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Role of intracellular accumulation in the prooxidant cytotoxicity of daunorubicin

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Oxidative stress partly contributes to the cytotoxicity of the anticancer anthracycline daunorubicin in FLK and HL-60 cells. However, daunorubicin is 100–300 times more cytotoxic than the model quinone danthron possessing a similar reduction potential. This discrepancy may be explained by the high cell/medium partition coefficient of daunorubicin, 570 ± 40 (HL-60 cells) and 230 ± 30 (FLK cells) calculated using the fluorescence quenching method. The cell/medium partition coefficient for danthron is below 10.

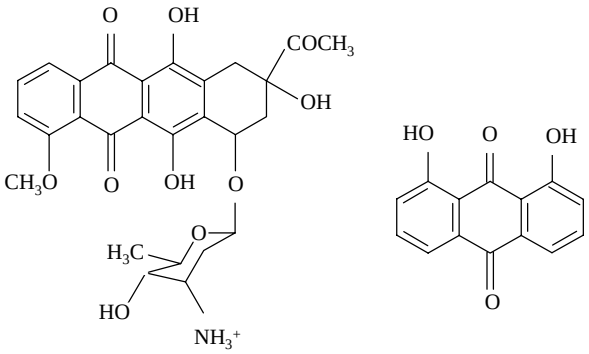
Key words: daunorubicin, intracellular accumulation, cytotoxicity, oxidative stress

INTRODUCTION

Daunorubicin (Figure) and the related anthracycline quinones are widely used in cancer treatment [1, 2]. DNA intercalation and topoisomerase inhibition are the universally accepted mechanisms of anthracycline cytotoxicity. However, the role of another mechanism, the oxidative stress resulting from enzymatic free radical formation and redox cycling of anthracyclines, is a subject of controversy ([2, 3], and references therein). Antioxidants partly protect from daunorubicin toxicity in HL-60 human promyelocytic leukemia cells [3], thus pointing to the involvement of oxidative stress. Another indication of oxidative stress is an increase in quinone cytotoxicity with an increase in their single-electron reduction potential, E^1_7 [3–6]. However, daunorubicin was much more cytotoxic than one may expect from its E^1_7 value, –0.34 V [3]. In this paper, using HL-60 cells and bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK), we demonstrate that an enhanced cytotoxicity of daunorubicin, partly realated to the prooxidant events, may be explained by its high intracellular accumulation.

MATERIALS AND METHODS

Daunorubicin (Minmedprom, Russia), 1,8-dihydroxyanthraquinone (danthron), 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), desferrioxamine, and *N,N'*-diphenyl-*p*-phenylene diamine (DPPD) (Sigma) were used as received. HL-60 and FLK cells, obtained from the former All Union Bank of Cell Cultures (St. Petersburg), were grown and maintained in RPMI-1640 or Eagle's media, respectively, with 10% fetal bovine serum at 37 °C [5]. In the cytotoxicity experiments, cells (3.0×10^4/ml, FLK, or 3.0×10^5/ml, HL-60) were grown in the presence of compounds for 24 h and counted using a hematocytometer with viability determined by exclusion of Trypan blue. Before the count, FLK cells were trypsinized. The accumulation of daunorubicin and danthron by HL-60 cells and by trypsinized FLK cells was examined fluorimetrically [7] in Hank's medium with 10 mM glucose at 25 °C, using a Hitachi MPF-4 spectrofluorimeter. The emission wavelength was 560 nm, the excitation wavelengths were 480 nm



Daunorubicin Danthron

Figure. Structural formulae of daunorubicin and danthron

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(daunorubicin) and 430 nm (danthron). The cell/medium partition coefficent (*P*) was calculated according to the equation:

$P = (I_o – I) / (I \times$ the volume fraction of packed cells in their suspension), where I_o is the initial fluorescence of the compound in the cell suspension, *I* is the residual fluorescence after addition of 0.02% Triton X-100. The cell volumes of trypsinized FLK cells in Hank's medium were measured using a hematocytometer grid as described [8]. Statistical analysis was performed using Statistica 4.3 (Statsoft Inc., 1993).

Table. **Effects of 2 μM DPPD, 300 μM desferrioxamine and 20 μM BCNU in the 24 h cytotoxicity of 2.2 μM daunorubicin and 100 μM 1,8-dihydroxyanthraquinone (danthron) in FLK cells**

Additions	Viable cells (%)[a]	
	a) daunorubicin	b) danthrom
Compound	36 ± 3	56 ± 2
Compound + DPPD	56 ± 2	82 ± 3
Compound + desferrioxamine	48 ± 3	87 ± 3
Compund + DPPD + desferrioxamine	58 ± 3	92 ± 3
Compound + BCNU	27 ± 2	35 ± 2

[a] The results as the mean ± standard deviation of 3–4 independent experiments. For 2–5 against 1, the differences are statistically significant with $P < 0.05$ according to Student's t test.

RESULTS AND DISCUSSION

The daunorubicin concentrations for 50% cell survival (cL_{50}) determined in this work were equal to 1.47 ± 0.22 μM (FLK) and 0.38 ± 0.07 μM (HL-60). They were 100–300 times lower than cL_{50} of a model anthraquinone danthron (Figure), 120 ± 15 μM (FLK) and 150 ± 15 μM (HL-60), whose single-electron reduction potential, –0.325 V, is close to E^1_7 of daunorubicin [5]. Daunorubicin cytotoxicity in FLK cells was partly prevented by the antioxidant DPPD and the iron ion chelator desferioxamine, and potentiated by BCNU which depletes intracellular reduced glutathione [5, 6] (Table). Analogous effects were characteristic of the cytotoxicity of danthron (Table). Cell viability in control experiments was 97 ± 2%. DPPD, desferrioxamine, and BCNU decreased cell viability by 1–3%. These data are in line with the previously reported protective effects of antioxidants towards daunorubicin cytotoxicity in HL-60 cells [3], thus showing that daunorubicin exerts a prooxidant effect on both cell lines investigated.

A significant accumulation of anthracyclines in various cell types has been reported [7, 9, 10], although not expressed in quantitative terms. The intracellular accumulation of anthracyclines and other fluorescent quinones may be monitored by their time-dependent fluorescence quenching in a cell suspension ([7], and references therein). In our typical experiment, the fluorescence intensity of 5 μM daunorubicin in the presence of 10^7/ml HL-60 cells decreased by 20% after 50 min without reaching the steady-state level. The subsequent addition of 0.02% Triton X-100, which causes cell permeabilization [7], decreased the fluorescence by 80% in 2 min. An analogous slow daunorubicin fluorescence quenching and fluorescence drop after addition of Triton X-100 was also characteristic of FLK cells. The cell/medium partition coefficients (see equation) were calculated from the final levels of 2–10 μM daunorubicin fluorescence in the presence of Triton X-100 and $0.5–1.0 \times 10^7$/ml HL-60 cells or $0.7–2.7 \times 10^6$/ml FLK cells (n = 8), using the volume of HL-60 cell, 0.7 pl [11] and the volume of FLK cell, 3.2 ± 0.2 pl determined in the present work (n = 17). The latter value is close to the value of Ha-ras expressing NIH 3T3 fibroblasts, 2.6 pl [8]. The obtained *P* values were equal to 230 ± 30 (FLK cells) and to 570 ± 40 (HL-60 cells). The latter value was close to $P \approx 800$, which was calculated by us using the available data on ^{13}C-daunorubicin accumulation by HL-60 cells [10]. Although the fluorescence quenching method determines mainly the amount of a DNA-intercalated compound [7], the ratio of the content of anthracyclines in the nucleus and in the other cell compartments is about 4:1 [9]. It shows that in other cell compartments the daunorubicin concentration may be increased 40–100 times. In contrast, the quenching of danthron fluorescence under identical conditions was negligible, giving us $P < 10$ for danthron accumulation in both cell lines. This is consistent with the weak DNA-intercalating activity of danthron [12]. It also points to its low extranuclear accumulation, which is in line with the few available data on other neutral policyclic quinones *e.g.*, 2,3-dimethoxy-1,4-naphthoquinone with $P = 1.5–10$ [13]. Since the oxidative stress exerted by anthracyclines and other quinones is mainly associated with their redox cycling in microsomes, cytosol and mitochondria [1, 6], the increased daunorubicin extranuclear accumulation may be partly responsible for its high cytotoxicity with the partly prooxidant character. The redox cycling of quinones by nuclear NAD(P)H-oxidizing flavoenzymes [14] is considered to be less important. However, taking into account the differences in the nuclear

accumulation of daunorubicin and danthron, the above mechanism may also contribute to their different cytotoxicity.

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VIDULÀSTELINËS AKUMULIACIJOS VAIDMUO PROOKSIDANTINIAM DAUNORUBICINO CITOTOKSIÐKUMUI

Santrauka

Oksidacinis stresas – prieðnavikinio antraciklino daunorubicino citotoksinio poveikio FLK ir HL-60 làstelëms dalyvis, taèiau daunorubicinas yra 100–300 kartø toksiðkesnis uþ artimo redukcijos potencialo modeliná chinonà dantronà. Ðie skirtumai gali bûti susieti su dideliu daunorubicino làstelës/terpës pasiskirstymo koeficientu (570 ± 40 (HL-60 làstelës), 230 ± 30 (FLK làstelës)), nustatytu fluorescencijos gesinimo metodu. Analogiškas dantrono parametras yra maþesnis nei 10.