Increased generation of cysteinyl leukotrienes in Kawasaki disease

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Abstract

Endogenous cysteinyl leukotriene synthesis was assessed in 10 patients with Kawasaki disease and 10 healthy controls by measuring excretion of leukotriene E₄ (LTE₄) in urine. LTE₄ was increased more than fivefold in patients with Kawasaki disease compared with controls (median (range) 55.3 (31.8-120.6) v 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.

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Kawasaki disease is an acute multisystem vasculitis of infancy and early childhood.¹ About 25% of patients develop coronary vasculitis severe enough to cause aneurysm formation, stenosis, and thrombosis of coronary arteries.² During the acute phase of the disease a number of immunoregulatory abnormalities have been demonstrated including pathologically endothelial cell damage.²

It has been suggested that the arachidonic acid pathway is activated in Kawasaki disease. An enhanced in vitro biosynthesis of thromboxane A₂ by platelets³ and increased in vitro production of leukotriene (LT) B₄, a powerful chemoattractant, by stimulated polymorphonuclear cells has been reported in patients with Kawasaki disease.⁴

The cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄ are potent endogenous proinflammatory 5-lipoxygenase products also derived from arachidonic acid acting at nanomolar concentrations.⁵ Because they induce increased vascular permeability by endothelial cell interaction, affect microvascular tone, and are released during episodes of myocardial ischaemia,⁶ a role of these lipid mediators in the pathophysiology of Kawasaki disease seems possible.

Tracer experiments have demonstrated that urinary LTE₄ can be used as an index metabolite to assess cysteinyl leukotriene synthesis in vivo.⁵ In this study, we measured urinary LTE₄ 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 bacteriuria 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 LTE₄ was measured essentially as described.⁷ Briefly, ³H-labelled LTE₄ (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 LTE₄ 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 LTE₄ content was determined by enzyme immunoassay using a specific antibody (Cayman). Radioactivity was measured by scintillation counting, and each LTE₄ value was corrected for (³H)LTE₄ recovery for that sample. Calculation of the standard curve regression and LTE₄ concentrations was carried out after a linear log-logit transformation.

The identity of urinary LTE₄ was demonstrated by gas chromatography-mass spectrometry (GC-MS), performed on a Finnigan MAT 95 system as described previously.⁷ Briefly, synthetic and isolated urinary LTE₄ were catalytically reduced and desulphurised to 5-hydroxyeicosanoic acid and derivatised to their pentafluorobenzyl ester trimethylsilyl ether derivatives.

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