INTRODUCTION

Systemic mastocytosis is a heterogeneous disorder characterized by the proliferation and accumulation of atypical mast cells in tissues, principally in the bone marrow and skin. Diagnosis of systemic mastocytosis can be difficult when typical cutaneous signs of mastocytosis are lacking, when patients present with primarily gastrointestinal symptoms or when mastocytosis imitates the disease course of other disorders (e.g. Crohn’s disease, hematologic disorders, gastrointestinalally mediated allergy) (1-3). In addition to histamine a array of mast cell mediators may be responsible for clinical symptoms in systemic mastocytosis (e.g. arachidonic acid metabolites, tryptase, chymase, platelet activating factor, TNF and several other cytokines (4, 5)). Mediators which are stored in the granula of the mast cells such as histamine, tryptase or heparin are rapidly released by degranulation of the mast cells activated by immunological and non-immunological stimuli and, hence, induce immediate symptoms of mast cell activation. On the other hand, arachidonic acid metabolites like leukotrienes, prostaglandines, platelet activating factor and thromboxanes are synthesized de-novo and are responsible for delayed developing symptoms of mast cell activation.

Currently, of more than 60 mast cell mediators only tryptase, histamine and its metabolites, heparin and prostaglandin D2 and its metabolites are used in the diagnostic and therapeutic monitoring of systemic mastocytosis (2-4). The question arises as to whether patients with systemic mastocytosis exhibit continuously increased urinary leukotriene excretion which could serve as an additional biomarker. In addition, the impact of the leukotrienes on clinical symptoms and disease activity has not yet been studied in systemic mastocytosis. Therefore, in the present study on patients with indolent systemic mastocytosis it was investigated whether a correlation between urinary excretion of leukotrienes and the intensity of the symptoms of systemic mastocytosis exists.

MATERIALS AND METHODS

The diagnosis of indolent systemic mastocytosis in the 9 patients of the present study (mean age 51.7±14.2; 6 male, 3 female) was established according to the World Health...
Organism criteria based on clinical, histological and immunological findings. The diagnosis of systemic mastocytosis requires the presence of multifocal dense mast cell infiltrates in one or multiple extra-cutaneous organs, mostly bone marrow (major criterion), plus at least one of the following minor criteria: a) abnormal morphology of extra-cutaneous mast cells (spindle-shaped cells); b) increased serum tryptase level (>20 ng/ml); c) abnormal expression of CD2 and/or CD25 on bone marrow mast cells; d) detection of a KIT mutation at codon 816 in extra-cutaneous organs. Diagnosis of systemic mastocytosis can also be made when at least 3 minor criteria are present (3-6). All patients presented with gastrointestinal and systemic symptoms. Histologically, 5 patients had bone marrow involvement, 3 gastrointestinal tract lesions with increased numbers of c-kit positive and CD25 positive mast cells and 1 patient had mast cell infiltrates in liver; cutaneous signs of mastocytosis were present in 5 of the 9 patients. Clinical symptoms of the patients included hypotension, recurrent anaphylactic reactions, abdominal pain, osteoporosis, hepatomegaly, diarrhea, flush, urticaria pigmentosa. Seven of 9 patients had no corticosteroid or immunosuppressiva treatment at the time of urine collection, while 2 patients were taking low dose prednisone (3 and 5mg/day). According to a standardized symptom score patients were divided into a group with high symptom intensity (5 patients, symptom score ≥10 points) and one with low symptom intensity (4 patients, symptom score <10 points (7)). The control group consisted of 11 healthy subjects with a mean age of 39±12 years (6 female, 5 male) who did not show signs of local or systemic allergy, food intolerance or skin lesions. All probands signed an informed consent document for detailed immunologic urine mediator analysis and the study was performed in accordance with the Helsinki declaration.

Leukotriene B4 and the cys-LTs were measured in 12-hours urine samples by an ELISA (IBL Immunobiological Laboratories, Hamburg, Germany) according to the manufacturers’ instructions (8). Cys-LTs are mostly excreted as LTE4 in human urine, but the cys-LTs ELISA used contained also specific antibodies to LTC4, LTD4 and LTE4 to detect metabolites of this leukotriene pathway (8, 9). Urinary methylhistamine (UMH) was measured by tandem mass spectrometry (Medical Laboratory Buchwald/Schulits, Weiden, German). The urine collection period was performed under an unrestricted diet until 2 p.m. at two subsequent days; the collection of urine samples from each day was then from 6 p.m. to 6 a.m. under overnight fasting (10, 11). As the UMH and the leukotriene values may be influenced by renal function, weight and body size, values were related to creatinine excretion and body size, values were related to creatinine excretion and body surface area (BSA) and ng/mmol creatinine x m² BSA, respectively (10-12). From each patient and controls at least two urine samples were collected and the mean values of the mediators analysed were used for statistical analysis. Analysis of data and statistical comparisons were made by Graph Pad Prism™, version 4.0 for Windows, and SPSS, version 11.5 for windows. Descriptive statistics is given by mean±SD and statistical comparisons were made by unpaired t-test. Correlations were performed by Pearson correlation.

RESULTS

Urinary excretion of cysteinyl-leukotrienes

The cumulative 12 hours urinary excretion of cys-LT LTC4, D4 and E4 in healthy controls amounted to 15.0±10.3 (ng/mmol creatinine x m² BSA). From the data obtained in the healthy volunteers we defined as upper edge of the normal range for cyst-LT excretion the mean plus 2 standard deviations, i.e. 35.6). Four of 9 mastocytosis patients (44.4 %) had cyst-LT levels above 35.6.

In patients with high symptom intensity urinary excretion of cys-LTs (51.8±36.2) was markedly higher than in the control group (p=0.003, Fig. 1). Even in patients with low symptom intensity urinary excretion of cys-LTs was significantly increased compared to the control group (38.9±39.8, p=0.037, Fig. 1). UMH values (µg/mmol creatinine x m² BSA) of patients with high and low symptom intensity were significantly elevated compared with the controls: 36.1±23.1 and 8.4±0.8, respectively, versus 4.6±1.9 (p<0.0001 and p=0.01, respectively). There was a positive correlation of the excretion of the cyst-LTs of the patients with the UMH values (r=0.503, p=0.084, Fig. 2).

Urinary excretion of leukotriene B4

The cumulative 12 hours urinary excretion of LTB4 in healthy controls amounted to 12.7±7. From the data obtained in the healthy volunteers we defined as upper edge of the normal range for LTB4 excretion the mean plus 2 standard deviations, i.e. 26.7. Four of 9 mastocytosis patients (44.4%) had LTB4 levels above 26.7.

![Fig. 1. Urinary excretion of the cysteinyl-leukotrienes (Cys-LT, mean±S.D.) in patients with indolent systemic mastocytosis with high (n=5) and low (n=4) symptom intensity (white and grey columns, respectively) and in healthy volunteers (n=11; black columns).](image1)

![Fig. 2. Correlation of urinary methylhistamine (UMH, µg/mmol creatinine x m² BSA) and urinary excretion of the cysteinyl-leukotrienes (Cys-LT, ng/mmol creatinine x m² BSA) in patients with indolent systemic mastocytosis (n=9).](image2)
Determine the individual disease- or mast cell activity, to monitor its course as well as to monitor the effect of therapy on mast cell activity in patients with systemic mastocytosis. In this view, the urinary excretion from overnight- or 24 hours urine has the advantage to present on the basis of stable metabolites the real mediator production of an individual patient including his disease state, extent of mast cell activation, neurovegetative regulation etc. as has been published for methylhistamine in food allergy and mastocytosis or 6-hydroxymelatonin in irritable bowel syndrome.

Thus, measurement of urinary cys-LTs excretion may have the potential to represent the individual activation of the eicosanoid metabolism in systemic mastocytosis, to explain certain symptoms pathophysiological and to express mastocytosis activity clinically, thus enabling a targeted mediator antagonistic therapy in future. Cyst-LTs can initiate, amplify, or dampen inflammatory responses and influence the magnitude, duration, and nature of subsequent immune responses.

REFERENCES


6. Keyzer JJ, de Monchi JG, van Doormaal JJ, van Voorst Vader PC. Improved diagnosis of mastocytosis by measurement of

DISCUSSION

When activated by specific antigen, complement, or other transmembrane stimuli, mast cells generate three eicosanoids from arachidonic acid: prostaglandin D2, leukotriene B4, and the cysteinyl leukotrienes LTC4, LTD4 and LTE4. In the present study we found that patients with systemic mastocytosis excreted significantly elevated amounts of leukotrienes, both of LTD4 and of the cys-LTs. This finding is in agreement with studies demonstrating an increased exocytosis of mediators from mast cells in systemic mastocytosis which is reflected by increased urinary excretion of methylhistamine and prostaglandin D2 and its metabolites.

In the present study, the amount of urinary excreted cys-LTs was in contrast to that of excreted LTD4 related to the intensity of the symptoms of systemic mastocytosis. Moreover, urinary excretion of cys-LTs paralleled UMH, an established biomarker of systemic mastocytosis. These findings indicate that the cyst-LTs represent the main products of the 5-lipoxygenase pathway in mast cells of mastocytosis patients and confirms an increased excretion of LTE4 in systemic mastocytosis patients (13). Although production of cyst-LTs is not specific for mast cells, since they can also be formed in other immune cells including neutrophils, eosinophils and macrophages, our findings in the present patients with systemic mastocytosis strongly suggest that elevated local and systemic levels of cys-LTs may contribute to clinical symptoms in systemic mastocytosis (2, 12-15).

Determination of leukotrienes is not part of the current WHO-criteria to define systemic mastocytosis (4). However, the measurement of stable leukotriene metabolites like urinary cys-LTs may serve as an additional, simple, non-invasive method to

![Fig. 3. Urinary excretion of leukotriene B4 (LT-B4, mean ±S.D.) in patients with indolent systemic mastocytosis with high (n=5) and low (n=4) symptom intensity white and grey columns, respectively and in healthy volunteers (n=11; black column).](attachment:image.png)


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