EFFECT OF TRITON X-100 ON ACCUMULATION AND THERAPEUTIC ACTION OF DOXORUBICIN IN MICE WITH LEUKEMIA P-388 AND WITH INDUCED RESISTANCE TO THE CYTOSTATIC

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Multiple drug resistance (MDR) of tumor cells is one of the main obstacles to chemotherapy of malignant neoplasms at the present time. An extensive search is currently in progress for modifiers to overcome this form of resistance. It has been shown that substances enabling MDR to be overcome belong to different classes: they include Ca^{2+}-channel blockers, calmodulin inhibitors, and detergents [4]. However, if these are used in vivo serious problems arise, due to the prolonged maintenance of an effective concentration of the modifier in the blood plasma. For instance, for effective use of the Ca^{2+}-channel blocker verapamil, its concentration in the blood plasma must be about 50 μg/ml, and its circulation time in the blood stream about 2 h. Meanwhile, with a concentration of verapamil of the order of 3 μg/ml, serious cardiovascular disturbances are observed, so that this drug cannot be used in higher doses [7]. Our previous investigations showed that another MDR modifier, the detergent Triton X-100 (TR), in very low concentrations (0.0001%) makes the membrane of resistant and sensitive cells more permeable for polycyclic compounds [1].

We therefore decided to study the possibility of using TR as a modifier of MDR in vivo in order to overcome resistance of P-388 leukemia cells to the polycyclic anthracycline antibiotic doxorubicin (DX).

EXPERIMENTAL METHOD

Leukemia P-388 cells with induced resistance to DX (P-388/DX) were obtained by selection from P-388 leukemia cells (P-388 is an original strain, obtained from the tumor strains bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) during treatment of animals with small doses of the antibiotic. Induction of resistance required 35 passages. To maintain leukemia P-388 and P-388/DX male DBA/2 mice aged 2-3 months were used. Tumor cells were transplanted intraperitoneally in a dose of $1 \cdot 10^6$ cells per mouse in 0.2 ml of medium 199. Leukemia cells were isolated from ascites fluid in Hanks' solution, and washed to remove erythrocytes by hemolytic shock. The cells were counted in a Goryaev's counting chamber.

The concentration of DX (pharmacopeal preparation) in leukemia P-388 and P-388/DX cells was estimated 30 min after intraperitoneal injection of DX in a dose 5 mg/kg, by the method described previously [2]. Triton X-100 ("Serva," West Germany), was injected intraperitoneally in a dose of 40 mg/kg 15 min before injection of DX. The fluorescence measurements were made on a Hitachi M-850 fluorescent spectrophotometer (Japan). Accumulation of the preparation in the cells was measured at least 3 times at different passages of the tumors. The intensity of fluorescence of DX was determined at 470 and 590 nm, at maxima of absorption and fluorescence of DX respectively.

The therapeutic action of DX against the background of TR administration was assessed on BDF_1 (DBA/2 × C57BL/6) mice aged 2-3 months, with transplanted leukemia, by calculating the average lifespan of the animals which died. Treatment was given 24 h after transplantation of the tumor.

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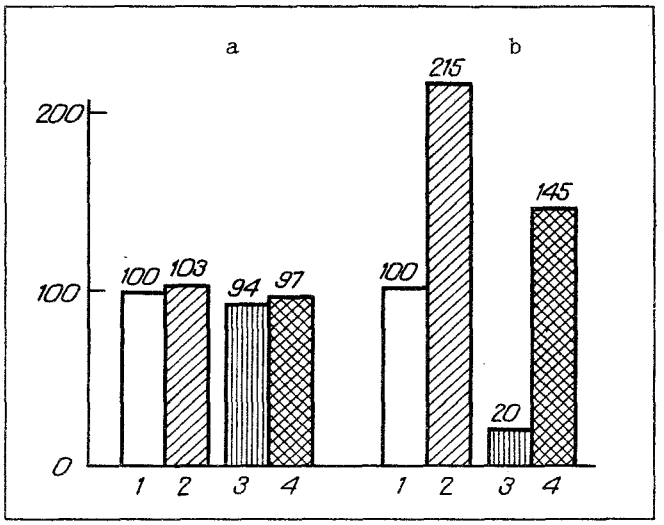

Fig. 1. Effect of TR on accumulation of DX in P-388 and P-388/DX leukemia cells a) Leukemia P-388, b) leukemia P-388/DX. Ordinate, accumulation of DX in cells (in % of initial level). 1) Initial level of DX accumulation in cells; 2) initial accumulation of DX superposed on administration of TR; 3) DX concentration in cells after 6 h; 4) DX concentration in cells after 6 h, superposed on injection of TR. Average of three measurements given.

The results were subjected to statistical analysis by the Fisher–Student test.
LD_{50} of TR was determined by Behrens' method.

EXPERIMENTAL RESULTS

The toxicity of TR was studied in experiments on mice LD_{50} of TR was found to be 153.6 ± 15.5 mg/kg and its maximal tolerable dose (MTD) was 80 mg/kg body weight. Thus TR was found to have a wide margin between concentrations at which the substance was toxic and in which it had a modifying action on accumulation of polycyclic compounds in cells (of the order of 1 mg/kg, on the basis of the results of experiments in vitro).

The effect of TR on DX accumulation was studied in P-388 leukemia cells, both sensitive and resistant. The dose of TR was 0.5 MTD, namely 40 mg/kg body weight. The concentrations of accumulated DX in P-388 and P-388/DX leukemia cells are shown in Fig. 1 as percentages of the initial level. The initial level (100%) was taken to be the quantity of DX in leukemia P-388 or P-388/DX cells 30 min after injection of the cytostatic. The choice of these time intervals was dependent on the pharmacokinetics of DX in tumor cells and in the whole animal, which we described previously [2]. TR left the initial accumulation of DX in sensitive cells virtually unchanged, whereas in resistant cells the detergent increased the value of this parameter to 215%. After 6 h the content of DX in sensitive cells was virtually unchanged, namely 94 and 97% without and with the TR modifier respectively. However, in leukemia P-388/DX cells only 20% of the initial level of the cytostatic remained after 6 h. Meanwhile TR disturbed the outflow of DX from the resistant cells and after 6 h the concentration of the cytostatic was 145% of its initial level.

In the next stage of the investigation the effect of TR was studied on the therapeutic action of DX in mice with leukemia P-388 and P-388/DX. The data in Table 1 show that injection of detergent alone did not affect the length of survival of animals with leukemia P-388 and P-388/DX. The use of DX in a dose of 5 mg/kg increased the mean length of survival of the animals with leukemia P-388 from 9.6 days in the control to 24.2 days. Meanwhile DX did not change the length of survival of the animals with leukemia resistant to the action of the cytostatic (P-388/DX) compared with that of the untreated animals. Infection of TR did not increase the efficacy of the cytostatic.

TABLE 1. Effect of TR on Therapeutic Effect of DX in Mice with Leukemia P-388 and P-388/DX (M ± m, n = 10)

Leukemia	Modifier	Dose of DX, mg/kg	Mean length of survival of dying animals, days
P-388	—	—	9,6±0,2
P-388	TR	—	9,6±0,3
P-388	—	5	24,2±1,2
P-388	TR	5	23,3±0,9
P-388/ DX	—	—	10,1±0,2
P-388/ DX	TR	—	10,0±0,1
P-388/ DX	—	5	11,4±0,3
P-388/ DX	TR	5	11,4±0,3

Thus rather contradictory results were obtained from this investigation: although DX accumulates in resistant cells to a greater degree when the modifier is used, its cytotoxic action is not increased under these circumstances. This disparity between accumulation of the cytostatic in the cells and its cytotoxic action is observed in atypical MDR and can be explained by a disturbance of activity of the enzyme DNA-topoisomerase II in this form of resistance [3]. Our own results can also be explained by blocking of DNA-topoisomerase II, which may be connected with a disturbance of the ionic composition of the cells (elevation of the Na^+ concentration) under the influence of TR [5]. Moreover, reduction of the cytotoxicity of DX when the detergent is used in vivo may be connected with the increased protein concentration in the ascites fluid compared with the incubation medium in vitro. For instance, it has been shown that another nonpolar detergent, Tween-80, in an in vitro system, completely abolishes the resistance of Chinese hamster cells to actinomycin D and daunomycin. However, with an increase the concentration of serum in the incubation medium of the cells from 10 to 20% the cytotoxicity of these antibiotics was reduced by half if the detergent was used. The mechanism of action of serum proteins.

Thus despite the modifying action of TR on accumulation of DX in leukemia P-388/DX cells, the detergent does not alter the pharmacological action of the cytostatic, which makes it impossible for it to be used as a modifier of MDR in vivo.

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