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DOSE DEPENDENT CONVERSION OF ESTRAGOLE IN THE RAT AND MOUSE TO THE CARCINOGENIC METABOLITE, 1'-HYDROXYESTRAGOLE

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The carcinogenic potential of a chemical is generally assessed from the results of life-time feeding studies in rodent species. However, extrapolation of the findings to man is frequently difficult for many reasons, and one of the more important of these is that human exposure generally occurs at much lower levels than ones used in animal studies. The metabolism of many chemicals is dose-dependent and this is of particular significance when biotransformation produces a carcinogenic metabolite. At low doses a compound may be safely disposed of metabolically whereas at higher doses these pathways become saturated and alternative routes become increasingly employed which lead to the formation of toxic metabolites.

Estragole (4-methoxyallylbenzene) is a major component of the oils derived from basil, fennel and tarragon and is also found in baked foods and carbonated beverages (1). The average adult human intake of estragole from dietary sources has been estimated to be 72μg/day. Like safrole (3,4-methylenedioxyallylbenzene), estragole is hepatocarcinogenic in weanling mice at doses of about 500mg/Kgs (2). The carcinogenicity is related to the formation of a reactive metabolite by hydroxylation of the C^1 position of the allyl side-chain to give 1'-hydroxyestragole, which is a more potent hepatocarcinogen than estragole itself.

In the mouse carcinogenicity tests, the dose was many times larger than those to which man is ordinarily exposed. We now show that the conversion of estragole to the carcinogenic metabolite is dose-dependent in mouse and rat, a fact which must be considered when rodent carcinogenicity studies are extrapolated to man.

MATERIALS AND METHODS

^{14}C-Estragole (4-[^{14}C-methoxyl]-allylbenzene), sp. act. g. 1mCi/mmol, radiochemical purity >99%, was a custom synthesis by the Radiochemical Centre, Amersham, U.K. 1'-Hydroxy-estragole (1-Hydroxy-1-(4'-methoxyphenyl)-prop-2-ene) was prepared by the method of Drinkwater et al. (2), and was identical in i.r., u.v., n.m.r. and mass spectra with that described (2). Thin layer chromatography (tlc) used Merck Silica gel F_{254} plates (0.2 mm thick on aluminium support), solvent n-hexane: diethyl ether (1:1 by vol.), and 1'-hydroxy-estragole had R_F 0.50. High pressure liquid chromatography (hplc) used a Waters Associates U6K valve loop injector and Model 6000 A pump and a Cecil 2012 u.v. detector set at 275 nm. The column was 300 x 4.9 mm packed with μBondapak C_{18}, and with a mobile phase of 33% aq. CH_3CN, flow rate 1ml/min, 1'-hydroxyestragole had retention time 8.6 min.

Mass spectrometry was performed in the electron impact mode at 70eV with a Varian-MAT CH5 instrument.

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Animals and Dosing Male CD-1 mice (body weight 20g) and female Wistar albino rats (body weight 200g) received ^{14}C-estragole in trioctanoin by i.p. injection. Mice received 2μCi/20g and 0.2ml/20g and rats 20μCi/200g and 0.2ml/200g. Rats were housed in Metabowls (Jencons Limited) and mice in MiniMetabowls, equipped to collect urine and faeces. Air from the metabowls was drawn with a pump through drying traps of $CaCl_2$ and $MgClO_4$ and then through two traps of 200 ml ethanolamine- 2-methoxyethanol (1:2v/v) to trap $^{14}CO_2$ in the expired air.

Radiochemical techniques ^{14}C in urine and other solutions was determined by liquid scintillation spectrometry (Packard TriCarb Model 3385) with quench correction by reference to an external standard. ^{14}C on chromatograms was located by scanning (Packard Model 7201).

Assay of 1'-hydroxyestragole in urine Urine samples (0.25ml) and 0.2M acetate buffer pH5 (0.5ml) were incubated with 5000 units bovine liver β-glucuronidase (Ketodase: General Diagnostics) at 37° for 24h and extracted with ether (15ml). 1'-Hydroxyestragole (5mg) was added as carrier to facilitate detection and prevent losses. The extract was dried (anhyd. Na_2SO_4), evaporated and the residue taken up in methanol (1 ml). Aliquots were counted for ^{14}C and subjected to tlc. After scanning for ^{14}C the area of silica gel corresponding to 1'-hydroxyestragole was scraped from the plate into vials, scintillator added and counted for ^{14}C.

RESULTS

Identification of 1'-hydroxyestragole as a metabolite of estragole in rat and mouse Urine of rats and mice dosed with ^{14}C-estragole was treated with β-glucuronidase as described, extracted with ether at pH5 and the extracts examined by tlc and hplc. In both cases a ^{14}C peak with the same chromatographic characteristics as those of 1'-hydroxyestragole was seen and the identity of this was confirmed by mass spectrometry. For this purpose, extracts not spiked with 1'-hydroxyestragole were used, and the peaks isolated had mass spectra identical with authentic 1'-hydroxyestragole. Similarly prepared extracts of urine not treated with β-glucuronidase contained no 1'-hydroxyestragole, indicating that this metabolite was excreted entirely conjugated with glucuronic acid.

Influence of dose size on the formation of 1'-hydroxyestragole Groups of rats and mice received ^{14}C-estragole as described in doses of 0.05, 5,500 and 1000 mg/Kg and urine and $^{14}CO_2$ in the expired air collected for 24h. The elimination patterns in the two species are shown in Table 1, which shows that as the dose increased, excretion of ^{14}C as $^{14}CO_2$ fell while that in urine rose. The fraction of urinary ^{14}C extracted into ether at pH5 after treatment with β-glucuronidase rose with increase in dose in the rat from 26% to 53% Corresponding figures in the mouse were 29% to 50%. The formation of 1'-hydroxyestragole, excreted as its glucuronide, was 0.9% of dose in rat and 1.3% in mouse at the 0.05 mg/kg dose 9.0% (rat) and 9.5% (mouse) at 1000 mg/kg. The relationship between the molar formation of 1'-hydroxyestragole and dose size in both species is also given in Table 1. With a 20,000 fold increase in dose, there occurs a 162,000 fold increase in the output of this metabolite.

Table 1

Influence of dose size on the elimination of ^{14}C and formation of 1'-hydroxyestragole after administration of ^{14}C-estragole to rats and mice

Dose (mg/kg)	% dose in 0-24h urine	% dose in 0-24h as $^{14}CO_2$	% urinary ^{14}C extracted at pH5.0	% dose as 1'-hydroxy-estragole in urine	1'-hydroxy-estragole formation (nmol/kg/24h)
Rat					
0.05	25 (11-31)	34 (22-42)	26 (21-32)	0.9 (0.4-1.5)	3.1
5	25 (18-39)	33 (27-38)	40 (32-50)	3.6 (1.4-6.0)	1224
500	40 (31-60)	19 (14-27)	59 (55-65)	7.5 (5.3-9.1)	255000
1000	52 (43-62)	20 (13-28)	53 (51-54)	8.0 (7.9-8.0)	544000
Mouse					
0.05	27 (25-29)	38 (36-41)	29 (28-32)	1.3 (1.2-1.4)	4.4
5	25 (17-30)	38 (35-40)	31 (22-38)	1.5 (0.6-2.2)	510
500	56 (40-72)	27 (26-29)	50 (47-52)	8.2 (7.7-8.6)	279000
1000	59 (51-73)	22 (8-30)	50 (45-55)	9.5 (8.0-11.4)	646000

Figures represent means of at least 3 animals with ranges in parentheses

DISCUSSION

Estragole is known to be metabolized along a number of pathways including O-demethylation (to give chavicol), epoxidation of the double bond, 1'-hydroxylation and oxidative degradation of the side-chain to carboxylic acids (3,4). Results of the present studies indicate that at least two of these pathways, namely, O-demethylation and 1'-hydroxylation exhibit dose-dependency in both the mouse and rat. Thus the proportion of the dose that undergoes O-demethylation declines in a dose-dependent fashion and this is accompanied by an increase in the proportion of the dose that undergoes urinary elimination. This change presumably arises from saturation of the enzyme systems responsible for O-dealkylation. The corollary of this is that at higher dose levels a relatively greater substrate level would be available for alternative metabolic reactions such as 1'-hydroxylation and this is in fact what seems to occur. Thus, 1'-hydroxyestragole formation accounts for only about 1% of dose at 0.05 mg/Kg and this increases to about 9% at 1000 mg/Kg. The increase is highly significant by the Spearman rank correlation test ($r_{(s)} = 0.89$; $P < 0.001$).

The significance of the data becomes particularly evident when it is considered in terms of actual amounts (nmol) of the carcinogenic metabolite produced at different dose levels. The amount of 1'-hydroxyestragole formed rose 162,000 fold over the dose range studied.

If the hepatocarcinogenicity of estragole is related to the formation of the 1'-hydroxy metabolite, as appears to be the case, then induction of tumours may also be dose-related. The data also indicates the importance of taking into account dose-dependent metabolic variations when assessing the significance of animal carcinogen bio-assays for the safety assessment of compounds to which man is exposed.

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