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SHORT COMMUNICATION

The reversible *N*-oxidation of the nitroimidazole radiosensitizer Ro 03-8799

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Ro 03-8799 (2-nitro-(piperidino-methyl)-1-imidazole ethanol; Fig. 1) is a potent, basic, nitroimidazole hypoxic-cell radiosensitizer. It is currently undergoing clinical trial and has a low total-body exposure as a result of both its rapid renal clearance and extensive oxidative metabolism to the *N*-oxide Ro 31-0313 (Fig. 1) [1]. We report here on the reversible nature of this *N*-oxidation in mice.

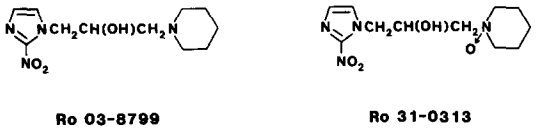

Fig. 1. Structures.

Materials and methods

Ro 03-8799 and the *N*-oxide Ro 31-0313 were supplied in powder form by Roche Laboratories (Welwyn Garden City), the former as the hydrochloride salt. Both drugs were dissolved in Hanks buffered salt solution (HBSS) and given i.v. at 0.01 ml/g body wt. All Ro 03-8799 drug doses and concentrations are reported as the free base. Experiments were carried out with inbred male C3H/He mice supplied by OLAC Ltd. (Bicester, U.K.) which were used at between 25 to 35 g body wt. For plasma pharmacokinetic studies, whole blood was removed by cardiac puncture into heparinised syringes and centrifuged at 4000 *g* for 15 min to obtain plasma. Metabolic studies were carried out on groups of 5–6 mice contained in a Urimax metabolism cage, and urine was collected frozen on dry ice for 24 hr after drug administration. The 24-hr *in vitro* stability of Ro-31-0313 and Ro 03-8799 in whole blood, plasma and urine at 37° was also determined. All samples were handled at 4° and stored at −20° before analysis. Drug concentrations in whole blood, plasma and urine were determined using the high-performance liquid chromatography (HPLC) method of Malcolm *et al.* [2], with minor modifications.

Curve fitting and kinetic parameters were determined using the subroutine VCO5AD of the Harwell Subroutine Library, which fits a linear combination of *n* decaying exponentials to *m* data points, by minimising the sum of squares of the residuals.

Results and discussion

Figure 2a shows a plasma time course for Ro 03-8799 in mice after a dose of 175 μg/g i.v. The plasma clearance of Ro 03-8799 was biphasic with a short distribution-phase half-life ($t_{1\alpha}$) of 8.5 min (4.9–33 min; ±2 S.E.) and a longer terminal-phase half-life ($t_{1\beta}$) of 28 min (23–35 min; ±2 S.E.). The latter was in good agreement with the data of Smithen *et al.* [3] for IP administered Ro 03-8799. At this dose the *N*-oxide metabolite Ro 31-0313 reached a peak concentration of approx. 6 μg/ml, occurring 20–40 min after drug administration (Fig. 2a).

Figure 2b shows the plasma clearance of injected Ro 31-0313 after 40 μg/g i.v. This dose produced *N*-oxide con-

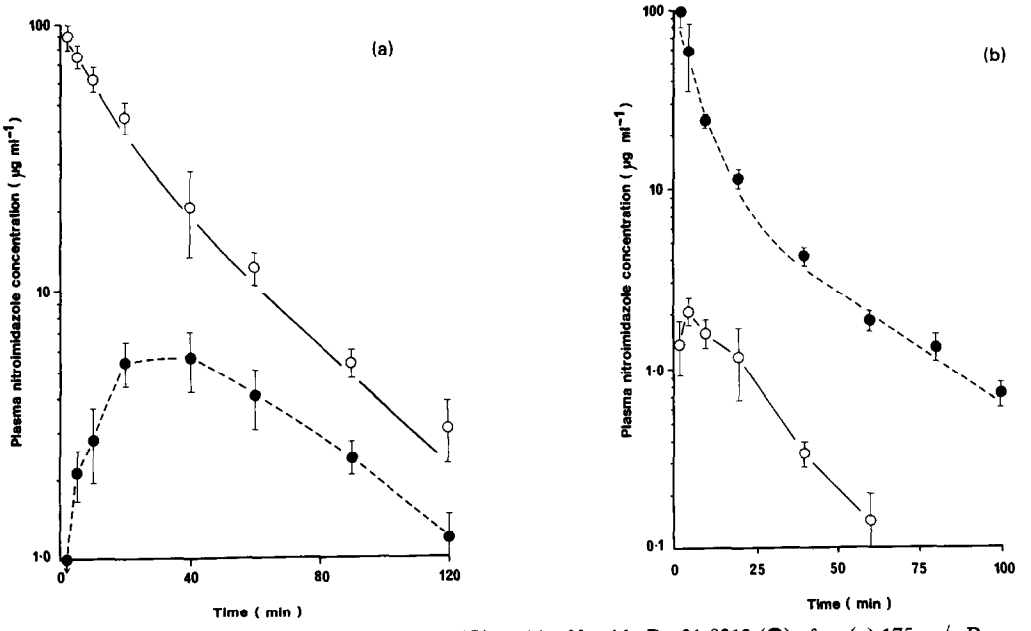

Fig. 2. Plasma concentrations of Ro 03-8799 (○) and its *N*-oxide Ro 31-0313 (●) after (a) 175 μg/g Ro 03-8799 i.v., and (b) 40 μg/g Ro 31-0313 i.v. Error bars indicate ± 2 S.E. Data from three independent experiments, with 3–4 mice per time point.

centrations of 5–7 μg/ml at 25–30 min, which is similar to the peak *N*-oxide metabolite levels occurring at 20–40 min after a dose of 175 μg/g Ro 03-8799 (Fig. 2a). Ro 31-0313 elimination was biphasic and similar to that seen for Ro 03-8799, with a $t_{\frac{1}{2}\alpha}$ of 4 min (3.3–5.2 min; ±2 S.E.) and a $t_{\frac{1}{2}\beta}$ of 24 min (22–28 min; ±2 S.E.). At the early time points Ro 03-8799 was readily detectable in the plasma as a metabolite. At 10 min the concentration peaked at 2 μg/ml and decreased thereafter to below the limit of detection at 80 min.

The data for the 24-hr urinary recovery experiments are shown in Table 1. After Ro 03-8799 administration, about 34% of the dose was recovered as parent compound and 6.7% as the *N*-oxide metabolite, with a further 60% of the total dose unaccounted for. About 50% of the i.v. administered Ro 31-0313 dose was recovered as unchanged drug with a further 1.8% recovered as Ro 03-8799 metabolite, the remaining 50% of the dose being unaccounted for. The low recovery of nitroimadazole compounds in the 24-hr urine (<60%) for both injected Ro 03-8799 and Ro 31-0313 indicates considerable additional metabolism. This might include nitroreduction and ring cleavage to smaller fragments as occurs with other nitroimidazoles [4].

Full kinetic stability studies were carried out at 37° over 24 hr, using plasma, whole blood and urine spiked with 10 μg/ml Ro 03-8799 or Ro 31-0313. The kinetic studies showed that Ro 31-0313 was stable for at least 4 hr in urine and plasma, and that the concentration of Ro 31-0313 remained constant in whole blood for at least 4 hr after an initial conversion of about 8% to Ro 03-8799. Table 2 shows the final percentage of Ro 31-0313 remaining and Ro 03-8799 formed after 24-hr incubation of Ro 31-0313 in whole blood, plasma and urine. Ro 31-0313 was degraded by 86% in whole blood, and by 30–40% in urine and plasma. Under these conditions no Ro 03-8799 was formed in urine and only some 6% produced in plasma; in contrast 28% reduction to Ro 03-8799 occurred in whole blood.

Table 2. Twenty-four-hour *in vitro* stability of Ro 31-0313 at 37° in whole blood, plasma and urine

Biological fluid	% Ro 03-8799 formed	% Ro 31-0313 remaining
Whole blood	28 ± 7.3	14 ± 11
Plasma	6.0 ± 6.1	61 ± 36
Urine	0.0	70 ± 36

Figures represent mean ± 2 S.E., N = 5. Pooled data from two independent experiments.

Similar stability studies using Ro 03-8799 were carried out in whole blood and plasma at 37°. Ro 03-8799 was very unstable in whole blood, (89% loss at 24 hr) but stable in plasma (no loss after 24 hr). Under these conditions Ro 03-8799 did not form Ro 31-0313.

Because of the possibility of *ex vivo* metabolism, whole blood Ro 31-0313 standards were prepared at the same concentration and time as *in vivo* samples were removed from Ro 31-0313 treated mice. In all cases the spiked standards contained either no Ro 03-8799 or considerably less than the equivalent *in vivo* samples.

These data show that Ro 03-8799 *N*-oxidation to Ro 31-0313 is a reversible reaction *in vivo*. A large number of *N*-oxide metabolites undergo reduction back to the parent compound [5] as reported here for Ro 03-8799. This reduction may be catalysed by haeme complexed iron as for imipramine *N*-oxide [6] both *in vivo* and *ex vivo*, and the stability data (Table 1) shows that whole blood is capable of reducing Ro 31-0313 *ex vivo*. Several enzymes have also been implicated in this type of reduction, including xanthine oxidase [7] and reduced cytochrome P-450 [8]. The precise mechanism involved in the reduction of Ro 31-0313 back to Ro 03-8799 remains to be elucidated.

In summary Ro 03-8799, a basic nitroimidazole radiosensitizer, is rapidly cleared from plasma and extensively metabolised after i.v. administration, and a major metabolite is an *N*-oxide, Ro 31-0313 [2]. The present results demonstrate that the *N*-oxidation of Ro 03-8799 is a reversible process as occurs with other *N*-oxide metabolites. The pharmacological and toxicological consequences of this are unknown. A practical consequence is the need for careful sample handling in metabolic and pharmacokinetic studies with Ro 03-8799.

Table 1. Twenty-four-hour urinary excretion of Ro 03-8799 and Ro 31-0313 after i.v. administration

Drug and dose	% Administered dose excreted in 24-hr urine*		
	Ro 03-8799	Ro 31-0313	Total
Ro 03-8799 175 μg/g	35, 32	6.7, 6.6	42, 39
Ro 31-0313 40 μg/g	1.7, 1.8	57, 45	59, 47

* Data are shown from two independent experiments, with 5–6 mice per experiment.

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