Phase I and Pharmacokinetic Study of Tirapazamine (SR 4233) Administered Every Three Weeks¹

Suresh Senan,² Roy Rampling, Martin A. Graham, Peter Wilson, Hernani Robin, Jr., Nils Eckardt, Nora Lawson, Alex McDonald, Reinhard von Roemeling, Paul Workman, and Stanley B. Kaye


ABSTRACT

Tirapazamine (SR 4233; 3-amino-1,2,4-benzotriazine-1,4-di-N-oxide) is a bioreductive agent exhibiting up to 200× greater toxicity for hypoxic cells as compared to oxygenated cells. In murine studies, a selective increase in tumor kill was observed when tirapazamine was coadministered with other agents, notably cisplatin. A Phase I study of single-agent tirapazamine administered i.v. every 3 weeks was conducted to determine the toxicity of a schedule for use with systemic chemotherapy. A total of 28 patients were given 50 courses of tirapazamine at doses ranging from 36–450 mg/m². No tumor responses were observed. Reversible deafness and tinnitus were dose-limiting, with ototoxicity observed in 1 of 6 patients treated at 330 mg/m², 1 of 4 patients treated at 390 mg/m², and 3 of 3 patients treated at 450 mg/m². Muscle cramps, nausea, and vomiting were also observed. Pharmacokinetic studies revealed a greater than dose-proportional increase in the area under the plasma concentration × time curve (AUCs) of the two major metabolites. Patients who developed ototoxicity generally showed higher plasma AUC values for the parent drug and metabolites. The mean plasma tirapazamine AUC at 330 mg/m² was 1026.5 μg/ml × min (range 863.8–1252.3), but no pharmacokinetic data are available for the solitary patient who developed ototoxicity at this dose level. These AUC values were in the (estimated) range required for therapeutic effect in murine studies. Ototoxicity was not observed when the AUC of tirapazamine was equal to or less than 1252 μg/ml × min. The dose of 330 mg/m² was therefore chosen as an appropriate level for combination chemotherapy studies.

INTRODUCTION

Tirapazamine (SR 4233; 3-amino-1,2,4-benzotriazine-1,4-di-N-oxide) is a bioreductive agent exhibiting 15–200× greater toxicity for hypoxic cells as compared to oxygenated cells (1). The one-electron activation of tirapazamine by cellular reductases including cytochrome P450 reductase (2–4) generates a nitrooxide radical (5) that, in the absence of oxygen, induces single- and double-stranded breaks in DNA. Further reduction leads to the formation of the inactive two-electron and four-electron reduction products SR 4317 and SR 4330, respectively (6). The coadministration of tirapazamine with other cytotoxins, notably cisplatin (7, 8), and also during fractionated radiotherapy (9) produces a selective in vivo enhancement of tumor kill. Bone marrow toxicity was seen in both mice (7, 10) and rats given tirapazamine (11), and necrosis of the olfactory nerve was observed in rats (11). Retinal rod cell degeneration was observed in dogs given an oral formulation of tirapazamine, but this finding was not seen after i.v. dosing at comparable study design and drug exposure levels.³

A Phase I study of single-agent tirapazamine administered i.v. every 3 weeks was conducted to evaluate the toxicity of a schedule for use with systemic chemotherapy. The aims of the study were to establish the toxicity profile and the MTD⁴ and to study the plasma pharmacokinetics of tirapazamine and its metabolites, and in turn, to correlate this with toxicity.

PATIENTS AND METHODS

Patient Selection. Patients with histologically proven cancer that was refractory to conventional therapy were eligible to participate in this study. The study was approved by the institutional ethics committee, and written informed consent was obtained from all patients before investigations to determine eligibility were performed. Eligibility criteria included: age over 18 years; a performance status of 0–2 on the Eastern Cooperative Oncology Group scale; a minimum life expectancy of 12 weeks; and normal organ function, including WBCs ≥ 100 X 10⁹/liter, platelet count ≥ 100 X 10⁹/liter, bilirubin ≤ 20 μmol.

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³ Sanofi Winthrop, data on file.
⁴ The abbreviations used are: MTD, maximal tolerated dose; AUC, area under the plasma concentration × time curve; CI, 95% confidence interval; Cmax, maximum plasma concentration; Cdm2, candelalm2; ECG, electrocardiogram; ERG, electroretinogram; inf, infinity; k, elimination constant; t½, plasma half-life; Vdm, volume of distribution (steady state).

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aspartate transaminase and alanine transaminase levels less than 2 × the upper normal range, prothrombin and partial thromboplastin times within normal limits, and a serum creatinine ≤ 120 μmol.

No patient received chemotherapy, radiotherapy, or immunotherapy in the 3 weeks before tirapazamine administration (6 weeks in the case of nitrosoureas and mitomycin C). Pregnant or lactating women were excluded, as were patients with serious active infections or a fever of greater than 38.3° C. After the development of ototoxicity, patients with preexisting hearing impairment that required the use of a hearing aid were also excluded.

**Drug Formulation and Administration.** Tirapazamine was supplied by Sanofi Winthrop in glass ampules containing 20 ml (0.7 mg/ml) of drug in an isotonic citrate buffer (pH 3.7-4.3). The infusion bags were prepared at the in-house pharmacy in Glasgow. Tirapazamine (0.7 mg/ml) was administered using an infusion pump by continuous i.v. infusion at a fixed rate of 5 ml (3.5 mg/min); consequently, the duration of the infusion increased with increasing dose.

**Study Design.** All patients had a baseline history, physical examination, full blood count, biochemistry, clotting profiles, urine microscopy, chest radiograph, and ECG. Tumor measurements were made (when possible) using appropriate clinical or radiological methods. Baseline and posttreatment audiograms were performed after ototoxicity was first observed. Ocular assessment was incorporated into patient evaluation at doses equal to and exceeding 330 mg/m². A baseline and day 14 testing of visual fields was performed using a Humphrey visual field analyzer. Color vision was assessed using the full 100-hue test of Farmsworth Munsell. ERGs were also performed at the same assessment, in accordance with the recommendations of the International Standardization Committee (12). Briefly, ERG rod recordings were performed after a 20-min dark adaptation, and a stimulus strength of 2.4 Cdm⁻²/s was used. ERG cone recordings were performed after a 10-min light adaptation to 14 Cdm⁻², and a stimulus strength of 1.54 Cdm⁻²/s was used. Either contact lens electrodes or gold foil electrodes were used.

Patients were hospitalized for each treatment and for at least 24 h after the end of tirapazamine infusion, during which time they were observed for adverse events. All concomitant medications were recorded. Patients were reviewed weekly on an outpatient basis to record toxicity, and investigations were repeated at each review, except for tumor measurements and ECG, which were repeated on day 22. Eye tests and audiograms were measured on two occasions posttreatment. Retreatment at the initial treatment dose (or the preceding dose level in the case of patients who experienced unacceptable toxicity) was offered to patients with stable or responding disease. Patients were withdrawn from the study if there was evidence of disease progression or life-threatening toxicity, or at the request of the patient.

The clinical study was initiated at a dose of 36 mg/m², a third of the toxic dose low in rats, and a modified Fibonacci escalation scheme was utilized. Drug-related toxicity was graded according to the Common Toxicity Criteria (from the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD). Dose escalation ceased when at least 50% of patients at a specific dose level developed either grade 3 non-myelosuppressive toxicity (except for nausea, vomiting, fever, alopecia, weight gain) or grade 4 myelosuppressive toxicity. The MTD was defined as the dose that produced significant yet manageable and reversible toxicity in at least two of six patients. Additional patients (up to six) were treated at the preceding dose levels to further characterize the toxicity and to select a relatively nontoxic dose for use with chemotherapy.

During the first course of tirapazamine, blood samples were collected for pharmacokinetic studies via a heparinized cannula in the opposite arm from the infusion site. Samples (5 ml) were collected in lithium heparin tubes before and at the following nominal time points: mid-infusion (or every half-hour for infusions lasting longer than 2 h); and end-infusion at 0, 10, 15, 30, 45, 60, 120, 240, and 360 min, and approximately 24 h. Samples were centrifuged immediately, and the separated plasma was stored at −70°C before analysis using a sensitive high-performance liquid chromatography assay for tirapazamine and its metabolites (13). Each high-performance liquid chromatography analytical run was comprised of: (a) a freshly prepared calibration curve; (b) recovery standards prepared over the same concentration range as the calibration curve; and (c) pharmacokinetic samples.

**Pharmacokinetic Analysis.** Clinical pharmacokinetic parameters were determined using the MASTER_PK program resident within the RS1 graphics package at Sanofi Winthrop Inc. Actual times were used for all pharmacokinetic determinations. The Cₘₐₓ of tirapazamine was determined from the steady-state plasma concentration at the end of drug infusion, and Cₘₐₓ values for SR 4317 and SR 4330 were determined from an inspection of the data. The t₁/₂ was determined from the terminal rate constant (kₑ), which was estimated by linear regression of the last portion of the plasma concentration/time profile. The AUC was calculated using the trapezoidal rule and extrapolated to inf by dividing the last quantifiable plasma concentration by the kₑ. The plasma Vₘₐₓ and plasma clearance values were calculated using the absolute dose expressed in the same units used for analysis [dose (mg/m²) × body surface area (m²)] × 1000 μg/ml.

**Statistical Analysis.** For the parameter AUC(0-inf), dose proportionality was assessed using regression analysis. For Cₘₐₓ and half-life, dose effects were assessed by one-way ANOVA, using kₚ values for the half-life analysis. For plasma clearance and Vₘₐₓ, dose effects were evaluated by simple linear regression in which body surface-adjusted values of dose (nominal dose × surface area) were used instead of nominal dose values.

**RESULTS**

A total of 28 patients were treated in the study, and patient details are given in Table 1. The starting dose was 36 mg/m², the highest dose administered was 450 mg/m², and a total of 50 courses of tirapazamine were administered (Table 2). No objective tumor responses were seen in this extensively pretreated patient population. The dose-limiting toxicity was reversible deafness and tinnitus. In addition, muscle cramps, nausea and vomiting, diarrhea, and nonspecific ERG changes were also observed.

**Ototoxicity.** Tinnitus and reversible hearing loss developed in 1 of 6 patients treated at 330 mg/m², 1 of 4 patients treated at 390 mg/m², and in all 3 patients treated at 450 mg/m².

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6 Sanofi Winthrop, data on file.
enzyme levels were not elevated in the four patients in whom cisplatin-containing chemotherapy (cumulative cisplatin dose = 640 mg) 3 months previously. This patient (patient 19) had no symptoms of ototoxicity or peripheral neuropathy after cisplatin administration. Except for a patient who was treated at 450 mg/m², the ototoxic symptoms resolved completely in all patients. In the case of the former (patient 22), acute symptoms resolved 17 h after onset, but tinnitus recurred 5 weeks after treatment and persisted until her death from progressive tumor 4 weeks later. Only 1 of 6 patients treated at the 330 mg/m² dose level developed tinnitus, and this occurred after only the first of 3 treatments at this dose. The audiograms of this patient showed reversible hearing loss only after her first course, and no pharmacokinetic data were available for this course. No evidence for cumulative ototoxicity was observed. With the exception of a single patient (patient 25) who was using 300 mg aspirin daily on a long-term basis, no patients received concurrent medication with potential for ototoxicity.

**Muscle Cramps.** Muscle cramps occurred in patients at all dose levels except 36 mg/m² (Table 4). The onset of cramps was generally between 2.4–24 h after the start of infusion, but it was delayed up to 5 days in 1 patient. Typically, cramps began on waking up in the morning, affected mainly the lower limbs, and were relieved by weight-bearing or stretching the affected muscle. The episodes were generally mild and transient and did not increase in severity with dose or after retreatment at the same dose. The duration of cramps varied from 1–14 days, and the cramps persisted longer in the 3 patients treated at 120 mg/m² (14 days in all 3 patients) than in those treated at 450 mg/m² (0, 1, and 1 day, respectively). Creatinine phosphokinase enzyme levels were not elevated in the four patients in whom enzyme levels were measured after the onset of cramps. The administration of diazepam did not influence the incidence of cramps. No electrolyte abnormalities were detected in patients with cramps, and no patient developed signs or symptoms of peripheral neuropathy.

**Visual Abnormalities.** One patient who was treated at 330 mg/m² and 2 who were treated at 390 mg/m² underwent pre- and posttreatment ocular evaluations, the results of which were normal. Pretreatment and sequential posttreatment visual tests were performed in 2 of the patients treated at 450 mg/m², and ERG abnormalities were seen for both, but only at the evaluation at 2 weeks posttreatment. One patient developed a 30% decrease in rod recordings in the left eye, which reverted to normal 4 weeks after treatment. This patient also complained of 2 episodes of transient blurring of vision that lasted for less than 5 min (at 187 and 230 min after the start of a tirapazamine infusion lasting 220 min). The patient received no further treatment. The second patient treated at 450 mg/m² developed a decrease in the B wave amplitude of the rod response in his left eye on ERG, which reverted to normal on reassessment at 4 weeks. No additional abnormalities were seen after this patient was twice retreated at 330 mg/m². No abnormalities of color vision were detected in patients.

**Gastrointestinal Toxicity.** Nausea and vomiting were observed at all dose levels, with the time of onset ranging between 40–190 min from the start of infusion. Dose escalation was not limited by nausea and vomiting, and the median duration of symptoms was 5 h in patients who had not previously received cisplatin-containing chemotherapy. Prophylactic antiemetics (i.v. ondansetron (8 mg) and i.v. dexamethasone (8 mg)) were subsequently administered to all patients treated at doses exceeding 180 mg/m². Symptoms settled rapidly after the administration of i.v. ondansetron (8 mg) to patients treated up to this dose. The most severe (grade 3) toxicity developed in four patients who had received prior chemotherapy that included cisplatin.

Seven patients had bowel movements occurring 100–300 min after the start of infusion, and six of these patients received doses of 250 mg/m² or higher. A patient with a colostomy developed loose stools after each of her six treatments. The diarrhea did not recur after the day of treatment.

**Other Toxicities.** Reversible (grade 1) thrombocytopenia was seen in a single patient after a dose of 450 mg/m². No leukopenia or thrombocytopenia developed in other study patients, including those who received higher cumulative doses of tirapazamine. Four patients who were treated at 120, 330, and 450 mg/m² experienced a distinctive “burning” smell coming on
Table 3  Tirapazamine ototoxicity: summary of data on individual patients

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>Patient no.</th>
<th>Toxicity grade</th>
<th>Time of onset</th>
<th>Duration of symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>330</td>
<td>24</td>
<td>Grade 2</td>
<td>24 h - “fullness” in ears</td>
<td>Resolution of tinnitus after 6 h</td>
</tr>
<tr>
<td>390</td>
<td>25</td>
<td>Grade 3</td>
<td>48 h - tinnitus and hearing loss</td>
<td>Partial improvement in hearing after 8 h</td>
</tr>
<tr>
<td>450</td>
<td>19</td>
<td>Grade 3</td>
<td>15 h - profound hearing loss and tinnitus</td>
<td>Resolution of tinnitus on day 8</td>
</tr>
<tr>
<td>450</td>
<td>22</td>
<td>Grade 3</td>
<td>15 h - intermittent tinnitus and hearing loss</td>
<td>Resolution of symptoms after 17 h</td>
</tr>
<tr>
<td>450</td>
<td>23</td>
<td>Grade 3</td>
<td>2.3 h - transient tinnitus</td>
<td>Partial improvement in hearing after 9 h</td>
</tr>
</tbody>
</table>

*Common Toxicity Criteria scale for ototoxicity: Grade 1, asymptomatic hearing loss; Grade 2, tinnitus; Grade 3, hearing loss, correctable with hearing aid; Grade 4, hearing loss, not correctable with hearing aid.

**Time from start of tirapazamine infusion.

Table 3 - continued

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>Patient no.</th>
<th>Toxicity grade</th>
<th>Time of onset</th>
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</tr>
</thead>
<tbody>
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**Time from start of tirapazamine infusion.

The bilateral hearing loss recorded on day 2 is apparent mainly in the 250 Hz-2 kHz range (the stippled line represents hearing in the left ear). Audiograms reverted to baseline on day 17, but recurrent tinnitus developed 5 weeks later and persisted until death (9 weeks after her single treatment).

**Fig. 1** Audiograms of a patient treated at 450 mg/m². The bilateral hearing loss recorded on day 2 is apparent mainly in the 250 Hz-2 kHz range (the stippled line represents hearing in the left ear). Audiograms reverted to baseline on day 17, but recurrent tinnitus developed 5 weeks later and persisted until death (9 weeks after her single treatment).
Fig. 2. Audiograms of a patient treated at 450 mg/m². Preexisting bilateral high-frequency noise-induced deafness was present (the stippled line represents hearing in the left ear), but the tirapazamine-induced hearing loss is most apparent from 250 Hz–2 kHz.

Table 4  Tirapazamine Phase I study: incidence of muscle cramps

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients treated</th>
<th>No. experiencing cramps</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>3</td>
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<tr>
<td>120</td>
<td>3</td>
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<tr>
<td>180</td>
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<td>3</td>
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<tr>
<td>250</td>
<td>3</td>
<td>2</td>
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<tr>
<td>330</td>
<td>6</td>
<td>5</td>
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<tr>
<td>390</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>450</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

an estimated 53.1-fold (CI, 33.7–83.6) increase in mean AUC(0-∞). For values of Cmax and k1, there was a significant dose effect (P < 0.001 and P = 0.002, respectively).

The 4-electron reduction product, SR 4330, was first detected at the 72 mg/m² dose level. The Cmax increased significantly with dose (P < 0.001), with peak plasma levels of 6–11.9-fold lower than corresponding SR 4317 levels. The AUC levels of SR 4330 were between 6–12.5-fold lower than those of SR 4317 and declined with a mean t½ of 242.8 ± 103.7 min. The mean AUC(0-∞) increased in a greater than dose-proportional manner (P < 0.001), with a 12.5-fold increase in tirapazamine dose producing an estimated 30.3-fold (CI, 12.9–71.1) increase in AUC.

DISCUSSION

When tirapazamine was administered every 3 weeks by continuous i.v. infusion, the MTD was 390 mg/m². The dose recommended for Phase II study with other cytotoxic agents is 330 mg/m². At this dose, a total of 14 courses of tirapazamine were administered, and only 1 of 6 patients developed (mild) ototoxicity. The other major toxicities included nausea and vomiting and muscle cramps. In contrast to the bone marrow observed toxicity in rats and mice (10, 11), mild thrombocytopenia was detected in only a single patient after a dose of 450 mg/m².

No tumor responses were observed for this selective hypoxic cytotoxin when administered as a single agent. This may be in keeping with the observation that hypoxic cells constitute only up to 20% of viable (i.e., clonogenic) cells in rodent tumors and human tumor xenografts (14). The oxic cell population that contributes to tumor growth is likely to be unaffected unless tirapazamine is combined with agents that target the oxic tumor population. Significant antitumor activity was seen in animal models when tirapazamine was coadministered with conventional cytotoxins (7, 8). Because the latter are commonly ad-
ministered every 3 weeks, it was necessary to establish the toxicity of tirapazamine by a similar schedule before clinical studies with chemotherapy.

The efficacy of cell kill by tirapazamine seems to be related to the AUC for the drug (15). Although pharmacokinetic studies were not performed in the preclinical studies of tirapazamine combined with fractionated radiotherapy (9) and chemotherapy (7, 8), data from our murine studies showed a linear relationship between tirapazamine dose and AUC in the dose range used for these combined studies (16). This dose-AUC relationship was used to estimate the AUC of tirapazamine, an approach that was shown to be valid by pharmacokinetic data from tumor-bearing mice (6). When tirapazamine (59 mg/m²) was administered with fractionated radiotherapy, a dose enhancement of 2.5–3 was observed in mice (6). Such a finding in patients with varying tumor loads suggests that systemic (i.e., nontumor) metabolism of tirapazamine predominates in both man and mouse. The limited nonlinear increases observed in the parent drug AUCs are unlikely to be of clinical significance. The marked nonlinear pharmacokinetics of the two metabolites are suggestive of saturable metabolic pathways for this rapidly metabolized agent, particularly at higher doses and plasma concentrations. Both these metabolites were nontoxic to cells under hypoxic or aerobic conditions (1), but their role in systemic toxicity has not been investigated in animal models.

Tinnitus and reversible hearing loss occurred only at doses equal to and exceeding 330 mg/m². Otoxicity was not seen in patients who were treated at lower dose levels, but whose cumulative doses exceeded 1 g (in two patients, this was 2 g). The ototoxicity was striking for its early onset and its reversibility at doses below 390 mg/m². An acute ototoxic insult can predispose to late damage (17), and longer-term follow-up is needed to exclude the development of late ototoxicity in tirapazamine-treated patients. An important study aim was to establish correlations between pharmacokinetics and toxicity. Muscle cramps did not show a dose-response relationship, and the relationship between pharmacokinetics and ototoxicity was evaluated. Complete pharmacokinetic data are available on 3 patients who developed ototoxicity (2 patients treated at 450 mg/m² and 1 patient treated at 390 mg/m²). These studies were not feasible in two patients with ototoxicity because of a lack of suitable venous access. In addition, the AUC values for SR 4330 could not be determined for 2 patients (treated at 390 and 450 mg/m², respectively) because plasma levels had not reached steady state by the end of sampling. Although this limits the power of the pharmacokinetic-toxicity correlations derived from this study, some useful conclusions can nevertheless be drawn.

Patients with ototoxicity had generally higher plasma AUC values for all three compounds. No ototoxicity occurred when the AUC of tirapazamine was equal to or less than 1252 μg/ml × min, the highest AUC value at 330 mg/m² (Fig. 6). At AUC values in excess of 1252 μg/ml × min (which was recorded in 3 patients), only 1 patient (with an AUC value = 1583.6 μg/ml × min) developed no ototoxicity. The lowest tirapazamine Cmax value in a patient with ototoxicity was 5.8 μg/ml, and this occurred in a patient without prior ototoxic drug exposure. However, two patients with higher Cmax values (7.9 and 6.6 μg/ml, respectively) also developed no toxicity. A greater overlap was seen between the AUC and Cmax values of SR 4317 and SR 4330 between patients with and without...
Further analysis of ototoxicity-pharmacokinetic correlations is required in Phase II/III studies, particularly because pharmacokinetic and/or pharmacodynamic interactions may occur with other cytotoxic agents.

With this schedule of tirapazamine administration, muscle cramps were transient and did not increase with dose. However, muscle cramping was dose-limiting when tirapazamine was administered on a daily schedule, making alternate-day administration the most feasible schedule of administration (18). In the absence of neural, muscular, or biochemical abnormalities, cramps rarely develop for the first time in patients with malignancy (19). Anticancer drugs such as vincristine, cisplatin, misodazole, and etanidazole have generally produced muscle cramping only in the setting of an established peripheral neuropathy (20–22). Exceptionally, cramping or muscle pain has been reported with experimental anticancer agents such as bryostatin 1, lonidine, and methyl-GAG in the absence of peripheral neuropathy and may provide clues as to the etiology of the toxicity seen after tirapazamine administration.

Muscle pain was dose-limiting in the Phase I study of bryostatin 1, an activator of protein kinase C (23). Decreases in muscle blood flow and mitochondrial function were observed in bryostatin-treated patients (24). Myalgia was also dose-limiting in the Phase I trial of lonidine [1–2 (dichlorobenzyl)-1H-indazole-3-carboxylic acid; Ref. 25]. Lonidine reduced efflux of lactate, producing ATP depletion and intracellular acidification in MCF-7 cells (26). In addition to the myalgia that developed in 68% of lonidine-treated patients, 4% of patients also developed diminished hearing (27). Similarly, varying degrees of lower limb pain, cramping, and weakness were seen after methyl-GAG (methylglyoxal bis(guanylhydrazone); NSC 32946) administration, and reversible tinnitus and vertigo also developed in a number of these patients (28). A number of the above-mentioned findings have been reported after the administration of tirapazamine. In MCF-7 cells, tirapazamine uncoupled oxidative phosphorylation, leading to tumour growth (29). Tirapazamine has also been shown to decrease blood flow in tumors (30, 31) and may damage vessels in normal tissues (32). The inner ear is supplied by an end artery, and sudden hearing loss is a recognized consequence of partial or complete occlusion of the cochlear vasculature (17). Both metabolic and vascular effects may thus mediate the tirapazamine-induced muscle cramps, but the exact mechanism remains to be elucidated.

Retinal abnormalities, involving mainly rod cells, were observed in dogs given a single high dose of an oral formulation of tirapazamine. Similar findings were not observed in studies with the i.v. formulation that used a comparable study design and resulted in comparable drug exposure levels. The significance of the ERG abnormalities in the 2 patients treated at 450 mg/m² is unclear, but these changes were nonspecific and reversible and were not observed at lower doses. One of these patients complained of transient ocular symptoms. In a Phase II trial of combined tirapazamine and cisplatin, transient visual “spots” were reported by patients after 11% of treatment courses (33). However, the details of any ocular assessments for these patients were not reported. The combination of drug-induced ocular and ear toxicity has been reported for a number of drugs including defereroxamine (34), nalidixic acid (35), cisplatin (36), and carboplatin (37). The presence of melanin pigment in the specialized receptor cells in both the retinal pigment epithelium and the stria vasularis of the cochlea may account for this pattern of toxicity. Melanins are excellent electron acceptors and have marked free-radical scavenging properties (38), which may be significant as tirapazamine is also activated to form a nitrooxide radical (5). Melanin granules also have a high affinity for some ototoxic drugs including aminoglycosides, antimalarials (quinine, quiniodone), and phenothiazines, and melanin may act as a reservoir from which free drug is released (39). These similarities in the pattern of eye, ear, and muscle toxicity for these diverse agents suggest that a common mechanism of toxicity may operate for these agents and tirapazamine.

In conclusion, we found that the dose-limiting toxicity of tirapazamine administered every 3 weeks by i.v. infusion is reversible tinnitus and hearing loss. The MTD was 390 mg/m², and the dose recommended in combination with other cytotoxins is 330 mg/m². At the latter dose, tirapazamine AUC levels were in the range at which efficacy was seen in murine studies. The etiology of the normal tissue toxicity with tirapazamine is unclear, and ototoxicity will be further characterized in ongoing Phase II/III clinical trials in combination with conventional cytotoxins. Serial evaluation of auditory and ocular function will be of particular importance when patients are treated with the combination of tirapazamine and cisplatin.

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