Increased generation of cysteinyl leukotrienes in Kawasaki disease

E Mayatepek, W D Lehmann

Abstract
Endogenous cysteinyl leukotriene synthesis was assessed in 10 patients with Kawasaki disease and 10 healthy controls by measuring excretion of leukotriene E_4 (LTE_4) in urine. LTE_4 was increased more than fivefold in patients with Kawasaki disease compared with controls (median (range) 55.5 (31.8-120.6) vs 10.2 (7.1-14.9) nmol/mol creatinine); this suggests that cysteinyl leukotrienes are involved in the pathophysiology of Kawasaki disease. Leukotriene synthetase inhibition or receptor antagonism may therefore offer a new potential therapeutic approach in children with this disease.

(Keywords: Kawasaki disease, cysteinyl leukotrienes, leukotriene E_4.

Kawasaki disease is an acute multisystem vasculitis of infancy and early childhood. About 25% of patients develop coronary vasculitis severe enough to cause aneurysm formation, stenosis, and thrombosis of coronary arteries. During the acute phase of the disease a number of immunoregulatory abnormalities have been demonstrated including pathologically endothelial cell damage.

It has been suggested that the arachidonic acid pathway is activated in Kawasaki disease. An enhanced in vitro biosynthesis of thromboxane A_2 by platelets and increased in vitro production of leukotriene (LT) B_4, a powerful chemoattractant, by stimulated polymorphonuclear cells has been reported in patients with Kawasaki disease.

The cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 are potent endogenous proinflammatory 5-lipoxygenase products also derived from arachidonic acid acting at nanomolar concentrations. Because they induce increased vascular permeability by endothelial cell interaction, affect microvascular tone, and are released during episodes of myocardial ischaemia, a role of these lipid mediators in the pathophysiology of Kawasaki disease seems possible.

Tracer experiments have demonstrated that urinary LTE_4 can be used as an index metabolite to assess cysteinyl leukotriene synthesis in vivo. In this study, we measured urinary LTE_4 excretion in 10 patients with Kawasaki disease and 10 healthy children to elucidate the potential role of cysteinyl leukotrienes in the pathogenesis of Kawasaki disease.

Patients and methods

Patients
We studied 10 patients (four girls) with Kawasaki disease hospitalised at the University Children's Hospital, Heidelberg, Germany; their mean age was 2.7 years (range 0.5-5.2 years). Each had at least five of the six diagnostic criteria for Kawasaki disease established by the Japanese Kawasaki Disease Research Committee in 1984, and other illnesses were excluded. Urine was collected before treatment during the acute phase (first 10 days) of the illness. Microscopy and culture of urine samples showed that no bacteria nor leucocyturia were present. The control group consisted of 10 age and sex matched children who had no sign of infection, haematological, connective tissue, lung, or cardiac disease.

Methods
Urine was obtained from spontaneous micturition and mixed with two volumes of 90% (vol/vol) aqueous methanol of pH 8.5 containing 0.5 mM EDTA, 1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, and 20 mM potassium hydrogen carbonate and stored at -80°C under argon until analysis. Urinary LTE_4 was measured essentially as described. Briefly, (3H)-labelled LTE_4 (Du Pont-New England Nuclear) was added as an internal standard. Samples were then acidified to pH 4.5 by addition of 0.1 hydrochloric acid homogenised, and pumped through activated Sep-Pak cartridges. Fractions having the same elution time as the synthetic LTE_4 were separated by reversed phase high performance liquid chromatography (HPLC) using a mixture of methanol/water (65:35, vol/vol) the aqueous part containing 0.1% acetic acid, 1 mM EDTA, and adjusted to pH 5.6 by ammonium hydroxide. The immunoreactive LTE_4 content was determined by enzyme immunoassay using a specific antibody (Cayman). Radioactivity was measured by scintillation counting, and each LTE_4 value was corrected for (3H)-LTE_4 recovery for that sample. Calculation of the standard curve regression and LTE_4 concentrations was carried out after a linear log-logit transformation.

The identity of urinary LTE_4 was demonstrated by gas chromatography-mass spectrometry (GC-MS), performed on a Finnigan MAT 95 system as described previously. Briefly, synthetic and isolated urinary LTE_4 were catalytically reduced and desulphurised to 5-hydroxyeicosanoic acid and derivatised to their pentafluorobenzyl ester trimethylsilyl ether derivatives.
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Results
IDENTIFICATION OF URINARY LTE4 BY GC-MS
The obtained mass fragments of urinary LTE4 was identical to that of synthetic LTE4 with the characteristic intensive mass fragments at m/z 399 (M−PFB)− and 309 (M−PFB−TMSOH)−. Identical mass spectra and retention times both on reversed phase HPLC and capillary column demonstrated unequivocally the presence of LTE4 in the urine of patients with Kawasaki disease and healthy controls.

EXCRETION OF LTE4 INTO URINE
In healthy children and patients with Kawasaki disease the excretion of LTE4 was log normally distributed. The patients with Kawasaki disease excreted more than fivefold higher amounts of LTE4 into urine than did the age and sex matched healthy controls (p<0:01). The median (range) was 55·3 (31·8–120·6) nmol/mol creatinine for the patients and 10·2 (7·1–14·9) nmol/mol creatinine for the controls (figure).

Discussion
In the present study, we have demonstrated a significantly increased excretion of LTE4 into urine in patients with Kawasaki disease compared with healthy children. The concentrations of urinary LTE4 in the healthy controls were similar to those reported recently.7 The identity of urinary LTE4 was demonstrated by GC-MS analysis.

Previous studies have shown an increased in vitro biosynthesis of thromboxane A2 and LTB4 by isolated blood cells suggesting an involvement of the arachidonic acid cascade in Kawasaki disease.3,4 However, in vitro data from isolated cells must be interpreted cautiously, as characteristically cells must be incubated with labelled arachidonic acid or appropriately stimulated to synthesise eicosanoids such as thromboxane or leukotrienes.

Urinary LTE4 excretion, however, is a reliable index metabolite to assess whole body synthesis of cysteinyl leukotrienes in vivo.5 The present findings therefore strongly suggest that cysteinyl leukotriene synthesis and generation is enhanced in Kawasaki disease.

It must be pointed out, however, that urinary LTE4 is not a specific marker for Kawasaki disease. In other diseases, such as asthma, cystic fibrosis, and juvenile rheumatoid arthritis, an enhanced urinary excretion of LTE4 has been demonstrated.5 This implies that LTE4 is not specific for a single disease but might provide a sensitive index of inflammation. In Kawasaki disease increased synthesis of cysteinyl leukotrienes might mediate certain symptoms associated with the disease. For example, it has been pointed out that cysteinyl leukotrienes are released during episodes of myocardial ischaemia providing evidence for their involvement during and after acute myocardial infarction and unstable angina attacks.6 Therefore and because of their potent vasoconstrictive capacity, cysteinyl leukotrienes might be involved in the origin of vasculitis and stenosis of coronary arteries in Kawasaki disease.

The definitive role of cysteinyl leukotrienes in Kawasaki disease has to be evaluated in further studies by the use of specific 5-lipoxygenase inhibitors or receptor antagonists. Measurement of urinary LTE4 provides a non-invasive and specific method useful to monitor the effect of these drugs on leukotriene synthesis in Kawasaki disease. Our results imply that leukotriene synthetase inhibition or receptor antagonism may offer a new potential therapeutic approach in children with Kawasaki disease.

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