METABOLIC FATE OF FCE 22716, A NEW ANTIHYPERTENSIVE AGENT, AND OF ITS N-OXIDE (FCE 24220) IN THE RAT

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SUMMARY

[^{3}H]-FCE 22716 and [^{3}H]-FCE 24220 were given both orally and intravenously to the rat. Radioactivity was mainly eliminated by the faecal route after oral administration in both cases. After intravenous administration, renal excretion was twice the faecal one in the case of FCE 22716, whereas for FCE 24220 the two routes were equal.

In urine FCE 22716 was eliminated almost completely unchanged after both oral and intravenous administration. FCE 24220 was extensively reduced to FCE 22716 after oral administration, whereas after intravenous treatment, this reduction, although important, was not complete.

KEY WORDS

FCE 22716, FCE 24220, N-oxides, absorption, excretion, metabolism, rat

INTRODUCTION

FCE 22716, 6-methyl-8β-(2,4-dioxo-1-imidazolidinylmethyl)-ergoline (Fig. 1), is a new ergoline derivative which has been shown to induce a dose-related fall in arterial blood pressure lasting for more than 6 h, both after acute and chronic oral administration in the rat (1-20 mg/kg) /1/.

FCE 22716 has a multitarget mechanism of action mainly involving blockade of α_1-adrenoreceptors and S_2-receptors. This drug appears to be the first ergoline selectively acting upon the cardiovascular system, without most of the side effects of these compounds /1/.

It is known that N-oxide derivatives are reduced back to the parent compound in several species, including the rat /2, 3/. The N-oxide FCE 24220 (Fig. 1), therefore, might be considered as a pro-drug of FCE 22716, as already demonstrated for amitriptyline N-oxide /4, 5/.

This paper reports on the excretion and metabolism in the rat of FCE 22716 in comparison with its N-oxide FCE 24220, in order to determine whether FCE 24220 can be reduced and FCE 22716 can be metabolized to its N-oxide derivative.

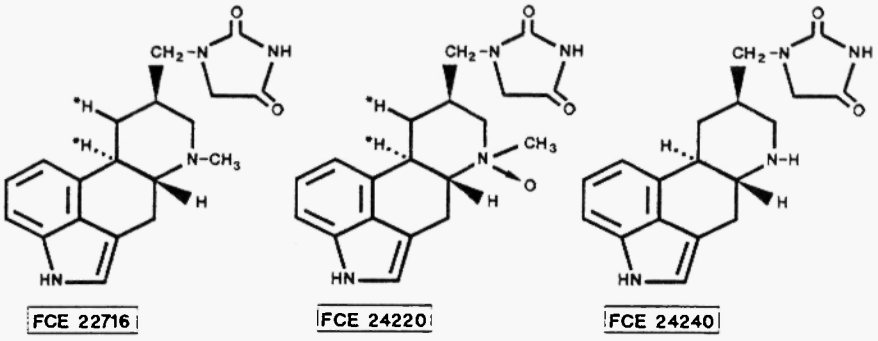

Fig. 1: Structures and labelling position (*) of [^{3}H]-FCE 22716, [^{3}H]-FCE 24220 and FCE 24240.

MATERIALS AND METHODS

Radioactive compounds and chemicals

[^{3}H]-FCE 22716 and the corresponding N-oxide ([^{3}H]-FCE 24220) were synthesized in the Radioisotope Laboratories of Farmitalia Carlo Erba (Nerviano, Italy). [^{3}H]-FCE 22716 was prepared via condensation of [9,10-^{3}H]dihydrolisergamine with 2-bromoacetic acid ethyl ester and successive imidazolidindione formation with potassium cyanate according to the method described for the unlabelled FCE 22716 by Mantegani *et al.* /6/. The purification of the final crude by TLC on silica gel F254 Merck plate (10x20 cm, 0.5 mm thick) using chloroform:methanol:30% ammonium hydroxide 90:15:3 by volume as elution solvent system, gave [^{3}H]-FCE 22716, 98% radiochemically pure, with a specific radioactivity of 8.32 GBq/mmol (24.58 MBq/mg) and 10% radiochemical yield. [^{3}H]-FCE 24220 was prepared via oxidation of [^{3}H]-FCE 22716 with 36% hydrogen peroxide solution. The compound was obtained as white precipitate, 97% radiochemically pure, with a specific radioactivity of 20.72 MBq/mmol (58.46 KBq/mg) and 50% radiochemical yield.

FCE 24240 was synthesized in the Chemistry Laboratories of Farmitalia Carlo Erba (Milan). [9,10-^{3}H]Dihydrolisergamine was supplied by Amersham Int. plc (UK).

Reagents and solvents were of analytical grade and obtained from Farmitalia Carlo Erba.

Animal studies

Three non-starved male Sprague Dawley rats (body weight 200-215 g) from Charles River Italia (Calco, Como, Italy) were treated with an oral dose of 5 mg/kg [^{3}H]-FCE 22716. The drug was suspended in 0.5% Methocel A-4C Premium (Dow Chemicals) and administered by gastric gavage.

A second group of three non-starved male Sprague Dawley rats (body weight 230-240 g) was treated with an i.v. dose of 5 mg/kg [^{3}H]-FCE 22716, dissolved as methanesulphonate salt in an isotonic saline solution and injected into the caudal vein.

Four non-starved male Sprague Dawley rats (body weight 190-230 g) were treated with an oral dose of 20 mg/kg [^{3}H]-FCE 24220, suspended in 0.5% Methocel.

An additional group of four non-starved male Sprague Dawley rats (body weight 230-235 g) was treated with an i.v. dose of 10 mg/kg [^{3}H]-FCE 24220, dissolved as methanesulphonate salt in saline solution.

All rats were housed individually in cages suitable for separate collection of urine and faeces. Animals had free access to food and water throughout the experiment.

Urine was collected at 6, 24, 48, 72 and 96 h in ice-cooled containers; faeces were collected daily for four days at room temperature. The metabolic cages were washed with a proper amount of water every 24 h. All the samples were stored at -20°C until assayed.

Quantitative determination of radioactivity

Aliquots of urine (0.2 to 1 ml) and of cage washings (4 ml) were mixed with 20 ml of Rialuma scintillation cocktail (Lumac B.V., Holland). Faeces were lyophilized, ground and successively analyzed by combustion technique (Oxidizer, model 306 Packard).

Determination of ^{3}H was made by liquid scintillation counting in a 300C Packard Spectrometer.

Separation and identification of urinary metabolites

Urine samples (0-6 and 6-24 h) of each animal were separately percolated through Amberlite XAD2 resin columns with a volume ratio resin/urine of about 0.40. The columns were washed with water, then the urinary organic products retained on the column were eluted with methanol.

Each organic eluate was evaporated to small volumes and chromatographed on TLC plates of silica gel F254 (0.5 mm thick, Merck, Darmstadt, FRG), in solvent systems (a): chloroform:methanol:30% ammonium hydroxide (80:20:1.5 by volume) and (b): chloroform:methanol:30% ammonium hydroxide (65:30:5 by volume). The compounds were observed under UV light at 254 nm and the indol ring was further revealed by spraying the plates with Ehrlich's reagent (solution of 1.5 g *p*-dimethylaminobenzaldehyde in a mixture of 75 ml methanol and 25 ml 37% hydrochloric acid) /7/.

The ^{3}H-labelled metabolites were detected using a radiochromatogram scanner (Berthold, LB282 TLC Analyzer interfaced with an Apple IIe Grappler plus personal computer) and identified

by comparison with standard reference compounds. Some methanol eluates of urine obtained after administration of FCE 24220 were also analyzed by HPLC. These analyses were performed on a Perkin Elmer Series 400 liquid chromatograph using a reverse phase μBondapak C18 Waters (10 μm) column, 30 cm x 3.9 mm int. diam., with a Corasil C18 Waters (35-70 μm) precolumn, 4.5 cm x 3.9 mm int. diam. and a mobile phase of acetonitrile (A) and KH_2PO_4 buffer 0.05 M, pH 7.4 (B) used isocratically with an A:B ratio of 30:70 by volume at a flow rate of 0.5 ml/min. Detection was performed by a combination of UV absorbance at 280 nm (Perkin Elmer LC 75 UV/VIS spectrophotometric detector) and radioactivity measurement (Packard TRACE 7150 interfaced with an IBM 3270 personal computer).

Stability of [^{3}H]-FCE 22716 in rat urine and of [^{3}H]-FCE 24220 in water and rat urine

[^{3}H]-FCE 22716 was dissolved in rat urine at a concentration of 1 μg/ml. [^{3}H]-FCE 24220 was dissolved in water and rat urine at a concentration of 100 μg/ml. The resulting solutions were divided into small aliquots, which were incubated at 37°C in closed vessels. Each aliquot was used for one analysis only.

Stability was checked at 0, 6 and 24 h by TLC (solvent system (a) for FCE 22716 and system (b) for FCE 24220), after processing the samples in the same way as the urine of the treated animals.

Mass spectrometry

Mass spectra in field desorption (FD) of FCE 22716 were carried out on a Varian MAT 311-A mass spectrometer equipped with a combined FI/FD/EI ion source, using benzonitrile-activate emitters. The total potential difference between the field emitter anode and the cathode was about 9KV; the emitter heating current was in the range 16-25 mA and the source temp. was 150°C.

RESULTS

Excretion of radioactivity

The urinary and faecal excretion of radioactivity after oral and intravenous administration of [^{3}H]-FCE 22716 and [^{3}H]-FCE 24220 to the rat is shown in Table 1.

After oral treatment, radioactivity was predominantly excreted in faeces, amounting to 53% of the dose at 96 h in the case of FCE 22716 and to 67% at 72 h for FCE 24220. Urinary excretion accounted for 29% and 24%, respectively, at the same times.

In contrast, after intravenous administration, the route of elimination was mainly the renal one for FCE 22716; the radioactivity recovered in urine was 61% of the dose at 96 h. For FCE 24220, the radioactivity recovered in urine was 46% of the dose at 72 h. Faecal excretion accounted for 33% and 48%, respectively, at the same times. After both oral and intravenous treatment, radioactivity elimination was almost complete within the first day for both drugs.

Urinary metabolic patterns

The urinary metabolites of FCE 22716 in each organic eluate from XAD2 resin were separated by TLC in solvent system (a), whereas metabolites of FCE 24220 were separated by TLC in system (b). Figure 2 shows typical radiochromatograms of the 0-6 h urine after oral and intravenous treatment with FCE 22716 and FCE 24220.

Unchanged FCE 22716 was virtually the only compound detected in rat urine after both oral and intravenous treatment with FCE 22716. Its amount was greater than 92% of the radioactivity recovered in the 0-24 h urine after both administration routes. The remaining radioactivity was not attributable to any definite peak, but to a dragging on the plate. Traces of radioactivity (Polar) were present at $Rf = 0$. The unchanged drug was identified both by comparison with the chromatographic behaviour of the reference compound (Table 2) and by mass spectrometry in field desorption. The mass spectra of a 0-6 h methanol eluate showed an intensive molecular ion at m/z 338 similar to that of authentic FCE 22716.

After oral administration of FCE 24220, the unchanged drug accounted for only 7% of the radioactivity excreted in the 0-24 h urine, the main metabolite being FCE 22716 (87% of the urinary

TABLE 1

Recovery of radioactivity after oral and i.v. administration of [^{3}H]-FCE 22716 and [^{3}H]-FCE 24220 to male Sprague Dawley rats. Data are expressed as mean cumulative percent of dose (SD, n=x).

Drug	**FCE 22716**				**FCE 24220**			
Dose (mg/kg)	**5**		**5**		**20**		**10**	
Route of administration	**os**		**i.v.**		**os**		**i.v.**	
n	**3**		**3**		**4**		**4**	
Time (h)	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
6	10.07[a]		43.41 (3.41)		5.06 (1.73)		17.92 (3.29)[b]	
24	27.35 (4.29)	46.77 (13.66)	58.77 (3.37)	30.61 (2.81)	22.12 (4.55)	60.44 (16.74)	37.27 (0.70)	42.51 (2.35)
48	28.66 (3.36)	51.40 (15.01)	60.06 (2.93)	32.22 (3.28)	23.23 (4.83)	66.76 (15.27)	44.42 (1.57)	47.37 (1.05)
72	29.11 (2.89)	52.35 (14.03)	60.35 (2.92)	32.44 (3.25)	23.52 (5.01)	67.13 (15.14)	45.70 (1.68)	48.03 (0.95)
96	29.24 (2.80)	52.76 (13.57)	60.58 (2.99)	32.57 (3.22)				
Cage washing (0-96, or 0-72 h)	4.21 (3.12)		3.47 (1.22)		2.09 (1.07)		1.16 (0.24)	
Total recovery	86.21 (12.53)		96.62 (0.30)		92.74 (11.94)		94.89 (1.90)	

a) n=2
b) n=3

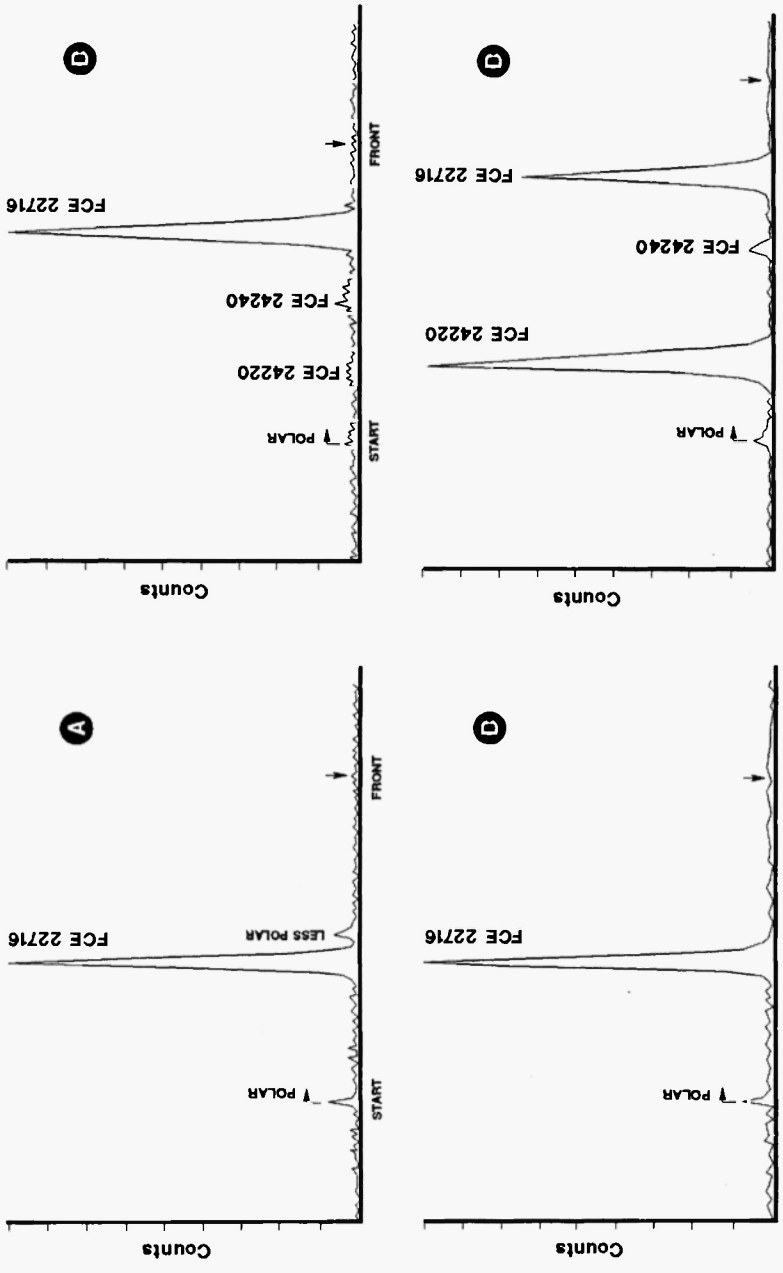

Fig. 2: Radio-TLC profile of methanolic XAD2 resine eluate of 0-6 h rat urine after oral (A, C) and intravenous (B, D) administration of [^{3}H]-FCE 22716 and [^{3}H]-FCE 24220, respectively.

radioactivity). The 6-demethyl derivative of FCE 22716 (FCE 24240; Fig. 1), was also found and accounted for 3% of the 0-24 h urinary radioactivity. Traces of radioactivity (Polar) were present at Rf = 0.

In contrast, after intravenous administration, unchanged FCE 24220 and FCE 22716 amounted to 52% and 32% of the 0-24 h urinary radioactivity, respectively. Traces of FCE 24240 and Polar were also detected after intravenous administration. These metabolites were identified by comparison with the reference compounds both by TLC and radio-HPLC (Table 2).

TABLE 2
Chromatographic behaviour of FCE 22716, FCE 24220 and FCE 24240

Compounds	TLC (Rf values in system)		HPLC (retention time, min)
	(a)	(b)	
FCE 22716	0.46	0.66	18.4
FCE 24220	0.06	0.20	10.3
FCE 24240	0.21	0.48	14.2

Table 3 reports the metabolites detected after oral and intravenous treatment with FCE 22716 and FCE 24220, expressed as mean percent of dose.

DISCUSSION

After oral administration of FCE 22716 and FCE 24220, radioactivity is predominantly eliminated by the faecal route, with a urine:faeces ratio of 1:1.8 and 1:2.8, respectively, at 72 h. Conversely, after intravenous administration of FCE 22716, renal elimination is about twice the faecal one, whereas after FCE 24220 the two routes are equally important. Statistical analysis shows no significant differences in urinary and faecal elimination between the two drugs after oral treatment. Conversely, there are statistically significant differences ($p < 0.01$, Student's t-test for impaired data) in the urinary and faecal elimination of radioactivity between the two compounds after

TABLE 3

Metabolites found by radio-TLC in 0-6 and 0-24 h rat urine following oral and i.v. administration of [^{3}H]-FCE 22716 and [^{3}H]-FCE 24220. Data are expressed as mean percent of dose.

Time	Drug	Route of administration	Metabolites					
			NA	Polar	FCE 24220	FCE 24240	FCE 22716	Less polar
0-6	FCE 22716	os	0 13	0.57	–	–	9.15	0 22
	FCE 22716	iv	0.14	2.16	–	–	40.87	0.24
	FCE 24220	os	0 25	0 15	0 86	0 15	3.65	–
	FCE 24220	iv	2.14	0.10	9 77	0.35	4 95	–
0-24	FCE 22716	os	0 31	1.03	–	–	25 35	0.66
	FCE 22716	iv	0 24	3.14	–	–	54 95	0.44
	FCE 24220	os	0.44	0 28	1.45	0.59	19 26	–
	FCE 24220	iv	3.46	1.53	19.29	0 90	12 15	–

NA = aqueous eluate from Amberlite XAD2 column

intravenous administration. The comparison between urinary excretion data after oral and intravenous dosing seems to indicate that about 50% of both compounds are absorbed after oral administration. Indeed, absorption must be much higher, if one takes into account the high faecal excretion observed after i.v. administration of both compounds.

After both oral and intravenous administration, FCE 22716 was found mostly unchanged in urine. This could be explained either in terms of lack of metabolism or of 'metabolic cycling', that is formation of the N-oxide derivative, rapidly followed by reduction to the parent drug.

Jaworsky *et al.* /8/, in a metabolic study of chlorpromazine N-oxide in man and dogs, found the presence of unchanged drug as well as the reduced derivative in these two species, whereas in the rat, only the reduced form was present. As dogs and man were found to be capable of oxidising chlorpromazine to its N-oxide derivative, the authors hypothesize a 'metabolic cycling' in these two species.

A clear example of regeneration of the parent drug from a metabolite has been reported by Al-Waiz *et al.* for trimethylamine N-oxide /9/.

After administration of FCE 24220, the main metabolic pathway is reduction of the N-oxide group to give the parent compound FCE 22716. This reduction is particularly extensive after oral administration. In fact only small quantities of unchanged drug are detected, whereas after intravenous dosing the ratio FCE 24220:FCE 22716 is 1:0.6.

This difference in the metabolism of FCE 24220 after the two administration routes could be ascribed to reduction of the N-oxide by the gut flora or to reduction by intestinal and/or liver enzymes during absorption, resulting in a substantial 'first-pass effect'.

Studies performed *in vivo* with indicine N-oxide /10/ show that also indicine can be detected in urine after both oral and intravenous administration. Both the liver and gut flora are responsible for the reduction of indicine N-oxide, although the gut flora appears to play the main role in the formation of indicine.

The involvement of liver and/or other tissues in the *in vivo* reduction of N-oxides has also been reported by Dajani *et al.* /3/ in a study on the reduction of nicotine N-oxide in the rat. It is well known that tertiary amine N-oxides can be reduced to the corresponding tertiary

amines by liver microsomal cytochrome P-450 under anaerobic conditions /11/. Low activities of N-oxide reductase have been found in microsomes from kidney and lung, and still lower in intestine, brain and heart /12/. Tertiary amine N-oxides can also be reduced by liver mitochondrial fractions to the corresponding amines, but the activity in mitochondria is less than that in microsomes /12/. Kitamura and Tatsumi /13/ report that aldehyde oxidase in cytosol acts as a major enzyme responsible for the reduction of tertiary amine N-oxides in mammalian liver. Further work is necessary to determine the relative importance of gut flora and the different enzyme systems in the reduction of FCE 24220.

The possibility of a chemical instability of FCE 24220 producing FCE 22716 can be excluded because the drug is stable both in water and rat urine at 37°C even after incubation for several hours.

A minor metabolic pathway of FCE 24220 is N-dealkylation with formation of the N-demethyl derivative FCE 24240, which was not detected after administration of FCE 22716.

The presence of both FCE 24220 and FCE 22716 after administration of FCE 24220 and the presence of only FCE 22716 after its administration seems to rule out the hypothesis of 'metabolic cycling' after administration of FCE 22716 but suggests rather a lack of metabolism of FCE 22716 in the rat.

It would be interesting to study whether or not FCE 22716 can be metabolized *in vitro* to its N-oxide.

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