Pharmacokinetics of Piroxantrone in a Phase I Trial of Piroxantrone and Granulocyte-Colony Stimulating Factor


ABSTRACT

Piroxantrone is an anthrapyrazole derivative with broad antitumor activity in vitro. In previous phase I trials, the dose-limiting toxicity of this agent was myelosuppression. Therefore, a phase I and pharmacokinetic study of a 1-h infusion of piroxantrone in combination with granulocyte-colony stimulating factor was conducted. In this article, we report the results of the pharmacokinetic analysis. Thirty-seven patients were studied over a dosage range of 150 to 555 mg/m². The plasma elimination of piroxantrone was biexponential with a mean (± SD) t½β of 3.2 ± 2.7 min and a mean (± SD) t½α of 82 ± 92 min. Clearance was 840 ± 230 ml/min/m². A limited sampling strategy was developed to allow the estimation of total drug exposure (area under the plasma concentration-time curve) from the plasma piroxantrone concentrations at 30, 60, and 120 min after the start of the infusion. The pharmacokinetic behavior of a presumed piroxantrone metabolite not previously described in plasma was also characterized. Based on in vitro cytotoxicity studies with partially purified extract of this compound, we do not believe that it contributes to the antitumor effects of piroxantrone at the concentrations observed in plasma. Finally, piroxantrone elimination was linear over the nearly 4-fold dose range studied, indicating that when dose adjustments are made, systemic drug exposure will remain predictable.

INTRODUCTION

Piroxantrone (oxantrazole, NSC 349174), an anthrapyrazole derivative, is one of a new class of highly active DNA-binding agents with broad preclinical antitumor activity both in the National Cancer Institute human tumor cell line panel and in s.c. implanted murine tumor models (1). In addition, the anthrapyrazoles demonstrate a decreased potential for cardiac toxicity compared with the anthracycline antibiotics in studies using the fetal mouse heart system (1–3).

In the initial phase I studies of piroxantrone, neutropenia was the dose-limiting toxicity, and the maximum tolerated dose was 160–190 mg/m² (4, 5). In order to determine whether higher doses of piroxantrone could be administered in combination with a cytokine, we initiated a phase I trial of piroxantrone as a 1-h infusion every 21 days.

Pharmacokinetic Analysis. Postinfusion concentration-time data were fit to both biexponential (n = 2) and triexponential (n = 3) equations with MLAB (7) using the formula:

\[ C(t) = \sum_{i=1}^{j} A_t e^{-\lambda_i t} \]

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2 The abbreviations used are: G-CSF, granulocyte-colony stimulating factor; HPLC, high performance liquid chromatography; AUC, area under the concentration-time curve; AGC, absolute granulocyte count.

where $C$ is the drug concentration at time $t$, $A_0$ is the intercept, and $A_1$ is the rate constant. Aikake’s information criterion was used to determine the best fit equation (8). The half-life for each phase of elimination was calculated by dividing 0.693 by the rate constant ($A_1$) for that phase. The AUC was calculated using the trapezoidal method, and the clearance was calculated from the equation: clearance = dose/AUC. The steady state volume of distribution was calculated from the area under the moment curve (9).

The relationships between percent change in WBC count, AGC, and platelet count from pretreatment baseline to nadir [i.e.: $(\text{baseline WBC} - \text{nadir WBC})/\text{baseline WBC} \times 100]$ were modeled with MLAB using the Hill equation: $\% \text{ decrease} = (100 \times \text{AUC}_5)/(\text{AUC}_5 + \text{AUC}_k)$, where $\text{AUC}_5$ is the AUC that produces 50% of the maximum effect, and $k$ is the Hill constant (10).

Limited Sampling Strategy. In order to determine the feasibility of a limited sampling strategy for piroxantrone, stepwise forward multiple regression analysis was performed using the plasma concentration at each time point versus AUC (11). The dose and AUC for each patient were normalized to the 355 mg/m² dose level. The $F$ test was used to select the optimal strategy.

RESULTS

Pharmacokinetics

Pharmacokinetic sampling was performed in a total of 37 patients at 7 dose levels. The plasma elimination of piroxantrone was biexponential with a mean (± SD) $t_{1/2a}$ of 3.2 ± 2.77 min and a mean (± SD) $t_{1/2B}$ of 82 ± 92 min (Fig. 1). Six patients (3 at the 355 mg/m² dose level, 2 at the 445 mg/m² dose level, and 1 at the 555 mg/m² dose level) had detectable but not quantifiable amounts of piroxantrone at 24 h postinfusion, suggesting that there may be an additional prolonged terminal phase of elimination. Clearance was 840 ± 230 ml/min/m². The pharmacokinetic parameters for each dose level are summarized in Table 1. Drug elimination was linear over the nearly 4-fold dose range studied (Fig. 2).

There was no correlation between piroxantrone AUC and percent change in WBC or AGC. The correlation between the percent change in platelet count and the AUC could be described by the equation: $\% \text{ decrease} = (100 \times \text{AUC}_{1.03}(486^{1.03} + \text{AUC}_{1.03})$, with an $r$ value of 0.41.

In addition to parent drug, another peak that absorbed light at 514 nm was apparent in the chromatograms of plasma from all patients. This peak had a retention time of 18 min and was not present in pretreatment plasma samples. It appeared by 15 min into the infusion in all patients, and attained maximum concentration between 30 and 60 min after the start of the infusion in the majority of patients, with 15 min being the earliest and 70 min the latest time to maximum concentration in any patient. The UV-visible light spectrum of this compound was similar to that of piroxantrone (Fig. 3). The AUC (in μM piroxantrone equivalents) of the compound increased linearly with dose (Fig. 4). For all dose levels, the mean AUC of the presumed metabolite was 20 ± 13% of the parent drug AUC. The plasma half-life of the metabolite was 39 ± 24 min.

Nineteen (±12)% of the total dose administered was recovered from the 24-h urine collection as unchanged parent drug. In addition to parent drug, 3 other peaks that absorbed light at 514 nm were also noted in the urine (Fig. 5). These peaks had retention times of 6, 9, and 18 min. The 18-min peak in urine appeared to be identical to that present in plasma.

The stepwise forward multiple regression analysis indicates that the piroxantrone AUC can be predicted with an $r^2$ of 0.92 using the concentrations from 30 min into the infusion, and 60 and 120 min after the start of the infusion by the equation $\text{AUC} = -6.6 + 22.4 \times C_{30} + 20.1 \times C_{60} + 515.4 \times C_{120}$, where $C_{30}$, $C_{60}$, and $C_{120}$ represent the plasma piroxantrone concentrations at 30, 60, and 120 min after the start of the infusion.
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Fig. 3. RV-visible light spectra of piroxantrone and the presumed metabolite eluting at 18 min. —, piroxantrone; — - - - - , metabolite.

Fig. 4. Relationship between piroxantrone dose and AUC of the presumed metabolite eluting at 18 min. The metabolite is measured in piroxantrone equivalents (see "Materials and Methods").

DISCUSSION

Piroxantrone pharmacokinetics have been previously reported in 2 phase I trials of this agent administered without growth factor support. The use of growth factors in the present study permitted the escalation of the piroxantrone dose to dose levels 2–3-fold higher than those previously studied in the absence of cytokines. As a result, several important new aspects of piroxantrone pharmacokinetics were elucidated. Over a broad dose range, there was no evidence of nonlinear pharmacokinetic behavior even at the highest doses studied. Elimination of piroxantrone was biexponential, with a rapid distribution phase, an elimination half-life of 82 min, and a clearance of 840 ml/min/m². The elimination and clearance observed in the present trial are consistent with the results of prior trials by Ames et al. (4), who reported a mean clearance of 1290 ml/min/m² and monoeponential elimination with a half-life of approximately 30 min, and by Hantel et al. (5), who reported a mean clearance of 720 ml/min/m² and biexponential elimination with half-lives of 2.9 ± 5.3 min and 18.7 ± 37 min.

There are several possible explanations for the moderate variability of the pharmacokinetic results both within and between studies. (a) Samples containing piroxantrone concentrations that are near the limit of detection for the assay are likely to show greater variability than those containing higher concentrations. Since the piroxantrone assay is relatively insensitive, many samples, whether from patients receiving lower doses or from the later time points after infusion, are likely to be subject to this sort of variability. (b) For a drug with a very short $t_{1/2\alpha}$, small differences in infusion duration and sampling times may create relatively large differences among patients in such parameters as peak plasma concentrations and $t_{1/2\alpha}$. (c) Genuine interpatient variability in drug handling appears to be considerable, not only for piroxantrone, but also for the related anthrapyrazole derivatives CI-937 (12) and CI-941 (13).

It is interesting to compare the pharmacokinetic behavior of piroxantrone with that of other anthrapyrazole analogues. For instance, CI-941, an agent that has shown promising activity against breast cancer in European trials, has a clearance of $420 ± 190$ ml/min/m², similar to that of piroxantrone, but a triexponential plasma disappearance curve with $t_{1/2\alpha}$ of 7.6 min, $t_{1/2\beta}$ of 65 min, and $t_{1/2\gamma}$ of 21 h (14). CI-937, when measured by a radioimmunassay that is 10-fold more sensitive than the HPLC methods used for other anthrapyrazoles, has a terminal half-life of almost 4 days (12). Although in our study the elimination of piroxantrone was best described by a biexponential equation, detection of drug at the 24-h time point in a few patients suggests that there may be a prolonged elimination phase for this drug that is below the limit of detection in the present assay system. Failure to detect terminal phases of elimination may result in underestimation of AUC, or overestimation of clearance.

An important application of pharmacokinetics is to establish the relationships between pharmacokinetic parameters and measures of toxicity and response. Although both WBC and AGC correlated with piroxantrone AUC in a previous trial in which G-CSF was not used (5), in our study, there was no correlation. This difference is probably due to the high starting dose in this trial, which resulted in profound neutropenia in almost all patients. In addition, the correlation between AUC and percent change in platelet count was too weak to be useful.

Another important result of this study was the identification of an apparent metabolite not previously described in plasma. The AUC of this compound was approximately 20% of the parent drug AUC and increased linearly with dose. This metabolite was also present in urine, where it accounted for the majority of piroxantrone-related com-
pounds excreted. By its UV-visible light absorption spectrum and retention time, this compound appeared to be identical to a presumptive metabolite previously identified in the urine of rhesus monkeys after piroxantrone administration. In that study, the metabolite appeared to be at least 1 log less cytotoxic than parent drug. Therefore, even though it is detectable in patient plasma in appreciable concentrations, it is unlikely to contribute significantly to the overall cytotoxicity of piroxantrone.

The stepwise forward multiple regression analysis shows that the AUC for piroxantrone after a 60-min infusion can be predicted with reasonable accuracy using only the 30-, 60-, and 120-min time points. These time points are convenient to obtain and, since the 60-min time point is the end of the infusion, also include the potentially important peak plasma level. In phase II studies, this limited sampling strategy should be validated in a prospective fashion.

In summary, the present study demonstrates that piroxantrone elimination is biexponential and dose-independent over a wide dose range. It is possible that there is an undetected prolonged terminal phase, which would require a more sensitive assay to confirm. A metabolite is detectable in human plasma and urine but probably does not contribute appreciably to the drug's activity. Finally, a limited sampling strategy has been developed that may permit the convenient estimation of the major pharmacokinetic parameters of piroxantrone in future trials.

REFERENCES
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