Clinical pharmacokinetic and in vitro combination studies of nolatrexed dihydrochloride (AG337, Thymitaq™) and paclitaxel

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Summary A clinical study of nolatrexed dihydrochloride (AG337, Thymitaq™) in combination with paclitaxel was performed. The aims were to optimize the schedule of administration and determine any pharmacokinetic (PK) interactions between the two drugs. In vitro combination studies were performed to assist with schedule optimization. Three patients were entered on each of three different schedules of administration of the two drugs: (1) paclitaxel 0–3 h, nolatrexed 24–144 h; (2) nolatrexed 0–120 h, paclitaxel 48–51 h; (3) nolatrexed 0–120 h, paclitaxel 126–129 h. Paclitaxel was administered at a dose of 80 mg m–2 over 3 h and nolatrexed at a dose of 500 mg m–2 day–1 as a 120-h continuous intravenous infusion. Plasma concentrations of both drugs were determined by high performance liquid chromatography. In vitro growth inhibition studies using corresponding schedules were performed using two head and neck cancer cell lines. In both HNX14C and HNX22B cell lines, synergistic growth inhibition was observed on schedule 2, whereas schedules 1 and 3 demonstrated antagonistic effects. In the clinical study, there was no effect of schedule on the pharmacokinetics of nolatrexed. However, patients on schedules 1 and 3 had a higher clearance of paclitaxel (322–520 ml min–1 m–2) than those on schedule 2 (165–238 ml min–1 m–2). Peak plasma concentrations (1.66–1.93 vs 0.86–1.32 μM) and areas under the curve (392–565 vs 180–291 μM min–1) of paclitaxel were correspondingly higher on schedule 2. The pharmacokinetic interaction was confirmed by studies with human liver microsomes, nolatrexed being an inhibitor of the major routes of metabolism of paclitaxel. Toxicity was not schedule-dependent. Nolatrexed and paclitaxel may be safely given together when administered sequentially at the doses used in this study. Studies in vitro suggest some synergy, however, due to a pharmacokinetic interaction, paclitaxel doses should be reduced when administered during nolatrexed infusion. © 2000 Cancer Research Campaign

Keywords: metabolism; interaction; schedule; inhibition

Nolatrexed dihydrochloride (AG337, Thymitaq™) was designed by Agouron Pharmaceuticals, La Jolla, CA, USA using knowledge of the three-dimensional structure of the active site of thymidylate synthase (TS) (Webber et al, 1993). It acts at the folate-binding site of the TS enzyme and, as a non-classical antifolate, the activity of nolatrexed is not dependent on activation by the enzyme folyl polyglutamate synthetase (FPGS) or a specific transport mechanism for cellular uptake. Previous studies with nolatrexed have shown that the optimal schedule of administration is as a continuous intravenous infusion over 5 days at a dose of 800 mg m–2 day–1 (Rafi et al, 1995, 1998). Clinical activity has been demonstrated in head and neck cancer (Belani et al, 1997a), hepatoma (Stuart et al, 1996), pancreatic carcinoma (Loh et al, 1997) and colonic carcinoma (Belani et al, 1997b). The main toxicities associated with nolatrexed are haematological and gastrointestinal (Rafi et al, 1998).

Paclitaxel, derived from the Pacific yew tree Taxus brevifolia, acts by stabilizing microtubules against depolymerization. Paclitaxel has demonstrated anti-tumour activity against a wide variety of different tumour types, most impressively ovarian (Goldspiel, 1997) and breast carcinoma (D’Andrea and Seidman, 1997; Goldspiel, 1997; Wilson et al, 1994). However, it has also shown significant activity against squamous cell carcinoma of the head and neck (Aisner and Cortes-Funes, 1997; Vokes et al, 1995). Major paclitaxel side-effects include myelosuppression, total alopecia and paraesthesiae (Rowinsky, 1997).

In view of the activity of both nolatrexed and paclitaxel in head and neck cancer and, with the exception of myelosuppression, their non-overlapping toxicities, the combination of the two drugs has been explored in clinical and laboratory-based studies. As the schedule of administration of a combination of drugs can play a major role in the toxicity and activity, three different schedules of drug administration were explored. On schedule 1, paclitaxel was administered prior to nolatrexed and on schedule 3 the order was reversed. On schedule 2, the paclitaxel was administered in the middle of the nolatrexed infusion. The pharmacokinetic results obtained for the patients on schedule 2 were of particular interest given that both paclitaxel and nolatrexed undergo metabolism mediated by the cytochrome P450 system.

The aims of the clinical study were:

1. To determine whether nolatrexed and paclitaxel could be safely given in combination
2. To assess the toxicity of the combination and to record any anti-tumour activity
3. To optimize the schedule of administration of the two agents
In vitro combination studies

The two head and neck cancer cell lines, HNX14C and HNX22B, were cultured at 37°C in a humidified atmosphere of 95% air/5% carbon dioxide in RPMI-1640 medium containing 10% v/v dialysed fetal calf serum, supplemented with glutamine and sodium bicarbonate. Both cell lines were passaged twice weekly using trypsin–EDTA. Initial studies were performed to determine the IC\textsubscript{50} for nolatrexed (120-h exposure) and paclitaxel (3-h exposure) for each cell line. For each experiment, exponentially growing cells were washed, trypsinized and seeded at a concentration of 5 × 10\textsuperscript{4} ml\textsuperscript{-1} (HNX14C) or 1 × 10\textsuperscript{4} ml\textsuperscript{-1} (HNX22B) into the wells of a 96-well plate. Cells were then left to adhere for 24 h before drug treatment. For each cell line, 3 different schedules of drug combination, corresponding to those used in the clinical study, were studied.

Schedule 1: paclitaxel 0–3 h, nolatrexed 24–144 h

After the initial 24 h, cells were exposed to varying concentrations of paclitaxel for 3 h. The drug was removed and the plates were then left for 21 h, after which varying concentrations of nolatrexed were added. The paclitaxel and nolatrexed drug concentrations used were as follows: 0.01 × IC\textsubscript{50} for the drug as a single agent, 0.1 × IC\textsubscript{50} \textsuperscript{95}, 0.3 × IC\textsubscript{50}, IC\textsubscript{50}, 3 × IC\textsubscript{50}, 10 × IC\textsubscript{50} \textsuperscript{95}. The ratio of drug concentrations at each dose was therefore the same. Cell growth inhibition was then assessed at 6 days (144 h) by the sulphorhodamine B (SRB) assay (Skehan et al, 1990). For each cell line, experiments were repeated on a minimum of three occasions.

Schedule 2: nolatrexed 0–120 h, paclitaxel 48–51 h

Cells were seeded as above and identical nolatrexed concentrations as those used in schedule 1 were added 24 h after cell adherence. At 48 h, the nolatrexed was removed and replaced by a combination of both nolatrexed and paclitaxel. This was replaced with nolatrexed alone 3 h later. Duplicate plates were used for this schedule and SRB assays were performed at day 6 (144 h) and day 8 (192 h). Experiments were repeated at least three times.

Schedule 3: nolatrexed 0–120 h, paclitaxel 126–129 h

Triplicate plates were set up and cells were exposed to nolatrexed for 120 h. The nolatrexed was then removed and replaced by paclitaxel 6 h later. At 129 h, the paclitaxel was removed. SRB assays were performed on days 6, 8 and 10 (144, 192 and 240 h). Experiments were repeated at least three times.

On every 96-well plate for each schedule, control columns of cells were exposed to medium alone, medium/DMSO (the paclitaxel was diluted in DMSO) and single-agent drug IC\textsubscript{50} concentrations.

The effects of the drug combinations were evaluated using the method of Chou and Talalay (1984). For each schedule of drug administration for both cell lines, curves were plotted of the log of the combination index (CI) versus the fraction of cells affected (Fa).

Table 1 Patient characteristics and outcome

| Patient no. | Age | Sex | Diagnosis          | Schedule | Prior chemo regimes | No. of courses | Response |
|------------|-----|-----|--------------------|----------|---------------------|---------------|----------|
| 3          | 52  | F   | Adrenocortical     | 1        | 3                   | 6             | SD       |
| 6          | 47  | F   | Colorectal         | 1        | 2                   | 1             | PD       |
| 9          | 65  | F   | Melanoma           | 1        | None                | 4             | SD       |
| 5          | 45  | F   | NSCLC              | 2        | 1                   | 4             | SD       |
| 7          | 40  | F   | *Adenocarcinoma    | 2        | None                | 2             | PD       |
| 10         | 72  | M   | Caecal             | 2        | 1                   | 1             | NE       |
| 2          | 59  | F   | Ovarian            | 3        | 2                   | 1             | NE       |
| 4          | 56  | F   | Ovarian            | 3        | 3                   | 2             | PD       |
| 8          | 44  | F   | Breast             | 3        | 1                   | 1             | NE       |

Patient 1 never commenced treatment due to deteriorating clinical condition. NSCLC = non-small-cell lung cancer. * = unknown primary site. SD = stable disease, PD = progressive disease, NE = not evaluable for response.
types were treated. Only two of the nine patients were chemotherapy naive. Twenty-two courses of treatment were administered in total. The study was approved by the Regional Ethical Committee. All patients gave written informed consent prior to registration on the study. Study exclusion and inclusion criteria were as described previously (Rafi et al, 1995), with the additional exclusion of patients having previously been treated with paclitaxel or nolatrexed. Although ultimately intended for use in patients with head and neck cancer, this initial study was performed in any patient with a solid tumour that was not amenable to conventional therapy.

**Patient monitoring**

Before treatment, a complete history was taken and a physical examination with evaluation of WHO performance status performed. A full blood count, urea and electrolyte concentrations and liver function tests were measured and, in addition, an electrocardiogram (ECG) and EDTA clearance determination were obtained. All but the latter two tests were repeated twice weekly during the first course of treatment and weekly thereafter. Although the primary purpose of the study was not to assess tumour response, patients with measurable or evaluable disease had representative lesions assessed at least every two courses by physical examination or appropriate radiological studies. Treatment courses were repeated every 3 or 4 weeks depending on the recovery of the patient from the previous course. Treatment was continued to a maximum six courses. Patients were withdrawn from the study in the event of progressive disease, or if it was felt by the investigator to be in the best interest of the patient. Common toxicity criteria (CTC) for toxicity and response were used.

**Clinical and drug metabolism studies**

Nine patients were randomized to one of three different schedules of administration of nolatrexed and paclitaxel:

Schedule 1: paclitaxel 0–3 h, nolatrexed 24–144 h
Schedule 2: nolatrexed 0–120 h, paclitaxel 48–51 h
Schedule 3: nolatrexed 0–120 h, paclitaxel 126–129 h.

Three patients were treated on each schedule.

Nolatrexed was administered as a continuous 120-h intravenous infusion via a central venous catheter at a dose of 500 mg m⁻² day⁻¹. The total dose was diluted in normal saline and slowly administered over the 5-day period using a Baxter pump (Baxter Healthcare, Newbury, Berks, UK). Paclitaxel was prepared according to the instructions of the manufacturer and administered as a 3-h intravenous infusion in 5% w/v dextrose at a dose of 80 mg m⁻². In order to prevent hypersensitivity reactions to the paclitaxel, patients were pre-medicated with dexamethasone, chlorpheniramine and cimetidine. Courses of treatment were repeated every 3–4 weeks depending on the recovery of the patient from the prior course.

Pharmacokinetic sampling was performed during the first course of treatment in all nine patients. The timing of samples was designed such that data from different schedules were comparable. Plasma samples were stored at −20°C until analysis by methods described previously (Rafi et al, 1995; Siddiqui et al, 1997).

In vitro studies investigating the effect of nolatrexed on the metabolism of paclitaxel were conducted using human liver microsomes (Keystone Skin Bank Exton, PA, USA). Ten individual human liver microsome preparations with P450 content (determined by CO difference spectra) greater than 0.3 nmol mg⁻¹ were pooled together based on equivalent milligrams of protein (determined by the Pierce Kit, Pierce Rockford, IL, USA). This pooling method ensured that there was equal contribution of the cytochrome P450 isoforms from each liver since the protein concentrations from each sample varied from 11.6 to 28.3 mg mL⁻¹.

Pooled human liver microsomes (1 mg protein per ml) were incubated with paclitaxel (for preliminary study 40, 80 and 200 μM and for Kᵢ determination 2.5, 5, 20 μM) and nolatrexed (for preliminary study 0–10 μM and for Kᵢ determination 0–20 μM) in 100 mM potassium phosphate buffer, pH 7.4, at 37°C in a shaking water bath for 5 min before initiating the reaction with NADPH (2 mM final concentration). A control sample containing all reagents except nolatrexed was also included. The final volume of each incubation was 0.5 ml. The reaction was terminated after 20 min by the addition of acetonitrile (5 ml). Samples were vortexed on a SP multi-tube vortexer (Baxter, McGraw Park, IL, USA) for 10 min, followed by centrifugation at 2000 g for 10 min at room temperature. The organic layer was removed and evaporated under a gentle stream of nitrogen using a Dri-Block sample concentrator (Techne, Princeton, NJ, USA) at 50°C. Samples were reconstituted in 200 μl in 60% acetonitrile/40% 10 mM ammonium phosphate buffer, pH 4.0.

Paclitaxel and 6α-hydroxypaclitaxel were measured by a previously published method (Jamis-Dow et al, 1995). Testosterone metabolism was monitored by high-performance liquid chromatography (HPLC), with UV detection at 254 nm (Lillibrige et al, 1998).

Pharmacokinetic parameters were estimated by non-compartmental analysis. Area under the concentration-time curve (AUC) for plasma was calculated using a combination of trapezoidal and log-trapezoid methods. Half-life was calculated from the terminal, log-linear portion of the plasma concentration–time curve.

**RESULTS**

**In vitro combination cytotoxicity studies**

From the single-agent in vitro studies performed, the following IC₅₀ values were calculated (mean (s.d.) n = 3): 3-h paclitaxel exposure; HNX14C 0.031 (±0.006) μM, HNX22B 0.038 (±0.005) μM; 120-h nolatrexed exposure; HNX14C 1.12 (±0.06) μM, HNX22B 0.66 (±0.06) μM.

For schedule 1 the combination of paclitaxel and nolatrexed in vitro demonstrated antagonistic effects for both cell lines, as demonstrated in Figures 1 and 2. For HNX14C the pattern on day 6 there was primarily antagonism, with some synergistic effects, but only at high levels of growth inhibition (Figure 1A). However, when growth was measured on day 8, there was marked synergism throughout the range of Fa (Figure 1A). For the HNX22B cell line treated with schedule 2, some synergism was again observed, but only at values of Fa greater than 0.5 (Figure 2A). Indeed the combination appeared to behave in an antagonistic manner below this Fa value. With schedule 3 and for Fa values of less than 0.75 in the HNX22B cell line, antagonism was observed for the HNX22B cell line regardless of the day on which cell growth was determined (Figure 2B). Similarly, for schedule 3 in the HNX14C cell line, the appearance of the CI/Fa graphs for growth inhibition on days 6, 8 and 10 are almost identical and reveal antagonism up
to a Fa value of approximately 0.75 after which synergistic effects predominate (Figure 1B). In conclusion, the in vitro data showed some evidence of synergy on schedule 2, particularly in the HNX14C cells. However, the effect was not sufficiently consistent to preclude clinical assessment of all three schedules.

**Clinical study**

**Pharmacokinetics**

Full pharmacokinetic sampling was performed on the first course for each of nine patients. The pharmacokinetic parameters of interest were peak plasma concentration, AUC, clearance and half-life of both nolatrexed and paclitaxel. In addition, the peak concentration and AUC of the two main paclitaxel metabolites was measured, i.e. those caused by 6-hydroxylation and 3′-phenylp-hydroxylation of the parent compound. Plasma concentration-time curves of nolatrexed and paclitaxel for a typical patient on each schedule are represented in Figure 3. The paclitaxel pharmacokinetic data are shown in Table 2 with the corresponding data for nolatrexed in Table 3.

Whilst nolatrexed pharmacokinetics were unaffected by schedule, a schedule-dependent interaction of nolatrexed with paclitaxel was observed. Typical pharmacokinetic parameters for paclitaxel when administered as a single agent (Keung et al, 1993; Gianni et al, 1995) are shown in Table 2. Patients on schedule 2 had a lower clearance of paclitaxel (165–238 ml min$^{-1}$ m$^{-2}$) when compared to patients on the other 2 schedules (322–520 ml min$^{-1}$ m$^{-2}$), with the exception of patient 8. The paclitaxel clearance on schedule 2 was comparable to that observed with a single-agent dose of 175 mg m$^{-2}$ and, as a result of the lower clearance on schedule 2, peak plasma concentrations (1.66–1.93 μM versus 0.86–1.32 μM) and AUCs (392–565 μM min$^{-1}$ versus 180–291 μM min$^{-1}$) were higher. The AUC achieved with 80 mg m$^{-2}$ paclitaxel on schedule 2 approaches that with a single-agent dose of 135 mg m$^{-2}$. The exception to the above generalizations was patient 8 who, although treated on schedule 3, had pharmacokinetic parameters similar to patients on schedule 2. This patient subsequently received single-agent paclitaxel (135 mg m$^{-2}$ as a 3-h infusion) and, in the absence of nolatrexed, impaired elimination of paclitaxel was still observed (CI = 127 ml min$^{-1}$ m$^{-2}$).
Paclitaxel metabolite peaks were identified by retention time (Huizing et al, 1993) and peak concentrations and AUCs were calculated. Paclitaxel metabolite AUCs were lowest in the patients treated on schedule 2. Patient 8, on schedule 3, had high levels of both metabolites, both when paclitaxel was administered alone and in combination with nolatrexed, suggesting a deficiency in secondary metabolism or biliary elimination of paclitaxel.

Figure 3 Plasma drug concentration versus time profiles for both paclitaxel and nolatrexed in a typical patient for each schedule of drug administration. Upper panel schedule 1, middle panel schedule 2, lower panel schedule 3.
In vitro metabolism studies

Nolatrexed inhibited the metabolism of paclitaxel to its 6-hydroxy-metabolite with a Ki of 0.64 μM. In addition, nolatrexed inhibited the metabolism of the model CYP3A4 substrate testosterone, with a Ki of 2.7 μM.

Toxicity

The toxicities experienced by the patients on all 22 courses of therapy are summarized in Table 4. Only two courses were complicated by myelosuppression. Patient 8 had grade 4 neutropenia, grade 3 leucopenia and grade 2 thrombocytopenia on her only course of treatment. She had metastatic breast carcinoma and had previously treated six cycles of epirubicin and cyclophosphamide. As shown in Table 3, it is highly likely that the toxicity can be explained by the pharmacokinetics of paclitaxel in this patient. Patient 10 suffered grade 3 neutropenia with his only course of treatment, although this did not require medical intervention. He had metastatic caecal carcinoma and had previously received 5-fluorouracil and folinic acid chemotherapy. Again, this toxicity appeared to be related to the pharmacokinetics of paclitaxel as this patient was treated on schedule 2.

From the viewpoint of gastrointestinal side-effects, nine of 22 courses were complicated by toxicity of grade 3 or greater. Patient

| Patient no. | Schedule | Total dose (mg) | Peak conc. (μg ml⁻¹) | AUC (mg ml⁻¹ min⁻¹) | Clearance (ml min⁻¹ m⁻²) | Half-life (min) |
|------------|----------|----------------|----------------------|---------------------|------------------------|----------------|
| 3          | 1        | 3940           | 6.2                  | 18                  | 137                    | 66             |
| 6          | 1        | 4575           | 6.9                  | 39                  | 64                     | 113            |
| 9          | 1        | 4100           | 6.7                  | 36                  | 70                     | 128            |
| 5          | 2        | 3575           | 6.4                  | 34                  | 73                     | 118            |
| 7          | 2        | 4575           | 6.6                  | 23                  | 108                    | 95             |
| 10         | 2        | 4625           | 5.0                  | 28                  | 91                     | 154            |
| 14         | 3        | 3875           | 6.7                  | 26                  | 99                     | 97             |
| 4          | 3        | 4225           | 8.5                  | 38                  | 62                     | 132            |
| 8          | 3        | 3800           | 5.6                  | 32                  | 79                     | 114            |

AUC = area under plasma concentration/time curve.

Table 4 Toxicity of the combination of paclitaxel and nolatrexed.

| Toxicity  | Grade | 1  | 2  | 3  | 4  |
|-----------|-------|----|----|----|----|
| WBC       |       | 1  |    |    |    |
| Platelets |       | 1  |    |    |    |
| Neutrophils|      | 1  | 1  |    |    |
| Nausea    |       | 8  | 3  | 3  |    |
| Vomiting  |       | 1  | 1  | 2  |    |
| Diarrhoea |       | 5  | 1  |    |    |
| Mucositis |       | 5  | 4  | 3  |    |
| Neuropathy|       | 6  |    |    |    |
| Rash      |       | 2  | 4  |    |    |
| Fatigue   |       | 1  | 15 |    |    |
| Alopecia  |       | 2  |    |    |    |
| Arthralgia|       | 4  | 2  |    |    |
| Others    |       | 6  | 7  | 3  |    |

Data from all three schedules have been pooled. (Figures represent the number of patient courses in which a particular grade of toxicity was experienced.) *Other toxicities included facial flushing, altered taste, dyspepsia, pruriitus and myalgia.
2 experienced grade 3 nausea and grade 4 vomiting and was taken off study because of this. This patient required intravenous anti-emetic therapy, but had experienced similar problems prior to receiving any treatment. There was no obvious association between the gastrointestinal toxicity and schedule of treatment.

A grade 2 rash was experienced in four patient courses and grade 2 fatigue in the majority of courses. Interestingly, neither of the two chemotherapy-naive patients experienced any toxicity greater than grade 2. Between them, they received six of the 22 courses of treatment.

**Efficacy**

Of the nine patients entered on the study, only six were evaluable for response, receiving a total of 19 courses of treatment. Three of these patients had stable disease after a minimum of four courses. The other three patients progressed whilst on treatment. No responses were observed on the study.

**DISCUSSION**

The aim of this study was to determine the optimum schedule and pharmacology of the combination of paclitaxel and nolatrexed in the treatment of solid tumours, particularly head and neck cancer. As well as potential interactions in terms of anti-tumour effect and host toxicity, the potential for pharmacokinetic interactions must also be considered.

The metabolism of paclitaxel has been widely studied in humans. Hepatic metabolism by the cytochrome P450 (CYP) system and biliary excretion play an important role in the removal of paclitaxel from the plasma (Kearns, 1997). The two major metabolites observed in humans are 6-hydroxy paclitaxel, whose formation is mediated by CYP2C8 (Cresteil et al, 1994) and 3'-phenyl-5-hydroxy paclitaxel mediated by CYP3A4 (Cresteil et al, 1994; Sonnichsen et al, 1995). Cytochrome P450 inducers such as phenytoin and phenobarbitone would be expected to increase the metabolism of paclitaxel and this has been shown in studies using human liver preparations (Harris et al, 1994) and in patients (Kuhn et al, 1997). Cytochrome P450 inhibitors, such as ketoconazole and fluconazole, have been shown to inhibit the metabolism of paclitaxel in vitro studies (Jamis-Dow et al, 1997). However, no metabolic interactions have been reported with the drugs commonly used in the paclitaxel pre-medication regime, notably dexamethasone, cimetidine and chlorpheniramine (Jamis-Dow et al, 1995; Schlichenmyer et al, 1995). Paclitaxel clearance is reduced when administered with multi-drug resistance reversal agents (Berg et al, 1995).

In the current study, patients on schedule 2 who received paclitaxel concurrently with nolatrexed had a lower clearance of paclitaxel with higher peak paclitaxel plasma concentrations and AUCs. In vitro studies demonstrated that nolatrexed was a potent inhibitor of CYP2C8-mediated metabolism of paclitaxel (K, 0.64 μM) and of CYP3A4 (K, 2.70 μM) which catalyse the two major routes of paclitaxel metabolism. Thus, nolatrexed apparently reduces the elimination of paclitaxel by inhibiting CYP450-mediated metabolism. There was no schedule-dependent effect on nolatrexed pharmacokinetic profiles when combined with paclitaxel.

On schedules 1 and 3, paclitaxel pharmacokinetics were in accord with previous data. The greater clearance of paclitaxel observed, compared to literature values at higher doses, is consistent with the reported non-linearity in paclitaxel pharmacokinetics.
Clinically, three different schedules of administration of nola-trexed and paclitaxel were studied, and all were well-tolerated. Myelosuppression was rare with the major side-effect being gastrointestinal toxicity. Fatigue was also common, affecting the majority of patient courses. No responses were seen, although three patients had stable disease.

In summary, the metabolism and hence clearance of paclitaxel is impaired when the drug is co-administered with nolatrexed. Further investigation of schedules and dosing was discontinued because the combination of in vitro and in vivo data indicated that the schedule most likely to be active produced a pharmacokinetic interaction. Further exploration of this drug combination would require careful dose optimization, especially given the non-linear pharmacokinetics of both drugs.

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