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Comparative Toxicity of Detorubicin and Doxorubicin, Free and DNA-bound, for Hemopoietic Stem Cells

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Summary. *We compared the toxicity of detorubicin (DET) and of doxorubicin (DOX) on the hematopoietic stem cells in $C_{57}BL_{6J}$ mice by means of the CFU_S and CFU_C assays. On an equimolar basis DET appears to be less toxic than DOX for both the pluripotent stem cells and the granulocytic progenitor cells. Moreover, the administration of these anthracyclines as DNA complexes leads to a decreased toxicity to the pluripotent stem cells, while no such attenuated toxic effect is observed in committed stem cells.*

Introduction

Recently, we have observed that the hematopoietic toxicity of daunorubicin (DNR) and of doxorubicin (DOX) can be significantly modified by giving these drugs as complexes with DNA [2, 10]. Moreover, when DNR is complexed to DNA its activity in vivo against the L1210 murine leukemia is enhanced [3].

We extended our study to a new anthracycline derivative, diethoxyacetoxy-14-daunorubicin (33 921 RP), and compared the effects on the pluripotent and committed stem cells of the free and DNR-associated forms of the drugs after IV injection.

Materials and Methods

$C_{57}BL_{6J}$ virgin female mice (Centre d'Animaux de Laboratoire, Heverlee, Belgium) weighing 18–21 g were used both as donors of bone marrow and as recipients for the assay of pluripotent stem cells. As the source of colony-stimulating factor we used sera of NMRI mice (Centre d'Animaux de Laboratoire, Heverlee, Belgium) previously treated with endotoxin by IP injection [6].

Bone marrow cells were obtained from a pool of five tibias whose tips were cut off, after which the marrow plugs were flushed out with 1 ml Hank's balanced salt solution supplemented with penicillin and streptomycin. Pluripotent stem cells (CFU_S) were assayed in vivo according to the method of Till and McCulloch [9]. The nucleated cells were counted in hemocytometers and diluted to obtain a cell concentration varying from 0.6×10^5 to 10×10^5 cells/ml; of this dilution 0.5 ml was injected into the tail vein of each of seven lethally irradiated mice (850 rads; Cesapan; Barazetti, Monza, Italy). The spleens were removed 8 days later and fixed in Bouin's solution; the macroscopic colonies were then counted. The contents in CFU_S, determined from the number of spleen colonies and the number of cells injected, were normalized with regard to the control values observed in untreated mice of the same batch.

Granulocytic progenitor cells (CFU_C) were assayed in vitro in soft agar as described by Quesenberry et al. [7]. In these experiments, postendotoxin serum was incorporated as a source of colony-stimulating factor [6] in the underlayer and $0.4–3 \times 10^5$ bone marrow cells were seeded by petri dish. Five replicate dishes for each pool were incubated at 37° C for 7 days in a fully humidified atmosphere of 10% CO_2 in air (Forma incubator, Ohio, USA). The content of CFU_C per tibia was determined from the mean number of colonies (cell aggregates containing 50 or more cells) per petri dish. The results were normalized to the control values.

Doxorubicin (DOX) and detorubicin (DET) hydrochlorides were given by Rhône-Poulenc (Paris). Herring sperm DNA (highly polymerized, type VII, Sigma, St Louis, Mo, USA) was dissolved in 0.15 *M* NaCl to a concentration of either 468 or 585 mg% w/v and autoclaved for 15 min at 120° C the day before an experiment. To prepare the DNA complex [10], the drugs were dissolved in triple-distilled water and mixed rapidly into the DNA to obtain a final molar ratio of DNA mononucleotide to drug of 20. The drug preparation was further diluted if necessary and injected IV 24 h before the assays. Five mice received each dose and five mice of the same age were kept uninjected as controls.

Linear regressions were fitted according to the method of least squares to give the general equation $\log y = ax + b$, where x was the dose of drug expressed in μM/kg and y the fraction CFU surviving/tibia. This equation was used to calculate the D_{10} and $D_{1/2}$ values. Slopes were compared by Fisher's F test for analysis of covariance.

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Results

Dose-survival Curves of Pluripotent and Committed Stem Cells

The CFU_S assay was used to determine the survival of the pluripotent stem cells 24 h after an IV injection of DET or DOX, free or complexed to the DNA. Figure 1 shows the fraction of CFU_S surviving per tibia as a function of the doses of drug injected.

For mice treated with DOX or DET the dose-survival curves of CFU_S are parallel, with a slightly lower toxicity of DET. The $D_{1/2}$ values for DOX and DET are, respectively, 5.3 and 5.7 μ*M*/kg ($P > 0.05$). DET-DNA is also slightly less toxic than DOX-DNA, the respective $D_{1/2}$ values being 7.3 and 6.3 μ*M*/kg ($P > 0.05$).

If CFU_S seem to be less sensitive to DOX-DNA than to DOX, they are significantly less depressed in mice treated with DET-DNA than in mice receiving DET in the free form ($P < 0.05$). This is also well illustrated in Table 1 by the D_{10} values, which are 21 or 25 μ*M*/kg for free or DNA-associated DOX, respectively, and 27 or 32 μ*M*/kg for DET or DET-DNA.

The fraction of CFU_C surviving 24 h after DOX or DET administered at different doses is shown in Fig. 2. The dose-survival curves of the granulocytic committed stem cells are very similar whether the drugs are free or complexed to DNA. However, an important difference is observed between DOX and DET, as shown by the $D_{1/2}$ values of 4.3 and 6.3 μ*M*/kg, respectively ($P < 0.01$), or 4.2 and

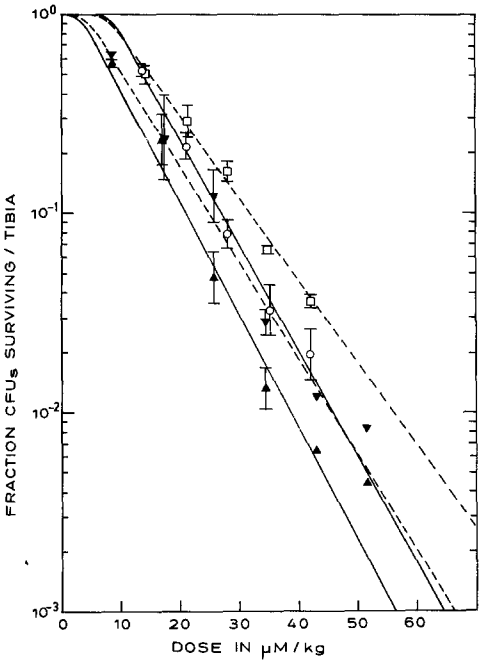

Fig. 1. Dose-survival curves for pluripotent stem cells (CFU_S). Groups of five mice were given different doses of DOX (▲——▲), DOX-DNA (▼---▼), DET (○——○), or DET-DNA (□---□). Their tibia marrows were assayed 24 h later for their content of CFU_S in irradiated host mice. The values shown are normalized to the untreated controls. Each point represents the geometric mean ± SEM of two or three separate experiments from a pool of five tibias per experiment

Table 1. In vivo sensitivity of hematopoietic stem cells to doxorubicin and detorubicin

Drug[a]	CFU_S		CFU_C	
	D_{10}	$D_{1/2}$	D_{10}	$D_{1/2}$
Doxorubicin	21	5.3	22	4.3
Doxorubicin-DNA	25	6.3	22	4.2
Detorubicin	27	5.7	34	6.3
Detorubicin-DNA	32	7.3	35	7.1

[a] The doses of the drug, expressed in μM/kg required to reduce the CFU population to 10% of the control values (D_{10}) and those required to reduce the survival of a CFU population by 0.5 on the linear part of an exponential curve ($D_{1/2}$) have been derived from the Figs. 1 and 2

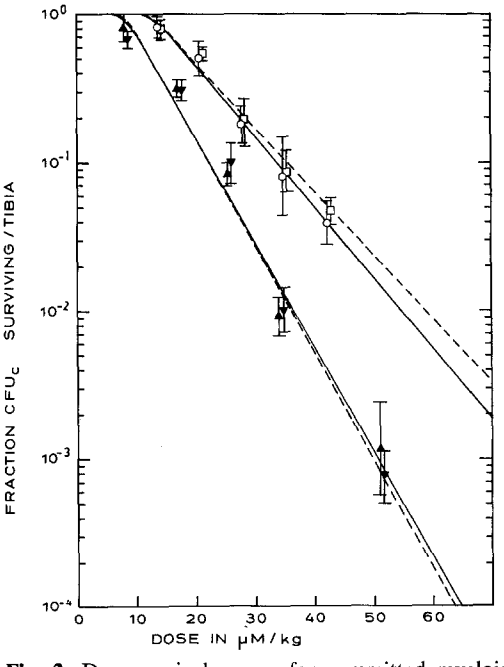

Fig. 2. Dose-survival curves for committed myeloid stem cells (CFU_C). Groups of five mice received different doses of DOX (▲——▲), DOX-DNA (▼---▼), DET (○——○), or DET-DNA (□---□) and their tibia marrows were assayed 24 h later for their content of CFU_C. The values shown are normalized to the untreated controls. Each point represents the geometric mean ± SEM of three to five separate experiments from a pool of five tibias per experiment

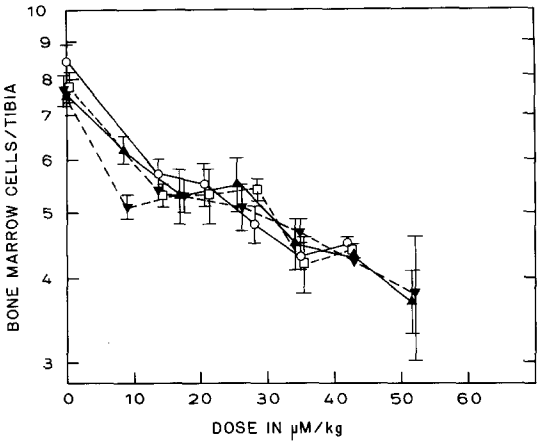

Fig. 3. Sensitivity of bone marrow cells. Groups of five mice were given different doses of DOX (▲——▲), DOX-DNA (▼– – –▼), DET (○——○), or DET-DNA (□– – –□) 24 h before and the nucleated cells counts were determined on each pool of five tibias. Each point represents the mean ± SEM of three or four separate experiments

7.1 μ*M*/kg for DOX-DNA and DET-DNA ($P < 0.005$).

Sensitivity of Bone Marrow Cells

The nucleated cell counts 24 h after administration of DOX, DOX-DNA, DET, or DET-DNA are presented in Fig. 3. All the drugs induce a reduction of the bone marrow cell count proportional to the amount injected. This decrease reaches 50% of the control values for doses of 50 μ*M*/kg and is similar for all drugs.

Discussion

We compared the survival of pluripotent (CFU_S) and committed (CFU_C) stem cells in $C_{57}BL_{6J}$ mice after an IV injection of DOX and DET, either free or complexed to DNA. This new semisynthetic anthracycline derivative has recently been described by Maral et al. [5]. Preliminary clinical trials have shown that DET is at least as effective as DNR or DOX in the treatment of malignant hemopathies [4]. The hematopoietic toxicity of DET in the CFU_S assay expressed as the $D_{1/2}$ value is 5.7 μ*M*/kg. This value is somewhat, but not significantly, higher than that of 5.3 μ*M*/kg observed after injection of DOX ($P > 0.05$). When complexed to DNA, DET becomes less toxic, with a $D_{1/2}$ of 7.3 μ*M*/kg, than DOX or even DOX-DNA (6.3 μ*M*/kg).

DET is significantly less toxic than DOX for the granulocytic committed stem cells ($P < 0.01$). These progenitors are, on the other hand, equally affected by the free and DNA-bound forms of each drug. The CFU_C $D_{1/2}$ values are 4.3 μ*M*/kg and 4.2 μ*M*/kg for free DOX and DOX-DNA while they are increased to 6.3 μ*M* and 7.1 μ*M*, respectively, for DET and DET-DNA.

By 24 h after injection the number of the bone marrow cells is depressed to the same extent for DOX and DET, whether free or complexed to DNA. The bone marrow cellularity does not reflect the differences observed on the CFU_S and CFU_C progenitor cells in this case.

The toxicity of DET-DNA and of DOX-DNA is lower for the pluripotent stem cells, whereas their toxicity for the granulocytic progenitors is equal to that of the free drug [2]. The toxicity of DET injected IV for the committed stem cells is significantly lower than that of DOX as estimated by the $D_{1/2}$ values, DOX being about 1.5 times more toxic for the CFU_C than DET.

Although recent results in our laboratory have shown that in the bloodstream DET hydrolyses very rapidly and generates DOX [1], our results indicate that DET could exert a lower toxicity on the hematopoietic system as far as the committed stem cells are concerned, as the results observed 24 h after administration of the drugs are not the consequence of differences in the mode of action of DOX and DET. These results are supported by recent observations in our laboratory indicating that while the chemotherapeutic activity of DET on DBA_2 mice bearing L1210 leukemia is 1.4 less significant than DOX given at equimolar doses, the hematopoietic toxicity of DET, assayed on the committed stem cells of the same mice up to 4 days after administration, is 2–2.5 weaker than that of DOX.

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