J. Pharm. Pharmacol. 1985, 37: 75–77
Communicated May 6, 1984

In-vivo metabolism in the rat and mouse of antrafenine to 1-*m*-trifluoromethylphenylpiperazine

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The analgesic antrafenine forms 1-*m*-trifluoromethylphenylpiperazine (mCF_3PP) during its biotransformation in the rat and mouse. At least 14 and 3% of an antrafenine dose (25 mg kg^{-1} p.o.) reaches the systemic circulation as mCF_3PP in the mouse and rat respectively. The metabolite easily enters the brain, reaching concentrations several times those in body fluids. This, together with the fact that mCF_3PP is known to produce several pharmacological effects compatible with a stimulatory action on 5-hydroxytryptamine postsynaptic receptors, suggests that this metabolite may contribute to the parent drug's pharmacological effects.

1-Aryl-piperazine formation is now known to be a common metabolic process for drugs with the aryl-piperazine moiety in the side-chain of their molecule (Caccia et al 1984). These metabolites are biologically active (Fuller et al 1980, 1981a; Maj & Lewandowska 1980; Rokosz-Pelc et al 1980) and their formation may therefore be a pharmacologically significant pathway for drugs undergoing extensive cleavage of the aryl-piperazine side-chain (Cervo et al 1981; Caccia et al 1982, 1983; Fong et al 1982).

Antrafenine, 2-[4-(3-trifluoromethylphenyl)-piperazine]ethyl-2-(7-trifluoromethyl-4-quinolyamino) benzoate (I), is an ester type non-narcotic analgesic, anti-inflammatory drug (Manoury et al 1979) which has not yet been studied from this metabolic point of view. It is reported to be efficiently absorbed after oral administration to man and animals and eliminated almost completely by biotransformation. The main metabolites are the acid and alcohol hydrolysis products, each of which retains analgesic activity. Other metabolites, formed in both man and animals, have not yet been identified (Dring et al 1978; Rovei et al 1977; Guinebault et al 1981).

In the present study the formation of 1-*m*-trifluoromethylphenylpiperazine (II, mCF_3PP) was investigated in antrafenine treated rats and mice. mCF_3PP, known to be a potent displacer of [^{3}H]5-HT binding to brain membranes (Fuller et al 1980) and to produce a number of behavioural and biochemical effects compatible with a stimulatory action on central 5-HT postsynaptic receptors (Fuller et al 1978; Fuller & Mason 1981) is formed in both animal species. To obtain preliminary information on the role of mCF_3PP in the effect of its parent drug, its disposition was investigated after oral antrafenine and intravenous mCF_3PP to rats and mice.

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Material and methods

Male CD-COBS mice, ca 25 g, and male CD-COBS rats, 200 g (Charles River, Italy) were treated orally with antrafenine (25 mg kg^{-1} or about 45 μmol^{-1}) or intravenously with mCF_3PP dihydrochloride (10 μmol kg^{-1} in terms of free bases) and were killed at various times after. Blood samples were collected in heparinized tubes, centrifuged and the plasma was stored at −20 °C. Tissues were removed immediately and stored at −20 °C. Urine was collected separately for 24 h in metabolic cages.

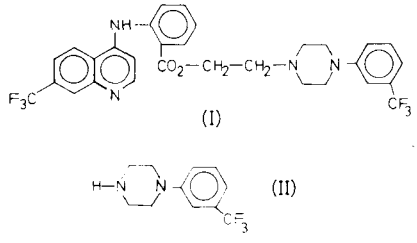
mCF_3PP was extracted from body fluids and tissues and detected by electron capture-gas liquid chromatography as previously described (Caccia et al 1984).

Distribution between red cells and plasma was assessed by incubating 2 ml of blood, with a predetermined haematocrit, for 30 min in a 37 °C water-bath with 10 μl of a 100 $\mu g\ ml^{-1}$ solution of mCF_3PP in 0·9% NaCl (saline). After centrifugation 0·5 ml of plasma was assayed for mCF_3PP.

The terminal elimination rate constant (β) was determined for each plasma or brain concentration time curve by non-linear least squares computer fit to either a mono- or a bi-exponential equation. The areas under the plasma or brain concentration-time curves (AUC) were calculated by the trapezoidal rule to the last measured concentration and then extrapolated to infinity. Half-life ($T_{\frac{1}{2}}$) and total body clearance (Clp) were determined by the usual equations. Estimates of steady-state volume of distribution (Vss) were calculated according to Benet & Galeazzi (1979).

Results and discussion

Analysis of body fluids and tissues of antrafenine treated rats and mice showed that biotransformation of



(I) antrafenine and (II) 1-*m*-trifluoromethylphenylpiperazine.

the drug in both species included the formation of mCF_3PP. Gas chromatography-mass spectrometry confirmed the presence of the metabolite in body fluids and tissues. The mass spectrum of mCF_3PP heptafluorobutyrate has been reported recently (Caccia et al 1984).

The plasma and brain concentration-time curves of mCF_3PP in mice and rats given oral antrafenine (25 mg kg^{-1}) are shown in Fig. 1. The concentration data represent the mean of 4 animals. In these experimental conditions peak plasma concentrations (C_{max}) of the metabolite were reached within 60 min in both species but values were about 5 times higher in mice (0·23 ± 0·05 nmol ml^{-1}) than in rats (0·04 ± 0·06 nmol ml^{-1}). Furthermore, as the metabolite persisted apparently longer in mice ($T_{\frac{1}{2}}$ 173 min) than in rats ($T_{\frac{1}{2}}$ not determinable because the plasma concentrations were below the sensitivity of the method) the plasma AUC of mCF_3PP in antrafenine-treated mice (57 nmol ml^{-1} × min) was about 10 times that in rats (5·6 mmol ml^{-1} × min). The whole-blood-to-plasma concentration ratio of mCF_3PP, however, was 2·6 in the mouse and 4·8 in the rat, implying a different distribution of the metabolite within the blood components of these species. Assuming that the haematocrit averaged 0·44 ± 0·01 in the mouse and 0·42 ± 0·01 in the rat, about 78 and 88% respectively of the metabolite blood concentrations would be found in the red cells of mice and rats. This may be related to species variations in plasma protein binding and/or affinity for red cell components of mCF_3PP. Thus, plasma AUC of the metabolite would be about 38% of the whole blood AUC in the mouse but only 21% in the rat.

Like other 1-aryl-piperazines (Caccia et al 1984), mCF_3PP was highly concentrated in the central nervous system, the brain-to blood concentration ratios being approximately 13–15 at the time of C_{max}. The time-course of the metabolite brain concentrations was almost parallel to plasma. Thus, the brain-to-blood AUC ratios were of the same order as those calculated on the C_{max} values (Table 1). Tissue distribution studies indicated that the metabolite also concentrated in lung, fat, liver, kidney and heart with tissue-to-blood ratios, from 40–50 (lung) to 6–8 (kidney, heart) 30 and 120 min after oral antrafenine in both species. Similar values were observed after intravenous injection of the metabolite (data not shown).

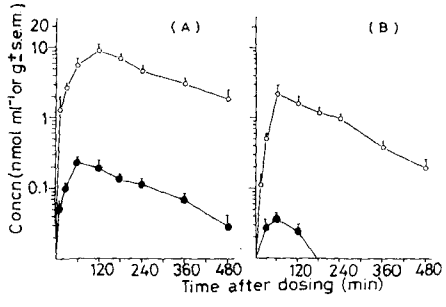


FIG. 1. Plasma (●) and brain (○)-time curves of 1-*m*-trifluoromethylphenyl-piperazine after oral antrafenine (45 μmol kg^{-1}) to mice (A) and rats (B).

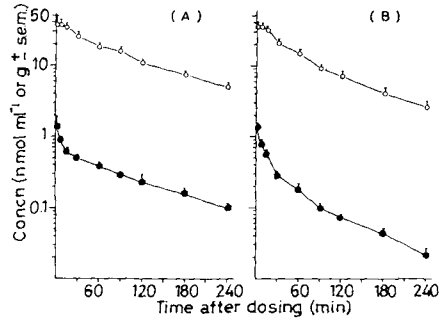


FIG. 2. Plasma (●) and brain (○)-time curves of 1-*m*-trifluoromethylphenylpiperazine after intravenous injection (10 μmol kg^{-1}) to mice (A) and rats (B).

These findings could be attributed to a number of different mechanisms including accumulation of mCF_3PP in fat components of the cells, tissue binding and active transport of the compound across cell membranes. Although mCF_3PP (pK_a = 8·7) would be largely in the ionized form at physiological pH, it is highly lipophylic (log P 2·43 as measured by buffer–octanol partition). Furthermore, active transport and tissue binding cannot be ruled out.

mCF_3PP was present in the 24 h urine of antrafenine treated rats and mice as a minor metabolite accounting for only trace amounts of the administered drug dose (25 mg kg^{-1} p.o.). Small amounts of mCF_3PP (less than 0·5% of the administered dose) were detected in the 24 h urine of both species intravenously injected with mCF_3PP (10 μmol kg^{-1}) indicating that the metabolite is almost entirely eliminated by biotransformation. In

Table 1. Kinetic parameters of 1-*m*-trifluoromethylphenylpiperazine (mCF_3PP) after oral antrafenine (45 μmol kg^{-1}) or intravenous mCF_3PP (10 μmol kg^{-1}) to mice and rats.

Parameter	Mouse	Rat
Plasma area under the curve (nmoles ml^{-1} × min)	86 (57)	40 (5·6)
Volume of distribution (litre kg^{-1})	14·2	16·2
Whole blood-to-plasma concentration ratio	2·6 ± 0·6	4·8 ± 0·4
Total blood clearance (ml min^{-1} kg^{-1})	45	52
Plasma half-life (min)	90 (165)	66 (N.D.)
Brain half-life	89 (144)	61 (130)
Brain area under the curve (nmol g^{-1} × min)	4160 (2320)	2826 (445)

Area under the curve (AUC) and half-lives of mCF_3PP after oral antrafenine to rats and mice are shown in parentheses.

AUC were calculated by the trapezoidal rule and extrapolated to infinity by using the β values of the i.v. experiments.

these experimental conditions the plasma concentrations of unmetabolized mCF_3PP (Fig. 2) showed a biphasic decline with an initial phase lasting 15–30 min followed by a second, slower phase with $T_{\frac{1}{2}}$ values of 90 and 66 min respectively in the mouse and rat (Fig. 2A, B), both shorter than those estimated in antrafenine experiments. Other kinetic parameters of mCF_3PP are summarized in Table 1. In agreement with findings that mCF_3PP has high affinity for tissues, V_{ss} greatly exceeded the total body water volume for these species. The total Clp values for mCF_3PP in the mouse (117 ml min^{-1} kg^{-1}) and rat (249 ml min^{-1} kg^{-1}) exceeded the known hepatic blood flow in these species (40–80 ml min^{-1} kg^{-1}).

Considering that mCF_3PP concentrates in the red cells, however, the values expressed on the basis of whole blood concentrations (Clb) would be about 60 and 80% smaller than the CLp values respectively in the mouse and rat and are thus approximate to the hepatic blood flow in these species. The relative large hepatic Clb in relation to the hepatic blood flow suggests that hepatic extraction of mCF_3PP is high.

As noted in the antrafenine experiment, mCF_3PP easily entered the brain, achieving C_{max} within 1–5 min of parenteral injection (Fig. 2). Then brain concentrations declined bi-exponentially as in plasma but at much higher concentrations. As in the antrafenine experiment, brain concentrations of mCF_3PP exceeded those in body fluids, expressed as AUC (Table 1).

The ratios of the AUC values of the metabolite after oral antrafenine to those after i.v. injection of mCF_3PP, calculated from plasma AUC values normalized to an equimolar dose were about 0·15 in the mouse and 0·03 in the rat. Therefore, in these experimental conditions the fraction of the antrafenine dose available to the general circulation as mCF_3PP was respectively 15 and 3% in the mouse and rat. This suggests that after pharmacologically effective doses of antrafenine, mCF_3PP formation may be significant process in the mouse and less important in the rat. These values, however, may be largely underestimated because they do not take account of first-pass metabolism of the metabolite when formed in-situ. This effect probably occurs since the Clb of mCF_3PP in both animal species is extensive. Investigation in animals have demonstrated that 1-aryl-piperazines such as 1-m-chlorophenyl-piperazine, quipazine and MK-212 (6-chloro-2-[1-piperazinyl]-pyrazine have analgesic activity in the hot plate test (Samanin et al 1976; Rochat et al 1982; Murray et al 1983) besides a variety of other pharmacological effects compatible with a stimulatory action on 5-HT receptors (Rodriguez et al 1973; Green et al 1976; Samanin et al 1977, 1979; Fuller et al 1981a). mCF_3PP produces some of the same effects of its structurally related compounds but is more active than its analogues in competing with [^{3}H]5-HT for binding to brain membrane receptors (Fuller et al 1978, 1981; Fuller & Mason 1981). It is thus likely that mCF_3PP is also active as an analgesic and that it contributes to the parent drug's pharmacological effects, at least in species which form significant amounts of the metabolite.

We thank L.E.R.S. Synthelabo for gifts of antrafenine. This work was supported by CNR, Rome, Italy, contract no. 83.02896.04.

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