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ORIGINAL ARTICLE

**Angela Michelutti · Raffaella Stocchi · Anna Candoni
Mario Tiribelli · Elisabetta Calistri · Domenico Russo
Renato Fanin · Daniela Damiani**

Effect of amifostine on the cytotoxicity of daunorubicin and daunoxome in tumor and normal cells

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Abstract Anthracyclines are powerful cytotoxic agents, used as first-line treatment of leukemias and many other tumors, but host-tissue toxicity is their main dose-limiting factor. However, their therapeutic effects depend not only on the toxicity, hence on the dose, but also on drug resistance. Among the mechanisms that can account for cell sensitivity to anthracyclines, there is an overexpression of drug transport proteins, like the transmembrane P-glycoprotein (PGP), the multidrug-resistance-related protein (MRP) and the lung-resistance-related protein (LRP). Attempts to reduce the toxicity of chemotherapeutic agents without affecting their efficacy have been made using liposomal anthracyclines or cytoprotective agents, as Amifostine. The aim of this study was to evaluate and compare the toxic effects of Daunorubicin, in normal or liposomal formulation, used in combination with WR1065, the active metabolite of Amifostine, against normal and tumor cells. In conclusion these data show that the preincubation with WR-1065 does not inhibit the drug toxic effect on blast cells and on tumor cell lines, independently by their multidrug resistance phenotype, but has a cytoprotective effect on stem cells causing a drug cytotoxicity reduction of 10–20%. This advantage is even higher using the liposomal formulation of DNR. Therefore, Amifostine can offer a chance of protecting normal cells from the toxicity of anthracyclines, in normal or liposomal formulation. The combination of liposomal anthracyclines with Amifostine can confer further advantages in management of leukemic patients, especially the elderly where treatment toxicity is a main problem. These patients may be candidates for alternative therapeutic strategies and the combination of DNX and Amifostine is an attractive treatment for these cases where a low nonhematological toxicity is required.

Keywords Amifostine · Daunorubicin · Daunoxome · Apoptosis · Stem cells · Blast cells

Angela Michelutti · Raffaella Stocchi · Anna Candoni
Mario Tiribelli · Elisabetta Calistri · Renato Fanin
Daniela Damiani (✉)
Division of Hematology, Department of Medical and Morphological Research, University Hospital,
P.le S. Maria della Misericordia, 33100, Udine, Italy
E-mail: Daniela.Damiani@uniud.it
Tel.: +39-432-559662
Fax: +39-432-559661

Domenico Russo
Chair of Hematology, University of Brescia, Italy

Introduction

Anthracyclines are powerful cytotoxic agents which are used as first-line treatment of leukemias, malignant lymphoma, and many other tumors. Three mechanisms of action are mainly ascribed to them: DNA intercalation, membrane binding, and lipid peroxidation [22]. Host-tissue toxicity (hematopoietic suppression, nausea and vomiting, mucositis, alopecia, cadiomyopathy) is the main dose-limiting factor. However, it is becoming increasingly evident that therapeutic effects of anthracyclines depend not only on the toxicity, hence on the dose, but also on drug resistance. Among the mechanisms that can account for cell sensitivity to anthracyclines there are an overexpression of drug transport proteins, like the P-glycoprotein (PGP), the multidrug-resistance-related protein (MRP) and the lung-resistance-related protein (LRP), the elevation of the anionic glutathione-S-transferase (GST) and the quantitative or qualitative defect of Topoisomerase II [4]. The mdr-1 gene is constitutively expressed to a variable extent in many normal cells but is expressed more frequently and at a higher level in several tumors, including leukemias and lymphomas [12, 25, 46]. The PGP is believed to work as an energy-ATP-dependent system which upregulates the efflux of anthracyclines and other unrelated compounds from the cells [44], and in doing so decreases the intracellular drug accumulation and reduces the sensitivity to cytotoxic agents [35].The overexpression of mdr-1-related PGP is one of the most important mechanisms contributing to the nonspecific multidrug resis-

tance (MDR), and a number of clinical studies have shown that the overexpression of PGP is negatively related to the outcome of chemotherapy [8, 31–33, 48, 49, 51]. These in vitro and in vivo findings led to develop strategies for reversing the effects of PGP. A large number of noncytotoxic compounds were found to be able to reverse the MDR phenotype in vitro through competitive inhibition of drug efflux [21, 23, 34]. These include calcium channel antagonists (e.g., Verapamil), cyclosporin (Cy-A) and its analog (SDZ PSC 833), and other miscellaneous agents. However, when these MDR-reversal agents were coadministered in vivo with the appropriate cytotoxic agents, increased host-tissue toxicities and dose-limiting side effects of the chemosensitizers themselves were observed [6, 26, 27, 29, 41, 50, 53]. Recent developments in supportive care for patients receiving cancer chemotherapy have focused on attempts to decrease the toxicity of chemotherapeutic agents without affecting the antitumor efficacy. Liposomal encapsulation of narrow therapeutic index drugs has been shown to be an optimal drug delivery system for cancer chemotherapy of solid tumors [3]. In fact, these drug carriers may make it possible to increase tumor captation of cytotoxic drugs while at the same time reducing damage to normal tissues and perhaps to overcome MDR [40, 58]. DaunoXome (DNX, Nextar) is a liposomal targeting system in which daunorubicin (DNR) is entrapped within the aqueous core of small lipid vesicles whose mean diameter ranges from 35 nm to 65 nm. These vesicles are composed of a single bilayer membrane of distearoylphosphatidylcholine and cholesterol (DSPC/chol) (2:1 molecular ratio) that shows a remarkable physical stability at body temperature which protect the entrapped drug from rapid and extensive uptake by the reticuloendothelial system and from diffusion to the majority of normal tissues [2]. Biodistribution experiments in animals showed that this liposomal formulation delivered about tenfold more DNR to solid tumors than to normal tissues when compared to free drug [16]. The principle responsible for the selective accumulation in tumor tissues is considered to be the abnormal disposition of endothelial cells in neoformed tumor vessels which allow liposome passage through large fenestrations [59]. Pharmacokinetics characteristics suggest that DNX may improve the therapeutic index of free DNR also in the treatment of acute leukemia, reducing DNR captation in normal tissues and allowing a significantly greater plasma availability for uptake by circulating and bone marrow leukemic cells [45]. Moreover, in vitro studies on drug resistant cell lines suggest that liposomal DNR can partially escape the pump action of PGP [38]. These findings make DNX a very attractive candidate for the treatment of acute leukemias, especially of elderly or secondary leukemias in which PGP is frequently overexpressed. Coincidental with these developments, the concept of cytoprotection of normal cells has further evolved. Amifostine (Ethyol) is a phosporylated aminothiol that protects bone marrow progenitors and other normal tissues from the toxicities of ionizing radiation and antineoplastics like alkylating agents, cisplatin, anthracyclines, and taxanes [14, 43, 60]. Normal tissues that can be protected include bone marrow, kidney, neural tissues, the heart, intestinal crypt cells, and pulmonary tissues [10]. This selective protection is based on differential dephosphorylation by alkaline phosphatase at the normal tissue site and the preferential uptake of the active thiol metabolite, WR-1065, by cells in normal tissue [9]. Moreover, the in vitro coincubation of amifostine or WR-1065 with different chemotherapeutic agents did not reduce the toxic effects of the anticancer drugs in several human cell lines [1, 18], either MDR-negative or MDR-positive [39]. Therefore, Amisfostine can offer a chance of protecting normal cells from the toxicity of anthracyclines and anthracenediones. The combination of anthracyclines entrapped in a targeting deliver system (liposomal DNR) with amifostine can confer further advantages in the management of leukemic patients.

The aim of this study was to evaluate and compare the toxic effects of DNR, in normal and liposomal formulation, and WR-1065, the active metabolite of Amifostine, against normal and tumor cells and in particular to verify if amifostine is able to maintain the advantage of DNX on blast cell toxicity and to reduce the toxicity on hematopoietic stem cells.

Materials and methods

Drugs

Daunorubicin (Pharmacia Spa, Milano-Italy) was dissolved in distilled water, aliquoted at 1 mg/ml and stored at −20°C. Daunoxome (Gilead, Foster City, California, USA) was dissolved in 5% dextrose, aliquoted at 1 mg/ml and stored at +4°C. WR-1065, the active metabolite of Amifostine supplied to us by Schering-Plough Spa, Milano-Italy, was dissolved in distilled water, aliquoted at 10 mg/ml and stored at −20°C.

Cell lines

We used three pairs of human tumor cell lines, each pair consisting of the parental sensitive line and of its drug-selected MDR subline: the acute myeloid leukemic cell line HL60 and its PGP overexpressing subline HL60 DNR, kindly provided by Dr. Grandi (Pharmacia Spa, Milano Italy), the human small-cell lung cancer cell line GLC4 and its MRP overexpressing subline GLC4/ADR, kindly provided by Dr. de Vries (Department of Internal Medicine, University Hospital, Groningen, The Netherlands) the human squamous lung cancer cell line SW1573 and its LRP overexpressing subline SW1573/2R120, kindly provided by Dr. Broxterman (Depart-

ment of Medical Oncology, Free University Hospital, Amsterdam, The Netherlands).

All cell lines were cultured at 37°C in a humidified atmosphere at 5% CO_2 and were maintained in exponential growth in RPMI 1640 (Biochem KG Seromed) supplemented with 10% heat-inactivated fetal calf serum (Biochem KG Seromed), 2 mM glutamine solution, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biochem KG Seromed).

The expression of PGP, MRP, and of LRP was evaluated by flow cytometry using the MRK-16 (Kamiya), the MRPm6 (Kamiya), and the LRP-56 (Kamiya) monoclonal antibodies, following the company guidelines as described elsewhere [36].

Results were expressed as the Mean Fluorescence Index, by calculating the ratio between the mean fluorescence intensity of cells incubated with MRK-16, MRPm6 or LRP-56, and the mean fluorescence intensity of the respective isotypic control. Cell-line characteristics are reported in Table 1.

Patients and healthy donors

Leukemic cells were obtained from the peripheral blood or bone marrow of eight patients with acute leukemia during diagnostic procedures. Normal lymphocytes were obtained from the peripheral blood of five healthy donors. CD34+ progenitor cells were obtained from 13 apheresis of nonleukemic patients during the mobilization procedures and of 4 healthy donors.

Cells, after a Ficoll sedimentation, were washed twice in PBS, checked for viability by using the tripan blue exclusion test and suspended in PBS. CD34-positive cells were obtained after positive selection with MiniMacs (Miltenyi Biotec) using the CD34 Isolation kit (Miltenyi Biotec) following the company guidelines.

Table 1 Three pairs of human cell lines were tested: the parental sensitive cell lines HL60, GLC4, SW1573 and their resistant sublines HL60 DNR, GLC4 ADR and SW ADR1573/2R120

Cell lines	Mean fluorescence index		
	MRK16 (PGP)	MRPm6 (MRP)	LRP56 (LRP)
HL60	3.7	1.0	1.0
HL60 DNR	33	2.2	2.0
GLC4	3.1	1.1	3.4
GLC4 ADR	2.2	8.5	4.9
SW1573	1.8	1.1	3.2
SW ADR1573/2R120	1.8	1.2	10.7

The table shows the reactivity (as mean fluorescence index) of each cell line to three monoclonal antibodies MRK16 (Kamiya) directed against the 170kd glycoprotein (PGP), MRPm6 (Kamiya) directed against the multidrug- resistance-related protein (MRP) and LRP56 (Kamiya) directed against the lung- resistance-related protein (LRP)

Apoptosis

Drug toxicity was determined with the evaluation of apoptotic cells after drug exposure.

After a continuous 24-h exposure with 300 ng/ml of DNX or free DNR, with or without WR-1065 5 μg/ml, apoptotic cells were evaluated by flow cytometry, using the AnnexinV-FITC Kit (Immunotech), following manufacturer instructions. Detection of apoptosis was based on the binding properties of AnnexinV to the phosphatidylserine lipids exposed on the external surface of cellular membrane as a consequence of a drug-induced damage. Necrotic cells are identified by a simultaneous test with propidium iodide (PI) that can enter and bind DNA only in necrotic cells [42]. The use of PI is customary to distinguish between apoptotic cells with an intact plasma membrane and those cells with a 'leaky' membrane that is undergoing secondary necrosis.

Clonogenic assay

The clonogenic activity of CD34 progenitor cells was evaluated plating 5×10^3 CD34 cells in 1 ml of the "complete" Methylcellulose Medium (H4434, Stem Cell, Vancouver, Canada) in the presence of DNR or DNX with or without WR-1065. CFU-GEMM, CFU-GM, BFU-E, CFU-E were counted at day 14.

Results were expressed as the percentage of drug toxicity calculated by dividing the number of colonies grown in complete medium alone by the number of colonies grown in the presence of DNR or DNX, with or without WR-1065 5 μg/ml.

Statistical analysis

Differences between two groups were compared using the Mann-Withney *U*-test.

Results

Cell lines

The apoptotic effects of 300 or 1,000 ng/ml of DNR in free or liposomal formulation, after a 24-h incubation with or without a preincubation with WR-1065 5 μg/ml, were tested on three pairs of human tumor cell lines (Table 2). In none of the used cell lines, neither sensitive nor resistant, WR-1065 protected the cells against the toxic effects of the anticancer drugs.

Blast cells

The apoptotic effect of 300 ng/ml of DNR in free or liposomal formulation, after a 24-h incubation with or without a preincubation with WR-1065 5 μg/ml, was

Table 2 Apoptosis after a 24-h incubation with Daunorubicin (DNR) or Daunoxome (DNX), at the concentration of 300 ng/ml, with or without a 15-min preincubation with WR-1065 5 μg/ml (WR) in the three pairs of tumor cell lines tested

	Percent apoptosis after 1-day incubation			
	DNR	DNR + WR	DNX	DNX + WR
HL60	32 ± 0.1	35 ± 0.5	33 ± 0.4	35 ± 0.5
HL60 DNR	7 ± 0.3	6 ± 0.4	13 ± 0.3	14 ± 0.7
GLC4	25 ± 0.5	24 ± 1.0	26 ± 0.6	26 ± 1.3
GLC4 ADR	15 ± 0.8	13 ± 1.1	19 ± 1.0	20 ± 0.9
SW	28 ± 1.0	28 ± 0.9	29 ± 0.9	30 ± 0.9
SW ADR	18 ± 0.6	20 ± 0.3	23 ± 0.6	23 ± 0.3

The percentage of apoptotic cells was determined by flow cytometry using a FITC antiannexin V antibody. Data on incubation of cell lines with DNR or DNX 1,000 ng/ml, not reported in these table, showed the same trend with no effect of WR1065 on the percentage of apoptotic cells

tested on blast cells obtained from bone marrow or peripheral blood of eight leukemic patients (Table 3). In accordance with the results obtained with tumor cell lines, the addition of the active metabolite of Amifostine did not affect drug toxicity and DNX was slightly but not significantly ($P = 0.44$) more toxic than DNR. WR-1065 did not significantly modify the percentage of apoptotic cells: from 35.5 to 33.8 for DNR ($P = 0.87$) and from 40.8 to 38.5 for DNX ($P = 0.71$).

Normal lymphocytes and stem cells

The apoptotic effect of 300 ng/ml of DNR in free or liposomal formulation, after a 24-h incubation with or without a preincubation with WR-1065 5 μg/ml, was tested on normal lymphocytes obtained from the peripheral blood of five healthy donors and on normal progenitor CD34+ cells obtained from 13 apheresis of nonleukemic patients during the mobilization procedures and from 4 healthy donors (Table 3).

DNX was more toxic than DNR, but this difference was not statistically significative ($P = 0.37$ for normal lymphocytes, $P = 0.17$ for healthy donors' stem cells and $P = 0.06$ for patients' stem cells). Differently from blast cells the presence of WR-1065 had a protective effect on normal cells reducing significantly the percentage of apoptotic cells. The active metabolite of Amifostine caused a reduction of the percentage of apoptotic cells from 47 to 36.4 for DNR ($P = 0.02$) and from 50.8 to 32.4 for DNX ($P = 0.002$) in normal lymphocytes, from 47.5 to 38 for DNR ($P = 0.003$) and from 52 to 28.5 for DNX ($P = 0.002$) in stem cells obtained from healthy donors, and from 43.9 to 36 for DNR ($P = 0.002$) and from 48.1 to 33.6 for DNX ($P = 0.0003$) in stem cells obtained from patients during mobilization procedures.

This protective effect was always higher for DNX than for DNR ($P = 0.001$ in normal lymphocytes, $P = 0.04$ and $P = 0.0003$ in normal progenitors obtained from healthy donors and patients, respectively). As an example, Fig. 1 shows the flow cytometric evaluation of the apoptotic effect of DNR in free and liposomal formulation on normal progenitors CD34+. The presence of WR-1065 protected the cells causing a reduction of the percentage of apoptosis: from 49 to 39% for DNR and from 55 to 38% for DNX.

Clonogenic assay

The protective effect of WR-1065 was evaluated on the clonogenic activity of CD34 progenitors (Table 4). DNX was always more toxic than DNR, reducing the number of colonies, but this difference was not significative ($P = 0.09$ for CFU-GEMM, $P = 0.12$ for CFU-GM, $P = 0.054$ for BFU-E, $P = 0.09$ for CFU-E). Moreover, these data confirmed the protective effect of WR-1065 on normal progenitors: the count of CFU-GEMM, CFU-GM, BFU-E, CFU-E was always higher in samples pretreated with the cytoprotective agent.

WR-1065 protected the cells reducing the percentage of drug toxicity from 50.2 to 33.8 for DNR ($P = 0.0000$) and from 52.6 to 34.5 for DNX ($P = 0.0000$) in CFU-GEMM, from 43.4 to 30.6 for DNR ($P = 0.0004$) and from 48.7 to 32.9 for DNX ($P = 0.0003$) in CFU-GM, from 46.9 to 31.5 for DNR ($P = 0.0000$) and from 51.7 to 36.1 for DNX ($P = 0.0000$) in BFU-E and from 45 to 29.8 for DNR ($P = 0.0000$) and from 50.2 to 32.3 for DNX ($P = 0.0000$) in CFU-E. This protective effect was not significantly higher on DNR in liposomal formulation, as observed for the apoptosis induction ($P = 0.89$

Table 3 Apoptosis after 1-day incubation with DNR or DNX, at the concentration of 300 ng/ml, with or without a 15-min preincubation with WR-1065 5 μg/ml (WR). The percentage of apoptotic cells was determined by flow cytometry using a FITC antiannexin V antibody

	Percent apoptosis after 1-day incubation					
	DNR	DNR + WR	*P**	DNX	DNX + WR	*P**
Leukemic blast cells ($n = 8$)	35.5 ± 16.1	33.8 ± 15.4	0.87	40.8 ± 12.9	38.5 ± 12.9	0.71
Normal lymphocytes (n = 5)	47 ± 6.0	36.4 ± 5.8	0.022	50.8 ± 6.8	32.4 ± 6.7	0.002
Normal progenitors CD34+ (healthy donors) ($n = 4$)	47.5 ± 3.1	38 ± 2.5	0.003	52 ± 4.7	28.5 ± 7.3	0.002
Normal progenitors CD34+ (patients) ($n = 13$)	43.9 ± 5.1	36 ± 6.6	0.002	48.1 ± 5.7	33.6 ± 7.2	0.0003

(*Mann-Withney *U*-test)

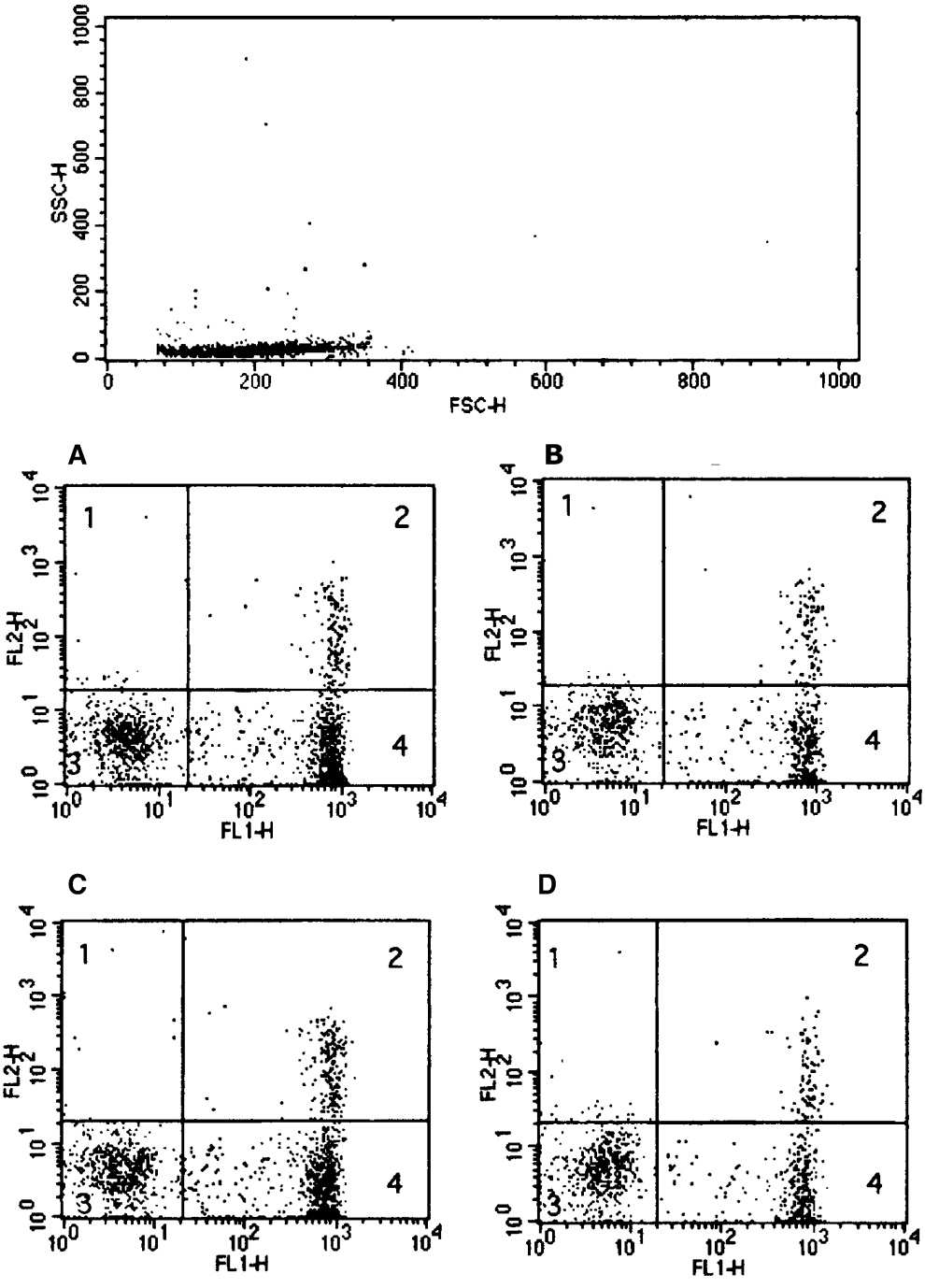

Fig. 1 Flow cytometric evaluation of apoptosis in normal progenitors CD34+. After a continuous 24-h exposure with 300 ng/ml of DNX or free DNR, with or without WR-1065 5 μg/ml, apoptotic cells were evaluated by flow cytometry, using the AnnexinV-FITC Kit (Immunotech), following manufacturer instructions. In each panel three clusters of cells can be identified: *quadrant 2*: secondary necrotic cells (high FL1 and high FL2 signal); *quadrant 3*: viable cells (low FL1 and low FL2 signal); *quadrant 4*: apoptotic cells (high FL1 and low FL2 signal). The presence of WR-1065 protected the stem cells causing a reduction of the percentage of apoptosis: from 49% to 39% for DNR and from 55 to 38% for DNX. **A** cells after incubation with DNR (apoptotic cells = 49%, *quadrant 4*) **B** cells after incubation with DNR and WR-1065 (apoptotic cells = 39%, *quadrant 4*) **C** cells after incubation with DNX (apoptotic cells = 55%, *quadrant 4*) **D** cells after incubation with DNX and WR-1065 (apoptotic cells = 38%, *quadrant 4*)

Table 4 Clonogenic assay

	Percent drug toxicity					
	DNR	DNR + WR	*P**	DNX	DNX + WR	*P**
CFU-GEMM	50.2 ± 2.4	33.8 ± 5.7	0.000002	52.6 ± 3.4	34.5 ± 4.6	0.00000
CFU-GM	43.4 ± 7.0	30.6 ± 6.2	0.0004	48.7 ± 7.7	32.9 ± 8.1	0.0003
BFU-E	46.9 ± 6.0	31.5 ± 4.9	0.000007	51.7 ± 4.1	36.1 ± 5.4	0.000001
CFU-E	45 ± 5.1	29.8 ± 3.7	0.000001	50.2 ± 7.5	32.3 ± 6.8	0.00002

CFU-GEMM, CFU-GM, BFU-E, CFU-E were counted after a 14-day incubation. Drug cytotoxic effect was calculated dividing the number of colonies grown in free medium by the number of colonies grown in the presence of DNR or DNX with or without WR-1065 (WR) (*Mann-Withney *U*-test)

for BFU-E, $P = 0.11$ for CFU-E, $P = 0.10$ for CFU-GM, $P = 0.40$ for CFU-GEMM).

Discussion

Host-tissue toxicity and drug resistance represent the major obstacles to effectiveness of anticancer chemotherapy. Over the past 10 years, significant progress has been made in defining the mechanisms of MDR even if many efforts to overcome them have been disappointing. In other words, it is possible to deliver higher doses of antineoplastic agents to tumor cells but, in doing so, it is nearly impossible to prevent the increase of toxicity against normal tissues. The main drug dose-limiting factors are host-tissue toxicity, such as hematopoietic suppression and mucositis, and the concomitant increase of infections. In the past years, a great deal of interest was pointed out on Amifostine, a prodrug with a broad spectrum of cytoprotective properties against different classes of anticancer drugs, including anthracyclines, taxanes, platinum derivatives, and a wide range of alkylating agents [10, 14, 28, 43, 60, 61]. This selective cytoprotection of Amifostine on normal tissues is based on its differential dephosphorylation by alkaline phosphatase at the tissue site and the preferential uptake of its active thiol metabolite, WR-1065, by normal tissue cells. Differences in the alkaline phosphatase concentration of normal versus malignant tissues result in greater conversion of Amifostine to WR-1065 in normal tissues, and consequently in selective cytoprotection [7, 10, 17, 47, 52, 55]. While the precise mechanism of this selective uptake remains to be fully elucidated the fact remains that Amifostine is concentrated rapidly in normal tissue and this accumulation forms a basis for the protective effect, while the limited dephosphorylation of Amifostine to WR-1065 at the tumor tissue site provides the first major barrier to its uptake and subsequent potential tumor protection.

This selective cytoprotection of normal tissues from the cytotoxic effects of anticancer drugs could be an alternative approach to deliver high doses of chemotherapy to tumor cells. Such a strategy has been investigated in preclinical models but clinical applications are still limited. In vitro studies have shown that in a wide series of human cell lines not selected for a drug resistance the coincubation of Amifostine or WR-1065 with chemotherapeutic agents does not reduce the toxic effects of anticancer drugs in human cell lines [1, 18, 39].

Several clinical studies [19, 20, 54, 56, 57] showed that Amifostine can be safely given, without excess of toxicity or nonengraftment. The major Amifostine-related side effects included hypotension, dehydration, nausea, and skin reaction. Blood pressure was monitored during the administration of Amifostine, but the majority of patients had little or no hypotension. These literature data showed that the use of Amifostine caused mild to moderate and always reversible side effects, indicating that the addition of Amifostine enables a potential escalation of drug dose providing an intensive regimen applicable to patients of all ages.

Moreover, Amifostine appeared to have a significant impact on duration of all grades of mucositis in patients who underwent stem cell transplantation procedures [20]. In particular of great interest was the observation that patients who received Amifostine had a significantly lower incidence of grade ≥ III infections ($P = 0.008$) and a shorter duration of antibiotic therapy ($P = 0.03$), probably due to a preservation of gut-mucosal integrity against intraluminal pathogens.

In this work three pairs of human cell lines with an MDR due to a PGP (HL60 system), an LRP (SW1573 system) or an MRP (GLC4 system) overexpression, blast cells obtained from leukemic patients and normal cells, lymphocytes and stem cells, were tested for the protective effects of WR-1065, the active metabolite of Amifostine, against DNR in free or liposomal formulation.

These data show that the preincubation with WR-1065 doesn't inhibit the drug toxic effect on blast cells and on tumor cell lines, independently by their MDR phenotype, but has a cytoprotective effect on stem cells causing a drug cytotoxicity reduction of 10–20%. This advantage is even higher using the liposomal formulation of DNR and the observation that this effect was obtained with WR-1065, the active metabolite of Amifostine, strengthened the data. Therefore, Amifostine can offer a chance of protecting normal cells from the toxicity of anthracyclines, in normal or liposomal formulation. The combination of liposomal anthracyclines (liposomal DNR) with Amifostine can confer further advantages in management of leukemic patients, especially the elderly where treatment toxicity is a main problem. In fact, patients aged more than 60 years affected by acute leukemia have usually a poor prognosis. Less than 50% of them can achieve a complete remission when treated with intensive chemotherapeutic regimens and less than 10% can survive longer [5]. The majority of elderly patients who do not respond well to standard treatment have a secondary leukemia, specific or complex cytogenetic abnormalities and a high expression of several proteins that interfere with drug sensitivity [13, 15, 24, 30, 31, 37]. For these cases standard treatment is more toxic than useful and there is very little evidence that treatment intensification can improve the results [11]. On the other hand, a significant proportion of patients cannot be treated with aggressive chemotherapy because of their performance status, presence of concomitant diseases or organ failure. These patients may be candidates for alternative therapeutic strategies and the combination of DNX and Amifostine is an attractive treatment for these cases where a low nonhematological toxicity is required.

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