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Pharmacokinetics and metabolism of the mixed-function hypoxic cell sensitizer prototype RSU 1069 in mice

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Summary. RSU 1069 is a leading compound in the class of mixed-function hypoxic cell sensitizers. Possessing an alkylating aziridine function as well as a nitro group, it represents an important prototype molecule for new sensitizer development. Using a novel HPLC assay for RSU 1069 and its metabolites with a cyanopropyl column, we studied the detailed pharmacokinetics and metabolism of this drug in mice. An i.v. dose of 100 mg kg^{-1} produced peak plasma concentrations of about 100 μg ml^{-1}. Absorption was rapid after i.p. injection but peak plasma concentrations were some three- to fourfold lower, giving an i.p. bioavailability of 55%. The elimination $t_{1/2}$ was route-dependent; e.g. after 50 mg kg^{-1} the $t_{1/2}$ was 37.2 and 22.4 min for the i.v. and i.p. routes respectively ($P < 0.001$). There was also an indication of dose-dependent kinetics, with a 37% increase in elimination $t_{1/2}$ when the i.p. dose was doubled from 50 to 100 mg kg^{-1}. Oral bioavailability was low. The volume of distribution was 0.65–1.31 ml g^{-1} at 50 mg kg^{-1}, but tissue penetration was limited. Brain/plasma ratios ranged from 9.3% to 66.8%, while the mean steady-state tumour/plasma ratio was 28.4%, a value considerably less than the 80%–100% ratios occurring with the neutral 2-nitroimidazole misonidazole. About 18% and 8% of a dose were excreted as the parent drug and the ring-opened hydrolysis product (RSU 1137) in the 8 h urine, indicating the likelihood of extensive metabolism via aziridine-ring removal and nitroreduction. RSU 1137 was also detected in mouse plasma and tissues and, in contrast to the aziridine ring-intact parent compound, gave tumour/plasma ratios of 100%. These studies should provide a pharmacokinetic basis for the evaluation and development of improved mixed-function sensitizers.

Introduction

Mixed-function hypoxic cell sensitizers contain both a sensitizing nitro group and an additional alkylating aziridine function within the same molecule. Developed by Adams et al. [1] and based on promising results obtained with the dinitrophenylaziridine CB 1954 [12], these compounds are of current interest because of their greater potency as radio- and chemosensitizers compared with simple nitroimidazoles such as misonidazole [1-(2-nitro-1-imidazolyl)-3-(1-methoxy)-2-propanol; MISO] [2].

RSU 1069 is an analogue of MISO in which an aziridine ring replaces the methoxy group in the side chain (Fig. 1). It exhibits greater sensitizing potency in hypoxic cells than would be predicted from its one-electron reduction potential, which is comparable with that of MISO [1, 2]. RSU 1069 also exhibits much greater hypoxic cell cytotoxicity than MISO [6, 18]. These properties are thought to result from the ability of RSU 1069 to act as a bifunctional alkylating agent under hypoxia [10, 11, 13].

In view of the important role of drug disposition in determining the activity of hypoxic cell sensitizers in vivo [20], we developed a sensitive and specific HPLC assay for RSU 1069 and its aziridine ring-opened metabolites in biological materials. Using this method we carried out a detailed evaluation of the pharmacokinetics and metabolism of RSU 1069 in mice. This represents an extension of our previous preliminary studies [22] and related investigations [4, 7] using the HPLC method described in detail here. We believe the results obtained from these studies will be useful in the continued development of mixed-function hypoxic cell sensitizers based on RSU 1069 as the prototype.

Materials and methods

Mice and tumours. Adult inbred BALB/c and C3H/He mice of both sexes were obtained from OLAC (Bicester, UK) or our own breeding colony. The animals were allowed food (PRD nuts; Labsure, Poole, Dorset, UK) and water ad lib and were used in experiments at 20–38 g body wt.

The KHT fibrosarcoma was grown i.m. in the hind leg of C3H/He mice as previously described [14]. Mice bearing tumours weighing in the range of 0.4–1.0 g were used.

Drug supply and administration. RSU 1069 [1-(2-nitro-1-imidazolyl)-3-(1-aziridinyl)-2-propanol; NSC 347503], its ring-opened hydrolysis product RSU 1137 [1-(2-nitro-1-imidazolyl-3-(1-ethanol)-2-propanol] and the aziridine ring-removed amine metabolite RSU 1137 [1-(2-nitro-1-imidazolyl)-3-(1-amino)-2-propanol] were supplied in powder form by Drs. IJ Stratford and I Ahmed of the Institute of Cancer Research (Sutton, Surrey, UK). The internal standard RGW 610 [1-(2-nitro-1-imidazolyl)-3-(1-phenyl) propane] was supplied by Drs. P Wardman and

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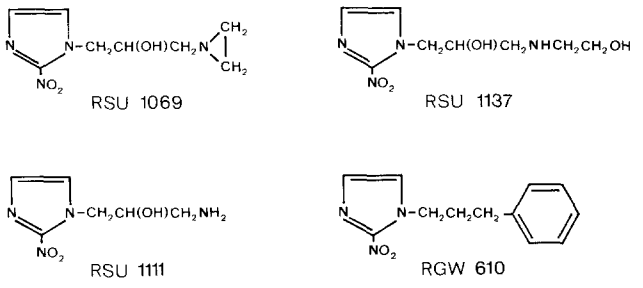

Fig. 1. Chemical structures

R Wallace of the CRC Gray Laboratory (Northwood, Middlesex, UK) and the University of Brunel (Uxbridge, Middlesex, UK). The chemical structures are shown in Fig. 1. RSU 1069 was given at a dose of 50 or 100 mg kg^{-1} in saline (0.9% NaCl) using a volume of either 0.01 or 0.04 ml g^{-1} i.p. or 0.01 ml g^{-1} i.v. via the tail vein, or 0.005 ml g^{-1} orally.

Sample preparation. Procedures for the collection and preparation of plasma, tissue and urine samples were similar to those described previously [17, 19, 21]. Briefly, nitroimidazoles were extracted from aliquots (100 µl) of plasma, urine and tissue homogenates (33% w/v) by the addition of either 2 vol. methanol alone or 10 vol. methanol containing internal standard (RGW 610 at 2.5 or 5 mg l^{-1}), and centrifuged at 2,000 *g* for 15 min at −15° C. Aliquots of the supernatant were either taken to dryness under a vacuum and resuspended in running buffer (50 µl) or injected directly into the HPLC apparatus for analysis. Samples were handled at 4° C and stored at −20° C for up to 2 weeks prior to analysis.

High-performance liquid chromatography (HPLC). Concentrations of RSU 1069 and its metabolites were determined by reverse-phase HPLC using equipment and columns supplied by Waters Associates (Milford, Mass. USA). The equipment used included a Model 410A automated sample injector (WISP), two Model 6000 A chromatography pumps, a Model 660 solvent programmer, a Model 420 data module, a Model 430 system controller, a Model 440 fixed-wavelength UV detector and either an RCM-100 or Z-module. Separations were carried out on a Waters Resolve cyanpropyl (CN) Rad-Pak column (8 mm × 10 cm; 10 µm beads) using either isocratic or gradient elution. The isocratic mobile phase consistent of 10% methanol (Rathburn, Walkerburn, Scotland) in 10 m*M* ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) adjusted to pH 3.0 with orthophosphoric acid (H_3PO_4). This was delivered at a constant flow rate of 3–4 ml min^{-1} as appropriate. Alternatively, a linear gradient was run over the first 5 min from 10%–50% methanol in 10 m*M* $NH_4H_2PO_4$ (pH 3.0) and held at the final concentration for a further 2 min before reequilibration under the initial conditions. Columns were eluted at a constant flow rate of 3–4 ml min^{-1}. RSU 1069, 1137 and 1111 were identified by co-chromatography with authentic material.

Pharmacokinetic parameters. The pharmacokinetic parameters were calculated as described elsewhere in detail [17, 21]. Briefly, elimination half-lives ($t_{1/2}$) with 95% confidence limits were estimated from post-peak concentration data using least-squares linear-regression analysis from the equation $t_{1/2} = \ln 2/k$, where k is the elimination rate constant given by ln concentration vs time. Plasma AUCs were calaculted using the expression $AUC_{o-\infty} = C_o/k$, where C_o is the extrapolated concentration at time 0. Alternatively, AUC_{o-t} from time 0 to t was calculated by Simpson's rule. The remaining AUC from t–∞ was given by C_t/t, where C_t is the plasma concentration at time t, and the $AUC_{o-\infty}$ was then obtained by the sum of AUC_{o-t} + $AUC_{t-\infty}$.

Results

Chromatography

Figures 2 and 3 show examples of the chromatographic separations obtained using the isocratic and gradient HPLC methods respectively.

Figure 2a shows a chromatogram of a methanol extract of plasma taken from a C3H/He mouse given saline i.p., in which there are no detectable peaks after the solvent front. Figure 2b shows a methanol extract of pooled C3H/He mouse plasma spiked with RSU 1111 (peak 1), RSU 1137 (peak 2) and RSU 1069 (peak 3). All three compounds were clearly resolved from each other. Figure 2c shows a typical chromatogram of a methanol extract of plasma taken from a C3H/He mouse treated 30 min earlier with 50 mg kg^{-1} RSU 1069 i.p. There is a large peak corresponding to RSU 1069 (peak 3). A much smaller peak corresponding to the aziridine ring-hydrolysis product RSU 1137 (peak 2) is also detectable, as is an unidentified minor metabolite. However, there was no evidence of the aziridine ring-removed amine metabolite RSU 1111 in plasma or any tissue examined.

Figure 3 shows chromatograms of methanol extracts of 1:10 diluted, pooled BALB/c mouse urine collected 8 h after drug vehicle (*a*) or RSU 1069 (*b*) respectively. The internal standard (peak 4) is clearly shown in Fig. 3a, together with a number of minor peaks. In Fig. 3b there are obvious peaks corresponding to RSU 1069 (peak 3), RSU 1137 (peak 2) and the internal standard, with a smaller peak representing RSU 1111 (peak 1) and an unidentified metabolite running close to the solvent front.

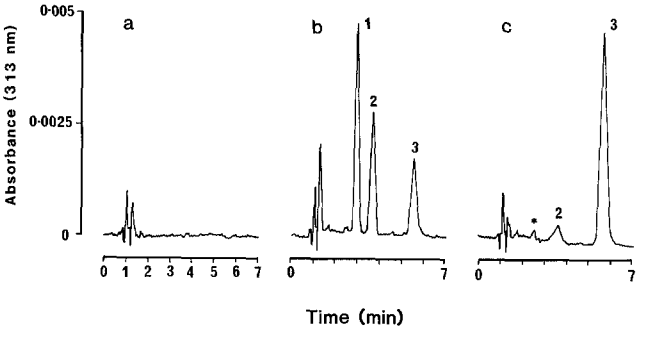

Fig. 2. Isocratic HPLC chromatograms. **a**: blank C3H/He mouse plasma; **b**: C3H/He mouse plasma spiked with 10 µg ml^{-1} RSU 1069 (*peak 3*), RSU 1137 (*peak 2*) and RSU 1111 (*peak 1*); **c**: C3H/He mouse plasma taken 30 min after 50 mg kg^{-1} RSU 1069 i.p. and containing RSU 1069 (*peak 3*), RSU 1137 (*peak 2*) and an unknown polar metabolite (*). Chromatographic conditions: column, Waters Resolve cyanopropyl (CN) Rad-Pak; mobile-phase, 10% methanol in 10 m*M* $NH_4H_2PO_4$ buffer (pH 3); flow rate, 3.5 ml min^{-1}; column pressure, 600 psi; temperature, ambient; detection, absorbance at 313 nm; injection volume, 30 µl

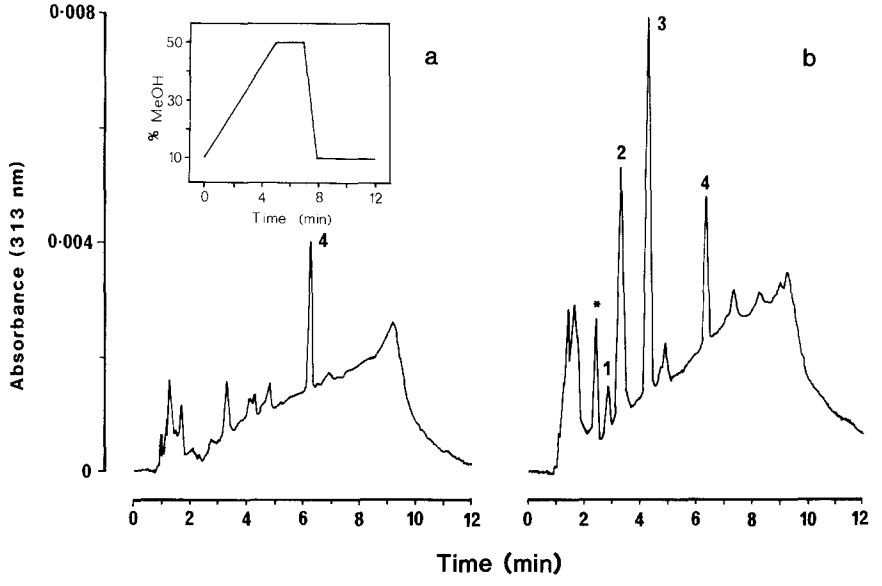

Fig. 3. Representative gradient HPLC chromatograms of pooled 1:10 diluted C3H/He mouse urine obtained 8 h after **a**: drug vehicle (saline) i.p.; and **b**: 50 mg kg^{-1} RSU 1069 i.p., *Peak 1*, RSU 1111; *peak 2*, RSU 1137; *peak 3*, RSU 1069; *peak 4*, internal standard (RGW 610 at 2.5 mg l^{-1} in methanol); * denotes an unidentified polar metabolite. Chromatographic conditions as in Fig. 2, except that the mobile phase was a 10%–50% methanol gradient (*inset*) in 10 m*M* $NH_4H_2PO_4$ buffer (pH 3) and the column pressure was 1,000 psi

In view of the shorter run time and more stable baseline obtained with the isocratic HPLC technique, this was used most frequently for drug analysis. To illustrate the performance of this assay the following details are given for the isocratic method. Calibration curves were linear over the concentration range 0.5–200 μg ml^{-1} for RSU 1069, 1137 and 1111 with zero intercepts. Coefficients of variation were 9.8%, 4.3% and 8.3%, respectively, for a concentration of 10 μg ml^{-1} in mouse plasma ($N = 6$). For an injection volume of 30 μl the lower limits of detection for RSU 1069, 1137 and 1111 were 0.5, 0.3 and 0.3 μg ml^{-1}, respectively. This was improved about threefold by drying down and resuspending samples in a small volume of running buffer. The corresponding on-column detection limit was 3–5 ng. The extraction efficiency for all three compounds was >90%.

Stability

RSU 1069 stability was monitored for 7 days in saline solution at 4°, 22° (room temperature) and 37° C. Hydrolysis of RSU 1069 was extensive at 37° C, with 88% loss and 43% RSU 1137 formation after 7 days in a dark environment. At room temperature only 13% hydrolysis was seen at 7 days, and there was no loss at 4° C in a dark environment. The drug was very stable over 24 h, with no loss at room temperature and <6% loss at 37° C. Similar results were obtained under artifical laboratory lighting.

Plasma pharmacokinetics

Figures 4a and 4b show the pharmacokinetics of RSU 1069 in the plasma of male BALB/c mice receiving 50 mg kg^{-1} RSU 1069 i.v. or i.p., respectively. Plasma clearance was clearly biphasic after i.v. drug injection. The very rapid distribution phase was essentially complete within 5 min, followed by a slower elimination phase with a half-life ($t_{1/2}$ with 95% confidence limits) of 37.2 (31.9–44.7) min. The volume of distribution (Vd_{ext}) was 1.05 ml g^{-1}.

RSU 1069 was absorbed rapidly after i.p. injection, giving a peak plasma concentration of 30–40 μg ml^{-1} at

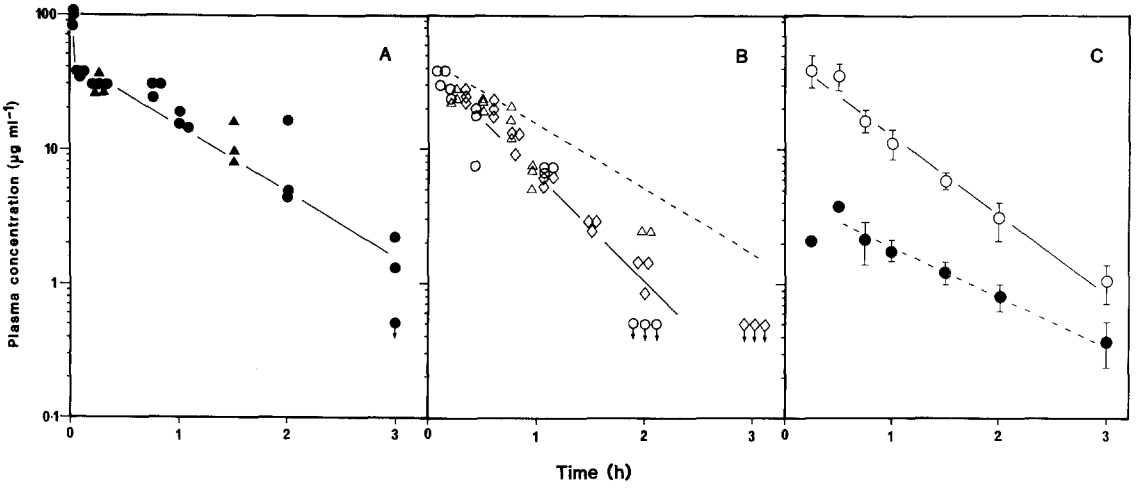

Fig. 4. Pharmacokinetics of RSU 1069 in BALB/c mice. **A**: After 50 mg kg^{-1} i.v: each symbol represents a single mouse and different symbols represent independent experiments. **B**: After 50 mg kg^{-1} i.p.; symbols as in *A*. *Dotted line* represents i.v. data from panel **A** (same experiment). **C**: After 100 mg kg^{-1} i.p. with *open symbols* representing RSU 1069 and *closed symbols*, RSU 1137 plasma concentrations; results are mean ± 2SE from a single experiment with five mice per point

the earliest sampling time (5 min post-injection). Peak concentrations were three- to fourfold lower than for the i.v. route, and the plasma clearance was considerably faster after i.p. dosing, with an elimination $t_{1/2}$ of 22.4 (20.6–24.6) min ($P < 0.001$). The corresponding Vd_{ext} was 1.13 ml g^{-1}, which was similar to that occurring after i.v. injection. The plasma $AUC_{0-\infty}$ values were 42.4 and 23.7 μg ml^{-1} h for the i.v. and i.p. routes respectively, resulting in an i.p. bioavailability of 55%.

Figure 4c shows the plasma pharmacokinetics of RSU 1069 and its aziridine ring-opened hydrolysis product RSU 1137 in BALB/c mice after the higher dose of 100 mg kg^{-1} RSU 1069 i.p. Peak RSU 1069 concentrations occurred at the earliest recorded time of 15 min and were between 30 and 50 μg ml^{-1}. The apparent Vd_{ext} was 2.0 ml g^{-1}, a value almost twice that occurring after 50 mg kg^{-1} i.p. The elimination $t_{1/2}$ was also significantly longer at 30.7 min (28.3–33.6; $P < 0.001$) than for the low-dose i.p. route. The $AUC_{0-\infty}$ was 36.9 μg ml^{-1} h. Figure 4c also shows that peak plasma RSU 1137 metabolite concentrations of about 4 μg ml^{-1} were seen 30 min after the high dose of RSU 1069. The apparent elimination $t_{1/2}$ was 48 min (41.9–56.2) and the corresponding $AUC_{0-\infty}$ was 3.9 μg ml^{-1} h.

RSU 1069 was also given to C3H/He mice at a dose of 50 mg kg^{-1}. The pharmacokinetic parameters for three separate experiments are shown in Table 1. Figure 5a shows the data for one experiment (A) where the RSU 1069 absorption was rather slow, resulting in a peak concentration of 30–40 μg ml^{-1} at 20 min. Peak concentrations of the metabolite RSU 1137 were also reached at 20 min and were about 5 μg ml^{-1}. In the other two experiments peak plasma concentrations occurred at the earliest measured time points (5 and 30 min) but were lower than for experiment *A*. An example is shown in Fig. 5b. Drug elimination was monoexponential and similar in all three experiments and the mean elimination $t_{1/2}$ was 23.9 ± 3.5 min (mean ± 2SE; $N = 3$). The mean Vd_{ext} was 1.07 ± 0.52 ml g^{-1} (mean ± 2SE; $N = 3$) and the mean $AUC_{0-\infty}$ was 26.1 μg ml^{-1} h (range, 16.3–31.3). These values were similar to those obtained in BALB/c mice at the same dose. The mean apparent elimination $t_{1/2}$ for RSU 1137 in these experiments was 41.8 ± 11.2 min (mean ± 2SE; $N = 3$).

Figure 5c shows the plasma pharmacokinetics of RSU 1069 and RSU 1137 in C3H/He mice after an oral dose of 50 mg kg^{-1}. Absorption was rapid but peak concentrations were somewhat lower than for the i.p. route. Drug elimination appeared to be biexponential, with a terminal $t_{1/2}$ of 49.8 min (36.2–81.6). The $AUC_{0-\infty}$ after oral dosing was 33% of that occurring after i.p. injection at 8.37 μg ml^{-1} h. Peak plasma concentrations of the hydrolysis product RSU 1137 were comparable to those produced after i.p. injection and remained relatively constant at between 0.4–0.6 μg ml^{-1} from 1–3 h after oral dosing.

Table 1. Summary of the plasma pharmacokinetics of RSU 1069 and its major metabolite RSU 1137 in C3H/He mice given 50 mg kg^{-1} RSU 1069 i. p.

Parameter	RSU 1069			RSU 1137		
Expt	A	B	C	A	B	C
Peak plasma concentration (μg ml^{-1})	35.4	18.0	23.3	5.07	1.18	1.45
$t_{1/2}$ (min)	24.5	20.6	26.5	32.7	40.6	52.0
Vd_{ext} (ml g^{-1})	0.649	1.54	1.03	NA	NA	NA
$AUC_{0-\infty}$ (μg ml^{-1} h)	31.3	16.3	30.8	7.19	1.49	2.76
P_{cl} (ml g^{-1} h^{-1})	1.59	3.07	1.62	NA	NA	NA

Each value was determined in a separate experiment with 3–6 mice per time point and 3–8 time points per experiment
NA, Not applicable

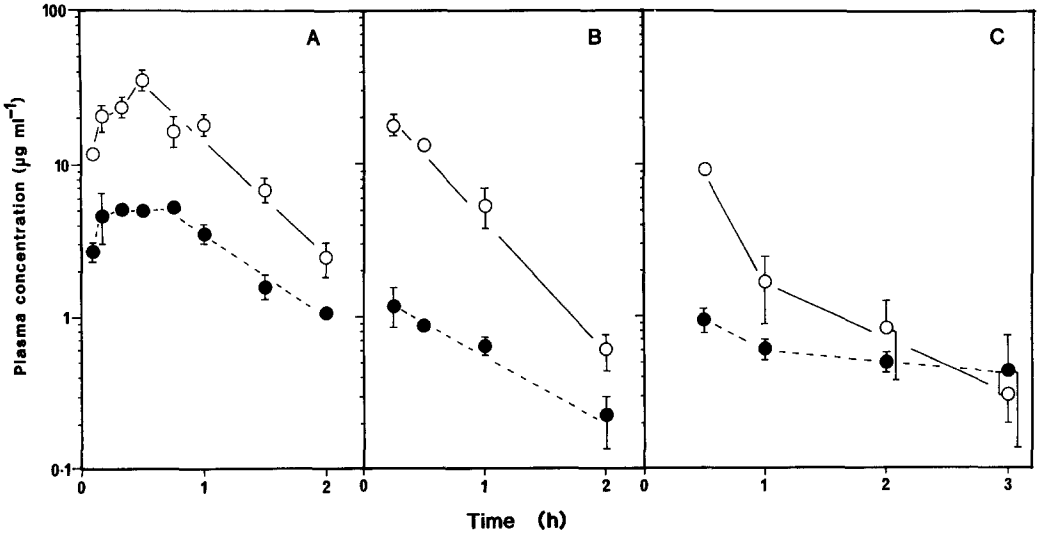

Fig. 5. Pharmacokinetics of RSU 1069 and its major metabolite, RSU 1137, in C3H/He mice. Panels **A** and **B** illustrate two of three repeat experiments at 50 mg kg^{-1} i.p.; absorption was slower in the experiment depicted in *A* than in the other two experiments. Panel **C** shows the results of an experiment in which the same dose was given orally. In all cases *open symbols* represent RSU 1069, and *closed symbols*, the metabolite RSU 1137. Results are mean ± 2SE, with 4–6 mice per point

Table 2. Tissue/plasma ratios for RSU 1069 and its major metabolite RSU 1137 in KHT tumour and brain tissue from C3H/He mice given 50 mg kg^{-1} RSU 1069 i. p.

Time (min)	Tumour/plasma (%)		Brain/plasma (%)	
	RSU 1069	RSU 1137	RSU 1069	RSU 1137
5	10.0± 3.8 ($N = 6$)	57.3±23.5 ($N = 6$)	BLD	BLD
10	17.1± 4.8 ($N = 5$)	67.2±19.3 ($N = 6$)	BLD	BLD
15	37.3±19.6 ($N = 4$)	122 ±44.7 ($N = 4$)	9.25± 1.3 ($N = 3$)	BLD
20	30.8± 6.2 ($N = 6$)	112 ±10.5 ($N = 6$)	12.8 ± 1.4 ($N = 6$)	30.6± 4.9 ($N = 6$)
30	31.2± 7.9 ($N = 10$)	157 ±27.8 ($N = 9$)	14.8 ± 2.5 ($N = 10$)	54.2±14.8 ($N = 6$)
45	27.9± 4.6 ($N = 6$)	101 ±16.8 ($N = 6$)	22.7 ± 2.8 ($N = 6$)	47.0± 6.9 ($N = 6$)
60	25.3± 5.0 ($N = 9$)	110 ±22.1 ($N = 9$)	19.7 ± 4.0 ($N = 10$)	78.9± 9.6 ($N = 6$)
90	21.9± 8.57 ($N = 6$)	173 ±64.7 ($N = 6$)	31.5 ± 6.1 ($N = 6$)	161 ±33.9 ($N = 6$)
120	58.2±40.5 ($N = 5$)	184 ±29.6 ($N = 6$)	66.8 ±36.0 ($N = 5$)	201 ±26.8 ($N = 6$)

Ratios are mean ± 2 SE of N mice using pooled data from two independent experiments
BLD, Below limit of detection

Tumour and brain penetration

Table 2 summarises the penetration of RSU 1069 and its ring-opened hydrolysis metabolite RSU 1137 into KHT tumours and normal mouse brain tissue after 50 mg kg^{-1} RSU 1069 i.p.

The penetration of RSU 1069 into KHT tumours was relatively modest, with tumour/plasma ratios considerably less than 50%. Steady-state conditions were reached after 15 min, resulting in a mean tumour/plasma ratio of 28.4%±3.4% (mean±2SE; N=41) over a 15–90 min period. Peak tumour concentrations occurred between 15 and 30 min and were 6.7 and 13.6 μg^{-1}, as determined in two separate experiments. RSU 1069 penetration into brain tissue was slower than for KHT tumours. Brain/plasma ratios did not reach steady state during the 2-h measured period but increased progressively to a value of 66.8% at 2 h.

Tumour concentrations of the metabolite RSU 1137 equilibrated with plasma after 15 min and remained stable for the remainder of the time course. In contrast to RSU 1069, steady-state tumour/plasma values for RSU 1137 were 130%±15.4% (mean±2SE; N=40), indicating good tissue penetration. Absolute peak tumour concentrations were 1.4 and 9.1 μg^{-1} in two experiments. Brain/plasma ratios for RSU 1137 increased steadily over the time course, reaching 201% after 2 h.

Urinary excretion

The 0 to 8 h urinary recoveries of RSU 1069 and its metabolites from C3H/He mice are shown in Table 3. Some 18% of the dose was eliminated as the unchanged parent drug, with a further 8% recovered as the aziridine ring-opened hydrolysis product (RSU 1137), a value consistent with the readily detectable concentrations of this metabolite in mouse plasma and tissues. Only small amounts of the aziridine ring-removed amine product (RSU 1111) were detected. The total urinary recovery of RSU 1069 and its metabolites was 26%.

Table 3. The urinary excretion of RSU 1069 and its aziridine ring-opened metabolites in C3H/He mice after a dose of 50 mg kg^{-1} RSU 1069 i. p.

Compound	% dose excreted in 0 to 8 h urine	
	Experiment* A	B
RSU 1069	16.1	19.5
RSU 1137	9.4	6.4
RSU 1111	<0.1	0.8

* Pooled urine from four mice per experiment

Discussion

The HPLC method described here is specific for RSU 1069 and its metabolites and is sensitive enough to allow accurate quantitation in biological materials, making this a useful technique for the detailed study of RSU 1069 pharmacokinetics and metabolism in mice. As well as being used for this purpose in the present study and in an earlier report from this laboratory [22], this method has also been used by others in pharmacokinetic studies in mice [4] and in a small clinical study of RSU 1069 pharmacokinetics in man [7].

RSU 1069 was shown to be degraded in saline solution in a temperature-dependent fashion, with 88% degradation after 7 days at 37° C compared with no loss at 4° C under similar conditions. RSU 1069 was stable for at least 24 h at room temperature, thus allowing standard drug preparation and administration techniques to be used. The major product was identified as the ring-opened hydrolysis product RSU 1137. Ring opening might be expected to be more rapid in vivo due to the presence of high concentrations of reactive nucleophiles.

After i.v. injection in BALB/c mice, RSU 1069 was eliminated biphasically; however, the distribution phase was extremely short and the kinetics could reasonably be described using a one-compartment model [5]. An i.v. dose of 50 mg kg^{-1} produced peak plasma concentrations of around 100 $\mu g\ ml^{-1}$. With i.p. injection, peak drug concentrations were rapidly achieved (usually within 5–15 min) but were three- to fourfold lower than with the i.v. route. RSU 1069 i.p. bioavailability was 55%, indicating the likelihood of some first-pass metabolism.

Oral administration of 50 mg kg^{-1} RSU 1069 to C3H mice resulted in peak plasma concentrations of only about 10 $\mu g\ ml^{-1}$. The oral bioavailability was estimated at 20% (assuming similar i.v. drug availability in C3H and BALB/c mice), possibly as a result of extensive acid hydrolysis in the stomach.

In BALB/c mice, RSU 1069 plasma elimination was clearly dependent on the route of administration, with the i.p. route producing twofold lower $t_{1/2}$ values than i.v. injection. Both strains of mice showed similar elimination

$t_{1/2}$s after the same i.p. dose. In addition, there was a suggestion of dose-dependent elimination in BALB/c mice, with a 37% increase in $t_{1/2}$ when the dose was doubled from 50 to 100 mg kg^{-1} i.p. ($P < 0.001$).

As with other nitroimidazoles of intermediate lipophilicity [21], RSU 1069 elimination was dependent on both renal clearance and metabolism. Urinary recovery of the unchanged drug was 16%–20%, with an additional 6%–9% recovered as the aziridine ring-opened metabolite RSU 1137 and <1% as the aziridine ring-removed amine metabolite RSU 1111, leaving some 74% of the dose unaccounted for. An unidentified polar metabolite was present on HPLC and further metabolism to non-UV-detectable imidazole ring-opened fragments is likely, as occurs with several other nitroimidazoles [9]. In addition, the alkylation of nucleophilic targets such as DNA through both the aziridine moiety and the reductive bioactivation of the nitro group may also account for some of this unrecovered material [8, 11].

The major plasma metabolite of RSU 1069 was shown to be the aziridine ring-opened hydrolysis product RSU 1137. This metabolite reached peak concentrations of between 1 and 5 μg ml^{-1} 15–30 min after 50 mg kg^{-1} RSU 1069 i.p. injection. The apparent elimination rate for RSU 1137 was some twofold slower than for the parent drug and did not appear to be dose-dependent. RSU 1111 was not detected in plasma, but an unidentified polar metabolite was seen on HPLC.

The volume of distribution ranged from 0.65 to 2.0 ml g^{-1}, depending on the dose, a value consistent with the expectation of good tissue penetration. However, tumour and brain penetration was generally limited. Tumour/plasma ratios after equilibration were between 20%–40% in the transplanted KHT fibrosarcoma. Brain/plasma ratios were even lower, at between 10%–30% under similar conditions. Other studies with the Lewis lung tumour in C57 mice have shown similarly low tumour/plasma ratios, with brain/plasma ratios always <100% [16]. In contrast, the B16 melanoma in C57 mice exhibited very high tumour/plasma ratios in the range of 370%–400%, although there was no evidence of drug accumulation in normal brain or other tissues [4]. Recent in vitro studies have shown that as the extracellular pH is increased from 5.4 to 8.4 B16 melanoma cells progressively accumulate RSU 1069 intracellularly, giving cell/medium ratios from 99% to 304% over this pH range [16]. In contrast, no such accumulation was evident in KHT and Lewis lung tumour cells. The partial exclusion of RSU 1069 from certain tissues and its accumulation in the B16 melanoma may relate to the weakly basic nature of the aziridine ring of RSU 1069 (pKa = 6.04) [15] and differences in intracellular pH regulation.

Other mechanisms may also contribute to the relatively low tissue/plasma ratios. Extensive plasma protein binding would reduce drug availability for tissue uptake. However, the closely related analogue MISO is only 5%–10% protein bound and shows tumour/plasma ratios of around 100% [19, 21]. The slightly lower lipophilicity of RSU 1069 compared with MISO (octanol-water partition coefficients, 0.22 and 0.43 respectively at pH 7.4) [15] might contribute to its poor brain penetration but is unlikely to be involved in the low tumour concentrations.

Interestingly, the major metabolite RSU 1137 gave steady-state tumour/plasma ratios of 100%, suggesting either that RSU 1069 is readily hydrolysed in this tissue or, perhaps more likely, that the removal of the protonatable aziridine ring allows the complete equilibration of this neutral metabolite with plasma.

By comparison, the mixed-function sensitizer CB 1954 (2,4 dinitro-5-aziridinylbenzamide) gave peak plasma concentrations similar to those occurring with RSU 1069 after i.p. injection in mice [22, 23]. The elimination half-life was four times longer for CB 1954 and the i.p. bioavailability some 50% greater than for RSU 1069. Tumour penetration was also more efficient, with tumour/plasma ratios between 50%–90% in EMT6 mammary tumours. CB 1954 was extensively metabolised in mice, with only 16%–25% of the parent drug recovered in the 24 h urine [23], a value similar to the 16%–20% recovery for RSU 1069 in 0 to 8 h mouse urine in this study.

Despite the fact that the pharmacokinetic behaviour of RSU 1069 allows scope for some improvement, active chemosensitizing and radiosensitizing concentrations are readily achieved with an i.p. dose of 80 mg kg^{-1} in mice [1, 2]. On the basis of our results and those of Deacon et al. [4], this dose would be expected to produce plasma concentrations of between 20–50 μg ml^{-1} 30 min after i.p. injection. Concentrations of 20–40 μg ml^{-1} RSU 1069 produced comparable radiation enhancement ratios of around 1.8 in Chinese hamster V79 cells in vitro [1, 2]. In contrast, plasma concentrations of RSU 1069 in man are limited to 1.5–3.5 μg ml^{-1} 30 min after drug infusion as a result of severe dose-limiting gastrointestinal toxicity [7]. Unfortunately, this concentration is unlikely to give appreciable hypoxic cell sensitization. However, the attractive molecular pharmacology of RSU 1069 has led to the continued pursuit of more potent and/or less toxic analogues [3]. We believe that the detailed pharmacokinetic data presented here for RSU 1069 should prove useful in the design and evaluation of improved analogues of this interesting mixed-function sensitizer.

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