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Inhibition of monoamine oxidase by furazolidone in germfree rats*

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Furazolidone, a nitrofuran with antimicrobial and antiprotozoic properties, is used in both human and veterinary medicine. The drug inhibits monoamine oxidase [MAO, EC 1.4.3.4; monoamino O_2 oxidoreductase (deaminating)] when administered either to humans [1] or to experimental animals such as the rat [2] and chicken [3]. Since MAO inhibition is not observed *in vitro* [1, 2], it has been suggested that it is not furazolidone but one of its metabolites which is responsible for the inhibition of MAO. Furazolidone is degraded *in vitro* by various rat tissues [4], but the metabolite putatively responsible for MAO inhibition has not been characterized. Because pretreatment with oxytetracycline prevents some of the effects of MAO inhibition by furazolidone, it was suggested that the formation of the inhibitory metabolite might occur in the intestinal flora [5]. This suggestion was supported by the observation that MAO inhibition due to orally administered furazolidone was diminished by prior treatment with neomycin [3]. As antibiotics may have effects other than suppression of the intestinal flora [6], we studied the effect of furazolidone on MAO activity in germfree rats. In this communication, we show that furazolidone has the same effect on MAO activity in both germfree and conventional rats and, therefore, that the flora can be excluded as the site for the formation of a furazolidone metabolite which is inhibitory for MAO.

Materials and methods

Furazolidone was a gift from the Eaton–Norwich Pharmaceutical Co. (Norwich, NY). Male germfree rats (weighing 180–350 g) and conventional rats (weighing 215–260 g) of the Sprague–Dawley strain (Charles River Breeding Laboratories, Inc., Wilmington, MA) were used throughout these experiments. Husbandry for germfree rats was as described previously [7] except that the diet was sterile rat chow (Charles River Breeding Laboratories).

Furazolidone was prepared as a suspension in 0.75% aqueous carboxymethyl cellulose for administration to conventional rats. For germfree rats it was suspended in 1% Tween 80 and autoclaved for 15 min at 121°. A single dose of furazolidone (50 mg/kg) was administered by gavage. Control animals received only the appropriate vehicle. After 16 hr the animals were killed by cervical dislocation and the brains and livers were removed immediately. These organs were homogenized in 5 vol. (w/v) of 20 mM Tris buffer (pH 7.4) containing 1.15% KCl at 0–4°. MAO activity was determined using radiolabeled tryptamine by a modification of the method of Wurtman and Axelrod [8]. Briefly, 25 μl of brain or liver homogenate containing 100–500 μg protein was incubated at 37° with 250 μl of 0.5 M Tris–HCl buffer (pH 7.4) and 25 μl of 10 μM [2-^{14}C]tryptamine bisuccinate (New England Nuclear Corp., Boston, MA; sp. act. 51.5 mCi/mmole) containing approximately 1000 cpm/μl. The reaction was stopped after 10 min by the addition of 0.2 ml of 2 N HCl. The deaminated product was extracted with 6 ml toluene, and the solution was clarified by centrifugation. A 4-ml aliquot of toluene was then assayed for radioactivity in 10 ml Aquasol (New England Nuclear Corp.). A correction was made for the small amount of [^{14}C]tryptamine which was also extracted into the toluene phase. This correction (about 5 per cent) was determined from a control which lacked tissue homogenate. Protein was determined by the method of Lowry *et al.* [9]

Results and discussion

Sixteen hours after a single oral dose (50 mg/kg) of furazolidone, the MAO activity was diminished significantly in both liver and brain of either conventional or germfree rats (Table 1). The decrease in MAO activity in conventional rats (25 per cent in brain and 67 per cent in liver) was similar to that observed in germfree rats (35 per cent in brain and 50 per cent in liver). How this finding relates to the postulated A and B forms of MAO [10] is unclear.

Table 1. Effect of furazolidone on MAO activity in brain and liver from conventional and germfree rats

	MAO activity* [pmoles products formed · min^{-1} · (mg protein)$^{-1}$]			
	Brain		Liver	
	Conventional	Germfree	Conventional	Germfree
Control	4.9 ± 0.6 (4)	5.6 ± 0.6 (6)	30.5 ± 7.2 (4)	21.2 ± 3.8 (6)
Furazolidone	3.7 ± 0.4† (4)	3.4 ± 0.4† (6)	10.2 ± 2.6† (4)	9.9 ± 3.0† (6)

* MAO activity in brain or liver homogenate was assayed with [2-^{14}C]tryptamine as described in Materials and Methods. Values are means ± S.D.; the number of animals used is in parentheses.
† Significantly different from control, $P < 0.05$.

These results with germfree rats exclude the flora as a site for the formation of a furazolidone metabolite responsible for the inhibition of MAO. This site of metabolite formation had been suggested by the finding that MAO inhibition by furazolidone was diminished when animals were pretreated with antibiotics [3, 5]. Antibiotics may have other pharmacokinetic effects, however, as they may decrease the absorption of other drugs, either by binding them within the gut or by increasing their intestinal transit times [6]. As these effects of antibiotics may change the

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access of a drug to sites of mammalian metabolism, experiments with germfree rats are a more dependable means to decide whether the flora is involved in reactions found in the conventional animal. Our results with germfree rats indicate that the metabolite of furazolidone which is inhibitory to MAO is of mammalian origin. In clarifying the origin of the inhibitory metabolite, attention should be given to the observation that this effect of furazolidone is potentiated in animals pretreated with the drug-metabolizing enzyme inducer 2-bis-(*p*-chlorophenyl)ethane (DDT) [5].

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