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Phase I/pharmacokinetic trial of the novel thioxanthone SR233377 (WIN33377) on a 5-day schedule

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Abstract *Purpose*: SR233377 (WIN33377) is a novel 4-aminomethyl thioxanthone derivative with promising preclinical activity against solid tumors at doses substantially below the MTD. We performed a phase I trial to determine a suitable phase II dose of SR233377 when administered as a 2-h intravenous infusion for five consecutive days. *Methods*: A group of 25 patients with a range of solid tumor diagnoses and good performance status received SR233377 at eight dose levels ranging from 4.8 mg/m^2 per day to 74.7 mg/m^2 per day. Cycles were repeated every 35 days and patients were evaluated for response following two cycles of treatment. Doses were escalated in cohorts of three using a modified Fibonacci scheme. Pharmacokinetic sampling was performed during the first cycle in all patients. *Results*: Toxicities of SR233377 on this schedule included neutropenia, fever, nausea, and dyspnea but all were mild and not dose-limiting. Asymptomatic prolongation of the corrected QT (QTc) interval during infusion in all patients monitored at the 74.7 mg/m^2 dose level prompted closure of the study. QT lengthening correlated with increasing plasma concentrations of SR233377. SR233377 C_{max} values increased linearly with dose, but substantial interpatient variability in SR233377 AUC, clearance, and half-life was noted. There was no evidence of drug accumulation when day 1 and day 5 AUC and C_{max} values were compared. Seven patients displayed tumor growth inhibition lasting for 4 months or more. *Conclusions*: We conclude that SR233377 administered on a 5-day schedule is associated with tolerable clinical symptoms and some activity against a range of solid tumors but dosing is limited by QTc prolongation, a condition that predisposes to ventricular arrhythmias. Phase II development on this schedule is not recommended based on the occurrence of this concentration-dependent effect. Further investigation of alternative schedules of administration and of SR233377 analogues is warranted.

Key words Thioxanthones · Phase I trial · Pharmacokinetics · Cardiac toxicity · QT interval

Introduction

N-[[1-[[2-(diethylamino)ethyl]amino]-9-oxo-9H-thioxanthen-4-yl]methyl]methanesulfonamide (SR233377) is a novel antitumor agent of the thioxanthone class (Fig. 1). These agents have not been widely developed as anticancer drugs in the clinic, though hycanthone and lucanthone have been used for many years in the treatment of schistosomiasis [3]. Early mechanistic work indicates that these compounds are DNA intercalators with the capacity to alkylate deoxyguanosine residues [6]. Subsequent DNAase I footprinting studies have indicated that lucanthone and hycanthone preferentially bind to AT-rich sequences in DNA [1]. Structure-activity investigations have indicated that DNA binding can be dissociated from antischistosomal activity [7]. Phase I trials of hycanthone have revealed hepatotoxicity to be dose-limiting [9, 10] and the drug was subsequently abandoned for this reason [5]. Continuing evaluation of structural analogues devoid of the hepatotoxicity of hycanthone yielded SR233377.

This compound was selected for development based upon its apparent selectivity for solid tumors over leukemia cell lines in an in vitro disk diffusion soft agar colony-formation assay and its ability to produce cures

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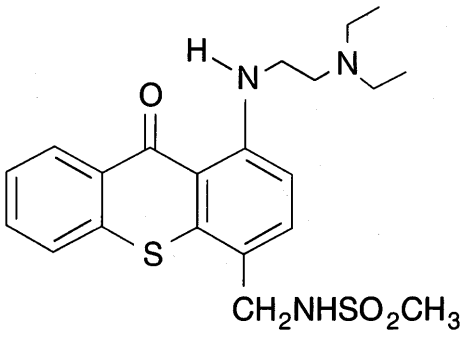


Fig. 1 Structure of SR233377

in murine Panc-03 and Colon-38 solid tumors in vivo at tolerable doses [2]. SR233377 is also active against MX-1 human mammary carcinoma xenografts in vivo at a dose of 48 mg/kg. Mechanistic studies have not definitively established a mechanism of cytotoxicity: inhibition of DNA synthesis, DNA intercalation and inhibition of topoisomerase II all result from SR233377 administration, but the relationship with cell death is unclear [20]. Animal toxicity studies have revealed SR233377 to be well tolerated with reversible effects mostly confined to the bone marrow and gastrointestinal tract [16]. No cardiotoxicity has been observed. The murine 10% lethal dose is 48 mg/m^2 per day on a 5-day schedule.

Based upon these promising preclinical findings, we performed a phase I trial to determine an appropriate phase II dose of SR233377 administered as a 2-h intravenous infusion for five consecutive days.

Materials and methods

Patient population

Eligible patients were at least 18 years of age with histologically confirmed solid tumors that were refractory to standard therapy or for which no effective therapy was available. A Karnofsky performance status of ≥70% and a life expectancy of ≥3 months were required. Patients had adequate bone marrow function (absolute neutrophil count of ≥2000/mm^3, platelet count ≥100 000/mm^3), hepatic function (bilirubin not more than twice normal) and kidney function (serum creatinine ≤1.5 mg/dl), and had either measurable or evaluable disease. Patients had recovered from all toxicity of prior treatment and had received no prior chemotherapy or radiotherapy within 3 weeks of entry onto this study (6 weeks for nitrosoureas and mitomycin). Patients with active infections or poorly controlled respiratory or neurologic disease were excluded from the study. All patients gave written informed consent in accordance with federal, state and institutional guidelines.

Prior to therapy a medical history, physical examination, complete blood count, biochemical profile, ECG, urinalysis, and chest radiography were performed. Patients were monitored with biweekly blood counts, and biochemical profiles. Physical examination and ECG were performed prior to every course. Doses were not escalated within patients. Dose modifications were not made for nausea and vomiting, alopecia or anemia. Toxicity was assigned according to the NCI Common Toxicity Criteria (Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland, 1988). Patients with measurable disease were evaluated for response after the first treatment course (usually by radiographic scan or X-radiography) and then every other course. Standard response criteria were applied [12]. Those exhibiting a response to SR233377 or stable disease continued on therapy.

Drug administration

SR233377 was provided by Sanofi Pharmaceuticals (Collegeville, Pa.) as a clear yellow solution in clear glass ampoules. Each ampoule contained 20 ml of solution containing 2.5 mg/ml SR233377 in isotonic citrate buffer (pH 5.5). Normal saline was added to the drug for doses < 50 mg to maintain peripheral infusions. The solution was administered via a Medfusion syringe pump (Becton Dickinson, Franklin Lakes, N.J.) over exactly 2 h daily for 5 days. Because of the photosensitivity exhibited by SR233377, the drug was protected from light during storage and administration.

The starting dose of SR233377 was 4.8 mg/m^2 per day. Subsequent doses were increased using a modified Fibonacci escalation scheme. Dose escalation did not occur until two patients at a given dose level had completed at least 14 days of observation and the third patient had been treated for 5 days without dose-limiting toxicity. Provision was made to expand accrual to a level upon encountering severe or unexpected toxicity. Patients were retreated following 4 weeks of observation, in the absence of progressive disease.

The endpoint of the study was to determine the maximum tolerated dose (MTD), here defined as the dose of SR233377 at which three or more of six patients demonstrated grade 2 or worse nonmyelosuppressive toxicity or grade 3 or worse myelotoxicity.

Pharmacokinetic sampling and analysis

Blood samples for the determination of SR233377 pharmacokinetics were obtained during the first cycle from all patients. Samples (3 ml) were collected into heparinized tubes and immediately placed on ice. Sampling times on days 1 and 5 were just prior to infusion, hourly during the infusion, and 10, 20, 30, 45, 60, 90, 120, 240, 360 minutes and 24 h postinfusion. Samples on days 2, 3, and 4 were drawn immediately prior to infusion and at the end of infusion. Plasma was frozen at −20 °C until analysis. SR233377 plasma measurements were performed using a column-switching HPLC assay as previously described by Drozd et al. [4].

Plasma SR233377 concentration versus time curves were analyzed by regression techniques to assess dose-proportional effects. Noncompartmental analyses were performed using the MASTER_PK Program resident in RS1 at Sanofi Pharmaceuticals. Actual sampling times were used for all pharmacokinetic calculations. C_{max} values were derived from inspection of the plasma concentration data. Individual plasma SR233377 AUC values were calculated on days 1 and 5 using the trapezoidal rule. Clearance terms were calculated using the equation Cl = D/AUC (where D is the absolute dose administered). Vd_{ss} was calculated using the equation $Vd_{ss} = Cl \cdot MRT$ where MRT is AUMC/AUC (AUMC defined as the area under the moment curve). Half-life ($t_{1/2}$) values were determined from the slope of the regression line from the terminal portion of the plasma SR233377 concentration-time curve.

Results

The characteristics of the patients enrolled on this study are summarized in Table 1. A group of 25 patients received a total of 68 cycles of SR233377 at eight dose levels from 4.8 mg/m^2 per day to 74.7 mg/m^2 per day. All were evaluable for toxicity and response. The median age was 59 years(23–76 years), and all had good performance status. All had received prior chemotherapy

Table 1 Patient characteristics

Total entered/evaluable	25/25
Male/female	12/13
Age (years)	
Median	59
Range	23–76
Performance status	
0	7
1	15
2	3
Primary sites	
Colorectal	3
Gastric	1
Sarcoma	3
Ovarian/endometrial	9
NSCLC	5
Pancreatic	2
Breast	1
Renal	1
Prior treatment	
Chemotherapy	15
Chemotherapy/radiotherapy	10

but were not extensively pretreated. A range of primary tumor types was represented.

Toxicity

Overall, SR233377 was well tolerated on this schedule. There was minimal hematologic toxicity noted. No patient experienced dose-limiting hematologic toxicity; two patients developed grade 3 anemia, but this occurred in the setting of preexistent anemia in both. All other episodes of anemia, thrombocytopenia, neutropenia, and leukopenia were grade 1/2. Nonhematologic toxicities were also mild; nausea/vomiting and transient fevers predominated but again were not dose-limiting.

Following the observation of cardiotoxicity (ventricular arrhythmias and torsades de pointes) in two patients on a separate phase I trial of SR233377 administration once every 28 days [11], we performed ECGs and continuous cardiac monitoring during infusion on patients at the 74.7 mg/m^2 dose level. The QT and QTc intervals were observed for prolongation. Two of the patients were in their second cycle of treatment with SR233377, while the third was monitored during cycle one of therapy.

All three patients monitored had consistent QT and QTc prolongation with each dose of SR233377. The mean prolongation of the QT was 60 ms. Mean QTc prolongation was 53 ms; in two patients the intervals were >500 ms at the end of the SR233377 infusion. One patient had an occasional premature ventricular contraction but otherwise there were no arrhythmias associated with this effect. Serum potassium and magnesium levels were normal in all three. Dose escalation was halted at the 74.7 mg/m^2 dose level following this observation and no further patients were accrued to the study.

Pharmacokinetics

SR233377 pharmacokinetic parameters are presented in Table 2. Mean C_{max} values increased linearly with dose, but there was substantial interpatient variation in AUC on days 1 and 5 (Fig. 2). No consistent drug accumulation was noted when AUC and C_{max} values on days 1 and 5 were compared. Mean $t_{1/2}$ and clearance values were 6.9 h (range 0.9–26.4 h) and 41.6 l/h (range 5.2 to 100.1 l/h), respectively, with high interpatient variability again noted. Vd_{ss} ranged from 75.7 to 451.9 l (mean 166.1 l).

Pharmacodynamics

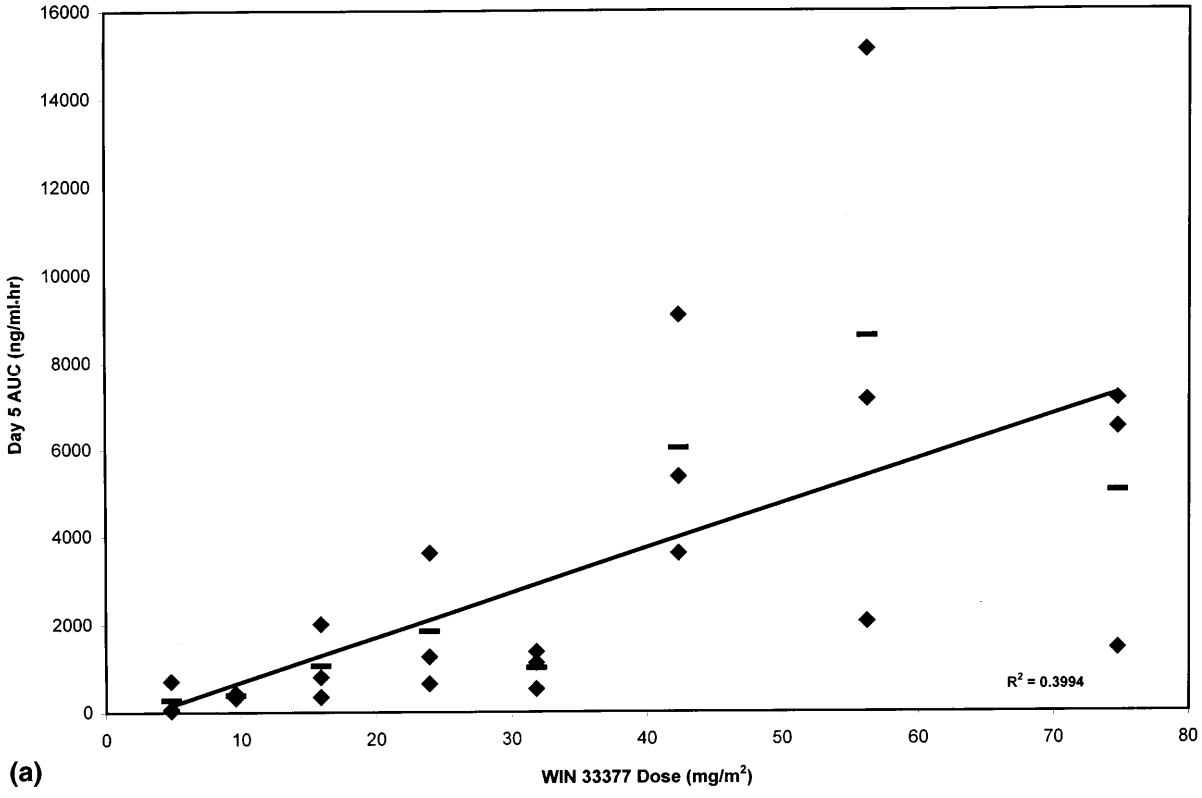
A clear relationship between plasma concentrations of SR233377 and the observed QTc prolongation in the monitored patients was demonstrated. The plasma concentration and QTc interval versus time curves for a patient treated with 74.7 mg/m^2 are shown in Fig. 3. An immediate and linear increase in QTc interval with increasing plasma SR233377 concentration was evident (Fig. 4).

Responses

There were no complete or partial tumor responses to SR233377 in this study but there was evidence of antitumor activity. A 59-year-old man with a leiomyosarcoma and multiple pulmonary metastases who failed

Table 2 Summary of mean SR233377 pharmacokinetic parameters by dose level. Values are means ± SD

Dose (mg/m^2/d)	*n*	C_{max} day 1 (ng/ml)	C_{max} day 5 (ng/ml)	$t_{1/2}$ (h)	AUC day 1 (ng/ml · h)	AUC day 5 (ng/ml · h)	Clearance (l/h)	Vd_{ss} (l/kg)
4.8	3	45.3 ± 9.7	59.2 ± 7.6	7.6 ± 8.1	141 ± 57	277 ± 374	48 ± 43.7	238.8 ± 148.4
9.6	3	162.9 ± 73.5	159.1 ± 42.8	2.6 ± 0.4	323 ± 47	385 ± 53	47.5 ± 6.7	123.9 ± 5.7
15.9	3	288.3 ± 21.8	223.4 ± 126.1	9.1 ± 9.7	1332 ± 1133	1051 ± 858	27.1 ± 20.8	118.2 ± 24.2
23.9	3	262.9 ± 139.6	389.3 ± 122.1	6.7 ± 2.7	1327 ± 1450	1839 ± 1566	30.1 ± 17.5	244 ± 180.3
31.8	3	489.8 ± 127.7	442.6 ± 248.1	2.8 ± 0.4	1076 ± 177	1001 ± 436	51.4 ± 12.7	118.7 ± 40.8
42.3	3	850.6 ± 386.2	974.7 ± 184	10.6 ± 10.3	3870 ± 694	6013 ± 2794	17.6 ± 4	189.5 ± 183.3
56.2	3	591.9 ± 262.1	1098.4 ± 759.9	12.3 ± 12.5	3790 ± 3293	8588 ± 9239	33.7 ± 27.5	244.9 ± 82.2
74.7	4	675.4 ± 289.6	872.8 ± 457.6	3.7 ± 2.5	1973 ± 773	5020 ± 3122	77.4 ± 29.8	266.1 ± 125.5



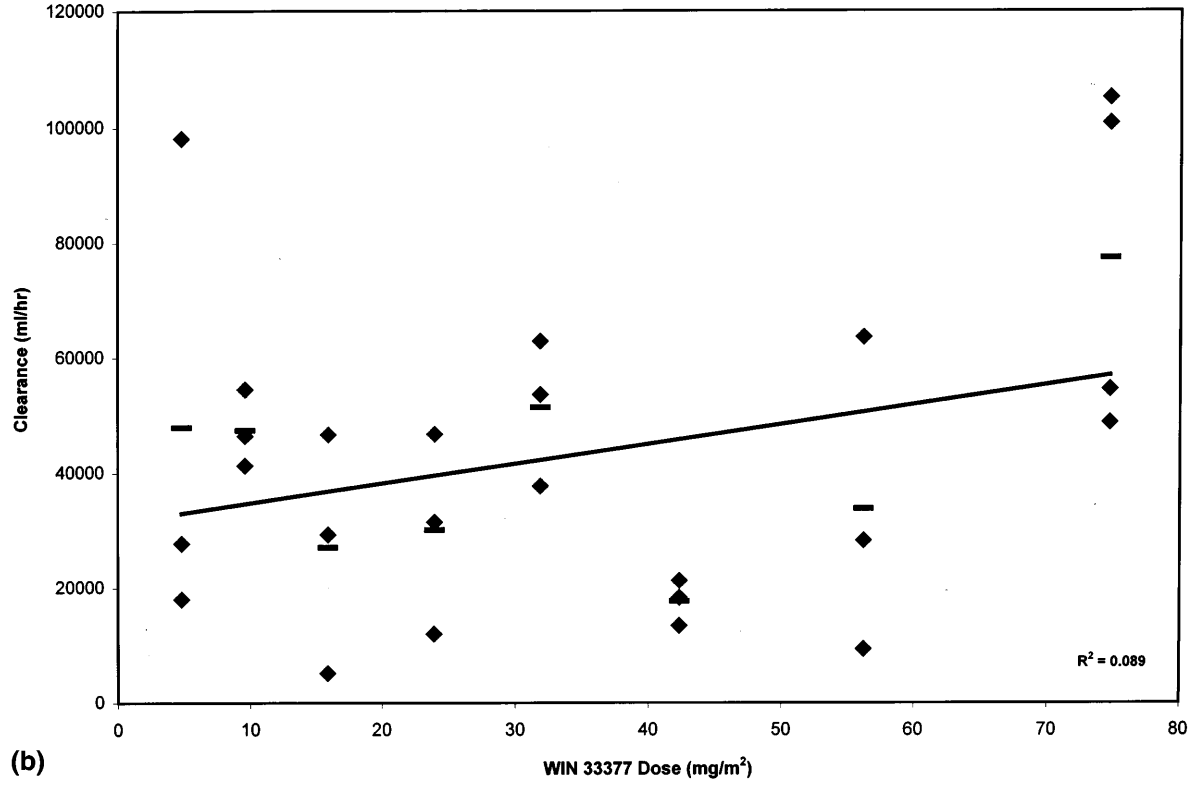


Fig. 2a,b Day 5 SR233377 AUC (**a**) and clearance (**b**) values for all patients per dose level (– mean value per dose level)

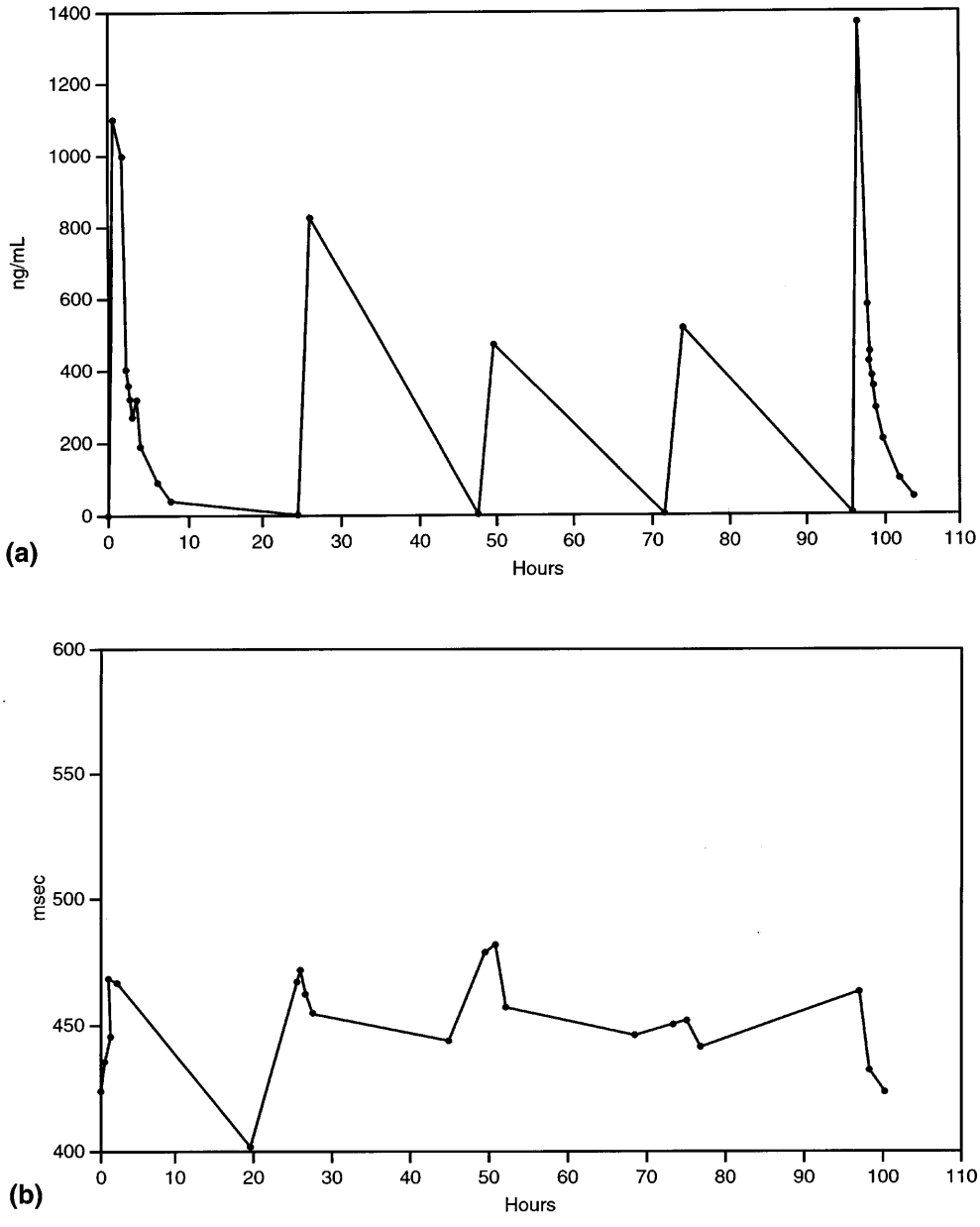


Fig. 3a,b SR233377 plasma concentrations (**a**) and QTc intervals (**b**) versus time during the treatment week in a patient at the 74.7 mg/m^2 per day dose level

previous doxorubicin therapy as well as two investigational agents had a 36% decrease in the size of his evaluable lesions after two cycles of SR233377. This response lasted through 6 months of therapy. Six other patients maintained stable disease (ovarian, colon, endometrial, and cervical cancers) for over 4 months, including a 72-year-old woman with ovarian cystadenocarcinoma metastatic to the peritoneum and inguinal lymph nodes who received five cycles of SR233377.

Discussion

SR233377 is one of a series of cytotoxic thioxanthones with excellent preclinical antitumor activity over a broad dosage range. DNA intercalation and topoisomerase II inhibition have been observed, but the major mechanism of cytotoxicity has not been established. Preclinical toxicology studies have shown side effects consistent with a topoisomerase II-directed mechanism [16]. However, QT interval prolongation, a condition associated with sudden death in patients treated with numerous otherwise innocuous drugs (most recently for example, astemizole [14]) has been observed in the initial clinical trials.

SR233377 administered as a 2-h infusion for five consecutive days produced no dose-limiting toxicity, but the QT/QTc interval prolongation observed in three patients at the 74.7 mg/m^2 dose level prompted closure of this study. While this prolongation was asymptomatic, the two episodes of ventricular arrhythmias observed in a parallel clinical trial of single-dose SR233377

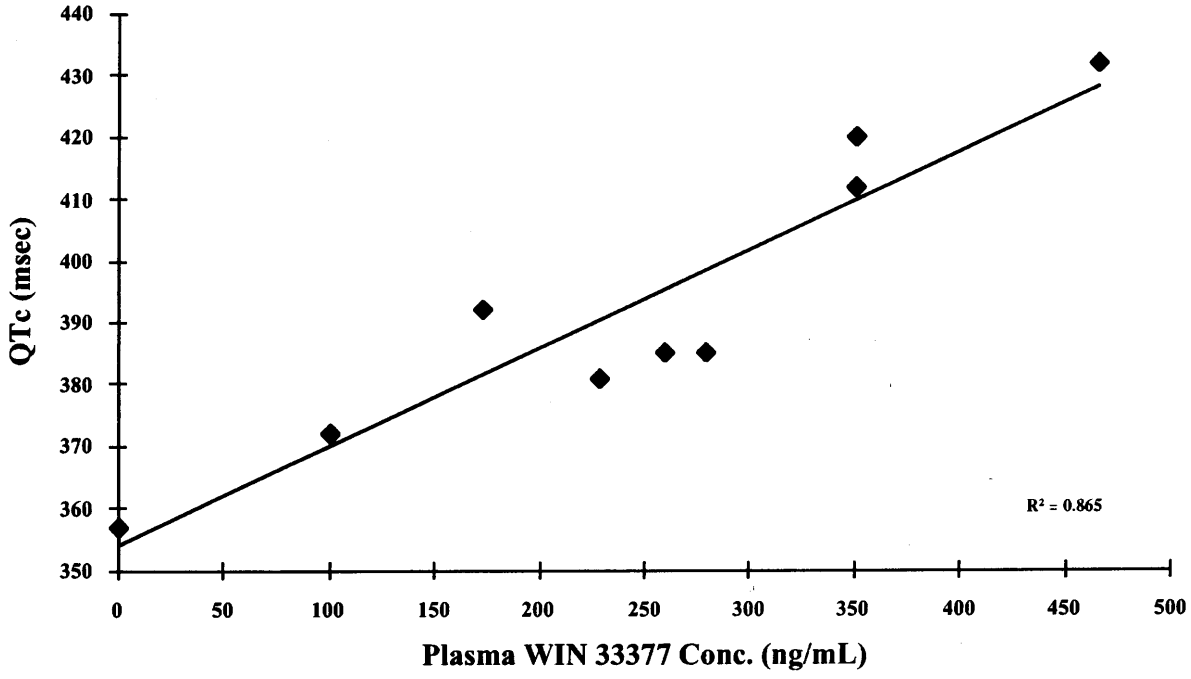


Fig. 4 QTc interval versus plasma SR233377 concentration during the first treatment day in a patient who received 74.7 mg/m^2 per day

are indicative of the potential life-threatening consequence of this effect. Prolongation of the QT interval represents a failure of normal repolarization of cardiac myocyte membranes caused by a reduction in outward potassium ion (K^+) current or enhanced inward flow of sodium (Na^+) or calcium (Ca^{++}) ions [17]. Membrane potential oscillation that spreads throughout the ventricular myocardium may then trigger polymorphic ventricular tachycardia (torsades de pointes) which can degenerate into ventricular fibrillation or resolve spontaneously. Drugs which block K^+, Na^+, or Ca^{++} ion channels (especially class IA and III antiarrhythmic agents) cause QT interval prolongation and predisposition to the development of tachyarrhythmias. This risk is magnified in the presence of hypokalemia, hypomagnesemia, bradycardia, or concomitant use of other drugs which prolong the QT interval [8].

The effect of SR233377 on the QT intervals in the patients we monitored was not predicted by preclinical studies. In vivo toxicology studies in rats have indicated that SR233377 concentrations in myocardium are seven times those in plasma, yet higher concentrations were achieved in other organs, including the spleen, kidney, and lungs (data on file, Sanofi Pharmaceuticals). QT intervals in the upper limit of the normal range have been reported in dogs, but none higher than normal. No cardiac toxicity was noted in preclinical evaluations of SR233377.

The mechanism by which SR233377 produces QT prolongation is unclear. As a DNA binding agent, an effect on ion channels or membrane repolarization is likely unrelated mechanistically. However, SR233377 produced concentration-dependent lengthening of the action potential in contractile tissues during in vitro electrophysiologic studies performed in conjunction with the single-dose study [11]. Cardiac toxicity with other DNA intercalating agents has been described. QT interval prolongation with amsacrine infusion has been reported in 12 of 12 patients in one study [15]. Arrhythmias in a larger analysis of amsacrine-treated patients occurred acutely in association with hypokalemia or concomitant use of drugs known to prolong the QT interval [19]. QT lengthening that was not dose-dependent or related to plasma concentrations has been described in two phase I trials of the DNA intercalator acodazole [13, 18]. Torsades de pointes occurred in one patient. Pazdur et al. reproduced their findings in a dog model and reported myocardial acodazole concentrations 53 times higher than those in plasma [13]. As with acodazole, cardiac myocytes may be sensitive to the preferential SR233377 accumulation noted in the dog studies.

The QT interval prolongation we observed in this trial correlated with rising plasma concentrations of SR233377. The relationship with plasma concentration indicates further that the wide variation in pharmacokinetic indices measured in this study would predict similar variability in the severity of this complication. Plasma SR233377 concentrations and AUC values on days 1 and 5 in the monitored patients at the 74.7 mg/m^2 dose level were not dramatically higher than those in unmonitored patients at lower dose levels: it is entirely likely that asymptomatic QT/QTc prolongation occurred at lower dose levels. We are therefore unable to definitively establish a dose level or plasma concentration at which this would not occur.

High inter- and intrapatient variability in plasma SR233377 AUC, clearance, and half-life values was noted among patients on this study. Preclinical evaluation showed that metabolism of SR233377 is rapid and extensive, and only 2.4% of drug is excreted unchanged in urine and feces [16]. Several patients in our trial exhibited plasma SR233377 half-lives in excess of 20 h, indicating that a polymorphism in a metabolizing enzyme such as cytochrome P450 2D6 may be involved [11]. In light of our findings, prolonged SR233377 plasma concentrations in a range that results in QTc prolongation could be especially hazardous. However, because the QTc effect is concentration-related and other toxicity (at least in preclinical models) appears AUC-related, a prolonged infusion may permit therapeutic doses to be delivered without exceeding the limits associated with QTc lengthening.

Based upon the QT/QTc interval prolongation we observed in this trial of SR233377, a phase II dose cannot be recommended on this schedule. Given the promising tumor growth inhibition noted in several patients with a range of primary tumors, chemical modification of SR233377 and further investigation of the thioxanthones as antitumor agents is warranted.

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