

THE EFFECT OF PHENOBARBITONE ON THE METABOLISM OF METHOXYFLURANE TO OXALIC ACID IN THE RAT

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SUMMARY

The effect of phenobarbitone on the metabolism of methoxyflurane in the rat was investigated. The conversion of methoxyflurane to oxalic acid was confirmed. Pretreatment with phenobarbitone resulted in a further increase in oxalic acid output indicating an acceleration of the biotransformation of methoxyflurane. If the nephrotoxicity of methoxyflurane is secondary to the products of its metabolism, increased microsomal enzyme activity resulting from prior exposure to drugs such as phenobarbitone would predispose to increased toxicity.

Most drugs undergo metabolic transformation in the body and the volatile anaesthetic agents prove to be no exception (Van Dyke, Chenoweth and Van Poznak, 1964). There is evidence that some of these anaesthetic biotransformations may result in toxic intermediates or end products. For example, high output renal failure following methoxyflurane (Penthrane) has been described (Crandell, Pappas and Macdonald, 1966; Pezzi, Frobes and Greenberg, 1966; Austin and Villandry, 1967; Panner et al., 1970; Mazze, Snell and Jackson, 1971). This toxic reaction has been correlated with metabolic degradation of methoxyflurane (Mazze, Trudell and Cousins, 1971). Identified products of this biotransformation in man include carbon dioxide, fluoride ion, dichloroacetic acid and methoxyfluoroacetic acid (Holaday, Rudofsky and Treuhaft, 1970). Calcium oxalate crystals have been found in the kidneys of patients dying with high output renal failure after methoxyflurane anaesthesia (Paddock, Parker and Guadagni, 1964; Austin and Villandry, 1967; Frascino, Vanamee and Rosen, 1970). Increased urinary excretion of oxalic acid after methoxyflurane anaesthesia was described by Mazze, Trudell and Cousins (1971).

Using 24-hour urinary oxalic acid excretion as an index of methoxyflurane metabolism, we investigated the effect of pretreatment with phenobarbitone—a known inducer of microsomal enzyme activity—on the putative biotransformation of methoxyflurane to oxalic acid in the rat.

MATERIAL AND METHODS

Forty-four male Sprague-Dawley rats weighing from 180 to 200 g, on a diet of Purina laboratory chow and water ad lib, were divided into 4 groups:

Group I. 10 rats were used as controls.

Group II. 10 rats received phenobarbitone sodium in 0.9% saline (30 mg/ml) at a dose of 75 mg/kg intraperitoneally, daily for 5 days.

Group III. 12 rats were exposed in a large airtight chamber for 2 hours, to methoxyflurane 0.3% in oxygen delivered by a calibrated anaesthesia vaporizer (Vernitrol, Ohio Medical Products).

Group IV. 12 rats received phenobarbitone sodium as in Group II, and were then exposed to methoxyflurane as in Group III.

The rats were then placed in metabolic cages, free of contact with possible environmental enzyme inducers and urine collected for the subsequent two 24-hour periods and the volumes measured.

Sprague-Dawley rats were a strain shown by Mazze and Cousins (1971), to have significant urine volume changes after methoxyflurane. The dose of phenobarbitone sodium, 75 mg/kg, i.p., was in the range previously shown capable of inducing hepatic microsomal enzymes in the rat (Orrhenius and Ernster, 1964; Van Dyke, 1966; Berman et al., 1971). The concentration of methoxyflurane, 0.3% in oxygen, only lightly anaesthetized the animals.

The quantity of oxalic acid in urine was determined by the spectrophotometric method of Zarembski and Hodgkinson (1965). A linear relationship was found between the extinction value on the spectrophotometer and the amount of oxalic acid in the sample, for values from 0 to 100 μg ($r = +0.996$).

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TABLE I

		Urine volume (ml)				Oxalic acid output (μg oxalic acid per mg creatinine)			
		First 24 hours		Second 24 hours		First 24 hours		Second 24 hours	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Group I	Control <i>n</i> =10	11.4	± 4.2	12.5	± 9.0	36.2	± 1.58	54.1	± 17.57
Group II	Phenobarbitone <i>n</i> =10	14.7	± 8.0	13.0	± 8.9	75.8	± 16.83	104.5	± 26.63
Group III	Methoxyflurane <i>n</i> =12	10.6	± 7.9	7.8	± 2.9	152.7	± 12.05	104.7	± 17.54
Group IV	Phenobarbitone and methoxyflurane <i>n</i> =12	20.6	± 8.6	14.4	± 8.2	377.8	± 51.07	236.6	± 30.62
						F=27.04		F=10.84	
						df=3 and 40		df=3 and 40	
						P<0.01		P<0.05	

TABLE II. *P* values for data given in table I (oxalic acid output for first 24 hours).

		Group I (Control)	Group II (Pheno- barbitone)	Group III (Methoxy- flurane)
Group I	Control	—	—	—
Group II	Phenobarbitone	P>0.05 n.s.	—	—
Group III	Methoxyflurane	P<0.01	P>0.05 n.s.	—
Group IV	Phenobarbitone and methoxyflurane	P<0.01	P<0.01	P<0.01

TABLE III. *P* values for data given in table I (oxalic acid output for second 24 hours).

		Group I (Control)	Group II (Pheno- barbitone)	Group III (Methoxy- flurane)
Group I	Control	—	—	—
Group II	Phenobarbitone	P>0.05 n.s.	—	—
Group III	Methoxyflurane	P<0.05 n.s.	P>0.05 n.s.	—
Group IV	Phenobarbitone and methoxyflurane	P<0.01	P<0.01	P<0.01

n.s. = not significant

Total oxalic acid excretion was computed from the concentration and urine volume. Creatinine excretion was determined by Folin's method with spectrophotometric measurement.

RESULTS

Urine volumes in the 4 groups are shown in table I and range from 7.8 ml to 20.6 ml/24 hr.

Oxalic acid excretion is expressed as a function of creatinine output, a constant related to the size of the muscle mass and relatively unaffected by large variations in urine volume (Moore, 1959). Results

are given in table I. Group results were compared by analysis of variance (Snedecor and Cochran, 1967) and the significance of the variance ratio was estimated by the use of F tables and are given in tables II and III.

The control group had mean values of 36.2 and 54.1 μg oxalic acid per mg creatinine for the respective two periods. Group II (phenobarbitone) showed no significant increase in oxalic acid excretion above the control group, mean values being 75.8 and 104.5 μg oxalic acid per mg creatinine. Group III (methoxyflurane) showed a significant increase in

oxalic acid excretion on the first day, the mean value being 152.7 μg oxalic acid per mg creatinine ($P < 0.01$) but there was no significant rise above the control group for the second 24 hours. Group IV (phenobarbitone plus methoxyflurane) excreted up to 10 times as much oxalic acid as the control group, the mean values being 377.8 and 236.6 μg oxalic acid per mg creatinine for the respective 2 periods. These differences are highly significant ($P < 0.01$). Analysis of variance showed there to be an interaction between phenobarbitone and methoxyflurane, highly significant ($P < 0.01$) but short-lived with $P > 0.05$ for the second 24 hours.

DISCUSSION

High output renal failure following methoxyflurane anaesthesia has been attributed to elevated levels of serum inorganic fluoride. Taves and associates (1970) suggested that concentrations of fluoride greater than 100 μM could produce renal changes. Mazze, Trudell and Cousins (1971) found mean peak serum inorganic fluoride concentrations of 190.4 $\mu\text{M}/\text{l}$. in patients showing clinically evident renal dysfunction, as well as increased oxalic acid excretion in all patients anaesthetized with methoxyflurane. Calcium oxalate crystals have been found in the renal substance of patients dying with high output renal failure after methoxyflurane anaesthesia (Paddock, Parker and Guadagni, 1964; Pezzi, Frobese and Greenberg, 1966; Austin and Villandry, 1967; Kuzucu, 1970; Panner et al., 1970; Frascino, Vanamee and Rosen, 1970).

Aufderheide (1971) found extensive renal tubular calcium oxalate crystal deposition, a rare lesion, with unanticipated frequency in his study of patients dying of postanaesthetic, non-oliguric uraemia following methoxyflurane anaesthesia. Hollenberg and associates (1972) described 3 patients who developed extremely prolonged renal failure after exposure to methoxyflurane. Renal biopsy in these patients revealed apparently viable tissue. Abundant crystalline birefringent material resembling oxalate crystals was present in the tubules, in the interstitium and occasionally in the adventitia of vessels. Although the nephrotoxic action of primary oxalosis usually results in anuria or oliguria, it is possible that precipitation of oxalate crystals in the renal parenchyma may be a factor in irreversible renal failure.

Metabolism of methoxyflurane *in vivo* was first reported by Van Dyke, Chenoweth and Van Poznak (1964) using ^{14}C labelled and ^{36}Cl labelled methoxyflurane. They went on to show *in vitro* that this

was accomplished by enzymes present in rat liver microsomes and involved two reactions, namely dechlorination and ether cleavage (Van Dyke and Chenoweth, 1965). Holaday, Rudofsky and Treuhaff (1970) using ^{14}C labelled methoxyflurane substantiated the observation, showing that dechlorination and ether cleavage of methoxyflurane occur in man and noted that if these two reactions occurred in the same molecule, oxalic acid could be another metabolite.

The stimulant effect of barbiturates and other drugs on liver microsomes was first noted by Remmer (1959) and Conney and associates (1960). Van Dyke (1966) using ^{14}C labelled methoxyflurane found that pretreatment of rats with phenobarbitone increased the percentage of the dose of ^{14}C methoxyflurane appearing as urinary metabolites and that phenobarbitone pretreatment increased both ether cleavage and dechlorination of methoxyflurane *in vitro*. Berman and associates (1971) reported that the metabolism of methoxyflurane was markedly enhanced in phenobarbitone treated rats as measured by an increase in concentration of inorganic fluoride in the liver and kidney.

Oxalic acid is normally formed endogenously, its precursors including glycine and ascorbic acid (Hockaday et al., 1964), and barbitone increases ascorbic acid formation through increased metabolism of glucose through the glucuronic acid pathway (Burns et al., 1960). The small increase in oxalic acid output in Group II, given phenobarbitone alone, probably reflects this increase in ascorbic acid metabolism. Our results show that oxalic acid excretion is increased about fourfold in rats given methoxyflurane ($P < 0.01$). When rats were treated with phenobarbitone before exposure to methoxyflurane there was significant interaction between phenobarbitone and methoxyflurane for the first 24 hours. This indicated that pretreatment with phenobarbitone accentuated the conversion of methoxyflurane to oxalic acid but this effect was short-lived. The interaction can be explained by the well-known effect of phenobarbitone in inducing microsomal enzymes. An *in-vitro* study confirming this reaction would be conclusive. With this in mind, liver homogenates from normal and phenobarbitone treated rats with the necessary co-factors were exposed to methoxyflurane 0.3% in oxygen for 1 hour in a metabolic incubator but the assay for oxalic acid proved negative. This result could be attributed to one or all of four possibilities: (i) the degradation to oxalic acid takes longer than the 1 hour time limit available under the experimental conditions;

(ii) only a very small fraction of methoxyflurane undergoes initial ether cleavage as a prelude to oxalic acid formation, i.e., 7–21% (Holaday, Rudofsky and Treuhaft, 1970); (iii) the reaction was inhibited by an excess of methoxyflurane; (iv) the dehalogenation is extrahepatic (Van Dyke 1966).

Renal toxicity due to methoxyflurane is probably a dose-related phenomenon. Important factors would include duration of anaesthesia and the amount of anaesthetic retained (Taves and associates, 1970). When Mazze, Trudell and Cousins (1971) found evidence of renal dysfunction after methoxyflurane, the total dosage of methoxyflurane was large and when they used a lower dosage this group reported no associated renal dysfunction (Cousins, Nishimura and Mazze, 1972). Rosen, Latto and Asscher (1972) found no evidence of renal dysfunction when only small doses of methoxyflurane were used in obstetric analgesia. Holaday, Rudofsky and Treuhaft (1970) found that excretion of inorganic fluoride depended not only on the length of exposure to methoxyflurane, but also that the maximum rates of excretion of the various metabolites were delayed. Part of this delay is thought to reflect a progressive increase in the capacity of the enzymes to metabolize the drug. When low dosage methoxyflurane anaesthesia was used, Cousins, Nishimura and Mazze (1972) found increases of mean serum inorganic fluoride concentrations to $45.7 \pm 4.2 \mu\text{mol/l.}$, not associated with renal dysfunction. However, this level of serum inorganic fluoride and the serum level of other metabolites such as oxalic acid would undoubtedly be exceeded if the rate of metabolism were accelerated. Our study shows that enhancement of the biotransformation of methoxyflurane can be produced by pretreatment with an enzyme inducer such as phenobarbitone.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Leroy D. Vandam, Harvard Medical School and Anesthesiologist-in-Chief, Peter Bent Brigham Hospital, for his helpful advice and criticism in the preparation of this paper and Dr D. R. Waud, Associate Professor, Department of Pharmacology, Harvard Medical School, for his help with the statistical analysis.

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EFFET DU PHENOBARBITAL SUR LA
TRANSFORMATION DU METHOXYFLURANE
EN ACIDE OXALIQUE CHEZ LE RAT

SOMMAIRE

L'effet du phénobarbital sur le métabolisme du méthoxyflurane a été étudié chez le rat. La transformation du méthoxyflurane en acide oxalique a été confirmée. Un traitement préalable par le phénobarbital a entraîné ultérieurement une augmentation de l'élimination d'acide oxalique, ce qui est en faveur d'une accélération de la biotransformation du méthoxyflurane. Au cas où la toxicité rénale du méthoxyflurane proviendrait des produits de son métabolisme, une activité accrue des enzymes microsomiques, consécutive à l'administration préalable de médicaments telles que le phénobarbital, devrait prédisposer à une toxicité accrue.

ÜBER DIE WIRKUNG VON PHENOBARBITONE
AUF DIE UMWANDLUNG VON
METHOXYFLURANE IN OXALSÄURE BEI DER
RATTE

ZUSAMMENFASSUNG

Die Wirkung von Phenobarbitone auf den Abbau von Methoxyflurane bei der Ratte wird untersucht. Die Umwandlung von Methoxyflurane in Oxalsäure wurde bestätigt. Eine Vorbehandlung mit Phenobarbitone ergab einen weiteren Anstieg der Ausscheidung von Oxalsäure. Dadurch wurde eine Beschleunigung in der Biotransformation von Methoxyflurane angezeigt. Wenn die Nephrotoxicität von Methoxyflurane demnach eine Folge seiner Stoffwechselprodukte ist, so würde die gesteigerte mikrosomale Enzymaktivität in Folge vorausgehender Verabreichung von Substanzen die Phenobarbitone zu gesteigerter Toxicität prädisponieren.

EFFECTO DE LA FENOBARBITONA SOBRE EL
METABOLISMO DE METOXIFLURANO EN
ACIDO OXALICO EN LA RATA

RESUMEN

Fue investigado el efecto de fenobarbitona sobre el metabolismo del metoxiflurano en la rata. Fue confirmada la conversión del metoxiflurano en ácido oxálico. El pretratamiento con fenobarbitona resultó en un ulterior incremento de la producción de ácido oxálico, indicando una aceleración de la biotransformación del metoxiflurano. Si la nefrotoxicidad de metoxiflurano es secundaria a los productos de su metabolismo, el incremento de la actividad enzimática microsómica a consecuencia de una exposición previa a medicamentos como la fenobarbitona predispondría a un aumento de la toxicidad.