Cancer Chemother Pharmacol (2001) 47: 355–360
DOI 10.1007/s002800000240

ORIGINAL ARTICLE

**Paola Della Torre · Arturo Podestà
Anthony R. Imondi · Donatella Moneta
Umberto Sammartini · Claudio Arrigoni
Andrea Terron · Marco Brughera**

PNU-159548, a novel cytotoxic antitumor agent with a low cardiotoxic potential

Received: 18 May 2000 / Accepted: 31 October 2000 / Published online: 17 February 2001

Abstract *Purpose*: PNU-159548 (4-demethoxy-3′-deamino-3′aziridinyl-4′-methylsulphonyl-daunorubicin), a derivative of the anticancer idarubicin, has a broad spectrum of antitumoral activity in vitro and in vivo attributable to its DNA intercalating and alkylating properties. The present study was conducted to determine the cardiotoxic activity of PNU-159548 relative to doxorubicin in a chronic rat model sensitive to anthracycline-induced cardiomyopathy. *Methods*: Young adult male rats were allocated to the following treatment groups: group 1, PNU-159548 vehicle control (colloidal dispersion); group 2, doxorubicin control (saline); groups 3, 4, 5, 6, and 7, PNU-159548 at 0.12, 0.25, 0.50, 0.75, and 1.0 mg/kg, respectively; and group 8, 1.0 mg/kg doxorubicin. Treatments were administered intravenously once weekly for 4 weeks (first sacrifice time) or for 7 weeks (rats killed at weeks 8, 12, 22, 27, or 35). Body weights, organ weights, serum chemistry, hematology, serum troponin-T, and cardiac histopathology were followed throughout the study. *Results*: Doxorubicin caused irreversible cardiomyopathy evident at week 4 in some rats and progressing in severity in all rats by week 8. There were also marked myelotoxicity, increased liver and kidney weights, testicular atrophy, and about 20% mortality by week 27 in doxorubicin-treated rats. The deaths were attributed to cardiomyopathy and/or nephropathy. PNU-159548 caused a dose-dependent myelotoxicity, with the dose of 0.5 mg/kg per week being equimyelotoxic to 1.0 mg/kg per week doxorubicin. PNU-159548 also caused an increase in liver weight that was reversible and a non-reversible testicular atrophy but, unlike doxorubicin, had no effect on kidney weight. At equimyelotoxic doses, the cardiotoxicity caused by PNU-159548, expressed as the mean total score, was less than one-twentieth of that induced by doxorubicin, and much less than that predicted on the basis of its content of idarubicin, which is in turn markedly less cardiotoxic than doxorubicin. *Conclusions*: The novel cytotoxic antitumor derivative, PNU-159548, is significantly less cardiotoxic than doxorubicin at equimyelosuppressive doses. The combination of intercalating and alkylating activities within the same molecule without the cardiotoxic side effects of anthracyclines makes PNU-159548 an excellent candidate for clinical development in oncology.

Key words PNU-159548 · Doxorubicin · Cardiotoxicity

P. Della Torre · A. Podestà · D. Moneta · U. Sammartini
C. Arrigoni · A. Terron · M. Brughera
Worldwide Toxicology, Pharmacia and Upjohn,
20014 Nerviano, Milan, Italy

A. R. Imondi (✉)
Battelle, 505 King Avenue, Columbus,
OH 43201-2693, USA
Tel.: +1-614-4247131
Fax: +1-614-4243716

Introduction

Anthracyclines are among the most commonly used anticancer agents for the treatment of a wide range of neoplastic diseases, including hematological and solid tumors. Although the emergence of drug-resistant tumors remains one of the principal obstacles to clinical success, chronic progressive cardiomyopathy is regarded as one of the major side effects associated with this class of compounds [7, 26, 27]. Therefore, a major effort has been directed toward the search for novel cytotoxics possessing a wide spectrum of antitumoral activity with a reduced cardiotoxic potential.

PNU-159548 (4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-DNR, formerly FCE 28729) is the lead compound of a novel class of cytotoxic antitumor agents (alkycyclines) characterized by a 4-demethoxydaunorubicin (idarubicin) backbone with an aziridinyl group in position C-3′ and a methylsulphonyl on position C-4′ of the amino sugar. As a consequence of the

structural changes made to the idarubicin moiety, PNU-159548 has a unique mechanism of action – DNA intercalation via the anthracycline backbone and DNA covalent bonding via the reactive alkylating group on the sugar [25]. This compound has been selected for clinical development on the basis of in vitro and in vivo results, which indicated that PNU-159548, because of its distinct mechanism of action, is highly cytotoxic with a spectrum and degree of antitumor efficacy comparable to or superior to that of many currently marketed antitumor agents [17]. Furthermore, PNU-159548 was active against cell lines expressing the MDR phenotype or resistant to alkylating agents [16].

Non-clinical safety studies indicate that the general toxicity of PNU-159548 is qualitatively similar to that of idarubicin and other anthracyclines, characterized by myelosuppression, liver, and gastrointestinal toxicities. Results of a single-dose study in the rat suggested that PNU-159548 is much less cardiotoxic than doxorubicin [17]. The purpose of this study was to evaluate the cardiotoxic potential of PNU-159548, using the multiple-dose cardiotoxicity rat model, developed in our laboratory for anthracyclines [28]. Doxorubicin was used as a reference compound for anthracycline-induced cardiotoxicity.

Materials and methods

Chemicals

PNU-159548 and doxorubicin, used as a reference compound for anthracycline-induced cardiotoxicity, were synthetized by Pharmacia and Upjohn, Milan, Italy. PNU-159548 was provided as a freeze-dried cake and reconstituted in Water for Injection as a colloidal lipid dispersion formulation (CDF). Doxorubicin was provided as the Adriablastin PFS formulation.

Animals and experimental design

All animal experimentation was conducted in strict compliance with EU and Italian Guidelines for Laboratory Animal Welfare. Sprague-Dawley [Crl:CD(SD)BR] male rats were obtained from Charles River Italy. Male rats were used since they are reported to be more sensitive than females to the cardiotoxic effects of anthracyclines [23]. The rats were housed in pairs in cages with sawdust bedding in a room with controlled temperature (21 ± 1.5 C) and humidity ($55 \pm 15\%$) and a 12-h light/dark schedule. They were allowed free access to water and 4RF21 pelleted food supplied by Mucedola (Milan, Italy).

At the time of the first treatment animals were about 7 weeks old and weighed 167–231 g. The rats were assigned randomly to treatment groups (36 rats/group) as follows: group 1, PNU-159548 vehicle (colloidal dispersion); group 2, doxorubicin vehicle (saline); group 3, 0.12 mg/kg PNU-159548; group 4, 0.25 mg/kg PNU-159548; group 5, 0.5 mg/kg PNU-159548; group 6, 0.75 mg/kg PNU-159548; group 7, 1 mg/kg PNU-159548; group 8, 1 mg/kg doxorubicin. Treatments were given once weekly by slow intravenous administration (1 ml/20 s) into the tail veins. Six animals/group were sacrificed after four treatments, whilst the remaining rats received seven treatments and were sacrificed at 8, 12, 22, 27, or 35 weeks after the first treatment. The rats were sacrificed by exanguination from the abdominal aorta under complete intraperitoneal sodium thiopental anesthesia.

Investigations

Mortality and individual body weights were recorded throughout the study. Hematology, clinical chemistry, and urinalysis were performed at sacrifice at weeks 4, 8, 12, 22, 27, and 35. Serum troponin-T levels were determined at these same time intervals using enzyme-linked immunosorbent assay (Boehringer Mannheim) [4]. Postmortem examinations included necropsy and weighing of heart, kidneys, testes, and liver. The heart was quickly removed and fixed in 4% buffered paraformaldehyde, dehydrated in ethanol, infiltrated, and embedded in methacrylate. Sections (1 μm) were microscopically examined after staining with alkaline toluidine blue. Histopathological evaluation of the hearts was performed using a scoring system described by Solcia et al. [29], in which the cardiomyopathy is expressed as a product of the severity and the extent of the damage. Severity (S) was defined as: grade 1, sarcoplasmic microvacuolations and/or inclusions, cellular edema, or intersitial edema; grade 2, as grade 1 plus sarcoplasmic macrovacuolations or atrophy, necrosis, fibrosis, endocardial lesions, and thrombi. Extent (E) was defined as: grade 0.5, less than 10 altered myocytes; grade 1, single scattered altered myocytes; grade 2, scattered small groups of altered myocytes; grade 3, several small groups of altered myocytes; grade 4, groups of altered and confluent myocytes; grade 5, most myocytes affected. The mean total score (MTS) for each group is Σ(SxE)/number of animals per group and is then rounded to the nearest 0.1.

Statistical analysis

Mean body weight, hematology, and clinical chemistry data were analyzed by Bartlett's test for homogeneity of variance, Fisher's and Dunnett's tests for homogeneous data, and Cochran and Cox's test for non-homogenous data. Organ absolute weights in treated groups were compared with those of the control groups by Dunnett's test on the adjusted means after the one-way model ANCOVA using the fasted body weight as covariate. The MTS and white blood cell counts of the treated groups were compared with controls using the Kruskal-Wallis test, followed by the Dunn one-tailed multiple comparison test. When cardiotoxicity scores of PNU-159548 were greater than zero, they were compared with that of the doxorubicin group using the Wilcoxon test.

Results

General toxicity

Mortality occurred sporadically and without a clear time or dose relationship up to the dose of 0.75 mg/kg per week PNU-159548. At the highest dose of PNU-159548, 1.0 mg/kg per week, mortality was 24% and was associated with marked myelosuppression, the probable cause of death. Mortality was also observed in animals treated with doxorubicin starting at week 19 (Table 1), which is consistent with results from previous studies on doxorubicin in the rat [11]. The delayed deaths following doxorubicin treatment were attributed to severe cardiotoxicity and/or nephropathy. At doses of 1.0 mg/kg per week, both PNU-159548 and doxorubicin inhibited body weight increase by about 30% (Fig. 1). Lower doses of PNU-159548 did not inhibit weight gains compared with the saline control.

Laboratory findings in rats given 0.25–1 mg/kg per week PNU-159548 included slight-to-marked dose-related decreases in leukocytes, ranging from 20% to 90%, with nadirs occurring during week 4. The time course

Table 1 Effect of PNU-159548 and doxorubicin on survival and organ weights in male rats

Compound	Dose[a]	Mortality		Organ weights[b]					
		%	Weeks of death	Liver g (SE)		Testes g (SE)		Kidneys g (SE)	
				Week 8	Week 35	Week 8	Week 35	Week 8	Week 35
PNU-159548 vehicle		0	–	8.8 (0.44)	14.3 (2.24)	3.3 (0.09)	3.7 (0.12)	2.8 (0.09)	3.5 (0.38)
Doxorubicin vehicle		0	–	9.2 (0.44)	16.0 (2.39)	3.2 (0.09)	3.6 (0.13)	2.6 (0.09)	3.5 (0.41)
PNU-159548	0.12	5	4–27	9.3 (0.43)	20.5 (2.47)	3.0 (0.09)	3.3 (0.14)	2.6 (0.09)	4.5 (0.42)
	0.25	8	6–19	9.4 (0.40)	15.7 (3.12)	2.5 (0.08)*	3.5 (0.17)	2.9 (0.09)	3.1 (0.53)
	0.50	3	30	10.7 (0.41)	16.2 (2.52)	1.6 (0.08)*	3.6 (0.14)	2.8 (0.09)	3.6 (0.43)
	0.75	8	21–25	12.1 (0.42)*	13.9 (2.81)	1.1 (0.09)*	2.0 (0.16)*	2.7 (0.09)	3.1 (0.48)
	1.0	24	6–11	13.6 (0.56)*	12.4 (3.82)	1.4 (0.12)*	1.1 (0.21)*	2.6 (0.12)	2.5 (0.65)
Doxorubicin	1.0	22	19–27	12.4 (0.40)*	17.2 (0.85)[c]*	1.3 (0.08)*	1.6[c] (0.23)*	3.2 (0.09)*	4.9[c]* (0.31)

*$P < 00.1$ vs. controls treated with doxorubicin vehicle (saline)
SE = standard error of the mean ($n = 4$–6)
[a] mg/kg once weekly for 7 consecutive weeks
[b] Organ weight as adjusted means after ANCOVA (g) at week 8 (the earliest sampling time showing relevant changes) and 35 (last sampling time)
[c] Value recorded at week 27 (last killing for doxorubicin-treated group due to high mortality)

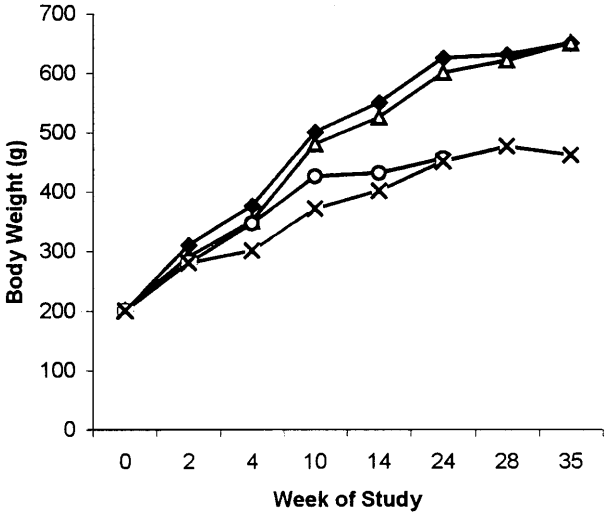

Fig. 1 Mean body weights of rats treated with either doxorubicin or PNU-159548 weekly for 7 weeks followed by a 28-week recovery period; ◆ saline control, ❍ doxorubicin (1 mg/kg), Δ PNU-159548 (0.5 mg/kg), ✕ PNU-159548 (1 mg/kg)

and the magnitude of the decrease in leukocytes seen at 0.5 mg/kg per week PNU-159548 (about 50%) were similar to that caused by 1 mg/kg per week doxorubicin (about 40%), indicating that these dose levels were equimyelotoxic (Table 2). No noteworthy changes in clinical chemistry parameters were seen after PNU-159548, while 1 mg/kg per week doxorubicin produced moderate-to-marked progressive and persistent increases in total cholesterol and triglycerides and, in a few animals, troponin-T levels (data not shown), a marker of myocardial ischemia/necrosis. A transient increase in mean liver weight ($P < 0.01$) was observed at 0.75 and 1 mg/kg per week PNU-159548 at week 8, while doxorubicin caused persistent increases in liver weight ($P < 0.01$) from week 8 through week 35 (Table 1). Testicular weight was decreased at 0.25 mg/kg per week of PNU-159548 at week 8 ($P < 0.01$), but recovered completely by week 35. At higher doses, marked decreases in testicular weight occurred as early as week 4 (data not shown) and complete recovery was observed only for the dose of 0.5 mg/kg per week at the end of the study (Table 1). A statistically significant non-reversible decrease in testicular weight ($P < 0.01$) and a non-reversible increase in kidney weight ($P < 0.01$) were seen after 1 mg/kg per week doxorubicin at week 8 (Table 1).

Cardiotoxicity

There were no treatment-related effects on mean heart weight after treatment with PNU-159548 or doxorubicin. Histological examination of heart from rats treated with PNU-159548 revealed microscopically similar but a much lower incidence and severity of myocardial changes than observed after doxorubicin (Table 2). The predominant light microscopy change consisted of multifocal vacuolar degeneration of myocytes, more evident in the left ventricle and in the interventricular septum.

At doses up to 0.5 mg/kg per week PNU-159548, only one of either five (0.12 mg/kg) or six rats (0.25 and 0.5 mg/kg) was minimally affected at a single sampling time. In each of these rats, the severity (S) was 2 and the extent (E) was 0.5, resulting in an MTS for their respective groups of 0.2, 0.17 (0.2), and 0.17 (0.2). At 0.75 mg/kg per week, cardiac lesions were present at weeks 12 and 22, showing a MTS of 0.3, while at 1 mg/kg per week the lesions were detected earlier, from week 8 to week 22, showing a maximum MTS of 0.8. The incidence of measurable cardiomyopathy at the dose of 0.75 mg/kg per week was 33% during weeks 12 and 22 and 0% at the other time points. At 1 mg/kg per week, the incidence of measurable cardiomyopathy ranged

Table 2 Myelotoxicity and cardiotoxicity of PNU-159548 and doxorubicin (*WBC* white blood cells, *MTS* mean total score of cardiac lesions, *D/E* damaged hearts/examined hearts, *ND* not determined due to high mortality)

Compound	Dose[a]	WBC[b] (SD)	Cardiomyopathy											
			Week of study											
			4		8		12		22		27		35	
			D/E	MTS	D/E	MTS	D/E	MTS	D/E	MTS	D/E	MTS	D/E	MTS
PNU-159548 vehicle	–	11.8 (4.9)	0/6	0	0/6	0	0/6	0	0/6	0	0/6	0	0/6	0
Doxorubicin vehicle	–	11.4 (3.1)	0/6	0	0/6	0	0/6	0	0/6	0	0/6	0	0/6	0
PNU-159548	0.12	11.7 (3.0)	0/6	0	0/6	0	0/6	0	0/6	0	0/5	0	1/5	0.2
	0.25	9.2 (2.6)	0/6	0	0/6	0	0/6	0	0/6	0	1/6	0.2*	0/3	0
	0.5	5.6 (1.5)	0/6	0	1/6	0.2*	0/6	0	0/6	0	0/6	0	0/5	0
	0.75	2.7 (0.7)**	0/6	0	0/6	0	2/6	0.3*	2/6	0.3*	0/5	0	0/4	0
	1	1.5 (0.9)**	0/6	0	2/5	0.4*	4/6	0.8*	3/5	0.8*	ND	ND	0/4	0
Doxorubicin	1	7.2 (2.0)	4/6	0.8	6/6	4.3	6/6	4.0	6/6	5.0	4/4	4.0	ND	ND

* $P < 0.01$ vs. doxorubicin using Wilcoxon test at scheduled times during which MTS > 0 for PNU-159548
** $P < 0.01$ vs. PNU-159548 vehicle; Kruskal-Wallis test followed by Dunn' s test (SD = standard deviation, $n = 6$)
[a] mg/kg/once a week for 7 consecutive weeks
[b] WBC ($\times 10^3/mm^3$) during maximum nadir (week 4)

from 40% to 67% (Table 2). Doxorubicin at 1 mg/kg per week induced cardiotoxicity which was slight at week 4 and marked at week 8, reaching the maximum MTS of 5.0 at week 22 (Table 2). The incidence of measurable cardiomyopathy was 100% at all time points, except at week 4 when it was 67%.

All MTS values observed after PNU-159548 were significantly ($P < 0.01$) lower than those observed after doxorubicin. At the equimyelotoxic doses of 0.5 mg/kg per week for PNU-159548 and 1 mg/kg per week for doxorubicin, PNU-159548 is estimated to be about 20 times less cardiotoxic than doxorubicin. Furthermore, at the markedly myelotoxic dose of 1.0 mg/kg per week, the cardiotoxicity of PNU-159548 was about one-sixth that of the same dose of doxorubicin.

All decedent rats and those killed in extremis following doxorubicin treatment showed generally moderate-to-marked vacuolar degeneration of myocytes, often associated with fibrosis and/or necrosis, while those receiving PNU-159548 did not show vacuolar degeneration, except for a single rat given 0.75 mg/kg per week killed moribund on day 154.

Discussion

Combination chemotherapy regimens consisting of anthracyclines and alkylating drugs are among the most widely used and effective treatments for breast cancer, lymphoma, and leukemia [6, 8, 14, 15]. Unfortunately, the risk of cardiotoxicity associated with anthracyclines limits their use.

The mechanism underlying anthracycline-induced cardiotoxicity has not been conclusively determined, although considerable evidence has accumulated indicating that cardiomyopathy may be due to iron-dependent free radical oxidative stress [9, 12, 18, 19]. Other mechanisms implicated in the myocardial damage include increases in cytoplasmic Ca^{2+}, inhibition of Na^+/Ca^{2+} exchange, dysfunction of sarcoplasmic reticulum, depression of the glycolytic pathway and intracellular ATP levels, base hydroxylation of DNA, loss of intracellular NAD^+, activation of poly (ADP-ribose) polymerase, and glutathione depletion [2, 13, 30]. Indeed, it is likely that the mechanism is multifactorial [23].

The histomorphology of anthracycline-induced cardiotoxicity has been well characterized in several animal species [1, 7, 20, 24] and closely resembles that in humans [3]. The cardiomyopathy is characterized by multifocal vacuolar degeneration of myocytes, which is generally more evident in the left ventricle and septum. Dilation of sarcoplasmic reticulum and transverse tubules has been described using electron microscopy. Controlled histopathological heart examination using a defined scoring system for the cardiomyopathy, such as the MTS described above, is essential for an accurate and reproducible evaluation of this lesion [3]. We have used semi-thin sectioning and the MTS scoring system in the chronic rat model routinely to compare the cardiotoxic potential of different anthracyclines [10, 28] and to demonstrate the cardioprotective effects of dexrazoxane [10, 11].

Results of the present study indicate that the new antitumor agent, PNU-159548, which has both DNA intercalating activity and alkylating properties, has a very low propensity for causing cardiotoxicity. In this chronic rat model, the severity and extent of the cardiotoxicity caused by PNU-159548 was less than one-twentieth of that caused by an equimyelotoxic dose of doxorubicin. Despite the marked differences in severity, the similarities in the histomorphology of the PNU-159548- and doxorubicin-induced cardiac lesions suggest that the lesions were caused by a similar mechanism(s). Serum troponin-T levels were elevated in a few doxorubicin-treated rats with evidence of myocardial necrosis, but not in rats that received PNU-159548. Herman

et al. [21] reported that circulating troponin-T levels were elevated in spontaneously hypertensive rats given 1 mg/kg per week doxorubicin for 7 weeks, the same treatment used in the present studies. Thus, it appears that serum troponin-T is a sensitive biomarker for severe cardiotoxicity when necrosis is present, but not for mild cardiomyopathy found with PNU-159548.

Idarubicin, the anthracycline moiety of PNU-159548, is less cardiotoxic than doxorubicin when used at clinically effective doses, which are about one-third those of doxorubicin [5]. Unpublished studies in the rat conducted in our laboratory indicate that 0.3 mg/kg of idarubicin is less cardiotoxic than an equimyelotoxic dose of doxorubicin (1.0 mg/kg). This is also true in the mouse, in which the incidence of cardiotoxicity with 1.0 mg/kg idarubicin is about 22% compared to 100% with an equimyelotoxic dose of 4 mg/kg doxorubicin [22]. These results suggest that the cardiotoxicity observed with these two anthracyclines is approximately proportional to the dose. In the case of PNU-159548, the myelotoxic dose is about one-third to one-half that of doxorubicin, yet the cardiotoxicity is 20-fold less or much lower than would be predicted on the basis of its idarubicin content (89%). The reason for this is not clear but may reflect the greater lipophilicity of PNU-159548, which is likely to result in a much-different tissue distribution compared with idarubicin.

Comparison of the two drugs at equimyelotoxic doses is considered appropriate because, clinically, cytotoxic antineoplastic drugs are generally dosed to a maximally tolerated myelosuppressive dose. Thus, in humans, at clinically effective doses, PNU-159548 is expected to be markedly less cardiotoxic than either doxorubicin or idarubicin. In addition, if its broad spectrum of activity against sensitive and resistant tumors is also demonstrated clinically, PNU-159548 should represent an important advance in the chemotherapy of cancer.

References

1. Ballerini L, Solcia E, Bellini O, Sala L, Bertazzoli C (1977) Experimental adriamycin cardiomyopathy in the rat. IRCS Med Sci 5: 431
2. Bienvenu P, Caron L, Gasparutto D, Kergonou JF (1992) Assessing and counteracting of anticancer drugs. In: Emerit I, Chance B (eds) Free radicals and aging. Birkhauser Verlag, Basel, p 257
3. Billingham ME, Mason JW, Bristow MR, Daniels JR (1978) Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat Rep 62: 865
4. Bleuel H, Deschl U, Bertsch T, Bolz G, Rebel W (1995) Diagnostic efficiency of troponin T measurements in rats with experimental myocardial cell damage. Exp Toxicol Pathol 47: 121
5. Borchmann P, Hubel K, Schnell R, Engert A (1997) Idarubicin: a brief overview on pharmacology and clinical use. Int J Clin Pharmacol Ther 35: 80
6. Bower M, Brock C, Gulliford T, O'Reilly SM, Smith DB, Newlands ES (1996) A weekly alternating chemotherapy regimen with low toxicity for the treatment of aggressive lymphoma. Cancer Chemother Pharmacol 38: 106
7. Bristow MR, Sageman WS, Scott RH, Billingham ME, Bowden RE, Kernoff RS, Snidow GH, Daniels JR (1980) Acute and chronic cardiovascular effects of doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. J Cardiovasc Pharmacol 2: 487
8. Coiffier B (1996) Ifosfamide in the treatment of lymphoma. Semin Oncol 23: 2
9. Davies KJ, Doroshow JH (1986) Redox cycling of anthracyclines by cardiac mitochondria. I. Anthracycline radical formation by NADH dehydrogenase. J Biol Chem 261: 3060
10. Della Torre P, Podesta A, Pinciroli G, Iatropoulos MJ, Mazue G (1996) Long-lasting effect of dexrazoxane against anthracycline cardiotoxicity in rats. Toxicol Pathol 24: 398
11. Della Torre P, Mazue G, Podesta A, Moneta D, Sammartini U, Imondi AR (1999) Protection against doxorubicin-induced cardiotoxicity in weanling rats by dexrazoxane. Cancer Chemother Pharmacol 43: 151
12. Demant EJ, Jensen PK (1983) Destruction of phospholipids and respiratory-chain activity in pig-heart submitochondrial particles induced by an adriamycin-iron complex. Eur J Biochem 132: 551
13. Earm YE, Ho WK, So I (1994) Effects of adriamycin on ionic currents in single cardiac myocytes of the rabbit. J Mol Cell Cardiol 26: 163
14. Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, Cruz AB Jr, Fisher ER, Wickerham DL, Wolmark N, DeCillis A, Hoehn JL, Lees AW, Dimitrov NV (1997) Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 15: 2483
15. Friedenberg WR, Spencer SK, Musser C, Hogan TF, Rodvold KA, Rushing DA, Mazza JJ, Tewksbury DA, Marx JJ (1999) Multi-drug resistance in chronic lymphocytic leukemia. Leuk Lymphoma 34: 171
16. Geroni C, Ripamonti M, Pennella G, Capolongo L, Marsiglio A, Caruso M, Suarato A, Marchini S, Grandi M (1998) PNU-159548(4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-DNR) a novel cytotoxic compound active in vitro and in vivo against tumors with different resistance mechanisms (abstract). Proc Am Assoc Cancer Res 39: 222
17. Geroni C, Ripamonti M, Marsiglio A, Pastori, A, Caruso M, Bargiotti A, Moneta D, Grandi M (1998) PNU-159548(4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-DNR) a novel cytotoxic agent with wide spectrum antitumor activity (abstract). Proc Am Assoc Cancer Res 39: 222
18. Gianni L, Corden BJ, Myers CE (1983) The biochemical basis of anthracycline toxicity and antitumor activity. Rev Biochem Toxicol 5: 1
19. Gutteridge JM (1984) Lipid peroxidation and possible hydroxyl radical formation stimulated by the self-reduction of a doxorubicin-iron (III) complex. Biochem Pharmacol 33: 1725
20. Herman EH, Schein P, Farmar RM (1969) Comparative cardiac toxicity of daunomycin in three rodent species. Proc Soc Exp Biol Med 130: 1098
21. Herman EH, Lipshultz SE, Rifai N, Zhang J, Papoian T, Yu ZX, Takeda K, Ferrans VJ (1998) Use of cardiac troponin T levels as an indicator of doxorubicin-induced cardiotoxicity. Cancer Res 58: 195
22. Imondi AR (1998) Preclinical models of cardiac protection and testing for effects of dexrazoxane on doxorubicin antitumor effects. Semin Oncol 25: 22
23. Imondi AR, Della Torre P, Mazue G, Sullivan TM, Robbins TL, Hagerman LM, Podesta A, Pinciroli G (1996) Dose-response relationship of dexrazoxane for prevention of doxorubicin-induced cardiotoxicity in mice, rats, and dogs. Cancer Res 56: 4200
24. Jaenke RS (1976) Delayed and progressive myocardial lesions after adriamycin administration in the rabbit. Cancer Res 36: 2958
25. Marchini S, Gonzalez Paz O, Ripamonti M, Geroni C, Bargiotti A, Caruso M, Todeschi S, D'Incalci M, Broggini M (1995) Sequence-specific DNA interactions by novel alkylating anthracycline derivatives. Anticancer Drug Des 10: 641

26. Mariani M, Capolongo L, Suarato A, Bargiotti A, Mongelli N, Grandi M, Beck WT (1994) Growth-inhibitory properties of novel anthracyclines in human leukemic cell lines expressing either Pgp-MDR or at-MDR. Invest New Drugs 12: 93
27. Plosker GL, Faulds D (1993) Epirubicin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cancer chemotherapy. Drugs 45: 788
28. Podestà A, Della Torre P, Pinciroli G, Iatropoulos MJ, Brughera M, Mazue G (1994) Evaluation of 4′-iodo-4′-deoxydoxorubicin-induced cardiotoxicity in two experimental rat models. Toxicol Pathol 22: 68
29. Solcia E, Ballerini L, Bellini O, Magrini U, Bertazzoli C, Tosana G, Sala L, Balconi F, Rallo F (1981) Cardiomyopathy of doxorubicin in experimental animals. Factors affecting the severity, distribution and evolution of myocardial lesions. Tumori 67: 461
30. Solem LE, Henry TR, Wallace KB (1994) Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration. Toxicol Appl Pharmacol 129: 214