ELEVATED PLASMA LEVEL OF LEUKOTRIENES IN BRONCHIAL ASTHMA PATIENTS: A POSSIBLE CLINICAL RELEVANCE

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SUMMARY

Plasma from bronchial asthma patients and healthy controls was investigated for the content of lipoxygenase products. After lipid extraction using SEP-PAK C18 Cartridges, the lipoxygenase products were measured by Enzyme-Immunoassay. Elevated chemotactic B4 was found in plasma from asthmatic patients with mean value (483±75) pmol/L, while the mean value in normal healthy donors was (140±12.1) pmol/L (M±SE). The levels of spasmodenic cysteinyl containing leukotrienes were also very high in the bronchial asthma patients. Elevations of leukotriene B4 and cysteinyl containing leukotrienes were detected during attacks of bronchial asthma. These results suggest that leukotriene B4 may be important in the pathogenesis of bronchial asthma and confirmed that peptidoleukotrienes play a role as chemical mediators during the asthmatic attack.

KEY WORDS   Leukotrienes C4 and B4 Bronchial asthma

INTRODUCTION

Stechschulte et al. (1973) reported the presence of a slow reacting substance (SRS-A) in the plasma of guinea pigs during anaphylaxis. Murphy et al. (1979) subsequently elucidated the structure of SRS-A and showed it to be composed of leukotrienes.

The first step in the biosynthesis of leukotrienes is oxidation of arachidonic acid at carbon atom 5 (Samuelsson et al., 1987), the reaction is catalyzed by 5-lipoxygenase enzyme forming the unstable epoxide leukotriene A4. This highly reactive intermediate can either be hydrolysed by LTA4 hydrolase, forming LTB4, or enzymatically conjugated with glutathione, (reaction catalyzed by LTC4 synthase) leading to LTC4 formation. The latter compound is metabolized to leukotrienes D4 and E4 by successive elimination of a glutamyl and glycine residue.

The cysteinyl-containing leukotrienes are potent bronchoconstrictors that increase vascular permeability in postcapillary venules and stimulate mucus secretion. Thus leukotrienes C4, D4 and E4, which are released from the lung tissue of asthmatic patients exposed to specific antigens, appear to play a pathophysiological role in immediate hypersensitivity reactions (Samuelsson, 1983).

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In contrast, LTB₄ causes adhesion and chemotactic movement of leukocytes, stimulating aggregation, enzyme release and superoxide anion generation in neutrophils. Less is known about whether LTB₄ is responsible for bronchial asthma and insufficient evidence is available to assess the true role of LTB₄ in this condition.

The aim of the present study was to add more information on the role of LTB₄ in asthmatic patients during wheezing attack, comparing the observed plasma levels with those in healthy controls.

MATERIALS AND METHODS

Subjects
34 Asthmatic patients who met the criteria for bronchial asthma and 10 healthy controls were studied. All the asthmatics were out-patients and when they came to the clinic because of a wheezing attack, arterial blood gas analysis was done to determine the severity of the attack. Blood samples were withdrawn both during the attack and between bronchial asthma attacks.

Extraction procedure
1 – 5 ml of blood was drawn in a heparinized tube (containing 20 ml of ethanol to stabilise cysteinyl-containing leukotrienes) from the asthmatic patients during and between wheezing attacks and from normal healthy individuals. All the samples were collected with the patients’ informed consent in accordance with approval of the project from the ethics committee of the Al-Azhar University. Immediately after collection, the blood samples were centrifuged (1500 xg for 15 min. at 4°C), then supernatant was stored at -80°C until assayed.
2 – After thawing to 4°C, the plasma was centrifuged at 15000 xg for 20 min. at 4°C.
3 – The supernatant was adjusted to pH 5.1 with 0.1 N HCl and passed through SEP-PAK C₁₈ cartridges (Water Associates, Millford, USA), pretreated with 20 ml of pure ethanol followed by 20 ml of distilled water. After the SEP-PAK was successively washed with 20 ml of ethanol-distilled water (1:9), the samples were eluted from the column with 10 ml of methanol.
The methanol fraction was then evaporated under nitrogen and the residue was dissolved in phosphate buffer saline.

Assay procedure
1 – All the reagents were brought to room temperature and mixed thoroughly without foaming before use.
2 – 100 μl of standard, buffer or sample was pipetted in duplicate into the antibody precoated wells.
3 – 100 μl of cysteinyl leukotrienes antibody was added to all wells, except the substrate blank and, in the case of LTB₄ determination, LTB₄ antibody was added also.
4 – The wells were covered with plate sealer and incubated overnight at 2–8°C.
5 – 100 μl of LTC₄ – or LTB₄ – Alkaline phosphatase conjugate was added to each well and the plate was incubated at 2–8°C for 3 hours.
6 – The sealer was removed and the liquid was aspirated from the wells which were washed three times with approximately 400 μl of wash buffer per well.
7 – 300 µl of Para-nitrophenyl phosphate substrate solution was added into all wells, and was incubated for 1 hour at 37°C.

8 – 50 µl stop solution were pipetted into all wells, including blank wells. The absorbance of the wells was read at 405 nm, versus substrate blank. The LTC₄ concentration as LTB₄ conc. were calculated as pmol/L from the horizontal axis.

**Statistics**

Student’s t-test was used for statistical analysis of differences between mean values of control and bronchial asthma patients, while t-test of paired sample was used for statistical analyses of differences between mean values during and between attacks for the same patient.

**RESULTS**

In bronchial asthma patients the plasma level of cysteinyl-containing leukotrienes ranged from 159 to 795 pmol/L, with a mean value 500±31 pmol/L (Mean ± SE) (n=31) while the level in healthy controls ranged from 75 to 150 pmol/L; mean value 112.1±8.27 (Mean ± SE) (n = 10) P < 0.0001 (Fig. 1).

The plasma level of spasmogenic cysteinyl leukotrienes during attack of bronchial asthma was significantly higher than the level between attacks (529±40 and 413±50.4) respectively (Mean ± SE) (n=11) P<0.002 (Fig. 2).

Immuno-reactive B₄ was detected in all plasma samples. Many of the asthmatic had raised levels ranging from 111 to 1488 pmol/L with mean (483±75) (n=29) while the amount for LTB₄ was lower in the plasma of healthy controls ranging from 59 to 208 pmol/L with mean (140±12.1) Mean ± SE (n=10) (Fig. 1).

The plasma from asthmatics during attacks had elevated LTB₄ compared to the same patients between attacks (432±62 and 325±109 pmol/L Mean ± SE respectively) (n=9) (Fig. 2).

**DISCUSSION**

In the present study we have demonstrated that spasmogenic cysteinyl leukotrienes and dihydroxy acid leukotriene B₄ can be measured in the blood of asthmatic patients by EIA technique after an extraction procedure.

Elevated levels of leukotactic B₄ in the plasma of bronchial asthma patients indicate the importance of LTB₄ as a mediator of bronchial asthma. A limited number of studies have previously addressed the question of whether leukotriene B₄ has a role in bronchial asthma. Uotila et al. (1986) and Shindo et al. (1990) demonstrated that the concentration of LTB₄ in arterial blood of asthmatic patients is higher than that of healthy controls (250 pmol/L in asthmatic patients; 28 pmol/L in normal subjects). Although the level of LTB₄ was low compared with our studies, the findings were based on only five patients and five controls. In the present study we have accumulated data on 34 asthmatic patients and 10 healthy controls.

There have been a few reports concerning the blood level of LTs using direct radio-immunoassay of plasma without prior extraction of HPLC. Hayes et al. (1983) reported that the level of LTC₄-like substance was more than 10 nmol/L, also Schonfeld et al. (1985) measured LTC₄ in plasma. Its mean value in asthmatics was 63.9 nmol/L and that
Figure 1. Plasma level of leukotrienes in bronchial asthma patients

*** P > 0.001
** P > 0.01

Figure 2. Plasma level of leukotrienes between and during attacks of bronchial asthma.

** P > 0.01
of normal subjects was 8–16 nmol/L. There are possibilities that direct radioimmunoassay without prior extraction may be affected by unknown cross-reacting substances in blood and that non-specific interference by proteins or lipids might take place.

Human plasma is known to degrade LTC_4 and LTD_4 to LTE_4 rapidly (Parker et al., 1980) indicating that LTE_4 is likely to be a significant product of SRS-A metabolism in vivo so the EIA reagents which we used in the present study include antibodies directed against all cysteinyl-containing leukotrienes.

Dahlen et al. (1980) reported that LTC_4, LTD_4, and LTE_4 were released from the lung tissues of asthmatic after antigen challenge. The amount of LTE_4 was less than LTC_4 and LTD_4. They also reported that human bronchi contracted on exposure to 6.2 nmol/L of LTD_4. The average level of spasmogenic cysteinyl leukotrienes in the present study is 500 pmol/L. The bronchoconstrictor concentration was thus approximately 12 times higher than the plasma level recorded in the present study. Since we sampled blood from the systemic circulation, there is substantial dilution of LTC_4 from the bronchiolar extracellular fluid. Therefore we think that the plasma level of cysteinyl leukotrienes in the present study is compatible with the in vitro study using human bronchi. Okubo et al. (1987) reported that there was no correlation between the level of plasma leukotrienes and pulmonary function tests such as forced expiratory volume (FEV) or airway resistance (Raw).

Leukotrienes are formed by leukocytes, Arachidonic acid is released and metabolized to leukotrienes in the leukocytes of asthmatic patients. LTB_4 has been reported to be formed in stimulated polymorphnuclear leukocytes and LTC_4 predominantly in stimulated eosinophils especially in the presence of glutathione (Verhagen et al., 1984; Borgeat et al., 1984). The precise relationship between the development of asthma, and the high concentrations of leukotrienes B_4 and C_4 present in arterial blood is however still unclear. It will be necessary to examine in more detail the changes in levels of leukotrienes C_4 and B_4 in arterial blood of the same patients throughout the clinical course of bronchial asthma, if this proves to be ethically acceptable.

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