Cancer Chemother Pharmacol (1997) 40: 1–10

ORIGINAL ARTICLE

**Martin A. Graham · Suresh Senan
Hernani Robin Jr · Nils Eckhardt · Dennis Lendrem
Jeffery Hincks · Dennis Greenslade · Roy Rampling
Stanley B. Kaye · Reinhard von Roemeling
Paul Workman**

Pharmacokinetics of the hypoxic cell cytotoxic agent tirapazamine and its major bioreductive metabolites in mice and humans: retrospective analysis of a pharmacokinetically guided dose-escalation strategy in a phase I trial

Received: 27 May 1996/Accepted: 30 September 1996

Abstract Tirapazamine (3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide; SR 259075) is a selective hypoxic cell cytotoxic agent that is bioreductively activated in tumours to a reactive-drug free radical. Preclinically the agent has been shown to possess additive and synergistic anti-tumour activity in combination with radiotherapy and chemotherapy regimens. In the present study the pharmacokinetics and metabolism of tirapazamine were investigated in mice and patients as part of pre-clinical and phase I investigations. The objectives of this work were twofold; firstly, to evaluate retrospectively the utility of a pharmacokinetically guided dose-escalation (PGDE) strategy for tirapazamine, and secondly, to investigate if pharmacologically relevant plasma concentrations could be achieved at tolerable doses. Pharmacokinetic studies for PGDE were conducted in mice at four dose levels ranging from one-tenth of the LD_{10} to the LD_{50}. The AUC at the LD_{10} (2932 $\mu g\,ml^{-1}$ min) was used to determine a target AUC value of 1173 $\mu g\,ml^{-1}$ min (equivalent to 40% of the mouse LD_{10} AUC) for clinical studies. A phase I study to investigate the tolerance of a single i.v. infusion of tirapazamine (once every 3 weeks) was initiated with close pharmacokinetic monitoring. The starting dose (36 mg/m^2) was based on toxicity data obtained in the mouse, rat and dog. Doses were escalated by increases in the volume and duration of infusion. A retrospective analysis of the pharmacokinetic and toxicity data was then made to determine the utility of a PGDE approach. The drug exhibited a steep dose-lethality relationship in mice (LD_{10} 294 mg/m^2, LD_{50} 303 mg/m^2). The major gross toxicities were body-weight loss (15–20%), pilo-erection and hypoactivity at all dose levels. Sporadic ptosis and conjunctivitis were observed at doses of >300 mg/m^2. The plasma elimination of tirapazamine fitted a mono-exponential open model, with rapid elimination from the plasma ($t_{1/2} = 36 \pm 0.65$ min) occuring at the LD_{10} dose of 294 mg/m^2. A 10.3-fold increase in dose resulted in a 25.0-fold increase in AUC. Clinically, doses were escalated over the range of 36–450 mg/m^2. Ototoxicity (tinnitus and reversible hearing loss) was dose-limiting at 450 mg/m^2 and the MTD was 390 mg/m^2 for this schedule. Pharmacokinetic analyses in patients revealed that the elimination of tirapazamine in patients

M.A. Graham · H. Robin Jr · N. Eckhardt · S.B. Kaye
P. Workman
Clinical Oncology and New Drug Development Group
CRC Deptartment of Medical Oncology, University of Glasgow,
Alexander Stone Building, Garscube Estate, Switchback Road,
Bearsden, Glasgow G61 1BD, UK

M.A. Graham · D. Greenslade
Department of Pre-clinical Drug Metabolism and
Pharmacokinetics, Sanofi-Winthrop, Alnwick,
Northumberland, England, UK

S. Senan · R. Rampling
Department of Radiation Oncology, Beatson Oncology Centre,
Western Infirmary, Glasgow, UK

D. Lendrem
Biostatistics, Sanofi-Winthrop, Alnwick, Northumberland, UK

J. Hincks
Department of Toxicology, Sanofi-Winthrop,
Great Valley Parkway, Malvern, Pennsylvania, USA

R. von Roemeling
Department of Clinical Oncology, Sanofi-Winthrop,
Great Valley Parkway, Malvern, Pennsylvania, USA

M.A. Graham (✉)
Department of Clinical Pharmacokinetics and Drug Metabolism,
Sanofi Pharmaceuticals Inc., 9 Great Valley Parkway,
P.O. Box 3026, Malvern, PA 19355, USA
Tel. 001 610 889 6025; Fax 001 610 889 6356

was generally bi-phasic, with low inter-patient variability being found in clearance. A 12.5-fold increase in dose resulted in a 19.0-fold increase in AUC. There was good quantitative agreement in metabolite formation between mice and humans with respect to the two- and four-electron bioreductive metabolites. AUC values recorded for tirapazamine at the MTD of 390 mg/m^2 (range 1035–1611 μg ml^{-1} min) were similar to the target AUC in mice. Importantly, these levels are consistent with the levels required for radiation-dose enhancement and effective combination with cisplatin in mice. Given (a) the similarities in plasma pharmacokinetics and metabolism observed at the target AUC/MTD in mice, rats, dogs and humans, (b) the similar degree of plasma protein binding seen between species and (c) the relatively low inter-patient variability noted in drug clearance, a successful PGDE approach should have been feasible. The results also indicate that potentially therapeutic levels of tirapazamine are achievable in patients at tolerable doses.

Key words Tirapazamine · Bioreductive agent · PGDE · Pharmacokinetics · Phase I study

Abbreviations *MTD* · Maximally tolerated dose · *AUC* area under the curve · *PGDE* pharmacokinetically guided dose escalation · C_{max} maximal concentration in plasma · t_{max} time to maximal plasma concentration · $t_{1/2}$ half-life · *Cl* clearance · K_{el} elimination constant · Vd_{ss} volume of distribution at steady state · LD_{10} 10% lethal dose · LD_{50} 50% lethal dose · LD_{90} 90% lethal dose · *HPLC* high-pressure liquid chromatography · *CI* 95% confidence interval

Introduction

The primary objective of a phase I study with a new anti-cancer agent is to determine the maximally tolerated dose by a particular route of administration or schedule. Conventionally, the phase I starting dose is based on a dose equivalent to one-tenth of the mouse LD_{10} (1/10 $MELD_{10}$, expressed on a surface-area basis) or, occasionally, one-third of the toxic dose low (1/3 TDL) in the rat or dog. These starting dose parameters are based on the retrospective analysis of toxicological data from the major classes of anti-tumour agents investigated in a variety of species since the early 1960s [7]. Conventionally, dose escalation is based on empirical schemes such as the modified Fibonnaci series (sequential increments of 100%, 67%, 50%, 40% and 30–35% for the remainder). However, this approach can be problematical, particularly if the starting dose extrapolated from mouse studies gives a falsely low prediction of human tolerance due to interspecies differences. In these circumstances it can be envisaged that completion of a phase I study may require multiple dose-escalation steps, resulting in a protracted trial. Additionally, large numbers of patients may be treated at potentially subtherapeutic doses.

Protracted phase I studies have given impetus to the development of more rational dose-escalation strategies based on pharmacokinetic principles. Pharmacokinetically guided dose escalation (PGDE) was originally proposed by Collins and co-workers [3] and subsequently refined by the EORTC Pharmacokinetics and Metabolism Group [5]. This approach is centered on the principle that disparities in drug tolerance between species may be due to differences in drug pharmacodynamics (e.g. intrinsic target cell sensitivity) or pharmacokinetics (e.g. species variation in plasma clearance). It may be possible to correct for pharmacokinetic differences between species and use mouse pharmacokinetic/toxicity data to guide dose increments rationally in patients (for reviews see [11, 12]).

The central hypothesis on which all PGDE strategies are based is that drug exposure (i.e. AUC) is equivalent at the respective maximally tolerated doses in mice and humans. If this hypothesis is correct, then it should be possible to select a dose level that results in a desired AUC value and level of toxicity. In turn, this may be used to guide the phase I dose escalation in a predetermined number of steps [3]. Retrospective and prospective studies of PGDE have indicated that the applicability of the approach is due (at least in part) to the cell-cycle specificity of an agent [8, 9]. PGDE appears to be effective for cell-cycle-phase non-specific agents such as platinum complexes, alkylating agents, DNA-complexing agents, antibiotics and topoisomerase inhibitors [5, 8, 9, 11, 12]. However, cell-cycle-specific drugs such as the antimetabolites and antimitotic drugs and certain compounds requiring metabolic activation for activity are considered poor candidates for PGDE [5, 8, 9, 11, 12], presumably due to species variability in drug metabolism and/or pharmacodynamic effects that complicate the extrapolation of exposure-effect relationships across species.

To date there has been no retrospective or prospective PGDE study involving a bioreductive agent. Therefore, this article describes the pharmacokinetics, metabolism and toxicity of tirapazamine in mice to evaluate retrospectively a PGDE scheme and to assess the utility of this approach for future bioreductive agents in this class.

Materials and methods

Chemicals and reagents

All chemicals and reagents were of analytical reagent grade or HPLC grade. Tirapazamine (3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide, SR 259075; formerly SR 4233; for animal use) was supplied as

a powder by Sanofi-Winthrop Inc. Tirapazamine (for clinical use) was supplied as a liquid formulated at 0.7 mg/ml in an isotonic citrate buffer with a pH of 3.7–4.3. The two-electron reduction product (SR 264012; formerly SR 4317), the four-electron reduction product (SR 260109; formerly SR 4330), and the internal standard (SR 259852) were obtained from SRI International, Life Sciences Division (Menlo Park, Calif., USA). For purposes of consistency with the published literature the two- and four-electron reduced metabolites are referred to as SR 4317 and SR 4330, respectively, throughout the text.

Preparation of dose solutions

For the toxicity studies in mice, tirapazamine was dissolved at 2.5 mg/ml in warm (40 °C) 0.9% saline. The stock solutions were diluted to 2.14, 2.01, 2.00, 1.91 and 1.87 mg/ml in saline. For the pharmacokinetic experiments in mice the 2.5 mg/ml stock solution was diluted to 2.01, 1.97, 0.985 and 0.197 mg/ml. Dose solutions for clinical use were drawn up directly from ampoules without further dilution and given at a fixed concentration (0.7 mg/ml).

Pharmacokinetics and toxicity studies in mice

Lethality determination

All animal studies were conducted in accordance with institutional and Home Office (UK) guidelines governing animal welfare. Groups of ten female BALB/c mice received tirapazamine as a single i.v. injection at 93, 96, 100, 101 and 107 mg/kg, equivalent to 279, 288, 300, 303 and 321 mg/m^2, respectively, using a mg/kg-to-mg/m^2 conversion factor of 3 for the mouse. Drug was injected over 0.5 min at 50 ml/kg. Control groups received an equivalent volume of saline. The LD_{10} and LD_{50} values were determined by probit analysis of the lethality data (Systat Software Ltd).

Pharmacokinetics studies in mice

For pharmacokinetics studies the drug was injected i.v. at four dose levels equivalent to one-tenth of the LD_{10}, one-half of the LD_{10}, the LD_{10} and the LD_{50}. At $t = 2$, 4, 6, 8, 10, 15, 20, 30, 45, 60, 120 and 240 min post-treatment, groups of three mice were ether-anaesthetized and blood samples were collected by cardiac puncture into heparinized tubes. Blood was also taken from three mice that received saline alone. The samples were immediately centrifuged for 5 min (1000 *g*) and the three samples obtained at each time point were pooled and stored frozen (-70 °C) until analysis.

Pharmacokinetics studies in patients

Phase I and pharmacokinetics studies were conducted with the written informed consent of the patient and with study approval from the local ethical review committee. Tirapazamine was given to sequential cohorts of patients by i.v. infusion at a rate not exceeding 5 ml/min. Doses were escalated from 36 to 450 mg/m^2 by progressive increases in the volume and duration of infusion. Infusion times ranged from 14 to 235 min. Blood samples (5- to 10-ml aliquots) were obtained from the opposite arm and collected into tubes containing lithium heparin anticoagulant. Samples were taken prior to drug administration and at the following nominal time points; mid-infusion (or hourly for infusions lasting longer than 2 h), end-infusion at 0, 10, 15 20, 30, 45, 60, 120, 240 and 360 min and at approximately 24 h. Plasma was obtained by centrifugation, then immediately frozen on dry ice. Samples were subsequently stored at -70 °C until analysis.

Pharmacokinetic analysis

Tirapazamine levels and the pharmacokinetics of the two-electron reduction product SR 4317 and the four-electron reduction product SR 4330 were assayed using a validated solid-phase extraction and HPLC method for mouse and human plasma [20]. Pharmacokinetic parameters in mice were determined by computer-generated iterative non-linear least-squares estimation (CURVEFIT program) [15, 17]. Clinical pharmacokinetic parameters were determined using the MASTER_PK program resident within the RS1 graphics package at Sanofi-Winthrop Pharmaceuticals. Actual times were used for all pharmacokinetic determinations.

The maximal plasma concentration (C_{max}) for tirapazamine was determined from the steady state plasma concentration at the end of infusion. C_{max} values for SR 4317 and SR 4330 were determined from inspection of the data. The terminal phase half-life ($t_{1/2}$) was determined from the terminal rate constant estimated by linear regression of the last portion of the plasma concentration/time profile. The area under the plasma drug concentration-time curve (AUC) was calculated using the trapezoidal rule and was extrapolated to infinity by division of the last quantifiable plasma concentration by the terminal rate constant [24]. The volume of distribution at steady state (Vd_{ss}) and plasma clearance (Cl) were calculated using the absolute dose expressed in the same units used for analysis [dose (mg/m^2) $\times$ body surface area (m^2) $\times$ 1000 mg/ml].

Statistical analyses

For the parameter AUC, dose proportionality was assessed using regression analysis. The parameter was assumed to follow a multiplicative power model of the form

$$y_{ij} = \alpha x_i{}^{\beta} \varepsilon_{ij},$$

where y_{ij} is the observed $AUC_{(0-inf)}$ value for the jth subject in the ith dose, where i is $1, 2 \ldots 10$ and j is $1, 2 \ldots n_i$, α is the slope parameter, β is the power parameter, x_i is the dose received by subjects in the ith group and $\varepsilon_{ij} \sim$ i.i.d. lognormal$(0, \sigma^2)$.

The above described model is equivalent to the log-transformed model,

$$ln(y_{ij}) = ln(\alpha) + \beta ln(x_i) + ln(\varepsilon_{ij}),$$

with parameters $ln(\alpha)$ and β being estimated via ordinary least-squares regression. Model lack of fit was evaluated by testing of the significance of the lack-of-fit error mean square against the pure-error mean square. Dose proportionality was assessed by testing of the hypothesis H_0: $\beta = 1$, and the dose-proportionality ratio estimate and 95% confidence interval were calculated as

$$[dose_i/dose_{i'}]^{b \pm t \times se(b)},$$

where $dose_i/dose_{i'}$ is the ratio of highest to lowest dose (12.5 for tirapazamine and SR 4317 and 6.25 for SR 4330 since no value was available for the lowest dose), b is the least-squares estimate of b, $se(b)$ is the standard error of b and t is the 97.5th percentile of the t distribution with degrees of freedom equal to the degrees of freedom from the mean square error of the regression analysis. Predicted AUC estimates and their confidence intervals for PGDE (see Table 3) were obtained by exponentiation of estimates from the regression of log AUC versus log dose.

For C_{max} and half-life, dose effects were assessed by one-way analysis of variance (ANOVA) using k_{el} values for the half-life analysis. For Cl and Vd, dose effects were evaluated by simple linear regression, where body-surface-adjusted values for

dose (nominal dose × surface area) were used instead of nominal dose values.

Results

Lethality determination in mice

Deaths occurred between days 3 and 8 post-treatment. The major toxicities encountered at all the dose levels were body-weight loss (15–20%), pilo-erection and hyperactivity. Tremors were observed in 10/10 mice treated at doses of $>300\ mg/m^2$. Sporadic ptosis (in one or both eyes), exudates and conjunctivitis were also observed in animals treated at doses of $>300\ mg/m^2$.

The lethality parameters were derived by probit analysis of the data and were calculated as: $LD_{10} = 294$ [95% confidence interval (CI) 288–300] mg/m^2, $LD_{50} = 303$ (CI 300–309) mg/m^2 and $LD_{90} = 312$ (CI 303–324) mg/m^2. From these data, four dose levels were selected for pharmacokinetic investigations: One-tenth of the LD_{10}, one-half of the LD_{10}, the LD_{10} and the LD_{50} (which corresponded to 29.4, 147, 294 and 303 mg/m^2, respectively).

Pharmacokinetics studies in mice

Pharmacokinetic investigations were conducted at four dose levels ranging from one-tenth of the LD_{10} and the LD_{50} (Table 1, Figs. 1, 2). The plasma elimination of tirapazamine fitted a monoexponential open model with a rapid half-life ($t_{1/2} = 36 \pm 0.65$ min) at 294 mg/m^2 (Table 1). Tirapazamine was rapidly converted to the two- and four-electron reduction products, consistent with previous investigations [26]. Peak plasma levels of 53.6, 26.7 and 3.4 μg/ml were attained for tirapazamine and the two-electron and four-electron reduced metabolites, respectively (Fig. 1). In mice the tirapazamine AUC increased in a greater than dose-proportional manner, as a 10.3-fold increase in dose resulted in a 25.0-fold increase in AUC. The AUC at the LD_{10} was 2939 $\mu g\,ml^{-1}$ min, from which a target AUC value of 1173 $\mu g\,ml^{-1}$ min was calculated for clinical studies.

Table 1 Pharmacokinetics and toxicity parameters determined for tirapazamine in BALB/c mice

Dose level	Dose (mg/m^2)	C_{max} (μg/ml)	$t_{1/2}$ (min ± SD)	AUC_{0-inf}[a] ($\mu g\,ml^{-1}$ min)
1/10 LD_{10}	29.4	4.4	21.2 ± 0.9	138
1/2 LD_{10}	147	23.9	35.0 ± 1.8	1184
LD_{10}	294	53.6	35.5 ± 0.6	2932
LD_{50}	303	55.6	41.8 ± 1.2	3451

[a] Target AUC for PGDE = 1173 $\mu g\,ml^{-1}$ min (40% LD_{10} AUC)

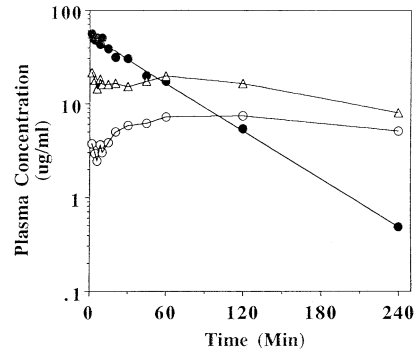

Fig. 1 Pharmacokinetics of tirapazamine (*black circles*) and the reductive metabolites SR 4317 (*open triangles*) and SR 4330 (*open circles*) as determined in BALB/c mouse plasma at the LD_{10} dose of 294 mg/m^2. LD_{10} AUC = 2932 $\mu g\,ml^{-1}$ min; 40% target AUC value for PGDE = 1173 $\mu g\,ml^{-1}$ min

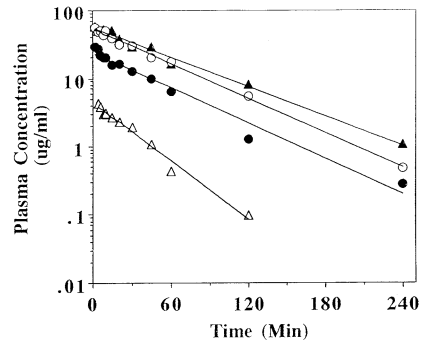

Fig. 2 Pharmacokinetics of tirapazamine in BALB/c mice between 1/10 LD_{10} and the LD_{50}. 1/10 LD_{10} = 29.4 mg/m^2 (*open triangles*), 1/2 LD_{10} = 147 mg/m^2 (*black circles*), LD_{10} = 294 mg/m^2 (*open circles*); LD_{50} = 303 mg/m^2 (*black triangles*)

Pharmacokinetics and dose limiting toxicity in patients

Doses were escalated using a modified Fibonnaci scheme from 36 mg/m^2 until dose-limiting toxicity was encountered at 450 mg/m^2 (Table 2). It was not possible to implement PGDE prospectively in this study, as regulatory approval and permission to implement PGDE was obtained only at the fourth dose level. Given the proximity of the actual AUC values to the target AUC, further savings in the number of dose-escalation steps would not have been possible by introducion of PGDE at this stage, and doses were escalated in fixed increments of approximately 32–39% up to the MTD (Table 3). Tinnitus and reversible hearing loss were initially observed in one patient treated at 450 mg/m^2 [21]. Further patients were then treated at doses ranging from 330 to 450 mg/m^2. Ototoxicity occurred following the administration of tirapazamine in 1/6 patients treated at 330 mg/m^2 and 1/4 patients treated at 390 mg/m^2 . In all, 3/3 patients developed tinnitus and reversible hearing loss at 450 mg/m^2 [21]. Non-dose-limiting toxicities included nausea and

Table 2 Summary of phase I pharmacokinetic parameters determined for tirapazamine

Patient number	Dose level (mg/m^2)	AUC ($\mu g\ ml^{-1}\ min$)	$t_{1/2}$ (min)	C_{max} ($\mu g/ml$)	t_{max} (min)	Cl (l/min)	Vd (l)
0001	36	85.3	41.5	1.498	14	0.578	30.3
0002	36	86.6	38.1	2.448	15	0.599	24.5
0003	36	91.6	47.3	1.701	20	0.696	40.6
Mean	36	87.8	42.3	1.882	16	0.624	31.8
0004	72	114.1	31.1	2.426	33	0.997	38.7
0006	72	165.9	53.2	1.927	19	0.816	47.5
Mean	72	140.0	42.1	2.176	26	0.906	43.2
0007	120	381.3	43.2	4.454	66	0.598	33.7
0008	120	247.3	40.6	3.457	64	0.898	40.2
0009	120	285.7	31.9	4.231	65	0.714	29.2
Mean	120	304.8	38.6	4.047	65	0.737	34.4
0011	180	510.2	50.7	4.700	77	0.529	40.6
0012	180	443.0	43.3	3.820	100	0.703	42.0
Mean	180	476.6	47.0	4.260	88	0.616	41.3
0013	250	729.5	44.5	4.496	120	0.685	41.4
0014	250	830.2	39.2	5.690	100	0.497	27.4
Mean	250	779.8	41.8	5.093	110	0.591	34.4
0016	330	1066.8	49.2	5.346	150	0.523	36.8
0017	330	1252.3	50.4	6.634	120	0.427	36.4
0018	330	923.2	44.2	4.950	150	0.629	39.0
0020	330	863.8	303.8	3.635	150	0.745	85.4
Mean	330	1026.5	47.9[a]	5.141	142	0.581	49.4
0025	390	1610.8	58.5	8.322	158	0.387	34.8
0027	390	1583.6	61.1	7.929	152	0.404	37.2
0028	390	1035.0	49.3	5.103	192	0.610	46.0
Mean	390	1409.8	56.3	7.118	167	0.467	39.3
0022	450	1348.7	44.0	6.022	150	0.557	24.5
0023	450	1405.2	71.1	5.842	210	0.503	43.6
Mean	450	1376.9	57.5	5.932	180	0.530	34.1
Group Mean $\pm$ SD	NA	NA	46.6 $\pm$9.53[a]	NA	NA	0.624 $\pm$0.157	39.0 $\pm$12.5

[a] Mean values excluding patient 20
(*NA* Not applicable)

vomiting and muscle cramping; the severity of the latter appeared not to increase with dose [21].

Tirapazamine was rapidly cleared from the plasma of patients, with the mean plasma clearance being 0.53 l/min and the mean Vd_{ss} being 34.1 l at 450 mg/m^2 (Table 2). In general, the pharmacokinetics of tirapazamine declined with a mean terminal half-life of 46.6 ± 9.53 min. In some individuals a short initial distribution phase and/or a prolonged terminal phase was evident that could not be accurately characterized.

Pharmacokinetic analyses demonstrated relatively low inter-patient variability in drug exposure (AUC) at a given dose level (Table 2, Fig. 3). The mean $AUC_{(0-inf)}$ increased with dose in a greater than dose-proportional manner (i.e. the hypothesis of $\beta = 1$ in the power regression model was rejected: $P < 0.001$). A 12.5-fold increase in dose was accompanied by an estimated 19.0-fold (95% CI 14.9- to 24.3-fold) increase in mean $AUC_{(0-inf)}$ (Fig. 3). There was no evidence of lack of fit for the power model ($P = 0.372$). C_{max} values significantly increased with dose ($P < 0.001$), although this increase was less than dose proportional due to the progressive increase in infusion time implemented during dose escalation. The half-life (k_{el}) of tirapazamine also significantly increased with dose ($P = 0.014$), which was accompanied with a slight but significant decrease ($P = 0.016$) in clearance. There was no significant dose effect on Vd_{ss} ($P = 0.282$; Table 2).

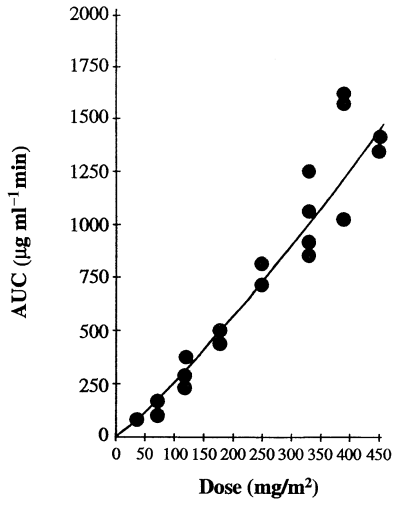

Fig. 3 Relationship between dose and AUC as determined for tirapazamine in patients

Two-electron reduced metabolite

The two-electron reduction product (SR 4317) was the major bioreductive metabolite detected in human plasma (C_{max} 3.0–4.1 μg/ml at a tirapazamine dose of 390 mg/m^2), with levels exceeding those of tirapazamine at later time points (Fig. 4). Levels of the two-electron reduction product declined relatively slowly, with the mean $t_{1/2}$ being 179 ± 83 min. The mean $AUC_{(0-inf)}$ increased with dose in a greater than dose-proportional manner (i.e. the hypothesis of $\beta = 1$ in the power regression model was rejected; $P < 0.001$). A 12.5-fold increase in dose was accompanied by an estimated 53.1-fold (95% CI 33.7- to 83.6-fold) increase in mean $AUC_{(0-inf)}$. There was no evidence of lack of fit for the power model ($P = 0.120$). For values of C_{max} and k_{el} there was a significant dose effect ($P < 0.001$ and $P = 0.097$, respectively).

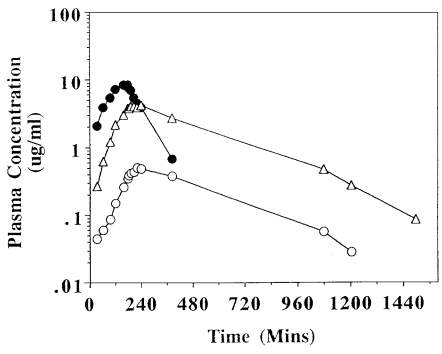

Fig. 4 Pharmacokinetic profile of tirapazamine (*black circles*) and the reductive metabolites SR 4317 (*open triangles*) and SR 4330 (*open circles*) as determined in a patient treated at 390 mg/m^2

Four-electron reduced metabolite

The four-electron reduced metabolite (SR 4330) was the second most abundant bioreductive metabolite detected in human plasma. C_{max} increased significantly with dose ($P < 0.001$). Levels ranging between 0.51 and 0.66 μg/ml were detected at 390 mg/m^2 (Fig. 4), which declined with a mean $t_{1/2}$ of 242.8 ± 103.7 min. The mean $AUC_{(0-inf)}$ increased with dose in a greater than dose-proportional manner (i.e. the hypothesis of $\beta = 1$ in the power regression model was rejected: $P < 0.001$). A 6.25-fold increase in dose was accompanied by an estimated 30.3-fold (95% CI, 12.9- to 1.1-fold) increase in $AUC_{(0-inf)}$. There was some evidence of lack of fit for the power model ($P = 0.058$). Inspection of the log-log plots (data not shown) revealed some curvature, suggesting that the true increase across the dose range may be even greater than that estimated from the multiplicative power model. There was no significant dose effect for k_{el} ($P = 0.039$).

Retrospective analysis of PGDE

Tirapazamine doses were escalated from 36 to 450 mg/m^2 in a total of eight escalation steps. Table 3 outlines the alternative PGDE approach for tirapazamine derived from a retrospective analysis of the data and provides estimates of drug exposure at each dose level. In this model, tirapazamine doses were escalated in 100% increments from 36 to 288 mg/m^2 in three steps. On the basis of the actual phase I pharmacokinetic data, this dose level would have resulted in an estimated AUC of 920 μg ml^{-1} min, i.e. just below the target AUC value of 1173 μg ml^{-1}min. On the achievement of plasma levels similar to the target AUC the remainder of the series would have been completed

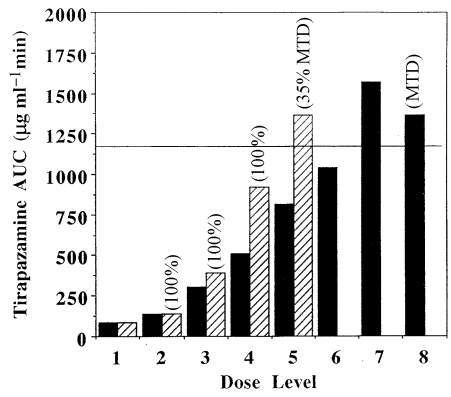

Fig. 5 Comparison of the actual phase I dose-escalation scheme (*black bars*) with the theoretical PGDE dose-escalation scheme (*dashed bars*). Percentages in parentheses represent the increments in dose. The line represents the target AUC (40% LD_{10} AUC = 1173 μg ml^{-1}min in mice)

Table 3 Retrospective analysis of PGDE[a]

Dose level	Actual dose (mg/m^2)	Mean actual AUC (range) in $\mu g\,ml^{-1}\,min$	Theoretical PGDE dose (mg/m^2)	Predicted PGDE AUC in $\mu g\,ml^{-1}\,min$ (95% CI)
1	36	89 (85–92)	36	64 (40–100)
2	72 (100%)	140 (114–166)	72 (100%)	142 (82–244)
3	120 (67%)	305 (247–381)	144 (100%)	316 (168–594)
4	180 (50%)	477 (443–510)	288 (100%)	705 (344–1447)
5	250 (39%)	780 (729–830)	389 (35%)	999 (468–2130)
6	330 (32%)	1027 (864–1252)	525 (35%)	1412 (639–3123)
7	450 (36%)	1376 (1349–1405)		
8	390 (− 13%)	1410 (1035–1611)		

[a] Percentages in parentheses represent the incremental rise in dose

with fixed 35% increments up to the MTD. Indeed, a single 35% increase in dose to 389 mg/m^2 would have reached the MTD in a total of five steps (Table 3, Fig. 5). This approach would have saved up to three dose levels, nine patients and approximately 6 months of development time without compromising safety.

Discussion

The pharmacokinetics and toxicity of tirapazamine have been studied in mice in parallel with a phase I clinical trial to investigate the tolerance of a single-infusion schedule once every 3 weeks in patients [21]. This paper deals with the pre-clinical and clinical pharmacokinetic analyses from these studies. There were two main objectives of this work. The first objective was to evaluate a pharmacokinetically guided dose-escalation (PGDE) strategy for tirapazamine and evaluate the utility of a PGDE approach for other bioreductive agents in this class. The second objective was to investigate if pharmacologically relevant plasma concentrations could be achieved at tolerable doses in patients. This is the first report to describe the pharmacokinetics of tirapazamine and its major metabolites in humans and also represents the first retrospective study to evaluate the utility of a PGDE approach in the clinical development of a bioreductive anti-tumour agent.

The first major objective was to investigate the phase I pharmacokinetics of tirapazamine in mice to make a retrospective analysis of a PGDE scheme in patients. Fuse et al. [8, 9] have proposed that cytotoxic agents can be classified into type 1 (cell-cycle-phase non-specific) and type 2 (cell-cycle-phase-specific) agents. Generally, PGDE appears to be applicable to type 1 agents, including platinum complexes, alkylating agents, DNA-complexing agents, antibiotics and topoisomerase inhibitors [8]. In contrast, type 2 agents such as the antimetabolites, antimitotic agents and drugs requiring metabolic activation for activity are considered not to be readily amenable to a PGDE approach [5, 8]. Although tirapazamine can be considered a type 1 agent, the observation that it requires metabolic activation for activity (6, 19, 24–27) could compromise a PGDE approach.

The applicability of PGDE was evaluated by murine pharmacokinetics and toxicity studies conducted in conjunction with the phase I clinical trial. From these and other pre-clinical safety studies in the mouse, rat and dog, the predicted dose-limiting toxicities in humans were myelosuppression and emesis [13, 23, 29]. Indeed, the dose-limiting toxicity for tirapazamine in patients was ototoxicity manifesting as reversible hearing loss [21]. Drug exposure in patients treated at 390–450 mg/m^2 was in the range 1035–1611 $\mu g\,ml^{-1}\,min$, similar to the 40% target AUC value (1173 $\mu g\,ml^{-1}\,min$) but lower than the mouse LD_{10} (2932 $\mu g\,ml^{-1}\,min$). It is noteworthy, however, that the target AUC value was based on conventional lethality estimates in the mouse (40% LD_{10} AUC) and that this relatively crude end point could have resulted in over-estimation of the target AUC. Indeed, this is probably reflected in the MTD/LD_{10} dose-ratio of approximately 0.5 found for tirapazamine. This result appears not to be unique to tirapazamine, as similar ratios have been reported for other type 1 agents such as nimustine (ACNU) (0.4), bleomycin (0.7) and mitomycin (0.7) [9]. The apparently lower exposure observed in patients at the MTD probably reflects the different end points used in the respective studies, namely lethality in mice (a relatively crude end point) and ototoxicity in patients [21]. The accuracy of PGDE predictions in future studies may thus be improved by consideration of the use of toxicity end points other than lethality in pre-clinical models.

Inter-species differences in drug metabolism have complicated the application of PGDE to other anti-tumour agents [11, 12], notably iodo-doxorubicin [10]. It was therefore important to determine if qualitative and/or quantitative differences in drug metabolism between mice and humans could have compromised a PGDE approach for tirapazamine. The hepatic metabolism of tirapazamine is considered the major site of biotransformation in rodents [16]. A variety of metabolic reactions are known to occur in

normal and tumour tissue, the major pathways being the formation of the two- and four-electron reduction products by cytochrome P450, cytochrome P450 reductase and DT-diaphorase [6, 16, 18, 19, 25–28]. These reduced metabolites also undergo secondary hydroxylation and conjugation reactions in vivo [16]. Although it was not possible to investigate the hydroxylated and conjugated metabolites in this study, there was good agreement between mice and humans with respect to the formation and clearance of the two- and four-electron bioreductive metabolites (Figs. 1, 4). With regard to the application of PGDE to tirapazamine, one of the most striking features was the lack of any substantial difference in metabolism between mice and humans with regard to SR 4317 and SR 4330. In mice the peak plasma concentration ratio recorded for tirapazamine: SR 4317:SR 4330 was approximately 2:1:0.06, which was in close agreement with the ratio noted for humans (approximately 2:1:0.08). In contrast to the established view that agents requiring metabolic activation for activity may not be good candidates for PGDE [3, 5], this study demonstrates that certain classes of bioreductive agents may be considered, provided that equivalence in drug metabolism can be demonstrated across the species.

In addition to drug metabolism, another important factor that should be considered in attempts at a PGDE approach is dose-proportional pharmacokinetics [11, 12]. Tirapazamine and the two bioreductive metabolites did exhibit non-linear pharmacokinetics in the mouse. The drug also exhibited a steep dose-lethality relationship in mice at doses $>294\ mg/m^2$ (Table 1). However, between 29.4 and $294\ mg/m^2$, i.e. the clinically relevant dose-range, drug toxicity was reversible and the pharmacokinetics, for all practical purposes, were reasonably linear. Although the pre-clinical studies highlighted the possibility of non-linear pharmacokinetics in patients, the degree of nonlinearity in animals was relatively small and should not have prevented the application of a PGDE scheme. Furthermore, the conservative target AUC value of 40% mouse LD_{10} AUC would also have provided a substantial safety margin for dose-escalation.

Species differences in plasma protein binding must also be considerd in the development of PGDE strategies for new agents [8, 11, 12]. The binding of [^{14}C]-tirapazamine has previously been described in both mouse and human plasma [20]. These studies showed that tirapazamine was not extensively bound to plasma protein and that the degree of binding was similar between the species [20]. Consequently, in the development of a PGDE strategy for tirapazamine there was no need to adjust the target AUC value on the basis of the unbound fraction of drug in mouse and human plasma [8, 9]. In addition, any inter-patient variability in tirapazamine clearance is unlikely to be due to a protein-binding phenomenon. Fortunately, there was low inter-patient variability in tirapazamine clearance at the starting dose ($36\ mg/m^2$) and subsequent dose-levels, which provided a firm basis for a pharmacokinetically guided approach. The low inter-patient variability in tirapazamine clearance is a favourable characteristic for the drug as it increases the chances of achieving reproducible drug, exposure whilst reducing the risk for idiosyncratic toxicities due to variable pharmacokinetics.

Phase I studies were initiated at $36\ mg/m^2$ and doses were escalated using a conventional scheme until toxicity was encountered at $450\ mg/m^2$. The dose was then de-escalated to $390\ mg/m^2$ (Table 2). The retrospective pharmacokinetic analysis reported in this manuscript, however, indicates that a PGDE approach would have been feasible for tirapazamine and could probably have saved two to three dose-levels. Table 3 and Fig. 5 illustrate the theoretical savings of a PGDE approach.

The first dose-escalation step in PGDE would have followed exactly the adopted approach, i.e. a 100% increment to $72\ mg/m^2$ (Table 3, Fig. 5). However, 100% increments could have been safely implemented for levels 3 and 4 up to $288\ mg/m^2$, at which point the predicted PGDE AUC ($705\ \mu g\,ml^{-1}\,min$; 95% CI $344–1447\ \mu g\,ml^{-1}\,min$) would have encompassed the target AUC of $1173\ \mu g\,ml^{-1}\,min$. On achievement of the target AUC, the series would subsequently have been completed in 35% increments. The first 35% increment to $389\ mg/m^2$ would probably have completed the series in a total of five steps, although a further additional step could have been evaluated, depending on clinical signs (Table 3, Fig. 5). In retrospect, this approach could have resulted in an estimated saving of two to three dose levels, six to nine patients and approximately 4–6 months of development time without compromising safety. PGDE may be a valuable study design for consideration in the development of second-generation compounds and other classes of bioreductive agents, provided that reasonable equivalence in drug metabolism and metabolite exposure can be demonstrated between animals and humans.

The second major objective of this work was to investigate the pharmacokinetics of tirapazamine in mice and patients to determine if potentially effective levels of tirapazamine could be achieved at tolerable doses. Although the plasma levels detected in patients were lower than the levels predicted from the mouse LD_{10}, drug exposure at the human MTD ($1035–1611\ \mu g\,ml^{-1}\,min$) was nonetheless in excess of the level predicted to result in effective radiation-dose enhancement on a multiple-dose schedule (approximately $80\ \mu g\,ml^{-1}\,min$) in mice [1, 2]. Importantly, recent preliminary clinical data indicate that tirapazamine can be given in doses in excess of $260\ mg/m^2$, three times a week for 4 weeks (i.e., a total of 12 doses) with a standard course of radiotherapy without causing dose-limiting toxicity [22]. The levels attained at the

MTD in the current study are also consistent with the levels predicted to be required for effective combination with cisplatin (approximately 1184 $\mu g\,ml^{-1}\,min$) in preclinical models [1, 4, 14].

In summary, these data show that PGDE would have been a feasible approach in the phase I development of tirapazamine and could have resulted in a substantial saving in patients and time without compromising safety. The accompanying pharmacokinetic and toxicity data gathered in mice and humans demonstrated comparable pharmacological effects between the species and demonstrated that target AUC values for phase I studies could be safely based on the mouse model. In addition, the low inter-patient variability in drug clearance and lack of species differences in metabolism would have facilitated a PGDE approach. Importantly, these studies also demonstrate that potentially therapeutic levels of tirapazamine can be attained in patients for combination therapy with radiation and chemotherapy.

Acknowledgements This work was conducted on behalf of the EORTC Pharmacokinetics and Molecular Mechanisms (PAMM) Group. The authors would like to acknowledge the assistance of Gale Kennedy and Sara Deehan in the analysis of pharmacokinetic data. This work was supported by grants from the Cancer Research Campaign (CRC), UK, and Sanofi-Winthrop Inc., USA. H.R. was a visiting Research Fellow sponsored by the Brazilian Medical Oncology Society.

References

1. Brown JM (1993) SR4233 (tirapazamine): a new anticancer drug exploiting hypoxia in solid tumors. Br J Cancer 67: 1163–1170
2. Brown JM, Lemmon MJ (1990) Potentiation by the hypoxic cytotoxin SR4233 of cell killing produced by fractionated irradiation of mouse tumors. Cancer Res 50: 7745–7749
3. Collins JM, Zaharko DS, Dedrick RL, Chabner BA (1986) Potential roles for preclinical pharmacology in phase-I clinical trials. Cancer Treat Rep 70: 73–80
4. Dorie MJ, Brown JM (1993) Tumor-specific, schedule-dependent interaction between tirapazamine (SR4233) and cisplatin. Cancer Res 53: 4633–4636
5. EORTC Pharmacokinetics and Metabolism Group (1987) Pharmacokinetically guided dose escalation in phase-I clinical trials. Commentary and proposed guidelines. Eur J Cancer Clin Oncol 23: 1083–1087
6. Fitzsimmons SA, Lewis AD, Riley RJ, Workman P (1994) Reduction of 3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide (tirapazamine, WIN 59075, SR 4233) to a DNA-damaging species; a direct role for NADPH: cytochrome P450 oxidoreductase. Carcinogenesis 15: 1503–1510
7. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper H (1966) Quantitative comparison of toxicity of anti-cancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother Rep 50: 219–239
8. Fuse E, Kobayashi S, Inaba M, Suzuki H, Sugiyama Y (1994) Application of pharmacokinetically guided dose escalation with respect to cell cycle phase specificity. J Natl Cancer Inst 86: 989–996
9. Fuse E, Kobayashi T, Inaba, M, Sugiyama Y (1995) Prediction of the maximal tolerated dose (MTD) and therapeutic effect of anticancer drugs in humans: integration of pharmacokinetics with pharmacodynamics and toxicodynamics. Cancer Treat Rev 21: 133–157
10. Gianni L, Vigani L, Surbone A, Ballinari D, Casali P, Tarella C, Collins JM, Bonadonna G (1990) Pharmacology and clinical toxicity of 4′-iodo-4′-deoxydoxorubicin: an example of successful application of pharmacokinetics to dose escalation in phase-I trials. J Natl Cancer Inst 82: 469–477
11. Graham MA, Kaye SB (1993) New approaches in preclinical and clinical pharmacokinetics. In: Workman P, Graham MA (eds) Pharmacokinetics and cancer chemotherapy. Cancer surveys. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 27–49
12. Graham MA, Workman P (1992) The impact of pharmacokinetically guided dose escalation strategies in phase-I clinical trials. Critical evaluation and recommendations for future studies. Ann Oncol 3: 339–347
13. Hincks JR, Greener Y, Olson HM, Barbolt TA, Brown RR, Wilkinson GE, Singer AW, Killinger JM (1992) Toxicity and pharmacokinetics of WIN 59075 (SR4233) in beagle dogs. Proc Amer Assoc Cancer Res 33: 550
14. Holden SA, Teicher BA, Ara G, Herman TS, Coleman CN (1992) Enhancement of alkylating agent activity of SR-4233 in the mouse FSaIIC murine fibrosarcoma. J Natl Cancer Inst 84: 187–193
15. Jennrich RI, Sampson PF (1968) Application of stepwise regression analysis to non-linear least squares estimation. Technometrics 10: 63–72
16. Johnson JA (1993) Further studies on the disposition of ^{14}C-tirapazamine following a single intravenous administration to male Sprague-Dawley rats. Sanofi-Winthrop Study Report 291. Sanofi-Winthrop, Malvern, Pa., USA
17. Ottaway JH (1973) Normalization in the fitting of data by iterative methods. Application to tracer kinetics and enzyme kinetics. Biochem J 134: 729–736
18. Rampling R, Cruickshank G, Lewis AD, Fitzsimmons SA, Workman P (1994) Direct measurement of pO_2 distribution and bioreductive enzymes in human malignant brain tumors. Int J Radiat Oncol Biol Phys 29: 427–431
19. Riley RJ, Hemingway SA, Graham MA, Workman P (1993) Initial characterization of the major mouse cytochrome P450 enzymes involved in the reductive metabolism of the hypoxic cytotoxin 3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide (tirapazamine, SR 4233, WIN 59075). Biochem Pharmacol 45: 1065–1077
20. Robin H Jr, Senan S, Workman P, Graham MA (1995) Development and validation of a sensitive solid phase extraction and high performance liquid chromatography assay for the bioreductive agent tirapazamine and its major bioreductive metabolites in mouse and human plasma for pharmacokinetically guided dose escalation. Cancer Chemother Pharmacol 36: 266–270
21. Senan S, Rampling R, Wilson P, Lawson N, Robin H, Murray LS, Workman P, Graham M, Kaye SB (1994) Phase I and pharmacokinetic study of tirapazamine (SR 4233), a highly selective hypoxic cell cytotoxin. Ann Oncol 5 [Suppl 5]: A252
22. Shulman L, Coleman CN, Buswell L, Riese N, Doherty N, O'Neil N, Von Roemeling R (1995) A phase I study of multiple dose tirapazamine (Tira, SR 4233) given concurrently with radiation therapy (RT). Proc Am Assoc Clin Oncol 14: 473
23. Spiegal JF, Spear MA, Brown JM (1993) Toxicology of daily administration to mice of the radiation potentiator SR 4233. Radiother Oncol 26: 79–81
24. Wagner JG (1975) Fundamentals of clinical pharmacokinetics. Drug Intelligence Publications, Hamilton, Illinois, pp 57–172
25. Walton MI, Workman P (1990) Enzymology of the reductive bioactivation of SR 4233, a novel benzotriazine di-*N*-oxide hypoxic cell cytotoxin. Biochem Pharmacol 39: 1735–1742

26. Walton MI, Workman P (1993) Pharmacokinetics and bioreductive metabolism of the novel benzotriazine di-*N*-oxide hypoxic cell cytotoxin tirapazamine (WIN 59075; SR 4233; NSC 130181) in mice. J Pharmacol Exp Ther 265: 938–947
27. Walton MI, Wolf CR, Workman P (1992) The role of cytochrome P450 and cytochrome P450 reductase in the reductive bioactivation of the novel benzotriazine di-*N*-oxide hypoxic cytotoxin 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR 4233, WIN 59075) by mouse liver. Biochem Pharmacol 44: 251–259
28. Wang J, Biedermann KA, Brown MJ (1992) Repair of DNA and chromosome breaks in cells exposed to SR 4233 under hypoxia or to ionizing radiation. Cancer Res 52: 4473–4477
29. Wimberly C, Gossett K, Cornacoff J, Greener Y, Hincks JR (1992) Single and multiple dose toxicity of a hypoxic cytotoxic agent (WIN 59075) in rats. Toxicologist 12: 154