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IN VIVO METABOLISM OF LEUKOTRIENE C_4 IN MAN:

Urinary excretion of leukotriene E_4

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Five - 20 nmoles of [5,6,8,9,11,12,14,15-$^3H_8$]leukotriene C_4 was injected into three male volunteers. Forty-eight percent of the administered ^3H was recovered from urine and 8% from feces, within a 72 hr period. Of the total urinary radioactivity 44% was excreted during the first hour after injection. This activity was mainly found in one compound, designated "I". The radioactivity excreted into urine later than one hour after injection, consisted partly of Compound I and two additional components, and partly of polar, non-volatile material. Compound I was identified as leukotriene E_4 by UV-spectroscopy and cochromatographies in three high performance liquid chromatography systems with synthetic reference compounds. A total of 13% of administered radioactivity was excreted in urine as leukotriene E_4. © 1985 Academic Press, Inc.

The metabolism of leukotriene C_4 or its 13,14-dihydro analog, leukotriene C_3 has been investigated *in vitro* (1) and *in vivo* in the mouse (2) and the monkey (3). These investigations showed that the glutathione part is susceptable to degradation whereas the fatty acid part of the molecule is much more resistant to metabolic alterations. Metabolites formed by elimination of the glutamic acid (leukotriene D) or the glutamic acid and the glycine residues (leukotriene E) were detected in blood of both species as well as in several mouse organs. It was also found that the predominating excretion route in rodents was via biliary excretion to feces (1,2). Recently, the structure of a fecal metabolite of leukotriene C_4 in the rat was reported (4). This

Abbreviations: ECG, electrocardiography; RP-HPLC, reverse-phase high performance liquid chromatography.

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compound was formed by elimination of the two terminal amino acid residues in leukotriene C_4 followed by acetylation of the amino group of leukotriene E_4. The present report is concerned with the excretion routes and the structure of an excreted leukotriene C_4 metabolite in man.

MATERIALS AND METHODS

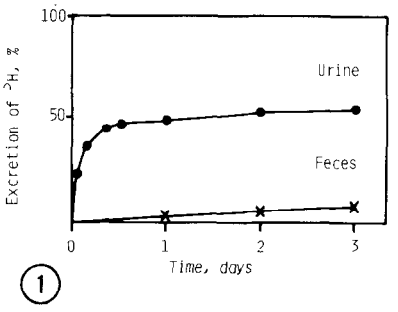
Chemicals. [5,6,8,9,11,12,14,15-$^3H_8$]Leukotriene C_4 was prepared as described (4). Synthetic leukotrienes C_4 and E_4 were kindly provided by J. Rokach, Merck-Frosst Canada Inc.. Tritium labeled leukotrienes were diluted with unlabeled compounds to the desired specific radioactivites and purified by RP-HPLC before use (4). N-Acetyl leukotriene E_4 isomers were prepared from leukotriene E_4 and 11-*trans* leukotriene E_4 as described (4).

Human experiments. Five - 20 nmoles of leukotriene C_4 (300-400 Ci/mol) was dissolved in 5 ml of sterile 0.9% NaCl (aq) + 3% ethanol and filtered through a 0.22 µM Millipore filter into a sterile glass ampoule. The solution was slowly injected intraveneously during 5-10 min, into the cubital vein of three 31-33 year old healthy volunteers. They had no history of any diseases, were on no medication and their ECG at rest was checked before the study. ECG was also continously recorded and blood pressure monitored at intervals (cuff method) during leukotriene infusions. Feces and urine were separately collected for three days. During the first day urine was collected in separate bottles for each micturation. All samples were kept on CO_2(s) until purifications began. Two volunteers who received higher leukotriene doses (12 and 20 nmoles) than the third volunteer developed a skin flush of the face during leukotriene C_4 infusions. No other adverse effects were observed.

Analytical methods. Urine was mixed with 4 volumes of ethanol, filtered, concentrated in vacuo, and desalted by Amberlite XAD-8 chromatography. The 80% ethanol eluate from the latter step was purified by silicic acid chromatography (4) and RP-HPLC. UV spectra were recorded on a Hewlett Packard model 8450A spectrophotometer. Fecal radioactivity was determined as described (4). To determine volatile radioactivity in urine, aliqouts of eluates from XAD-8 chromatographies were evaporated to dryness, dissolved in water, and analyzed by liquid scintillation counting. The amount of ^3H remaining after evaporation was compared to the amount present in the same volume of eluate which had not been evaporated and any difference in radioactivity was interpreted as representing volatile ^3H-metabolites.

RESULTS

Excretion of ^3H from [$^3H_8$]leukotriene C_4 and purification of ^3H-labeled metabolites. After intravenous administration of leukotriene C_4, labeled with tritium in the fatty acid part, 56% of the radioactivity was excreted within 72 hours. Eighty-three percent of the recovered tritium appared in urine and the rest in feces (Fig. 1).



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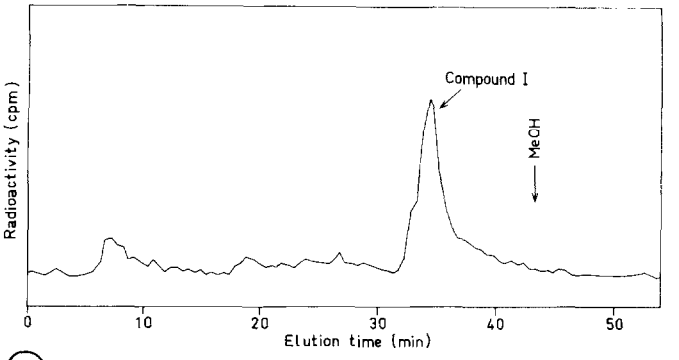

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Fig. 1 Excretion of tritium in urine and feces after intravenous administration of [5,6,8,9,11,12,14,15-$^{3}H_{8}$]leukotriene C_4.

Fig. 2 RP-HPLC of tritium labeled metabolites in urine collected 0-1 hour after i.v. injection of [$^{3}H_{8}$]leukotriene C_4. Conditions: column, 500x10 mm Polygosil C_{18}; mobile phase, methanol/water/acetic acid/o-phosphoric acid 70:30:0.07:0.03 (v/v/v/v) adjusted to pH 5.4 with NH_4OH; flow rate, 4.5 ml/min.

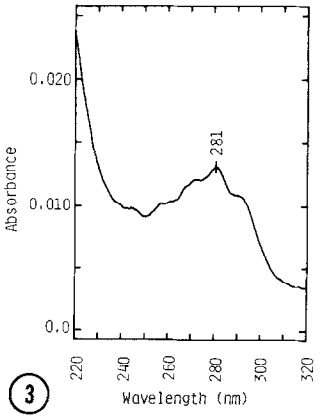
Of the urinary radioactivity 44% was excreted during the first hour and 33% during the period from 1 hour to 8 hours after injection.

Urine collected during different time periods was separately purified. When the 0 to 1 hour specimen was purified on Amberlite XAD-8, 15% of the tritium was eluted with water and the rest with 80% ethanol. The material in the latter eluate was purified by silicic acid chromatography. After eluting the column with methanol/ethyl acetate 15:85 (v/v), 83% of the radioactivity applied to the column was released with methanol/ethyl acetate 7:3 (v/v) . This material was analyzed by RP-HPLC. The chromatogram revealed essentially only one component, Compound I (Fig. 2). Urine collected between 1 and 8 ($\pm$ 1) hours was purified in the same way. Sixty-eight % of the radioactivity was eluted with water in the XAD-8 chromatography step, 27% was eluted with 80% ethanol, and 5% was retained on the column. When the ethanol eluate was fractionated by silicic acid chromatography 88% of the tritium appeared in the methanol/ethyl acetate 7:3 fraction. Analysis of this fraction by RP-HPLC showed three components: compound I and two minor components constituted 26, 6, and 4% of the radioactivity

applied to the column, respectively. Small amounts of Compound I were detected in urine up to 20 hours after administration. Altogether, 13.4 ± 2.4% of the administered ^{3}H or 27 ± 2% of the urinary radioactivity were found in compound I.

The radioactivity eluting with water on XAD-8 chromatography was checked for volatile components as described under Materials and Methods. No loss of radioactivity was observed, excluding that volatile ^{3}H-labeled metabolites (e.g $^{3}H_2O$) had been formed.

Structure of Compound I. Compound I had a retention time of 1.62 relative to leukotriene C_4, when analyzed by RP-HPLC. Fig. 3 shows a UV-spectrum of Compound I, which had a λ_{max} at 281 nm and shoulders at 270 and 292 nm. The spectrum was similar to the UV-spectrum of leukotriene C_4 suggesting that Compound I has retained the conjugated triene with an allylic thioether substituent of the injected substance. Compound I cochromatographed with leukotriene E_4 on RP-HPLC (Fig. 4). However, leukotriene E_4 was not well separated from N-acetyl-11-trans leukotriene E_4. Compound I was therefore also analyzed



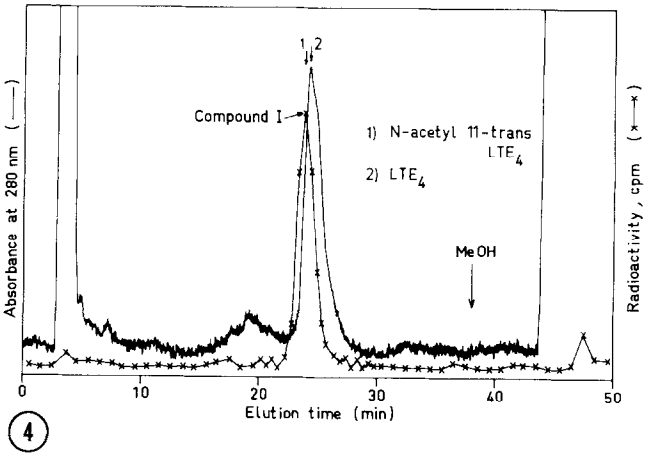


Fig. 3 Ultraviolet spectrum of compound I (see Fig. 2).

Fig. 4 Cochromatography by RP-HPLC of compound I, leukotriene E_4, and N-acetyl leukotriene E_4. Conditions: column, Nucleosil C_{18}; mobile phase, as in Fig. 2, flow rate, 1ml/min.

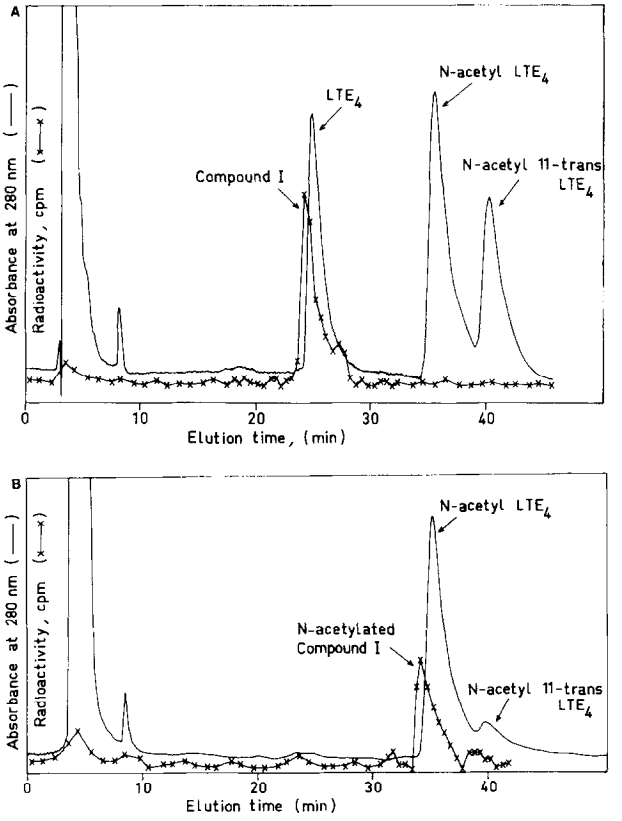


Fig. 5 Cochromatography by ion-pair RP-HPLC of (A) compound I, leukotriene E_4, N-acetyl leukotriene E_4, and N-acetyl 11-trans-leukotriene E_4 and (B) N-acetylated compound I, N-acetyl leukotriene E_4 and N-acetyl 11-trans-leukotriene E_4. Conditions: same as in Fig. 4 except that the NH_4OH in the mobile phase was replaced by 2.5 mM 1-pentane sulphonic acid which was used as counter ion.

together with synthetic reference compounds by ion-pair RP-HPLC (Fig. 5A). Compound I cochromatographed with leukotriene E_4 also under these conditions. Finally, a mixture of Compound I and leukotriene E_4 were N-acetylated and rechromatographed by ion-pair RP-HPLC (Fig. 5B). The chromatographic behaviour of the tritium labeled metabolite and the unlabeled reference compound changed in the same way. Based on these results, compound I was identified as leukotriene E_4.[1]

[1] The slight separation of radioactivity and UV absorbance in Figs. 4 and 5 is due to isotope discrimination between $^3H_8$ and protium species of leukotriene molecules (Murphy, R.C., Hammarström, S., and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76: 4275-4279).

DISCUSSION

Following i.v. injection of [5,6,8,9,11,12,14,15-$^{3}H_{8}$] leukotriene C_4 into three healthy male volunteers, the predominant way of tritium excretion was by the urinary route. This is different from results obtained with guinea pigs (1), mice (2), and rats (4) in which species the main route for elimination of leukotriene C metabolites was by fecal excretion.

The radioactive material appearing in urine during the first hour after injection consisted of mainly one metabolite. This compound was identified as leukotriene E_4 based on UV spectroscopy, cochromatography with synthetic leukotriene E_4 in two RP-HPLC systems and chemical transformation to N-acetyl leukotriene E_4 which was identified by ion-pair RP-HPLC cochromatography.

The rapid appearance of 13% of injected leukotriene C_4 radioactivity as urinary leukotriene E_4 suggests that efficient mechanisms exist for the uptake and metabolism of leukotriene C_4 by the kidneys. Approximately 20% of the cardiac output goes to the kidneys and they will therefore be exposed to a significant fraction of the injected leukotriene. More than 80% of the primary urine formed in the glomeruli is reabsorbed. Therefore, filtration alone can probably not explain the extensive excretion of leukotriene E_4 in man. The present results agree with a previous study using perfused rat kidney where a significant fraction of tritium from leukotriene C_3 was recovered from renal tissue as leukotriene E_3. In the rat kidney, however, urinary excretion of tritium was minimal (6).

Several groups have reported that cysteine-containing leukotrienes are metabolized to 6-*trans*-leukotriene B_4 and 6-*trans*-12-*epi*-leukotriene B_4 *in vitro* (7-9). These metabolites were not detected in a recent study, concerning *in vivo* metabolism of leukotriene C_4 in the rat (4). Since leukotriene B_4 and stereoisomers of this compound are largely metabolized by primates to urinary $^{3}H_2O$

in vivo (10) the lack of formation of volatile ^{3}H-labeled metabolites in the present experiments seems to exclude that any major conversion of the injected leukotriene C_4 to 6-*trans* leukotriene B_4 isomers occurred.

The results presented above suggest that quantitative measurements of leukotriene E_4 in human urine may provide a means of determining endogenous leukotriene C_4 formation in man in health and disease. Such investigations are in progress in our laboratories.

ACKNOWLEDGMENTS

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REFERENCES

1. Hammarström, S. (1981) J.Biol.Chem. 256, 9573-9578.
2. Appelgren, L.-E. and Hammarström, S. (1982) J. Biol. Chem. 257, 531-535.
3. Hammarström, S., Bernström, K., Örning, L., Dahle'n, S.E., Hedqvist, P., Smedegård, G., and Revenäs, B. (1981) Biochem. Biophys. Res. Commun. 101, 1109-1115.
4. Örning, L., Norin, E., Gustafsson, B., and Hammarström, S. (1985) J. Biol. Chem. in press.
5. Ziltener,H.J., Chavaillaz,P.-A. and Jörg,A. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1029-1037.
6. Ormstad, K., Uehara, N., Orrenius, S., Örning, L., and Hammarström, S. (1982) Biochem. Biophys. Res. Commun. 104, 1434-1440.
7. Goetzl, E.J. (1982) Biochem. Biophys. Res. Commun. 106, 270-275.
8. Henderson, W.R. and Klebanoff, S.J. (1983) J. Biol. Chem. 258, 13522-13527.
9. Lee, C.W., Lewis, R.A., Tauber, A.I., Mehrotra, M., Corey, E.J., and Austen, K.F. (1983) J. Biol. Chem. 258, 15004-15010.
10. Serafin, W.E., Oates, J.A., and Hubbard, W.C. (1984) Prostaglandins 27, 899-912.