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Effect of different routes of administration of cedrene on hepatic drug metabolism

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WHEN mice or rats are exposed to cedarwood bedding, they exhibit an increase in the metabolism of certain drugs.[1–3] Wade *et al.*[3] showed that this increase was due to the induction of hepatic microsomal enzymes by cedrene, the major constituent of cedarwood oil, and suggested that the main route of administration was by inhalation; however, they provided no estimate of the dose of cedrene required for induction. The present investigation was undertaken to estimate the dose of cedrene absorbed via inhalation and to compare the effect of different routes of administration of cedrene on hepatic drug metabolism.

Female, Sprague–Dawley rats (150–200 g) were obtained from Hormone Assay (Chicago, Ill.). Various doses of cedrene in corn oil (1 ml/kg) were injected orally or intraperitoneally once a day for 3 days. Control animals received corn oil (1 ml/kg). Rats were sacrificed 24 hr after the last oral or intraperitoneal administration of cedrene. When cedrene was administered to rats by inhalation, the rats were maintained in the inhalation chamber continuously except for 1 hr a day during which time the chamber was cleaned.

The system employed for the inhalation studies consisted of a drying tower ($CaCl_2$), an air flow meter, two gas washing bottles containing the cedrene, an inflow air reservoir, an inhalation chamber, and an outflow air reservoir (Fig. 1). A standard 15-l. desiccator was used for the inhalation chamber,

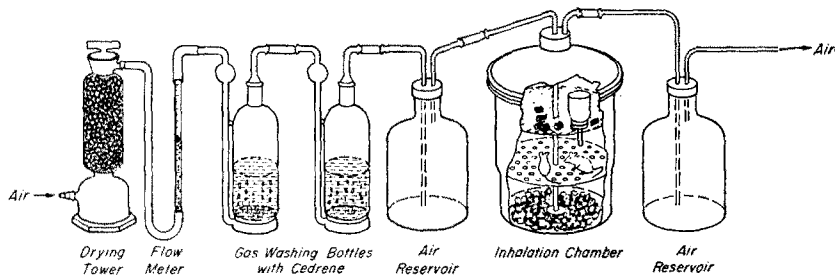

FIG. 1. Schematic diagram of inhalation system.

TABLE 1. ABSORPTION OF CEDRENE VIA INHALATION*

Inhalation period (days)	No. of rats in chamber	Av. body wt. (g)	Air flow (l./min)	Cedrene concentration†		Cedrene absorbed (mg/kg/day)
				Inflow reservoir	Outflow reservoir	
2	3	163	1·0	1·05 ± 0·08 (15)‡	0·62 ± 0·08	60 ± 11
6	4	192	1·5	1·21 ± 0·09 (29)	0·76 ± 0·08	61 ± 12

* Values are given as means ± standard deviations.
† Microgram of cedrene per 20 ml of air in reservoir.
‡ Numbers in parentheses indicate the number of measurements performed.

TABLE 2. EFFECT OF CEDRENE ADMINISTERED BY DIFFERENT ROUTES ON HEPATIC MICROSOMAL ACTIVITIES*

Pretreatment				Ethylmorphine metabolism†		Aniline metabolism†		P-450 content‡	
Route	Dose§	Length (days)	No. of animals	Control	Treated	Control	Treated	Control	Treated
Intraperitoneal	50	3	6	17·4 ± 2·5	27·2 ± 3·5‖ (1·6)	6·2 ± 0·9	8·0 ± 1·2	0·056 ± 0·004	0·065 ± 0·004‖ (1·2)
Intraperitoneal	100	3	5	12·8 ± 1·6	21·1 ± 3·6‖ (1·7)	4·6 ± 1·0	5·9 ± 1·1	0·045 ± 0·004	0·057 ± 0·006‖ (1·3)
Intraperitoneal	200	3	5	12·3 ± 3·1	23·0 ± 4·0‖ (1·9)	7·2 ± 0·8	8·9 ± 1·5	0·051 ± 0·005	0·077 ± 0·007‖ (1·5)
Oral	200	3	6	11·4 ± 2·5	29·6 ± 6·6‖ (2·6)	7·3 ± 2·0	8·0 ± 1·7	0·053 ± 0·006	0·075 ± 0·011‖ (1·4)
Untreated¶	0	2	3	14·6 ± 1·0		9·0 ± 0·8		0·060 ± 0·001	
Inhalation	60	2	3	15·0 ± 2·4	26·3 ± 3·0‖ (1·8)	11·5 ± 1·4	10·0 ± 1·0	0·054 ± 0·001	0·070 ± 0·003‖ (1·3)
Inhalation	60	6	4	14·6 ± 3·5	31·9 ± 3·9‖ (2·2)	9·1 ± 0·5	11·3 ± 0·3‖ (1·2)	0·056 ± 0·006	0·071 ± 0·004‖ (1·3)

* Values are given as means ± standard deviations.
† Formaldehyde or *p*-aminophenol formed in nanomoles per milligram of microsomal protein per 10 min.
‡ Optical density per milligram of microsomal protein.
§ Dose in milligram per kilogram per day.
‖ Ratio of treated over control appears in parentheses only in cases when the results are statistically significant at $P < 0{\cdot}01$.
¶ Rats were maintained in a regular cage and not placed in the inhalation chamber. Enzyme assays were carried out simultaneously with the 2-day inhalation experiment.

and the rate of air flow through the system was 1·0–1·5 l./min. Calcium chloride was placed under the perforated floor of the chamber to remove moisture, and the animals were allowed free access to food and water. The inhalation chamber was flushed with cedrene overnight before three or four rats were introduced into the chamber. Control rats were treated in a similar inhalation system without gas washing bottles and air reservoirs.

The concentration of cedrene absorbed by the animals via inhalation was measured by gas–liquid chromatography. Samples (20 ml) of air were removed from the inflow and the outflow reservoirs and injected into the column (4 ft, OV-17 on Chromsorb W, 80–100 mesh; flow rate = 90 ml/min; temperature = 107°) of a Barber Coleman model 10 gas chromatograph equipped with an argon ionization chamber. The difference in concentration between the inflow and outflow reservoirs was assumed to be due to absorption of cedrene by the animals, since no difference in concentration was found when the animals were not in the inhalation chamber. A steady-state concentration of cedrene in the inhalation system was obtained during the first 3 hr of inhalation. Cedrene assays were performed two to four times a day thereafter during the time when the cedrene concentration in the inhalation system was in a steady state. Each assay was duplicated. Table 1 illustrates the amount of cedrene absorbed via inhalation per kilogram of body weight per day.

The preparation of liver microsomes and enzyme assays were conducted as described previously by Davis *et al.*[4]

The effect of cedrene on the hepatic metabolism *in vitro* of ethylmorphine and aniline and on cytochrome P-450 content after the administration of cedrene by the oral, intraperitoneal, and inhalation route is shown in Table 2. When 50, 100, or 200 mg/kg of cedrene were injected intraperitoneally into female rats once a day for 3 days, ethylmorphine *N*-demethylase activity increased 60 per cent at the lowest dose to 90 per cent at the highest dose and cytochrome P-450 content increased about 20 and 50 per cent respectively. Oral administration of cedrene (200 mg/kg once a day for 3 days) produced a 160 per cent increase in ethylmorphine metabolism and a 40 per cent increase in cytochrome P-450 content. When cedrene was administered by the inhalation route in a dose of 60 mg/kg/day for 2 or 6 days, there was an 80–120 per cent increase in ethylmorphine *N*-demethylase activity and a 30 per cent increase in cytochrome P-450 content. The effect of cedrene appears to be rather selective, since it had little or no effect on aniline hydroxylase activity. By contrast, the activities of the liver microsomal enzymes were not significantly changed in rats kept in a control inhalation chamber.

The total daily dose of cedrene absorbed during the period of inhalation was sufficient to cause nearly maximal induction as assessed by either oral administration or by injection intraperitoneally. Indeed, the effects of cedrene on hepatic drug metabolism were comparable when cedrene was administered intraperitoneally or by inhalation. Thus, it appears that cedrene exerts similar effects on the hepatic drug-metabolizing system regardless of its route of administration.

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