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ORIGINAL ARTICLE

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# Phase I clinical and pharmacological study of oral methoxymorpholinyl doxorubicin (PNU 152243)

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**Abstract** *Purpose*: The methoxymorpholinyl doxorubicin analogue PNU 152243 was brought into clinical studies because of preclinical observations of its non-cross-resistance in mdr tumor cells, dose-limiting neutropenia, lack of cardiotoxicity, and antitumor activity after oral administration. *Methods*: PNU 152243 was given orally every 4 weeks to 21 adults with a variety of solid tumors at doses ranging from 59 to 940 μg/m$^2$. Antiemetic prophylaxis with 5-HT3 antagonists and steroids, given i.v. on day 1 and orally on days 2–8, was required beginning with the dose of 118 μg/m$^2$. The plasma pharmacokinetics of PNU 152243 were determined by an HPLC method with fluorescence detection. The in vitro myelotoxic effects on granulocyte macrophage-colony forming cells (GM-CFC) of the plasma from 11 patients, obtained 4 and 6 h after treatment at all dose levels, were also assessed. *Results*: Neutropenia was the main hematologic toxic effect and the maximum tolerated dose (MTD) for myelotoxicity was 940 μg/m$^2$, with neutropenia grade 3–4 in two of three patients. Dose-dependent nausea and vomiting were dose-limiting and the MTD for gastrointestinal toxicity was fixed at 820 μg/m$^2$, with grade 4 vomiting in one of two patients. Other frequent toxic effects were diarrhea and fatigue. Peak levels of PNU 152243 were achieved 4 h after dosing. Dose-dependent Cmax and AUCExp, and significant interpatient variability of the main pharmacokinetic parameters were found. Very low levels of the 13-dihydrometabolite PNU 155051 were detected only at the highest doses. The hematotoxicity tests showed a $<70\%$ colony growth inhibition with no correlation between the growth inhibition effect and the degree of myelotoxicity in the same patient. Plasma concentrations of PNU 152243 were 1000 times lower than the concentration inhibiting the growth of 70% of colonies. No objective tumor responses were seen. *Conclusions*: Owing to the occurrence of severe and prolonged nausea and vomiting, the clinical development of oral PNU 152243 was discontinued. The higher-than-expected neutropenia and its lack of relationship with plasma levels of PNU 152243 and its 13-dihydroderivative PNU 155051 might be related to the formation of potent cytotoxic metabolites present in human plasma at undetectable concentrations and with prolonged half-life, as suggested by hematotoxicity tests performed with plasma from patients in GM-CFC assays.

**Key words** Phase I · Methoxymorpholinyl doxorubicin · Anthracycline analogs · Hematotoxicology · Oral chemotherapy

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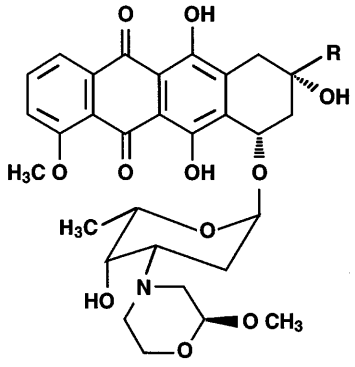
## Introduction

3′-Desamino-3′[2(s)-methoxy-4-morpholinyl]doxorubicin (PNU 152243), a lipophilic doxorubicin (Dx) derivative with a methoxymorpholinyl group at position 3′ of the sugar moiety (Fig. 1), was brought into clinical development because of its broad antitumor activity, and its observed antitumor activity against murine leukemia resistant to Dx, L-PAM and CDDP [4, 10]. Other preclinical features relevant for development were its lack of cardiotoxic effects and, after oral administration, its antitumor activity and 30% bioavailability [3].

Comparison of doses of PNU 152243 active in vivo and in vitro shows that this drug differs from most anthracyclines in being more potent when administered in vivo than in vitro. These findings, and the observation




**PNU 152243** **R = - CO $CH_2$ OH**

**13-dihydrometabolite** **R = - CH (OH) $CH_2$ OH**

**Fig. 1** Chemical structure of methoxymorpholinyldoxorubicin (PNU 152243)

that the cytotoxicity of PNU 152243 is increased in vitro in the presence of liver microsomes, suggest that the molecule is transformed into more toxic metabolites. After incubation in rat liver microsomes, highly cytotoxic metabolites of PNU 152243 can be identified. Among these, PNU 159682 is about 3700-fold more cytotoxic in vitro than the parent drug, while the other metabolites show similar or less cytotoxicity [4, 5, 6]. The main metabolite in rats is the 13-dihydrometabolite, PNU 155051, of which plasma levels about 10% those of the parent compound have been found. The finding that PNU 152243 metabolites are active against both sensitive and Dx-resistant tumor models suggests that they could be an important component of its pharmacological profile.

The main toxicities of PNU 152243 in mice, the most sensitive species, are myelosuppression and liver impairment, while gastrointestinal (GI) toxicity has been reported in dogs and rats. In the first phase I study with PNU 152243 administered as an i.v. bolus every 3 weeks, myelosuppression was dose limiting with a maximum tolerated dose (MTD) of 1500 $\mu g/m^2$ and a recommended phase II dose of 1250 $\mu g/m^2$ [11]. Additional toxicities are delayed and prolonged nausea and vomiting, early transient elevation of transaminases, fatigue and diarrhea.

Pharmacokinetic studies have shown linear kinetics of PNU 152243 and PNU 155051, a mean terminal half-life of the parent compound of 40 h and a considerable interpatient variability of the area under the concentration vs time curve (AUC).

The administration of PNU 152243 on a daily $\times 3$ schedule is associated with better GI tolerability and a lack of delayed and protracted nausea and vomiting. The recommended phase II dose is 500 $\mu g/m^2$ per day [9].

In the present phase I study with the oral formulation, a low starting dose of 59 $\mu g/m^2$ and a single (rather than a daily $\times 3$) schedule of treatment were selected owing to the lack of information on the extent of liver activation in humans that could be important after oral administration. In addition, cycles were scheduled every 4 weeks because of the late appearance of myelosuppression in the previous study. Pharmacokinetic and in vitro hematotoxicology studies with plasma samples of patients [7] were also performed, the latter with the specific intent of determining the presence of highly cytotoxic metabolites other than PNU 155051.

## Patients and methods

### Patients

Adult patients, aged 18 to 75 years, with microscopically confirmed diagnoses of solid tumors not amenable to conventional local or systemic treatments, were eligible for the study. Patients must have recovered from all acute toxic effects of prior therapy and a minimum of 4 weeks must have elapsed from the end of their last chemotherapy or radiotherapy (6 weeks in case of nitrosoureas and mitomycin C). Eligibility criteria included: an ECOG performance status (PS) of 2 or less; life expectancy of 3 months or more; a maximum cumulative dose of prior anthracyclines of 250 $mg/m^2$ of Dx equivalents; a left ventricular ejection fraction (LVEF) as assessed by echo or MUGA scan above the lower limit of normal; adequate bone marrow (neutrophil count $\geq 2.0 \times 10^3/\mu l$, platelet count $\geq 100 \times 10^3/\mu l$), liver (bilirubin, ALT/AST within normal limits) and renal function (serum creatinine within normal limits); alkaline phosphatase not more than 2.5 times the upper limit of normal (unless secondary to malignancy, for which a value not more than 2.5 times the upper limit of normal of ALT/AST was acceptable); feasibility and willingness to participate in pharmacokinetic studies; and the provision of written informed consent.

Criteria for exclusion were: previous high-dose chemotherapy requiring bone marrow transplantation or blood stem cell rescue; myocardial infarction within the last year; uncontrolled hypertension; irreversible arrhythmia; serious concomitant medical disease; GI disorders likely to hamper drug absorption; and concomitant chronic treatment with corticosteroids. One measurable or evaluable lesion was required in patients who were treated at the MTD or at the level immediately below.

### Study design

A starting dose of 59 $\mu g/m^2$, corresponding to one-tenth of the mouse equivalent $LD_{10}$ ($MELD_{10}$), was selected. The dose was increased by 100% increments in the presence of grade 1 or less toxicity and by 20–30% in instances of drug-related grade 2 or more toxicity. At least three patients, and six in instances of toxicity, were treated at each dose, with a 3-week period of observation between the treatment of the first and the second patient and between the treatment of the last patient at one dose level and the treatment of the first patient at the subsequent dose level.

Chemistry (including electrolytes, calcium, creatinine, urea, total protein, and albumin) and urinalysis were repeated before each cycle, while CBC with differential, hepatic enzymes and bilirubin were measured weekly, and more often in case of toxicity. Resting LVEF by MUGA scan or ultrasound, the same method used throughout the study, was repeated every two cycles starting during the fourth course (during the second in patients pretreated with anthracyclines).

Toxicity was evaluated according to the National Cancer Institute (NCI) Common Toxicity Criteria[1] (CTC). When no CTC criteria were available, a score of mild, moderate or severe was

[1] Common Toxicity Criteria from the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, Bethesda, Md.

assigned. Patients were asked to report their symptoms, which were mainly GI, and any concomitant medications by filling in a chart. Patients were evaluable for hematologic toxicity if at least weekly CBC counts with differential were available for a minimum of 4 weeks. Retreatment cycles at lower doses were not included in the toxicity analyses.

Dose limiting toxicities (DLT) were a grade 4 neutropenia lasting 5 days or more or of any duration associated with grade ≥ 3 infection, grade ≥ 3 thrombocytopenia, grade ≥ 3 nonhematologic toxicity, or grade ≥ 2 neurotoxicity. The MTD was defined as the dose at which two or more of three patients or three or more of six patients developed DLTs after the first administration of PNU 152243. At least three additional patients then had to be treated at the next lower dose level, which was the one to be recommended in the subsequent phase II study, to fully characterize the toxicity pattern.

In patients with measurable or evaluable disease, tumor response was assessed after two cycles and classified according to WHO criteria [8].

### Drug administration

PNU 152243 was supplied by Pharmacia & Upjohn, Milano, as hard gelatine capsules containing 0.1, 0.2, or 0.5 mg of active drug substance. The capsules were packaged in light-resistant vials and stored at 2–8 °C. The capsules were swallowed whole with 200–300 ml of water after overnight fasting to be continued for 2 h after treatment. PNU 152243 was administered as a single dose on day 1 to be repeated every 4 weeks. Prophylactic antiemetics were not initially allowed during the first cycle of treatment; however, because of the universal occurrence of nausea and vomiting, antiemetic prophylaxis was introduced at 118 μg/m$^2$ with i.v. thiethylperazine (regimen 1, Table 3), replaced at 470 μg/m$^2$ by 5-HT3 antagonists in combination with steroids given i.v. on day 1 and orally for 2 days (regimen 2), and then at 940 μg/m$^2$ by i.v. 5-HT3 antagonists, steroids, thiethylperazine and oral haloperidol on day 1, followed by oral 5-HT3 antagonists and steroids for 7 days (regimen 3). On day 1 i.v. antiemetics were given immediately before PNU 152243 administration. Concomitant oral benzodiazepines and ranitidine were given starting on the day before treatment. At the highest doses patients were hospitalized for at least 72 h; in instances of vomiting, i.v. fluids and i.v. haloperidol 5 mg continuous infusion were started. Oral loperamide was prescribed for diarrhea.

### Pharmacokinetics

#### *Sampling procedures*

Blood samples (5 ml) were collected from all patients after the first administration via an indwelling cannula from the antecubital vein into tubes containing heparin as anticoagulant. Samples were taken before (time 0, predose) and 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h after administration. A sampling schedule limited to 24 h postdosing was adopted up to 470 μg/m$^2$. The blood was immediately placed in an ice bath, centrifuged at 4 °C (1200 *g* for 10 min), and the separated plasma was stored at −20 °C in labelled cryotubes until dosing.

#### *Drug assay*

PNU 152243 and PNU 155051 were assayed in plasma using a previously reported and validated HPLC method after solvent extraction [10]. Briefly, 1 ml plasma was added with daunorubicin as internal standard, and 1 ml borate buffer, pH 8.4, and extracted with a diethyl ether/*n*-butanol mixture, and finally back-extracted into 0.3 *M* phosphoric acid. An aliquot of 160 μl was injected using a 717 WISP refrigerated autosampler (Waters, Milford, Mass.) into a model 501 HPLC (Waters). Chromatographic separation was carried out with a hypersil ODS (C18) 3 μm, 150 × 4.6 mm column (Alltech, Deerfield, Ill.) with a precolumn packed with Pellicular C18 37–53 μm (Whatman, Clifton, N.J.). The mobile phase was acetonitrile/tetrahydrofuran/0.05 *M* $KH_2PO_4$ brought to pH 2.7 with 1 *M* $H_3PO_4$ (18/10/72, v/v/v) at a flow rate of 1 ml/min. The system was equipped with a 474 fluorescence detector (Waters) set at excitation and emission wavelengths of 506 and 560 nm, respectively.

Daily calibration curves were produced in the range 0.1 to 5 ng/ml with a coefficient of correlation *r* always more than 0.995. The method was found to be highly precise (coefficient of variation ≤ 5%) and accurate (103%) with a lowest limit of quantitation of 0.1 ng/ml.

#### *Pharmacokinetic analyses*

The concentrations of PNU 152243 and PNU 155051 in plasma from each subject were evaluated using standard noncompartmental methods. The following pharmacokinetic parameters were determined: Cmax, maximum plasma concentration; Tmax, time at which Cmax is reached; AUCExp, area under plasma concentration-time curve up to the last experimental point available; AUCinf, area under plasma concentration-time curve extrapolated to infinity; t1/2, plasma half-life of the terminal phase. Cmax and Tmax were determined from the analytical data. AUCExp was determined by the trapezoidal rule. AUCinf was determined by extrapolating AUCExp to infinity from the last experimental point using the constant of elimination Ke, which is the slope of the terminal phase of the concentration vs time curve based on at least three data points. The terminal half-life was derived from the equation $t1/2 = 0.693/Ke$.

### Hematotoxicology studies

Plasma samples, drawn from the patients during pharmacokinetic studies, were collected before and 2, 4, 6 and 8 h after administration. In selected patients plasma was also collected at 12 and 24 h. Plasma was frozen at −20 °C until analysis. Between 4 and 8 months later plasma samples were thawed and 1-ml aliquots were incubate with $1 \times 10^5$ human umbilical cord blood (hCB) cells for 1 h at 37 °C. Cells were subsequently washed twice, then plated for clonogenic assay and granulocyte macrophage colony-forming cells (GM-CFC) were scored on day 14 as previously described [5]. GM-CFC inhibition induced by posttreatment plasma was then calculated as a percentage of that induced by baseline pretreatment plasma or medium as control.

For comparison, the in vitro myelotoxic activity of PNU 152243 and of its rat liver microsome-activated products as well as that of the highly cytotoxic metabolite PNU 159682 were measured by GM-CFC inhibition experiments, to enable the dose inhibiting 70% of the colonies to be derived from dose-response curves.

## Results

Entered into the study were 21 patients with an ECOG PS of 0–1 (Table 1), of whom 12 had been pretreated with chemotherapy which in 3 of them included anthracyclines. Five had received prior chemotherapy and radiotherapy, while three, treated at 118, 235 and 940 μg/m$^2$, respectively, were given PNU 152243 as initial treatment.

### Hematologic toxicity

All patients were evaluable for hematologic toxicity for a total of 43 cycles (Table 2). In absence of grade 2 toxicity, the dose was doubled to 470 μg/m$^2$ and then to

**Table 1** Patient characteristics

| Characteristics | No. of patients |
|---|---|
| Total patients | 21 |
| Male/female | 14/7 |
| Age, years | 59 (42–75) |
| Performance status (ECOG) | |
| 0 | 11 |
| 1 | 10 |
| Tumor types | |
| Colon | 5 |
| Pancreas | 2 |
| Other | 14 |
| Prior therapy | |
| Chemotherapy alone | 12* |
| Chemotherapy + radiotherapy | 5** |
| Radiotherapy | 1 |
| None | 3 |

* Previous Dx in 2 pts; ** Previous Dx in 1 pt

940 μg/m$^2$. At this dose the first patient, a 73-year-old man with bladder cancer pretreated with four courses of M-VAC (methotrexate, vinblastine, doxorubicin, cisplatin) developed grade 4 neutropenia of 8 days' duration and grade 1 thrombocytopenia. Of the other two patients treated at the same dose, one, a 42-year-old woman with renal cell cancer pretreated with interferon, developed grade 3 and grade 1 neutropenia after the first and second cycles, respectively, while the other, a 59-year-old man with thyroid carcinoma pretreated with radiation only, developed no hematologic toxicity. Three patients previously treated with chemotherapy were then entered at 700 μg/m$^2$, corresponding to a 50% increment from 470 mg/m$^2$, without the occurrence of myelotoxicity. The dose was then finally increased to 820 μg/m$^2$ in the last two patients treated, without the occurrence of myelotoxicity.

Overall, neutropenia was the main hematologic toxicity. It was grade 4 in only one patient, and was characterized by high interpatient variability, with a steep dose-response curve between 820 and 940 μg/m$^2$, and with the lowest neutrophil count occurring between days 15 and 28. Grade 1 thrombocytopenia was reported only in the patient who developed dose-limiting neutropenia at 940 μg/m$^2$. Hemoglobin levels were not significantly affected and grade 3–4 hemoglobin decreases were not reported.

**Table 2** Median neutrophil nadir (×10$^3$/μl) and median time to nadir (days) per dose level

| Dose (μg/m$^2$) | No. of eval. pts/cycles | Neutrophil nadir (×10$^3$/μl) | | Time to nadir (days) | |
|---|---|---|---|---|---|
| | | median | (range) | median | (range) |
| 59 | 3/8 | 5.5 | (4.7–7.9) | 15 | (21–27) |
| 118 | 3/6 | 3.9 | (2.9–6.0) | 28 | (10–33) |
| 235 | 4/12 | 2.5 | (1.6–7.5) | 21 | (8–30) |
| 470 | 3/5 | 3.7 | (1.8–6.0) | 25 | (18–32) |
| 700 | 3/5 | 2.5 | (2.0–3.3) | 22 | (18–31) |
| 820 | 2/2 | 3.7, 6.5 | | 18, 11 | |
| 940 | 3/5 | 1.6 | (0.28–3.0) | 18 | (15–20) |

## Nonhematologic toxicities

Dose-dependent nausea and vomiting was the dose-limiting toxic effect of oral PNU 152243 (Table 3). Because of the occurrence of this side effect at 118 μg/m$^2$, antiemetic prophylaxis was introduced, first with i.v. thiethylperazine and then, because of increased degree and duration at 470 μg/m$^2$, with combinations of 5-HT3 antagonists and steroids, given i.v. the day of treatment and then orally for up to a total of 7 days. Patients received PNU 152243 as inpatients and remained in hospital for up to 72 h to facilitate administration of the best antiemetic treatment. Nevertheless, 86% and 71% of cycles given at doses of 820 μg/m$^2$ or greater were associated with nausea and vomiting, respectively, of grade 2 or more. Two of three patients treated at 940 μg/m$^2$ and one of two at 820 μg/m$^2$ presented vomiting of grade 3 or more, thereby defining the MTD. Nausea and vomiting appeared late, starting about 12 h after dosing, with a median duration of 3 days for vomiting and 5 days for nausea. Two patients, one treated at 700 μg/m$^2$ and the other at 820 μg/m$^2$, refused any further admin-

**Table 3** Non hematologic toxicity

| Dose (μg/m$^2$) | No. of eval. pts/cycles | Antiemetic prophylaxis | No. of cycles with toxicity grade | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nausea | | | Vomiting | | | | Diarrhea | | | Fatigue | | |
| | | | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 1 | 2 | 3 |
| 59 | 3/8 | | | | | | | | | | | | | | |
| 118 | 3/6 | * | 1 | 1 | | | 4 | | | 2 | | | | | |
| 235 | 4/12 | | 6 | 2 | 1 | 3 | 2 | 1 | | 1 | | | 2 | 2 | |
| 470 | 3/5 | ** | 1 | 1 | 1 | 1 | | 1 | | 2 | 1 | | 1 | 1 | |
| 700 | 3/5 | | | 2 | | 1 | 1 | 2 | | 2 | | | 2 | 2 | |
| 820 | 2/2 | | | 1 | 1 | 1 | | | 1 | 1 | | | | | 1 |
| 940 | 3/5 | *** | | 2 | 2 | 1 | 2 | 2 | | 2 | 3 | | 3 | 2 | |

* Regimen 1 IV thiethylperazine, 6.5 mg t.i.d d1
** Regimen 2 IV 5 $HT_3$ antagonists and steroids d1, PO 5 $HT_3$ and steroids days 2–3
*** Regimen 3 IV 5 $HT_3$ steroids, thiethylperazine and PO haloperidol d1, PO 5 $HT_3$ and steroids d2–8

istration of PNU 152243 after the first cycle because of prolonged nausea and vomiting.

Diarrhea of grade 2 or less, appearing within 4 days, was reported during 60% of the cycles given at 940 μg/m$^2$. In one patient treated at 940 μg/m$^2$ melena and hematemesis were reported 3 days after the first dose; no ulcers or erosions were evident at gastroscopy and ranitidine and omeprazole were prescribed, with resolution of the symptoms. A Mallory-Weiss-type lesion was suspected and judged possibly drug-related. The patient suffered from moderate vomiting of short duration after the second administration of PNU 152243 which was followed after 2 days by severe abdominal cramps, vomiting and hemorrhagic diarrhea. Complete control of the symptoms was achieved within 24 h with antiemetics, antispasmodics and antidiarrheic drugs. Other GI side effects were epigastric pain and loss of appetite in three patients and one patient, respectively. Fatigue was reported by all patients treated at 940 μg/m$^2$. No significant changes of LVEF or of liver function tests were observed.

No objective responses were observed.

## Pharmacokinetic results

Measurable plasma levels of PNU 152243 and PNU 155051 were first achieved at 470 μg/m$^2$. Figure 2 shows the plasma pharmacokinetic profile of the parent compound in three patients treated at 940 μg/m$^2$.

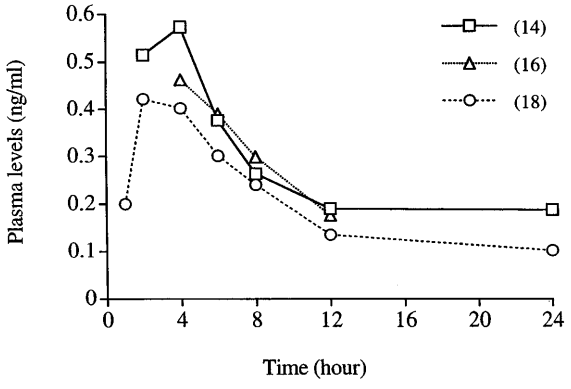



**Fig. 2** Decay curves of PNU 152243 in plasma from three patients treated at 940 μg/m$^2$

The main pharmacokinetic parameters are listed in Table 4. For both the parent compound and the metabolite, Cmax was achieved within 4–6 h with a median value of 4 h. The 13-dihydrometabolite was detectable in only six patients with concentrations approximately two times lower than those of the parent compound.

Significant relationships were found between the dose administered and Cmax ($r = 0.81$, $P < 0.01$; Fig. 3) and AUCExp ($r = 0.79$, $P < 0.01$; Fig. 4), while no relationship was found with AUCinf. Plasma decay of PNU 152243 showed a high interpatient variability, with a terminal half-life (mean ± SD) in eight patients of 11.1 ± 10.8 h (range 3.1–36.8 h).

## Hematotoxicity test results

In GM-CFC clonogenic assays of cells treated with the parent compound PNU 152243 and with the metabolite PNU 159682 the mean $ID_{70}$ values (concentrations inhibiting the growth of 70% of colonies) of PNU 152243 were 1150 ng/ml and 420 ng/ml for a 1-h and a 4-h exposure, respectively, and of PNU 159682 were 0.7 ng/ml and 0.24 ng/ml for a 1-h and a 4-h exposure, respectively. Thus PNU 159682 showed a more than 1000 times greater myelotoxic effect than PNU 152243. Table 5 shows the myelotoxic effects of the 4-h and 6-h plasma samples from 11 patients expressed as inhibition of colonies, the actual plasma concentrations of the parent compound at 4 h and 6 h and the neutrophil count nadir of the patients. The growth of GM-CFC in medium and in plasma from untreated patients was comparable. It appears that the myelotoxic effect was significant only at doses of 700 μg/m$^2$ or greater but that this was achieved with plasma concentrations of PNU 152243 almost 1000 times lower than the $ID_{70}$. The inhibitory effect was still present in plasma samples collected 8, 12 and 24 h after treatment (data not shown).

## Discussion

The clinical development of the lipophilic Dx derivative PNU 152243 was prompted by its antitumor activity in murine tumors resistant to Dx and alkylating agents, by

**Table 4** Main pharmacokinetic parameters of PNU 152243 and PNU 155051 (values in brackets)

| Pt. no. | Dose (μg/m$^2$) | Cmax (ng/ml) | Tmax (h) | AUCExp (ng/ml · h) | AUCinf (ng/ml · h) | Terminal half-life (h) |
|---|---|---|---|---|---|---|
| 11 | 470 | 0.147 | 2 | – | – | – |
| 15 | 700 | 0.404 (0.157) | 4 (4) | 2.64 (1.48) | 5.49 (4.45) | 11.6 (17.8) |
| 17 | 700 | 0.194 (0.129) | 4 (6) | 1.18 | 1.78 | 4.2 |
| 19 | 700 | 0.266 | 4 | 1.16 | 1.65 | 3.1 |
| 20 | 820 | 0.291 (0.139) | 4 (4) | 2.26 (0.75) | 3.79 | 7.6 |
| 21 | 820 | 0.360 | 2 | 2.78 | 4.71 | 9.3 |
| 14 | 940 | 0.573 (0.286) | 4 (2) | 6.60 (4.8) | 16.60 (6.8) | 36.8 (13.3) |
| 16 | 940 | 0.463 (0.21) | 4 (6) | 3.42 (2.7) | 4.85 | 5.6 |
| 18 | 940 | 0.422 (0.158) | 2 (2) | 4.65 | 6.16 | 10.3 |

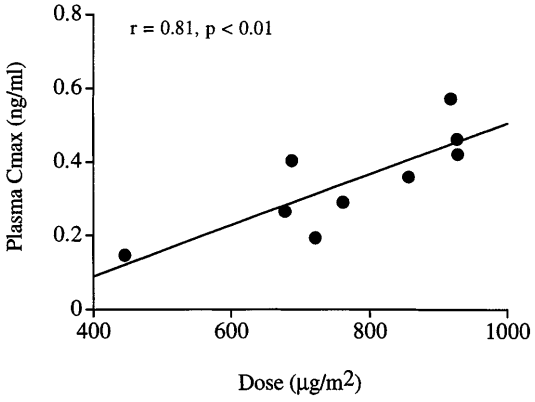



**Fig. 3** Relationship between dose of PNU 152243 and Cmax

its favorable toxicity profile with no cardiotoxicity and by its good activity when given by the oral route, suggesting an acceptable bioavailability [3, 10]. It has also been shown that PNU 152243 is activated in vitro by liver microsomes to highly cytotoxic products, at least partly responsible of its higher potency than the parent Dx [6].

The preclinical toxicological features, with myelosuppression, GI and hepatic toxicities as the main side effects, were confirmed in the first clinical study, in

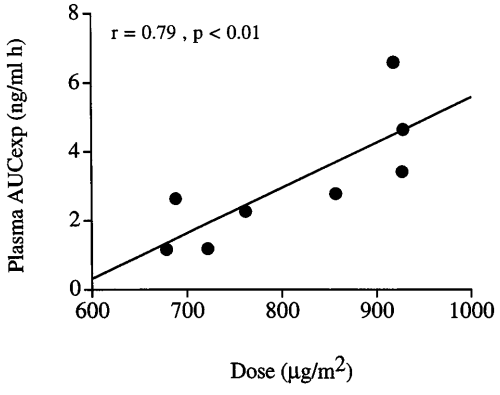



**Fig. 4** Relationship between dose of PNU 152243 and AUCExp

which PNU 152243 was administered i.v. on a single intermittent schedule every 3 weeks [11]. In that study, neutropenia was dose-limiting, the MTD was 1500 $\mu g/m^2$ and the recommended phase II dose was 1250 $\mu g/m^2$. Pharmacokinetic studies have shown a linearity of the plasma kinetics of PNU 152243, a high interpatient variability of the pharmacokinetic parameters and the presence of detectable levels of 13-dihy-drometabolite at doses of 675 $\mu g/m^2$ or higher.

In the present study, the pattern of toxicity observed with single oral dosing was qualitatively similar to that observed after i.v. administration with dose-dependent neutropenia, prolonged nausea and vomiting and fatigue as the main side effects. Transient elevations of hepatic transaminases and thrombocytopenia, however, which were of clinical relevance after i.v. administration, did not occur after oral treatment. In spite of intensive antiemetic treatment, patients suffered from delayed and prolonged nausea and vomiting, which became dose-limiting at 820 $\mu g/m^2$ and 940 $\mu g/m^2$, and was the main reason for discontinuing the clinical development of the oral formulation.

From a quantitative point of view, the profile of myelotoxicity seen is different after oral and i.v. administration. Neutropenia appeared at lower doses in the study with oral administration, where it was first observed at 235 $\mu g/m^2$. After i.v. administration neutropenia was first observed at a dose of 1250 $\mu g/m^2$, and only in patients heavily pretreated. The dose response curve of neutropenia was very steep with both routes of administration within different dose ranges: between 820 and 940 $\mu g/m^2$ after oral administration and between 1250 and 1500 $\mu g/m^2$ after i.v. administration. The higher degree of neutropenia observed with the oral formulation could not be explained by differences in the selection of patients or in the characteristics of their prior treatment; in fact, a higher number of previously untreated patients were entered in the i.v. study but almost all of them were treated at doses of 1250 $\mu g/m^2$ or higher.

The higher degree of neutropenia observed with the oral formulation did not appear to be directly related to

**Table 5** In vitro myelotoxicity of plasma samples

| Pt. No. | PNU 152243 dose ($\mu g/m^2$) | Neutrophil nadir ($\times 10^3/\mu l$) | Plasma samples | | | |
|---|---|---|---|---|---|---|
| | | | %GM-CFC survival (4 h) | %GM-CFC survival (6 h) | PNU 152243 concentr. (ng/ml) (4 h) | PNU 152243 concentr. (ng/ml) (6 h) |
| 2 | 59 | 4.9 | 90 | | | |
| 4 | 118 | 4.7 | 83 | 65 | | |
| 5 | 118 | 4.2 | 101 | 118 | | |
| 9 | 235 | 2.8 | 121 | 93 | | |
| 13 | 470 | 4.6 | 77 | 83 | | |
| 11 | 470 | 6.0 | 111 | 88 | 0.10 | 0.10 |
| 15 | 700 | 2.0 | 104 | 77 | 0.40 | 0.24 |
| 19 | 700 | 3.3 | 46 | 54 | 0.27 | 0.12 |
| 20 | 820 | 3.7 | 153 | 79 | 0.29 | 0.25 |
| 14 | 940 | 0.3 | 82 | 54 | 0.57 | 0.38 |
| 16 | 940 | 0.7 | 90 | 76 | 0.46 | 0.39 |

the plasma levels of PNU 152243, which were very variable, as previously reported after i.v. administration [1, 11]. After oral administration the 13-dihydro metabolite was also detected only in small amounts and after doses of 700 $\mu g/m^2$ or higher. The ratio of the AUC of the 13-dihydro metabolite of PNU 152243 to that of the parent compound was higher than that reported after i.v. administration [11], suggesting some first-pass formation of the metabolite. This finding does not explain the relatively high hematotoxicity seen after oral dosing as 13-dihydrometabolite has been reported to be less cytotoxic than the parent compound [5].

The results of the in vitro hematotoxicity tests could provide a partial explanation for the higher-than-expected degree of neutropenia observed after oral treatment. The concentrations of PNU 152243 found in plasma of patients 4 and 6 h after oral administration were 1000 times lower than those found to be myelotoxic in vitro. Since the exposure of GM-CFC to the patients' plasma caused growth inhibition, at least in some cases it is reasonable to suppose that the plasma of patients contained cytotoxic compounds other than PNU 152243.

A comparison between the bone marrow toxicity observed in vitro and in vivo showed that there was no correlation between the percentage of growth inhibition and the degree of neutropenia in the individual patients, even though the highest growth inhibition of 54% was observed in the patient who showed grade 4 neutropenia at 940 $\mu g/m^2$.

These results strongly suggest that myelosuppression after oral administration might be related to the production of highly cytotoxic metabolites present in plasma at low concentrations. Recently, Geroni et al (manuscript in preparation) have isolated and characterized 3′-deamino-3′,4′-anhydro-[2″(S)-methoxy-3″-hydroxy-4″morpholinyl]doxorubicin (PNU 159682) as a metabolite of PNU 152243. To our knowledge this is the most potent cytotoxic anthracycline derivative known and thus, even if present at low concentrations, may play a role in the pharmacological effects of PNU 152243.

Whether the higher toxicity of PNU 152243 after oral administration is due to a high formation of PNU 159682 is still unproven, since no studies have been carried out determining this metabolite in human plasma after i.v. or oral administration.

PNU 159682 is formed by a 3A isoform from a P450-dependent reaction which can be induced by steroids. Therefore we cannot exclude the possibility that, in the present study, the prolonged use of steroids as antiemetic prophylaxis, as frequently used in daily practice, might not have influenced the metabolic pathway of PNU 155243.

In conclusion, the severe GI toxicity found in the present study discouraged the continuation of the clinical development of oral PNU 152243. The pharmacological and myelotoxicity studies performed at preclinical and clinical level highlighted the importance of the metabolism of PNU 152243 in its activity and toxicity while the in vitro evaluation of the myelotoxicity of PNU 152243 and of its metabolites was essential in orientating its further clinical development.

The delayed GI and hematologic toxicity and the low response rate reported in a phase II study of the i.v. formulation in patients with tumors with intrinsic chemotherapy resistance did not support a broad clinical development of the compound [1]. On the other hand, the liver metabolism into active species has been taken into account in planning further well-defined disease-oriented clinical studies.

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