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Detection of Leukotrienes in the Serum of Asthmatic and Psoriatic Patients

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Purified serum samples from asthmatic and psoriatic patients and healthy controls were analysed by high-pressure liquid chromatography (HPLC) and the amounts of leukotrienes were measured from the corresponding HPLC fractions by specific radioimmunoassays. In the serum of healthy controls the amounts of leukotrienes B_4, C_4 and D_4 were very small or negligible. Rather great amount of leukotriene B_4 was, however, detected in the serum of many asthmatic and psoriatic patients. The amount of leukotriene B_4 was in the serum of asthmatic patients 120±20 pmol/ml (n=11, mean±SEM) and in that of psoriatic patients 100±10 pmol/ml (n=10). The amounts of leukotrienes C_4 and D_4 were rather small in the serum of most patients. The amount of leukotriene C_4 was, however, very high (250 pmol/ml) in the serum of a psoriatic patient. Significant amount of leukotriene D_4 was also detected in the serum of this patient. The present study indicated that leukotrienes are formed during blood clotting in the leukocytes of asthmatic and psoriatic patients and that the rate of formation is so high that leukotrienes may have a role in these diseases. *Key words: Asthma; Psoriasis.* (Received October 30, 1985.)

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Leukotrienes are a new group of biologically active compounds which have obviously a role in immediate hypersensitivity reactions and inflammation (1). They are formed by the 5-lipoxygenase enzyme from arachidonic acid and two other polyunsaturated fatty acids. When arachidonic acid is released from cellular phospholipids, it can be metabolized by the cyclo-oxygenase enzyme to prostaglandins and thromboxanes or by different lipoxygenases to hydroxy acids and leukotrienes (1, 2).

Arachidonic acid is metabolized by the 5-lipoxygenase enzyme first to unstable 5-HPETE, which can be converted into leukotriene A_4 (LTA_4) or 5-hydroxy eicosatetraenoic acid (5-HETE). LTA_4 can be hydrated into LTB_4, a dihydroxy acid, or conjugated with glutathione to LTC_4 which can be metabolized to LTD_4 and LTE_4 (1). LTB_4 causes adhesion and chemotactic movement of leukocytes and stimulates the aggregation of neutrophils (1). LTC_4, LTD_4 and LTE_4 are potent bronchoconstrictors and they increase vascular permeability in postcapillary venules (1, 3).

As the 5-lipoxygenase enzyme is usually not active, leukotrienes are not generally formed. Leukotrienes were first identified from stimulated leukocytes (1, 4, 5). Now they have been detected also in allergen stimulated lung tissue of asthmatics (6), and after allergen challenge in the tear fluid (7) and nasal washes (8, 9) of allergic subjects as well as in the skin of psoriatic patients (10, 11). As some leukotrienes are rapidly metabolized in the circulation (cf. 12) it has not been possible to detect leukotrienes in the plasma of asthmatic patients (13).

Arachidonic acid is release from phospholipids by the action of phospholipases (14, 15). Because the phospholipases are activated in platelets during blood clotting, arachidonic

acid is release from platelet phospholipids and is subsequently metabolized in platelets to thromboxane A_2 and 12-HETE during clotting (16). We suggested that a similar activation of phospholipases could occur also in leukocytes during clotting and that this could result in the formation of leukotrienes if the 5-lipoxygenase enzyme is active. Therefore we have analysed the amount of leukotrienes from the serum of asthmatic and psoriatic patients and healthy controls.

PATIENTS AND METHODS

Eleven asthmatic patients (aged 20 to 59 years; 2 female and 9 male) and ten psoriatic patients (aged 14 to 71 years; 3 female and 7 male) and seven healthy subjects (aged 21 to 56 years; 1 female and 6 male) were involved in this study. Seven asthmatic patients had an intrinsic type and four an extrinsic type of asthma. The blood samples were taken when the patients were on conventional long term treatment for asthma or psoriasis. Two asthmatic patients had an inhaled steroid.

Blood samples were taken from the antecubital vein into glass tubes. The blood samples from control and asthmatic subjects were allowed to clot for one hour at 37°C in a shaking water bath and those from psoriatic patients at room temperature for two hours. When serum was separated it was stored at −20°C until analysed.

The serum samples (1 ml) were mixed with ethanol (2 ml) and prostaglandin B_2 ("internal standard", 5 µl, 20 µg/ml), and were then centrifuged to remove precipitated material. Then 2 ml of the supernantant was mixed with 9 ml of phosphate buffer (pH 8.0) and this sample was purified using a SEP-PAK C18 column (Waters, Milford, MA USA) (17). The SEP-PAK column was activated with ethanol (15 ml) and washed with water (15 ml) before the sample in phosphate buffer (containing ethanol) was applied twice to the column using reduced pressure. After washing the column with 15 ml of ethanol : water (1 : 9) the sample was eluted from the column with 10 ml of methanol. This methanol fraction was evaporated to dryness under nitrogen and was redissolved in 50 µl of methanol : water (65 : 35) and was then analysed in a high-pressure liquid chromatography (Shimadzu LC-4A, Kyoto, Japan) using a reverse-phase column (Bondapak C18, 2 mm×30 cm, Waters) and an UV-detector (Shimadzu SPD-2AS). A representative HPLC chromatogram is shown in Fig. 1. Two eluting solvents were used: Solvent A: methanol : water (65 : 35, pH 6.5) and solvent B: methanol-: water (68 : 32, pH 5.6). The flow rate was 0.3 ml/min. After the injection the compounds were first eluted with solvent A for 30 min. Then the solvent was changed during one minute by a linear gradient to solvent B which was used until the end ot the analysis (90 min). The effluent was monitored by a spectrophotometer first at 280 nm (to detect conjugated trienes) and after 42 min at 235 nm (to detect monohydroxy acids). The elution of refernce compounds (LTB_4, LTC_4, LTD_4, 5-HETE, PGB_2) was checked every day. When the serum samples were analysed the effluent fractions corresponding to the reference compounds as well as some intermediate fractions were collected for radioimmunoassays.

Appropriate amounts of the HPLC effluents were taken for the radioimmunoassays. The effluent fractions were first neutralized and then evaporated to remove methanol. LTB_4 radioimmunoassay was performed as described earlier (18). The antiserum for LTB_4 and unlabelled LTB_4 were from Wellcome (RP93, Dartford, England) and ^{3}H-LTB_4 was from New England Nuclear (Boston, MA, USA). The cross-reactivities of the antiserum for LTB_4 were according to the manufacturer (18): 12-HETE 2%, LTC_4 0.03%, LTD_4 0.03%. Detection limit for LTB_4 was 0.03 pmol in the radioimmunoassay corresponding to 3 pmol/ml in the serum samples. The radioimmunoassay kit for LTC_4 was purchased from New England Nuclear (NEK-030). The cross-rections of the antiserum for LTC_4 were according to the manufacturer: LTB_4 0.006%, LTD_4 55%. The detection limit for LTC_4 was in the radioimmunoassay 0.1 pmol corresponding to 5 pmol/ml in the serum samples. As the cross-reactivity of the LTC_4 antiserum was 55% with LTD_4, it could be used to measure also the amount of LTD_4 from the corresponding HPLC fraction. Unlabelled LTD_4 was a generous gift from Dr J. Rokach (Merck Frosst Canada Inc., Canada).

RESULTS

When purified serum samples from control, asthmatic and psoriatic subjects were analysed by HPLC, the main peak in the chromatogram was due to 12-HETE (UV absorbance at 235 nm). A peak corresponding to LTB_4 standard was usually detected in the chromatogram (UV absorbance at 280 nm) of asthmatic and psoriatic patients. As this peak may be

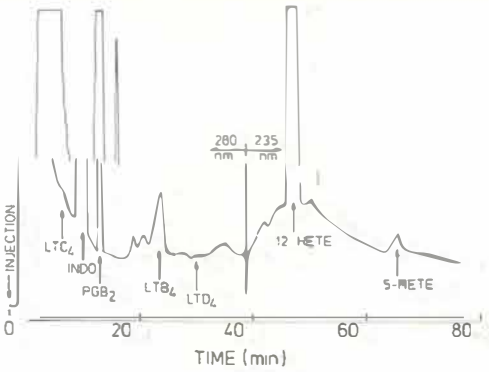

Fig. 1. Reversed-phase HPLC separation of the leukotrienes in serum samples. The effluent was monitored first at 280 nm and after 42 min at 235 nm, the effluent fractions corresponding to the reference compounds were collected for radioimmunoassay.

due to LTB_4 or its isomers, the amount of LTB_4 was measured from this HPLC fraction by a specific radioimmunoassay. Immunoreactive LTB_4 was detected in these HPLC fractions of all serum samples, but not in other fractions. Many asthmatic and psoriatic patients had a rather high concentration of LTB_4 and both the HPLC chromatogram and the radioimmunoassay indicated that the amount of LTB_4 was very small or negligible in the serum of healthy controls (Fig. 2). The serum concentration of immunoreactive LTB_4 was 120±20 pmol/ml (mean±SEM, n=11) for asthmatic patients, 100±10 pmol/ml (n=10) for psoriatic patients and 20±3 pmol/ml (n=7) for healthy controls.

Using HPLC analysis and the radioimmunoassay only small amounts (5–10 pmol/ml) of LTC_4 were detected in the serum of all control and asthmatic subjects and most psoriatic patients. Under the detection limit (5 pmol/ml) the amount of LTC_4 was in the serum of two out of ten control and three out of eleven asthmatic subjects and in none of the psoriatic patients. In the HPLC chromatogram of one psoriatic patient a clear LTC_4 peak was detected. The radioimmunoassay from this HPLC effluent fraction indicated that the concentration of LTC_4 was in the serum of this patient as much as 250 pmol/ml. The total leukocyte count of this patient was 6.0×10^6 per ml of blood, and the proportional amount of eosinophils was 3%. The highest level of immunoreactive LTD_4 was detected also in the serum of this patients (60 pmol/ml). The amount of LTD_4 was in the serum of other psoriatic patients very low (5–10 pmol/ml) or below the detection limit (5 pmol/ml). In the serum of asthmatic and control subjects the amount of immunoreactive LTD_4 was usually below the detection limit.

A distinct 5-HETE peak was detected in the HPLC chromatogram (235 nm) of six psoriatic and three asthmatic patients. The 5-HETE peak was usually clearly smaller than that of 12-HETE but greater than the LTB_4 peak. No 5-HETE peak was detected in the HPLC chromatograms of healthy controls.

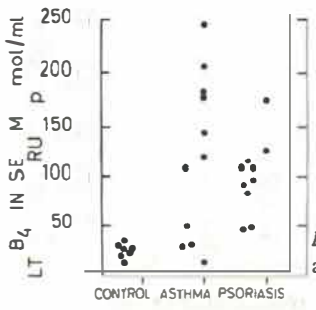

Fig. 2. The concentration of immunoreactive LTB_4 in the serum of asthmatic and psoriatic patients and healthy controls.

DISCUSSION

The present study suggests that leukotrienes are formed during blood clotting in astmatic and psoriatic patients. Plasma levels of leukotrienes B_4 and C_4 have been in our pilot studies (unpublished) consistently below the detection limits. Therefore, it is apparent that the leukotrienes detected in the serum samples have been formed during blood clotting. As the 5-lipoxygenase enzyme is present in leukocytes but not in platelets (19), leukotrienes were obviously formed in leukocytes. Thus, the present study suggests that the activation of phospholipases occurs also in leukocytes during blood clotting, and that arachidonic acid is released and metabolized to leukotrienes in the leukocytes of asthmatic and psoriatic patients. As no significant amounts of leukotrienes or 5-HETE were detected in the serum of healthy controls, it is obvious that the 5-lipoxygenase enzyme was not active in the leukocytes of control persons.

LTB_4 was the main leukotriene detected in the present study. LTB_4 has been reported to be formed in stimulated polymorphonuclear leukocytes and LTC_4 predominantly in stimulated eosinophils, specially in the presence of glutathione (20, 21). In the present study significant amounts of leukotrienes C_4 and D_4 were detected in the serum of only one psoriatic patient whose leukocyte count and the proportional amount of eosinophils were, however, normal.

As a great amount of 12-HETE is formed in platelets during blood clotting (16) and 12-HETE has a cross-reactivity of 2% in the LTB_4 radioimmunoassay (18), LTB_4 was separated from 12-HETE by HPLC before the radioimmunoassay. As the LTB_4 peak was rather distinct in the HPLC chromatogram of many asthmatic and psoriatic patients and the amount of immunoreactive LTB_4 was also great in these HPLC fractions, but not in other fractions, the results can be considered to be reliable.

Leukotrienes have earlier been detected in stimulated lung tissue of asthmatics (6) and in psoriatic skin (10, 11). Our study indicates that a part of the leukotrienes detected in the lung tissue and psoriatic skin could have been formed in leukocytes present in the tissues. Irrespective of their cellular origin, the detection of significant amounts of leukotrienes in the tissues and body fluids of asthmatics and psoriatics points to a possible significance of these inflammatory mediators in these diseases.

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