernatants.

2.3. *Isolation and purification*

The ethanol solutions from each incubation were filtered and evaporated to dryness directly following the incubations [13,14]. Samples were purified as in [12]. Briefly, ethanol fractions from incubations were subjected to alkaline hydrolysis at pH 12 and then immediately purified on Amberlite XAD-8 (Rohm and Haas, Philadelphia, PA) and silicic acid chromatography (cc7, Mallinckrodt). Samples were further purified on reverse phase HPLC using a Polygosil 0.60–10 C_{18} (10 × 100 mm) column and acetonitrile/H_2O, 2:70, buffered to apparent pH 5.7 with ammonium hydroxide as a mobile phase (flow rate 1 ml/min). UV detector was set as 280 nm.

2.4. *Analytical methods*

UV spectra were obtained on a Hewlett-Packard 8450A spectrophotometer. Bioassay was performed on isolated guinea pig ileum in a 7 ml cuvette with Tyrode's buffer containing mepyramine maleate (10^{-6} M) and atropine sulfate (10^{-6} M) as in [13]. Radioimmunoassay for LTC_4 was performed as in [14]. Here 1-ml suspensions of cells were incubated with crystals for 15 min at 37°C. Cells were treated with colchicine (10^{-5} M) for 1 h, 37°C, before addition of MSU. Control incubations without MSU were included in each experiment. Incubations were stopped by rapid freezing of the samples at −70°C. After thawing samples were centrifuged (1400 × g) and assayed without further purification.

3. RESULTS AND DISCUSSION

Human leukocytes exposed to crystals of MSU (but not urate in solution) produced slow-reacting substance as determined by the contractile response of isolated guinea pig ileum (fig.1). The response to synthetic LTC_4 on the same ileum is

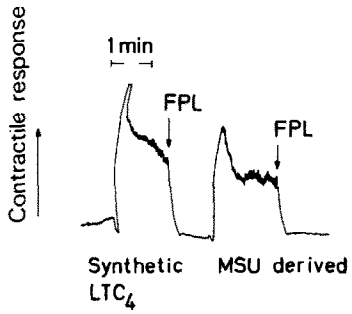

Fig.1. Guinea pig ileum bioassay. The contractile response of freshly prepared guinea pig ileum [13] was measured with synthetic LTC_4 and material derived from incubation of MSU crystals (2 mg/ml) with human leukocytes (100×10^6 cells/ml; 50 ml) 15 min, 37°C following extraction [12]. MSU crystals added directly to the ileum did not provoke contraction. The responses are representative of those obtained in three separate experiments.

shown for purposes of comparison. The contractions induced by both synthetic LTC_4 and the substance derived from incubation of human leukocytes with MSU crystals were rapidly reversed upon addition of FPL55712 (10^{-7} M) an antagonist of SRS [13]. When exogenous arachidonic acid was added to incubations containing MSU crystals SRS production was increased several fold (table 1).

The major component eluting in the HPLC system described in section 2.3 comigrated with synthetic LTC_4. The UV spectrum was essentially identical to that of synthetic LTC_4, with a maximum at 280 nm and shoulders at 270 and 292 (fig.2). The more polar materials eluting after the solvent front did not show UV spectra indicative of the presence of conjugated triene moieties. Unlike ionophore A23187, MSU crystals did not stimulate the formation of appreciable quantities of either 11-*trans*-LTC_4 [12] or LTD_4 [15].

In view of the clinical efficacy of colchicine in the treatment of gouty arthritis [16], we have examined the action of this drug on MSU-induced formation of LTC_4 using a sensitive radioimmunoassay. Leukocytes incubated with colchicine (10 μM, 37°C, 1 h) then exposed to MSU showed an impaired ability to form LTC_4 (table 1). A similar effect was noted when LTB_4 and its ω-oxidation products were examined [8]. The ratio of LTB_4 to LTC_4 formed by leukocytes exposed to MSU crystals was approximately 2:1. With respect

Table 1

Formation of LTC_4 by leukocytes exposed to MSU crystals: effect of colchicine

Incubation	LTC_4 (pmol/ml)
Cells (alone)	0.8
Cells + MSU crystals	5.1
Colchicine-treated cells + MSU	2.3
Cells + MSU crystals + exogenous arachidonic acid	14.8

Cells were exposed to MSU crystals (2 mg/ml, 15 min, 37°C) or pretreated with colchicine (10 μM, 37°C, 1 h) before exposure to crystals. Supernatants were assayed by RIA [14]. Results represent the mean of 2 separate experiments

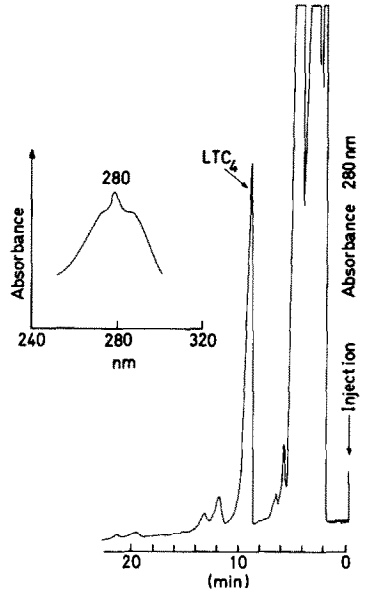

Fig.2. Reverse-phase HPLC chromatogram of products obtained following incubation of human leukocytes with MSU crystals (2 ng/ml), 15 min, 37°C. The incubation was stopped with ethanol and the lipids were purified by chromatography on Amberlite SAD-8 resin followed by CC-7 silicic acid, before HPLC. The polygosil C_{18}-column was eluted with acetonitrile/H_2O (30:70, v/v), pH 5.7, 1 ml/min. The inset shows the UV spectrum of the product eluting in this system.

to LTC_4 formation, MSU crystals proved to be approximately 2-log order less potent [14] than ionophore A23187. Nevertheless, when compared to other particulate stimuli encountered in pathology, such as opsonized microbes [17], MSU crystals appeared to be of equal potency with respect to LTC_4 formation. Since mixed leukocyte suspensions were used for these studies it is not possible to assign the principal cell type(s) responsible for the production of LTC_4 upon exposure to crystals.

These studies therefore indicate that MSU crystals stimulate the release of endogenous arachidonic acid and its subsequent transformation to LTC_4 in human leukocytes. Moreover, they suggest that LTC_4, a potent SRS [10] which constricts pulmonary airways and increases vascular permeability [18,19], may play a role in the pathophysiology associated with crystal-induced diseases.

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