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THE EXCRETION OF LEUKOTRIENE E_4 INTO URINE FOLLOWING INHALATION OF LEUKOTRIENE D_4 BY HUMAN INDIVIDUALS

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Healthy volunteers underwent bronchial challenge with increasing doses of nebulized leukotriene D_4 (0.007 - 200 nmol) at 15 min intervals. Total amounts of 200 nmol (females) and 400 nmol (males) were inhaled, corresponding to approximately 100 nmol and 200 nmol deposited in the lung, respectively. Of the latter amounts 3 ± 1% (mean ± S.E.M., n=5) was found to be excreted as leukotriene E_4 into the urine within 12 h. No further excretion after this period was observed. Approximately 50% of the total urinary leukotriene E_4 was excreted during the first 2 h. These results suggest that a possible formation of sulfidopeptide leukotrienes in the lung in vivo can be monitored by measuring leukotriene E_4 excretion into the urine. © 1987 Academic Press, Inc.

The sulfidopeptide leukotrienes (C_4, D_4 and E_4) can induce severe airway obstruction, tissue oedema and an increase in the bronchial mucus production (1). Recently, we reported on the specific formation of leukotriene C_4 by human eosinophils in vitro (2,3,4,5). As these cells have been shown to infiltrate into the lung at the beginning of the late asthmatic reaction (6), a major role for leukotriene C_4 or its metabolites in this reaction has been suggested (7). The evidence for such a role will become more substantial if the formation of sulfidopeptide leukotrienes can be demonstrated during an (induced) asthmatic attack. However, direct measurement of leukotrienes in the lung in vivo, is not feasible for technical and ethical reasons. Consequently, leukotriene formation in the lung can only be monitored in body fluids into which leukotrienes or their metabolites are excreted. In the lung the leukotrienes C_4 and D_4 are probably rapidly converted into leukotriene E_4 (8). The enzymes

ABBREVIATIONS:
RP-HPLC : Reversed-Phase High-Performance Liquid Chromatography

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γ-glutamyltranspeptidase and dipeptidase, which convert the leukotrienes C_4 and D_4 into leukotriene E_4, are also present in blood (9). Recently, Örning et al. (10) showed that when tritium-labelled leukotriene C_4 was injected intravenously into human individuals, 48% of the administered label was excreted into the urine within 72 h. Of the total urinary radioactivity 27% was derived from leukotriene E_4.

In the present study, we investigated whether and to what extent leukotriene D_4 when brought into the lung, is excreted as leukotriene E_4 into the urine. Therefore, healthy volunteers were challenged with amounts of leukotriene D_4 that caused severe bronchoconstriction and their urines were analyzed for leukotriene E_4 by RP-HPLC.

MATERIALS AND METHODS

Synthetic leukotriene D_4 was a gift of Merck-Frosst Laboratories (Dorval, Quebec, Canada). The methylester of prostaglandin B_2 was prepared from prostaglandin B_2 (Sigma, St. Louis, USA) by treatment with diazomethane. 4-hydroxy-2,2,6,6,-tetramethylpiperidinooxy free radical was purchased from Sigma. Octadecyl solid-phase extraction columns (6 ml) were from Baker (Phillipsburg, USA). Methanol and water were of HPLC quality and obtained from Merck (Darmstadt, FRG). HPLC sample filters (pore size: 0.45 µm) were from Nihon Millipore Kogyo KK (Yonezawa, Japan).

challenge experiments

Leukotriene D_4 was administered to 5 healthy volunteers (3 females and 2 males, age between 26 and 56 years) by means of aerosol inhalation, as published elsewhere (11). As one of the aims of this investigation was to determine the maximal response of the airways to inhaled leukotriene D_4, increasing doses were given instead of one combined dose. A total amount of approximately 200 nmol leukotriene D_4 was administered in doses of 0.007, 0.07, 0.7, 1.5, 3, 6, 12, 25, 50 and 100 nmol at 15 min intervals. The 2 male volunteers received an additional dose of 200 nmol. All individuals experienced chest tightness and wheezing. No other adverse effects were observed. The efficiency of the recently developed delivery system (12) was determined in 3 healthy volunteers using a ^{99m}Tc-labelled diethylene-amine-penta-acetate aerosol. A mean deposition into the lungs of 53% of the inhaled activity was found. Therefore, the total amount of leukotriene D_4 deposited into the lungs can be estimated to be approximately 100 nmol and 200 nmol for the female and male volunteers, respectively. Urine samples were collected during 24 h and stored at -80°C until sample preparation and RP-HPLC analysis.

sample preparation

After thawing, the urine samples were centrifuged at 45000 x g for 10 min at 4°C. The methylester of prostaglandin B_2 was added to the supernatants as an internal standard. The lipid material was extracted by octadecyl solid-phase extraction as described previously (13). The extraction columns were conditioned by washing first with 10 ml methanol, then with 5 ml water and finally with 5 ml of an 0.5% EDTA solution (w/v, pH 5.5). Washing with EDTA was found to be necessary for a quantitative recovery of the sulfidopeptide leukotrienes (13). The supernatant (approximately pH 6) was applied to the column and polar material removed by washing the column first with 10 ml water and then with 5 ml methanol/water (60:40, v/v). The methanol/water eluate was stored at -80°C to be analyzed for possible polar arachidonic acid metabolites. Finally, leukotrienes were eluted with 3 ml methanol. Recovery of the sulfidopeptide leukotrienes was found to be better than 90%. After addition of the radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical (2 µg/3 ml), the eluates were stored under nitrogen at -80°C until analysis by RP-HPLC.

analysis by RP-HPLC

Prior to analysis, the methanol eluates were evaporated to approximately 300 µl and filtered through a sample filter (pore size: 0.45 µm). The solutions were then further evaporated to approximately 50 µl and injected onto a CP Spher 8C18 column (250 x 4.6 mm, Chrompack, Middelburg, The Netherlands) protected by a CP Spher C18

guard column (75 x 2.1 mm). The columns were kept at 45°C during chromatography. A Hewlett-Packard HP 1090 solvent-delivery system was used and an HP 1040A diode-array detector. The solvent system was methanol/water/acetic acid (62:38:0.1) which had been brought to pH 4.6 with ammonium hydroxide. The aqueous phase contained 0.1% EDTA which improves the recovery of the sulfidopeptide leukotrienes by preventing binding of cations to the column (14). A flow-rate of 1.0 ml/min was maintained and the effluent was monitored by recording one spectrum between 210 and 350 nm per second. Data were processed with an HP 310 SPU workstation. Peak areas at 280 nm were quantified by relating them to that of the internal standard, using molar absorption coefficients of 40000 M^{-1} cm^{-1} and 28650 M^{-1} cm^{-1} for the leukotrienes and the methylester of prostaglandin B_2, respectively.

RESULTS AND DISCUSSION

In Fig. 1 an illustrative example of the RP-HPLC profile of the methanol fraction of the solid-phase extract of an urine sample is shown. The presence of leukotriene E_4 (compound I) and small amounts of leukotriene D_4 (compound II) was established by coelution of these compounds with synthetic standards and by their characteristic ultraviolet spectrum (insert Fig. 1). Sulfidopeptide leukotrienes could not be detected in the urine before bronchial challenge with leukotriene D_4. Although most of the polar compounds were removed by the washing procedures during the octadecyl solid-phase extraction of the eicosanoids, quantitation at 280 nm of the generally used internal standard prostaglandin B_2 (retention-time: 16 min) appeared to be very difficult because of the presence of many interfering compounds. Therefore, we used the methylester of prostaglandin B_2 (retention-time: 30 min) which elutes much later than most of the interfering compounds. In Fig. 2 a time-course is shown of the excretion of leukotriene E_4 into the urine of 5 healthy volunteers. As can be seen almost all of this

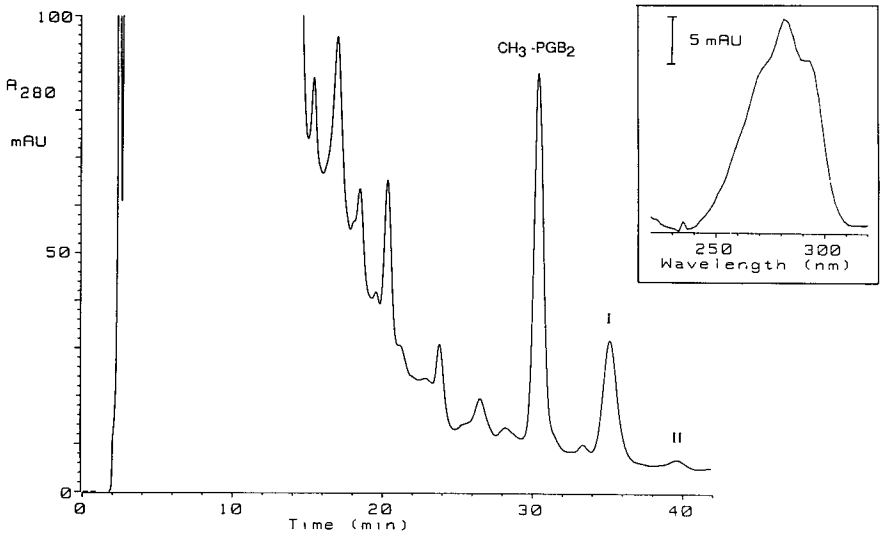

Fig. 1. RP-HPLC profile of the methanol fraction of the octadecyl solid-phase extract of the urine of volunteer 4, collected 2.3 h after challenge with the highest dose (100 nmol) of leukotriene D_4. Sample preparation and chromatographic conditions were as described under Materials and Methods. Insert: ultraviolet spectrum of compound I.

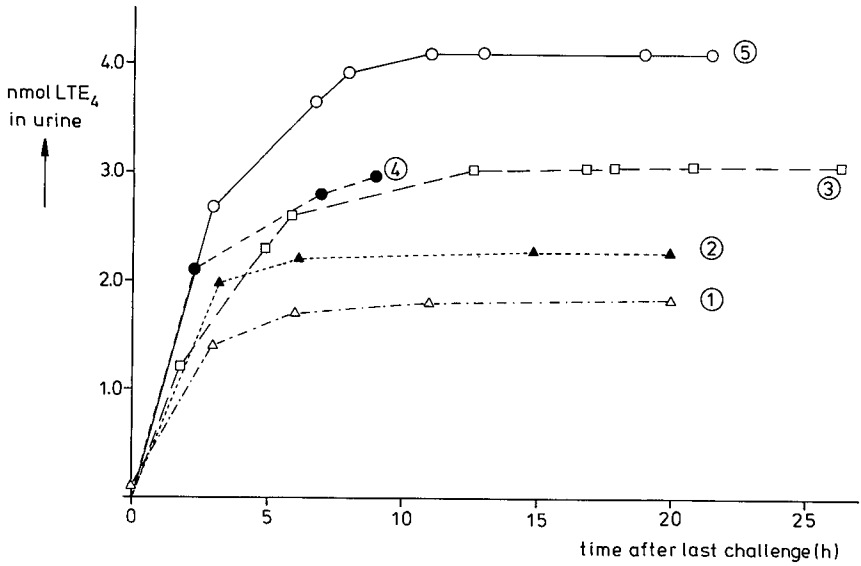

Fig. 2. Time-course of the excretion of leukotriene E_4 into the urine of 5 healthy volunteers (females: 1,2 and 4, males: 3 and 5). For easy comparison, the amounts of leukotriene E_4 excreted by the volunteers 3 and 5, which were challenged with twice as much leukotriene D_4 as the others, are represented as half the actual amounts. Therefore, the represented amounts are all related to an estimated amount of100 nmol of leukotriene D_4 deposited into the lung. Curves were drawn assuming that the excretion at t=0 was negligible. However, since the challenges were performed with increasing doses of leukotriene D_4, small amounts of leukotriene E_4 can be expected to be excreted already at t=0. Only in case of volunteer 1, urine could be collected at t=0. As can be seen the excreted amount of leukotriene E_4 at t=0 represents only a few percent of the total urinary excretion of this leukotriene.

leukotriene present in the urine was excreted within 12 h after the last bronchial challenge. It represented 3 ± 1% (mean ± S.E.M., n=5) of the total estimated amount of leukotriene D_4 deposited into the lungs. In Fig. 3 a time-course is shown in which the excreted amounts of leukotriene E_4 are represented as mean percentages of the total amounts of this leukotriene excreted into the urine. Of the total urinary leukotriene E_4, 46% appeared to be excreted during the first two hours. Beside leukotriene E_4, only

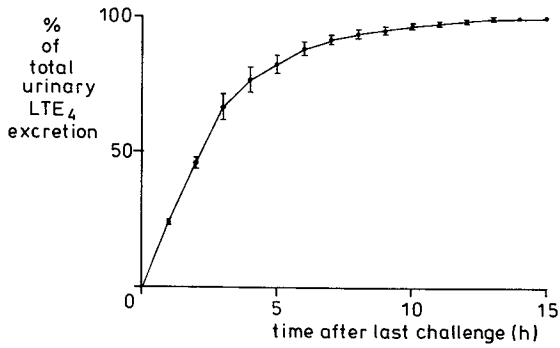

Fig. 3. Time-course representing the excretion of leukotriene E_4 into the urine as mean percentages of the total amounts of leukotriene E_4 excreted into the urine (± S.E.M, n=4).

small amounts of other arachidonic acid metabolites possessing a conjugated triene system were detected in the methanol fractions of the urines of the volunteers 2, 3, and 5. However, in the urine of volunteer 1, the only smoker in this study, and also in that of volunteer 4 substantial amounts of leukotrienes were found, having ultraviolet absorption maxima at 270 nm as well as at 280 nm, which did not coelute with the leukotrienes B_4, C_4, D_4 or E_4. Also in the methanol/water (60:40) fractions of all volunteers, considerable amounts of compounds were detected having the ultraviolet spectrum of a conjugated triene. These compounds are probably, at least in part, metabolites of leukotriene E_4, because their relative proportion was found to increase in time. Investigations are in progress to identify these leukotriene D_4/E_4 metabolites.

In summary, these results show that it is possible to measure the excretion of leukotriene E_4 following bronchial challenge with leukotriene D_4. Our findings suggest that the formation of sulfidopeptide leukotrienes in the lung, for example upon allergen challenge in allergic asthmatics, can be monitored by measuring the amount of leukotriene E_4 excreted into the urine. Preliminary analyses of the urines of allergic asthmatics collected during an asthmatic attack, show the presence of a compound which coelutes with leukotriene E_4.

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