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Lack of stereoselectivity in the pharmacokinetics and metabolism of the radiosensitizer Ro 03-8799 in man

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Summary. During a clinical toxicity study it was possible to obtain urine samples from six patients receiving either the R-(–)- or S-(+)-stereoenantiomeric forms of the developmental 2-nitroimidazole radiosensitizer Ro 03-8799 (pimonidazole). Paired plasma samples were also obtained from four patients. The pharmacokinetic data were compared with those for the racemic mixture in the same individuals. The results revealed no major differences in the plasma pharmacokinetics, urinary clearance or *N*-oxidation of the individual enantiomers as compared with the racemic mixture. A similar lack of stereoselectivity with respect to the acute dose-limiting CNS toxicity syndrome suggests that this may not involve a specific CNS receptor interaction.

Introduction

Ro 03-88799, α-[(2-nitro-1-imidazolyl)methyl]-1-piperidine-ethanol hydrochloride (pimonidazole, Roche Products), is a developmental hypoxic cell radiosensitizer that has undergone clinical evaluation both alone [13, 14] and in combination with SR 2508 (etanidazole) [1, 9, 10]. As compared with the original lead 2-nitroimidazole misonidazole, Ro 03-8799 has a higher electron affinity [17], is extensively protonated rather than being uncharged at physiological pH (pKa 8.7) [17] and exhibits an ability to accumulate in cells and tissues [1–4, 9, 10]. These features explain the improved radiosensitizing properties of Ro 03-8799 over misonidazole in vitro and in vivo [2, 3, 22]. Tumour concentrations achieved in man are consistent with significant hypoxic cell radiosensitization [1, 3, 4, 9, 10, 13, 14]. On the adverse side, however, the drug produces an acute CNS syndrome comprising dizziness, nausea, taste abnormalities, paraesthesia and a feeling of detachment; this limits individual doses to 0.75–1 g/m², and between 15 and 20 doses are tolerable [13, 14]. The pathogenesis of the syndrome remains to be elucidated.

Fig. 1. Stereostructures of the enantiomers of Ro 03-8799 (pimonidazole)

Ro 03-8799 normally exists as a racemic mixture of the R-(–)-and S-(+)-enantiomeric forms (Fig. 1). Prepared separately, these were shown to possess equivalent hypoxic cell-sensitizing activity and chronic aerobic cytotoxicity (Jones and Watts, personal communication). It was considered possible, however, that the two enantiomers might exhibit pharmacodynamic differences with respect to CNS-interactive properties, as do arylalkanolamines and arylalkylamines related in structure to pimonidazole [12, 15]. The identification of an appreciable difference in the ability to invoke the acute CNS response would favour the clinical use of the less toxic enantiomer, which might be given at higher doses than the racemic mixture, thus enabling the potential for a greater radiosensitizing effect.

Behavioural studies have failed to reveal a significant difference in toxicity between the two enantiomers (Holmes, personal communication). However, as no appropriate animal model directly equivalent to the human acute syndrome was available, a comparative study was carried out in man [11]. It was possible to obtain plasma and urine specimens from some of the patients, and the results of these pharmacokinetic studies are presented herein. These were considered to be particularly important since a number of racemic drugs show stereoselective differences in drug handling between enantiomeric forms [5], including misonidazole [21].

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Table 1. Patients' characteristics

Patient	Diagnosis	Sex	Age (years)	Surface area (m^2)	Enantiomer	Dose[a] ($g\ m^{-2}$)
A	Squamous carcinoma of lung (stage III)	F	66	1.4	R	0.25
B	Squamous carcinoma of lung (stage III)	M	65	1.8	R	0.75
C	Squamous carcinoma of lung (stage III)	M	69	1.6	R	0.75
D	Squamous carcinoma of lung (recurrent)	F	70	1.8	S	0.5
E	Adenocarcinoma of lung (stage II)	F	64	1.6	S	0.75
F	Squamous carcinoma of lung (stage III)	M	45	1.8	S	1

[a] Dose at which pharmacokinetic studies were performed

Table 2. Plasma pharmacokinetic parameters for Ro 03-8799 racemic mixture and R- or S-enantiomers

Patient	Dose ($g\ m^{-2}$)	Doe number[a]	Form	$t_{1/2}$ (min)	$AUC_{0-\infty}$ ($\mu g\ m^{-1}h^{-1}$)	V_d ($l\ m^{-2}$)	C ($l\ m^{-2}h^{-1}$)	Peak Ro 31-0313 ($\mu g\ m^{-1}$)
A	0.25	1	M	5.74	18.43	157	18.99	0.72
	0.25	2	R	5.74	14.53	200	24.09	0.37
B	0.75	5	M	6	69.05	169	19.55	1.75
	0.75	6	R	6.09	80.82	147	16.7	1.32
D	0.5	3	M	5.61	39.68	184	22.68	3.46
	0.5	4	S	4.15	45.78	118	19.66	4.34
F	1	7	M	4.21	52.61	208	34.22	2.77
	1	8	S	5.01	50.91	255	35.35	4.02

[a] In escalating dose schedule M, Racemic mixture

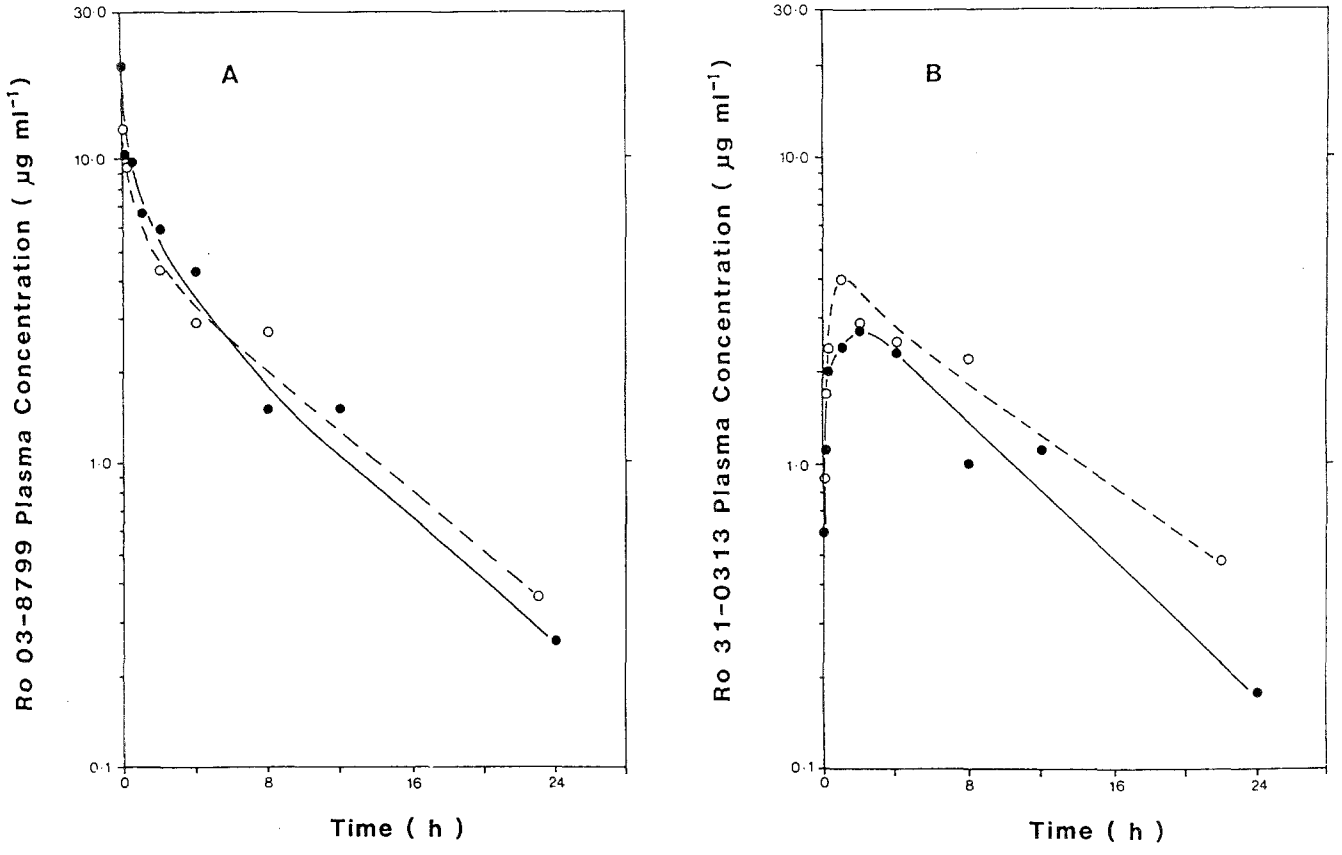

Fig. 2 A, B. Plasma pharmacokinetics of **A** Ro 03-8799 and **B** its *N*-oxide metabolite Ro 31-0313 in patient F following the administration of 1 g/m^2 Ro 03-8799 racemic mixture (■) or the S-enantiomer (○)

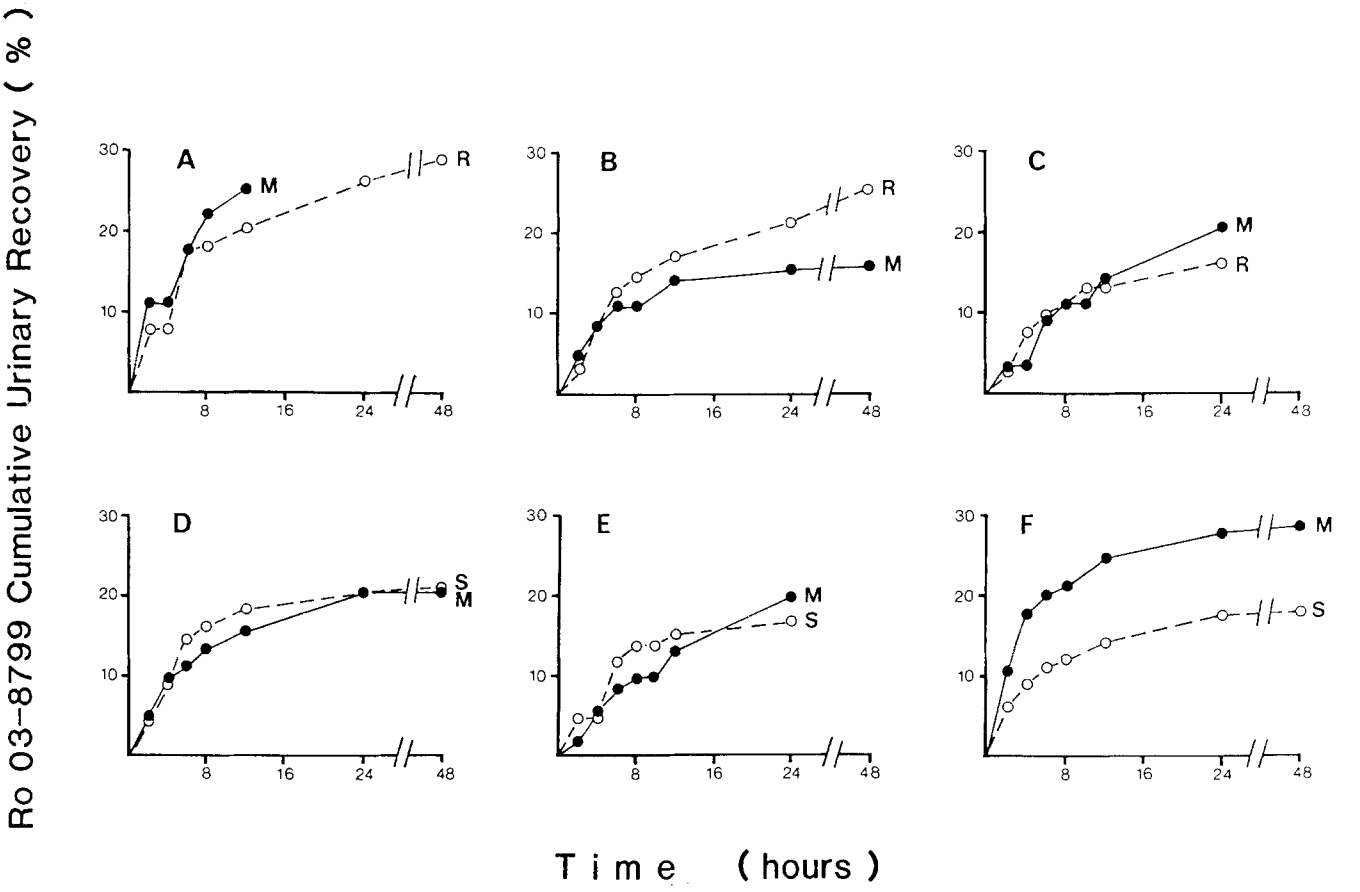

Fig. 3. Cumulative urinary recoveries for Ro 03-8799 following the administration of Ro 03-8799 racemic mixture (*M*, ●) or the R- or S-enantiomer (○). Individual results are shown for patients *A* – *F*

Patients and methods

Synthesis of enantiomers. Ro 03-8799 racemic mixture was prepared from racemic epichlorohydrin [16]. Both enantiomers were obtained either by optical resolution of the racemic mixture by diastereomeric salt formation with pure enantiomers of chiral acids or by the kinetic method of stereoselective *N*-oxidation [8, 11, 20]. *N*-Acyl derivative of α-amino acids are particularly useful as resolving agents for racemic bases such as Ro 03-8799 because both enantiomers of the amino acid are readily available in high optical purity, at least one of the diastereomeric acid-addition salts is highly crystalline and easily purified by recrystallisation, and subsequent treatment of the purified salt in a conventional manner results in liberation of the corresponding enantiomer of the base and recovery of the resolving agent. Thus, *N*-phthaloyl-L-phenylalanine gave the pure (–)-enantiomer of Ro 03-8799, $[\alpha]_D$ –6.0° ($c = 1 \cdot 0$, MeOH). The absolute stereochemistry of the enantiomers of Ro 03-8799 was established by stereospecific syntheses from the known enantiomers of epichlorohydrin, using the methods previously described by Smithen [16]. Thus, S-(+)-epichlorohydrin obtained from D-mannitol gave the R-(–)-enantiomer of Ro 03-8799 with >90% enantiomeric purity as judged by both optical rotation and chiral nuclear magnetic resonance (NMR). The S-(+)-enantiomer was obtained in similar enantiomeric purity. Each enantiomer was finally converted into the corresponding hydrochloride salt.

Patients. Hospital Ethical Committee approval was granted for the study, and informed consent was obtained from all subjects. Patients received the racemic mixture and either the R- or the S-enantiomer. Drugs were given on alternate days to enable clearance of previous doses of drug. Dosage began at 0.25 g/m^2 racemic mixture, followed by the same dose of the appropriate enantiomer on a double-blind basis. The dose was escalated within patients through 0.5 and 0.75 to 1 g/m^2, with each individual patient receiving the same enantiomer throughout. Drugs were infused i. v. in 50 ml normal saline over 10 min. A total of 12 patients were entered in the toxicity study, 6 from each of 2 centres [11]. The present paper describes the pharmacokinetic results obtained during the Cambridge series, brief details of which are given in Table 1. All doses refer to the hydrochloride salt.

Pharmacokinetic analysis. Fractional urine collections were made for all six patients, and paired plasma samples were obtained from four patients at the same dose of both the racemic mixture and the R- or S-enantiomer. Samples were stored at –20°C until analysis. Concentrations of Ro 03-8799 and its *N*-oxide metabolite Ro 31-0313 were determined by the paired-ion reverse-phase high-performance liquid chromatography (HPLC) method of Malcolm et al. [7], with minor modifications [19]. Identification of the analytes was made on the basis of co-elution with authentic standards using both the above method and a second, independent chromatographic system, together with the demonstration of identical multidiode-array spectra. The *N*-oxide was also confirmed by mass spectrometry. Concentrations refer to the hydrochloride salt.

Plasma Ro 03-8799 data were fitted to a one-compartment model by non-linear regression analysis using Subroutine VCO5AD of the Harwell Subroutine Library, and pharmacokinetic parameters were derived from standard equations [18]. No correction was necessary for infusion time according to the criteria of Freedman and Workman [6].

Results

Figure 2 shows the plasma pharmacokinetics for a typical patient and Table 2 summarizes the plasma pharmacokinetic parameters determined for all four subjects on whom

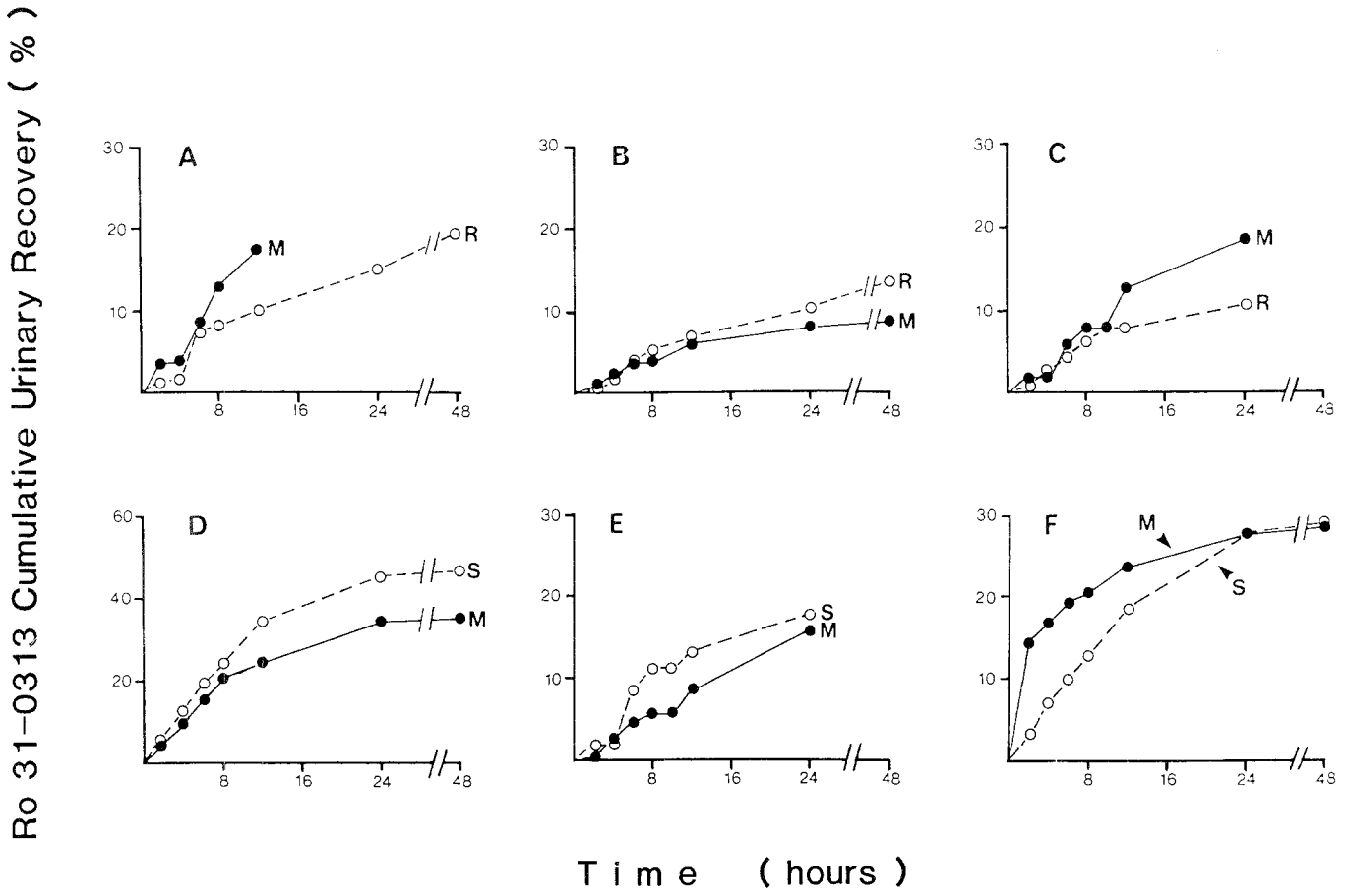

Fig. 4. Cumulative urinary recoveries for the *N*-oxide metabolite Ro 31-0313 following the administration of Ro 03-8799 racemic mixture (*M*, ●) or the R- or S-enantiomer (○). Individual results are shown for patients *A* – *F*

complete data were available. When the results achieved using the racemic mixture are compared with those obtained for the R- or S-enantiomer within the same patient, it is clear that the findings are extremely similar in each case. Matched cumulative urinary recoveries of Ro 03-8799 racemic mixture and the R- or S-enantiomer are shown for the six individual patients in Fig. 3, and data for the corresponding *N*-oxide metabolite are illustrated in Fig. 4. No consistent differences between the racemic mixture and either enantiomer are evident from these data, and the similarity is confirmed by the mean cumulative recoveries presented in Fig. 5.

Discussion

Although the number of patients who could be examined in this study was necessarily restricted by the limited supply of the enantiomer preparations, the comparison of results *within patients* enables us to conclude with reasonable confidence that there are no major differences in the plasma pharmacokinetics, urinary clearance or *N*-oxidation of the individual R- and S-enantiomers of Ro 03-8799 as compared with the racemic mixture. A similar lack of stereoselectivity was also apparent when the toxicity of the preparations was compared [11], suggesting the possibility that the acute toxicity syndrome may not involve a specific CNS receptor interaction. It should be mentioned, that we did not exclude the possibility of epimerization of Ro 03-8799 in vivo via oxidation of the clinical secondary alcohol to the ketone and subsequent reduction. However, we believe this to be unlikely, as there was no evidence of a ketone metabolite on HPLC; moreover, if reduction were to occur, it would be most likely to involve the nitro group preferentially.

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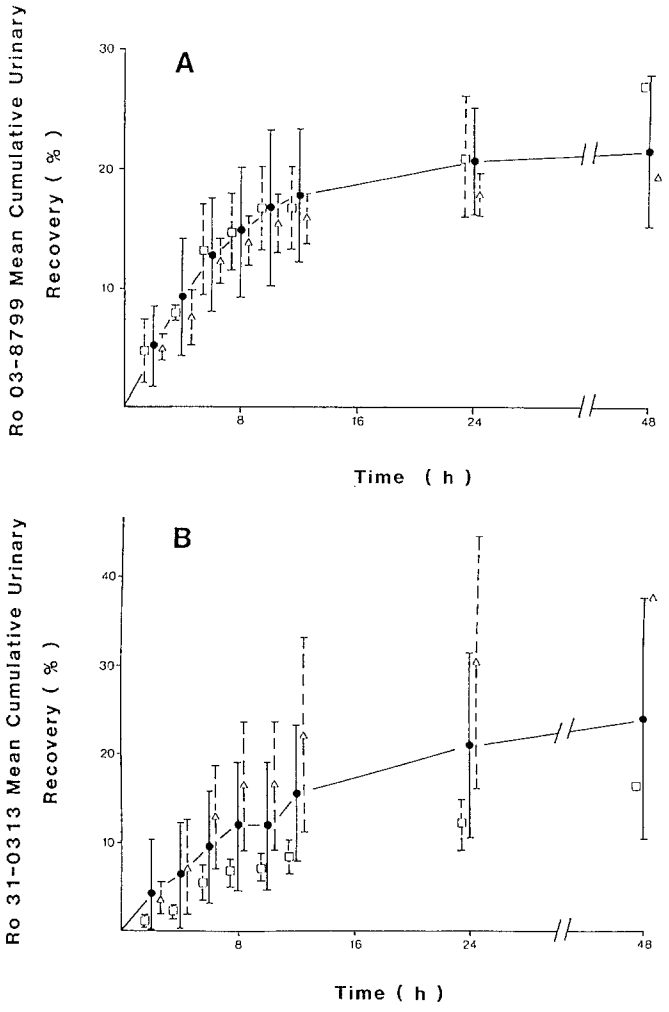

Fig. 5 A, B. Mean cumulative urinary recoveries for **A** Ro 03-8799 and **B** its *N*-oxide metabolite Ro 31-0313 following the administration of Ro 03-8799 racemic mixture (●) or the R- (□) or S-enantiomer (△). Average recoveries were calculated from the individual results presented in Figs. 4 and 5 and are shown ± SD, except for the R- and S-enantiomers at 48 h, at which time only two values were available in each case

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