Identical photosensitizing activities of a sulfonated aluminium phthalocyanine on human erythroleukemia cell lines susceptible or resistant to the cytotoxic activity of doxorubicin

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Abstract

In this paper we report that a human erythroleukemia cell line made 100 times more resistant than the parental line to the cytostatic activity of doxorubicin and spontaneously 500–1000 times more resistant to the cytostatic activity of an unrelated drug, namely taxol, exhibits on the other hand unchanged susceptibility to the photosensitizing activity of a sulfonated phthalocyanine.

Keywords: PDT, phthalocyanines, multiple resistance, doxorubicin, taxol.

1. Introduction

In a search for photosensitizing molecules which would absorb light strongly at longer wavelengths (*i.e.* greater than 600 nm) and therefore allow better tissue penetration than do the hematoporphyrin derivatives, such as Photofrin II, presently used in the photodynamic therapy (PDT) of some cancers, we observed the significant photosensitizing activities of a number of sulfonated metallophthalocyanines and naphthalocyanines, with maximal light absorption at 680 nm [1, 2].

Among preclinical studies which are envisaged for such molecules, there is a need to determine whether or not they exhibit the phenomenon of multiple drug resistance (MDR), which is observed with many of the cytotoxic drugs used in conventional anticancer chemotherapy.

The MDR phenomenon, a serious obstacle to effective therapy with a single drug or with drug combinations, appears to be chiefly due to a decrease of the global cellular uptake of the drugs or to an increased rate of drug excretion, primarily resulting from excessive production by the resistant cells of a glycoprotein (gp P) at their surface.

We chose as a model for this investigation a sulfonated aluminium phthalocyanine (AlPcS) and two lines of human erythroleukemia cells, namely K 562-S (normally susceptible to the cytotoxic activity of doxorubicin *in vitro*) and K 562-R (about 100 times more resistant to this drug than the latter, from which it had been derived through serial passages in the presence of increasing concentrations of doxorubicin). The original K 562 cell line consists of Philadelphia chromosome-positive cells obtained from a patient with chronic myelogenous leukemia in terminal blast crisis [3].

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2. Materials and methods

The K 562-S and K 562-R cell lines, kindly provided by Dr. H. Tapiero (Institut de Cancérologie et d'Immunogénétique, Villejuif, France) were grown as suspensions in RPMI 1640 medium containing 10% fetal calf serum and, in the case of the 562-R cells, a 5×10^{-8} M concentration of doxorubicin (Adriblastine, injectable solution, purchased from Laboratoire Roger Bellon, Neuilly-sur-Seine, France). Stock cultures were performed in 25 cm^2 Nunc plastic flasks containing 5 ml of medium and incubated horizontally in 5% CO_2–95% air at 37 °C; passages were performed twice weekly by diluting the cultures with fresh medium so as to contain initially 100 000 cells ml^{-1}. Within 3 days this concentration reaches on the average around 800 000 cells ml^{-1} in the S line, 600 000 ml^{-1} cells in the R line.

The cytostatic activities of doxorubicin and of taxol (batch prepared at this Institute) for the K 562-S and 562-R cells were determined as follows: 50 000 cells from a fully grown stock culture, in 1 ml of growth medium, were placed in Nunclon plastic tubes endowed with a flat surface on one side (Leighton type) and various concentrations of the drug tested were added immediately to the tubes under a volume of 0.1 ml. The cultures were then incubated for 3 days at 37 °C in 5% CO_2–95% air, following which cell viability was determined by the MTT colorimetric assay described by Mosmann [4], in which a tetrazolium salt (purchased from Sigma) is reduced to a colored formazan product by reducing enzymes present only in living, metabolically active, cells. The cytostatic activity of a given concentration of drug is expressed as the percentage of cell growth inhibition by comparison with control cultures, incubated under the same conditions in the absence of drug.

The photosensitizing activities of AlPcS for the K 562-S and 562-R cells were determined as follows: 200 000 cells were placed in each of the 12 wells of a Costar plate in 2 ml of medium, containing various concentrations (from 0.5–5 $\mu g\ ml^{-1}$) of AlPcS from a 1 mg ml^{-1} stock solution in PBS stored at 4 °C.

The plates were incubated 3 h in the dark at 37 °C in 5% CO_2–95% air; they were thereafter exposed during 1 h to the light (90 J cm^{-2}) from a 1000 W halogen lamp fitted with a filter cutting below 600 nm. Thereafter, the plates were incubated for 18 h at 37 °C in 5% CO_2–95% air, following which cell viability was determined by the MTT assay. The photosensitizing (cell killing) activity of a given concentration of AlPcS is expressed as the percentage of cell viability inhibition, by comparison with control cultures illuminated but not exposed to AlPcS. Other controls consisted of cultures incubated 3 h in the dark in presence of AlPcS but not exposed subsequently to light.

The synthesis of sulfonated aluminum phthalocyanines was performed according to the method of Vogler and Kunkely [5] and then sulfonated through a slight modification of the technique of Linstead and Weiss [6] which has been reported [1]. The salted solution was deionisated by Sephadex G 10 (Pharmacia).

3. Results

Figure 1 shows representative dose–effect curves of the cytostatic activity of doxorubicin for K 562-S and K 562-R cells. Calculation of the ID_{50} (inhibitory dose 50%) of this drug showed that it was roughly 50–100 times higher (from 7×10^{-6} M to 10^{-5} M *vs.* 10^{-7} M to 2×10^{-7} M) for the resistant cells than for the susceptible ones.

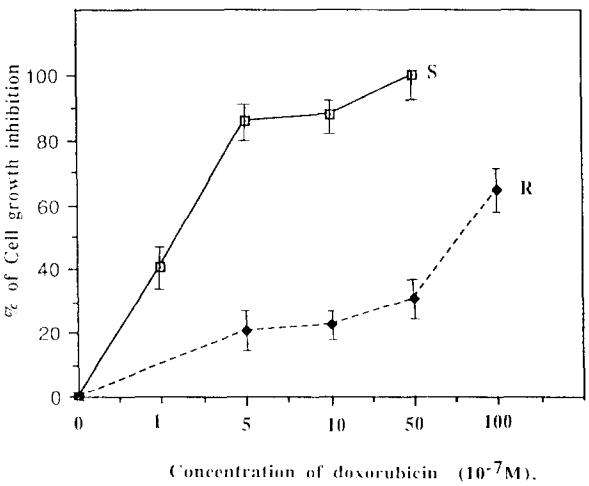

Fig. 1. Dose–effect curves of the cytostatic activity of doxorubicin for K 562-S and K 562-R cells.

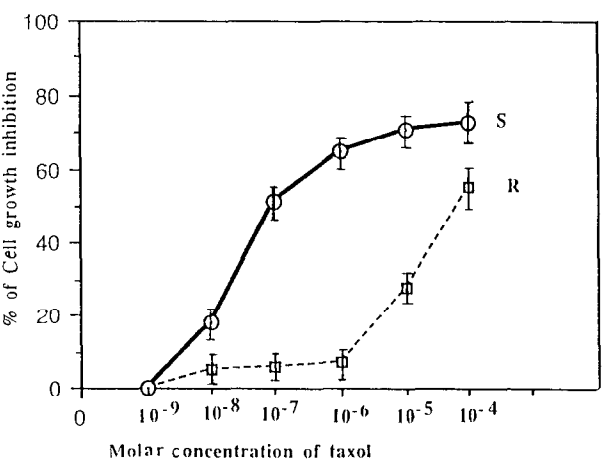

Fig. 2. Dose–effect curves of the cytostatic activity of taxol for K 562-S and K 562-R cells.

Figure 2 shows representative dose–effect curves of the cytostatic activity of taxol, an antimitotic drug acting on the microtubule system to which the K 562-R cells had never been exposed: nevertheless, the ID_{50} of taxol for the R cells was in successive experiments, approximately 500–1000 times higher than for the S cells.

Table 1 summarizes the results of two consecutive tests in which the photosensitizing activities of AlPcS were determined for K 562-S and 562-R cells. It is clear from the table that, if anything, the K 562-R cells were more susceptible than their S counterparts to this photosensitizing activity. The LD_{50} of AlPcS was around 0.9 μg ml^{-1} for the S cells, 0.6 μg ml^{-1} for the R cells, a non-significant difference. In addition, the highest concentration of AlPcS tested (5 μg ml^{-1}) was devoid of toxicity for both cell lines when treated cultures were not exposed to light.

TABLE 1

Comparative photosensitizing effects of AlPcS on doxorubicin-susceptible and resistant K 562 human erythroleukemia cells

Concentration of AlPcS (μg ml^{-1})	Percentage of cells killed			
	K 562-S line		K 562-R line	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0.5	10	18	12	25
1.0	58	58	77	76
1.5	n.d.	75	n.d.	84
2.0	83	84	92	84
5.0	96	n.d.	97	n.d.

4. Discussion

Our results show that a human erythroleukemia cell line made 100 times more resistant than the parental line to the cytostatic activity of doxorubicin and spontaneously 500–1000 times more resistant to the cytostatic activity of an unrelated drug, namely taxol, exhibits on the other hand unchanged susceptibility to the photosensitizing activity of a sulfonated phthalocyanine.

As stated in the introduction, the MDR phenomenon reflects itself in a decreased cellular uptake of the drugs or in an increased rate of drug excretion. In the case of doxorubicin, its incorporation into tumor cells is thought to be the result of a passive diffusion influx and an active outwards efflux [7]. The P glycoprotein, overexpressed in MDR + cells, has been shown to be an ATP-binding and -consuming drug transporter [8]. The absence of resistance of the K 562-R cell line to the photosensitizing activity of our sulfonated phthalocyanine could be readily explained if this molecule did not have to penetrate into the cytoplasm to exert its effects. Studies by fluorescence microscopy performed in our laboratory [2] have demonstrated however that the phthalocyanine does indeed penetrate into the cytoplasm and is localized in a perinuclear area, possibly the Golgi apparatus. Cell destruction is mediated through the *in situ* production of singlet oxygen, as was demonstrated by the reversal of AlPcS phototoxicity when histidine, a known scavenger of singlet oxygen, was present at 5 mM in the culture medium [2]. Vitamin E, another scavenger, did not exert this reversal, probably because its lipophilic character causes it to be localized in the cell membrane, whereas the hydrophilic histidine penetrates into the cytoplasm.

With respect to the porphyrin derivative Photofrin II, Marchesini *et al.* [9] have shown that a MDR+human breast carcinoma cell line, 300 times more resistant to doxorubicin than its parental line, was only 10 times more resistant to the photosensitizing activity of Photofrin II. Gomer *et al.* [10] showed that normal and heat-resistant (45 °C) clones of Chinese hamster fibroblasts or of mouse fibrosarcoma cells exhibited comparable levels of porphyrin uptake and photosensitivity and concluded that members of the 70 kD heat-shock protein family (which are elevated in the thermal-resistant cells and probably associated with the heat-resistant phenotype) do not play a significant role in modulating PDT sensitivity. It has been shown [11] that an association exists between the heat-resistant phenotype and the MDR+phenotype.

Kessel [12], on the other hand, showed that some anthrapyrazoles, a new class of antineoplastic agents which bind to DNA, also exhibit photodynamic effects, due to singlet oxygen production. This author examined the photodynamic effects of a number of anthrapyrazoles on the P 388 murine lymphoblastic leukemia cell line and on a doxorubicin-resistant (P 388/ADR) cell line and demonstrated that, whereas the latter was about 100 times more resistant to the cytotoxic activity of doxorubicin than the original susceptible line, it was only 10–15 times more resistant to the cytotoxic effects (in the dark) of these anthrapyrazoles as well as to drug-induced photodamage.

In conclusion, and pending confirmation by *in vivo* experiments, it may be assumed that photodynamic therapy of cancers may show efficacy even against tumors which, either spontaneously or following therapy with conventional anticancer drugs, exhibit cross-resistance of the MDR+type, to a wide range of such drugs. In this respect, sulfonated phthalocyanines may be particularly interesting.

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