Liposome encapsulated daunorubicin doubles anthracycline toxicity in cell lines showing a non-PGP related multidrug resistance

Sir,

DaunoXome (DNX) is a liposomal formulation of daunorubicin (DNR) developed by NeXstar with the aim of targeting the drug to tumors since liposomes have a better chance of penetrating the leaky vasculature of neoplastic tissues than the vasculature of normal tissues.[1] In a previous work it was observed that in cell lines showing PGP overexpression, DNX produced more DNR accumulation and higher toxicity than the conventional drug (free DNR) suggesting that liposomal anthracyclines could be suitable for treatment of PGP-positive leukemias.[2] In order to test the capacity of DNX to counteract DNR-resistance mechanisms associated with defects in cellular anthracycline accumulation other than PGP overexpression, a couple of drug-selected cell lines showing a multidrug resistance (MDR) and multidrug resistance protein (MRP) (GLC4-ADR150) or lung resistance-associated protein (LRP) (SW1573/2R120) overexpression were tested. The cellular time- and dose-related accumulation of DNX against free DNR were assayed by flow cytometry while the drugs' toxicity was tested by a MTT-microcultured tetrazolium colorimetric assay.[2] After 7 days of continuous exposure to the drugs, the toxicity of DNX was about 2.5 fold higher than that of the free DNR in both the drug-selected lines (Table 1 and Figure 1 A and B). In contrast to data observed in PGP-overexpressing cell lines,[2] cellular accumulation of the anthracycline was comparable when the LRP-overexpressing line SW1573/2R120 was treated with DNX or with free DNR. The MRP-overexpressing GLC4-ADR150 showed only a trend towards a greater DNR accumulation when exposed to DNX compared to free DNR (Figure 1 C and D). According to data reported by Forssen *et al.*, it could be hypothesized that the mechanism of the higher toxicity of DNX in cell lines with non-PGP related MDR could be a shift in nucle-

Table 1. Inhibition dose 50 (ID_{50}, ng/mL, mean ± 2SD of at least three tests) for liposomal DNR (DNX) and conventional (free DNR) daunorubicin. The cells' growth was assayed by the MTT tetrazolium colorimetric test after a 7-day culture in the presence of increasing doses of the anticancer drug with or without the addition of MDR modifiers. The ID_{50} was calculated from the dose-response curves. The resistance index (RI) was calculated by dividing the ID_{50} of the MDR sublines SW1573/2R120 and GLC4-ADR150 by the ID_{50} of the respective non -MDR lines SW1573 and GLC4.

	GLC4 ADR 150	*GLC4*	*RI*	*SW1573/ 2R120*	*SW 1573*	*RI*
Free DNR	708.9±12.3	7.1±1.0	100	98.6±6.9	5.1±0.4	19
+DVP	556.3±20.6	7.3±0.6	76	75.4±8.6	5.7±1.0	13
+PSC	520.8±13.9	6.8±1.6	72	74.1±7.6	4.9±0.9	15
DNX	265.1±9.8	5.6±0.8	47	40.1±4.3	5.0±0.7	8
+DVP	248.3±11.2	5.4±0.9	46	34.0±3.9	4.8±0.9	7
+PSC	227.5±14.5	5.1±1.2	45	38.6±4.9	4.8±0.7	8

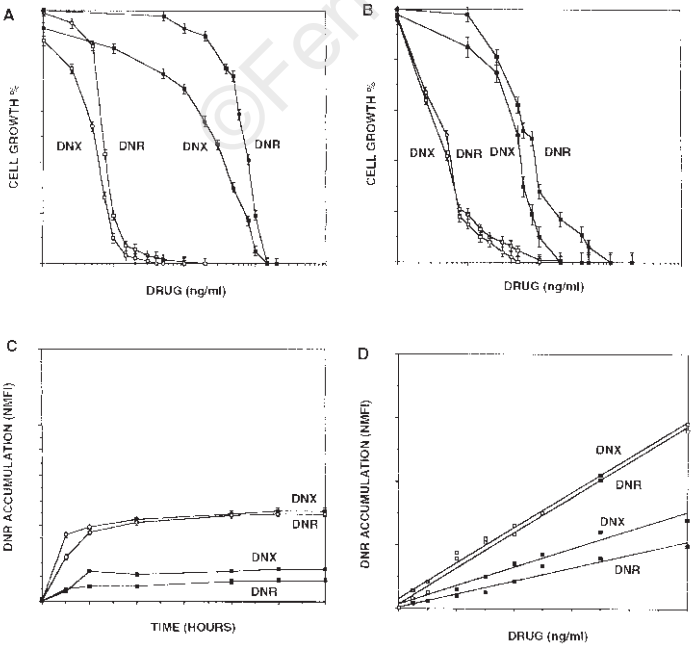

Figure 1. Dose-response curves for the two cell lines GLC4 ADR 150/ GLC4 (panel A) and SW1573/2R120/ SW1573 (panel B). In the two non-MDR lines GLC4 and SW1573 (open squares) the toxicity of daunoxome (DNX) parallels that of free DNR. In the two MDR variants GLC4-ADR 150 and SW1573/2R120 (closed squares) DNX is about 2.5 fold more toxic than the free drug (DNR). Panels C and D show the time- and dose-dependent cellular drug accumulation in the MRP overexpressing GLC4-ADR150 (closed squares) and in its parental non-MDR GLC4 cell line (open squares) assayed by flow cytometry. To test the relationship between the time of exposure to the drug and its accumulation, the cells were exposed for 24 hours to a fixed dose (300 ng/mL) of free DNR or DNX and the DNR-associated fluorescence was assayed at intervals (panel C). To test the relationship between exposure to the drug and its accumulation, the cells were exposed to increasing concentrations of free or liposomal DNR and the anthracycline-associated fluorescence was assayed after 24 hours of incubation (panel D).

us /cytoplasm distribution.[3] In fact, differently from PGP, LRP is primarily located in the cytoplasm[4] whereas MRP has been found both at the surface of the cellular plasma membrane, in the cytoplasm and in the Golgi region.[5,6] An additional part of this study was to compare the toxicity of DNX to that of a combination of free DNR plus MDR modifiers, in cell lines showing a non-PGP related MDR. In these MDR cell lines, the addition of MDR modifiers, such as D-verapamil or SDZPSC833, to free DNR only marginally increased the drug's toxicity. Moreover, this increase was smaller than that observed by substituting free DNR with DNX (Table 1). In conclusion, this work shows that the liposomal formulation of DNX doubles DNR toxicity in cell lines with an MDR associated overexpression of MRP or LRP. The increase in DNR toxicity due to the liposome encapsulation is higher than that produced by adding SDZPSC833 or D-verapamil to free anthracycline. These data support the ongoing research into the use of liposomal anthracyclines for the treatment of acute non-lymphocytic leukemia, considering that, even at disease onset, myeloid blast cells often show defects in drugs accumulation associated with co-overexpression of both PGP and LRP.[7-10]

Mariagrazia Michieli, Daniela Damiani, Anna Ermacora, Paola Masolini, Angela Michelutti, Michele Baccarani

Division of Hematology, Department of Medical and Morphological Research, Udine University Hospital, Italy

Key words

LRP, MRP, lung resistance-associated protein, multidrug resistance related protein, acute leukemia, liposome, daunorubicin, multi drug resistance.

Acknowledgments

This work was supported by the AIRC Milan and by MURST 1998 grant "Biological and molecular bases of therapy of acute non-lymphoid leukaemia and pharmacology and toxicology of anticancer anthracyclines: mechanisms and molecular basis for novel therapeutical strategy". We are grateful to Dr. MJ Broxterman (Dept. of Medical Oncology, Free University Hospital, Amsterdam, The Netherlands) and to Dr. EGE de Vries (Dept. of Internal Medicine, University Hospital, Groningen, The Netherlands) for supplying us with the LRP (SW-1573/2R120) and MRP (GLC4-ADR150) overexpressing cell lines.

Correspondence

Mariagrazia Michieli, M.D., Division of Hematology, University Hospital, p.le S. Maria della Misericordia, 33100 Udine, Italy. Phone: international +39-0432-559662-4 – Fax: international +39-0432-559661.

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Chronic myeloid leukemia in chronic phase with a partial trisomy 9 mosaicism

Sir,

We report a case of CML in chronic phase in a 72-year old woman with a previous history of heart disease and atrial fibrillation. She had a pronounced leukocytosis with a WBC count of 254×10^9/L, 1% of blasts and 10% of basophils. Her hematocrit was 33% and platelet count 370×10^9/L. Neutrophil alkaline phosphatase (NAF) activity was absent. Bone marow cellularity was increased and the myeloid/erythroid ratio was 1/2. The marrow contained 1% of blast cells.

Cytogenetic study, applying G-band techniques, of the bone marrow revealed the presence of two different cell lines: 60% of the metaphases analyzed were 46,XX,Ph while the remaining 40% had 47 chromosomes and Ph, the excess chromosome being a chromosome 9 with an interstitial deletion in its long arms.

Fluorescence *in situ* hybridization (FISH) was performed on chromosome preparations with two differently labeled bcr/abl translocation DNA probes (Vysis LSI bcr/abl tp). A total of 100-150 cells (metaphase and interphase) were counted. The existence of three signals was proved by the ABL probe, one hybridized to chromosome 9, another to the deleted 9 and the classic ABL/BCR fusion, in its nor-