CHANGES IN GLUTATHIONE-S-TRANSFERASE ACTIVITY DURING INDUCTION OF RESISTANCE IN LEUKEMIA P 388 AND ERHLICH'S ASCITES TUMOR CELLS TO DOXORUBICIN

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Overcoming acquired multiple drug resistance is one of the basic problems in the treatment of cancer. Mechanisms of onset of this phenomenon and the role of various factors in its development have been studied and discussed: the vascularization and oxygenation of the tumor, selection of tumor cells initially resistant to the cytostatic, phenotypic changes induced by the drug in the cells, and so on. More recently, particular attention has been paid to the mechanisms of elimination of the preparation form the cells, DNA repair, and detoxication. Glutatione and glutathione-dependent enzymes play a key role in drug detoxication. In particular, there have been reports of the role of SH-compounds in the activation of the semiquinone radicals of doxorubicin (DX), which is accompanied by a decrease in the cytotoxicity of the anthracycline antibiotic [3-5].

There is information in the literature on increased glutathione-S-transferase (GT) activity and a raised glutathione level in tumor cells resistant to DX. The use of these parameters also has been suggested to determine the sensitivity of a tumor to chemotherapy in clinical practice [8, 9]. In this case, it is important to assess correlation between GT activity and resistance of the cell to the cytostatic. Incidentally, most studies of GT activity have been carried out in a system in vitro on tumor cells with high levels of resistance (hundreds or thousands of times), whereas in clinical practice such levels of resistance are not found. It therefore seemed appropriate to study changes in GT activity in cells with resistance to DX induced in vivo.

EXPERIMENTAL METHOD

Reagents and preparations: glutathione, 1-chloro-2,4-dinitrobenzene were obtained from "Serva," West Germany. Doxorubicin and Finoptin were pharmacopoeial preparations. Leukemia P 388 cells, sensitive to DX (P 388, Tumor Strain Bank, All-Union Oncologic Research Center, Academy of Medical Sciences of the USSR), P 388 leukemia cells with induced resistance to DX (P 388/DX), and leukemia P 388 cells with induced resistance to a combination of Finoptin (FP) and DX (P 388/FP + DX) were transplanted intraperitoneally at the rate of $1 \cdot 10^6$ cells in 0.2 ml medium 199, into male DBA/2 mice aged 2-3 months [1, 2]. Cells of Ehrlich's ascites tumor, sensitive to DX (strain ELD, Tumor Strain Bank, All-Union Oncologic Research Center, Academy of Medical Sciences of the USSR) and resistant to DX (ELD/DX) were transplanted intraperitoneally at the rate of $2 \cdot 10^6$ cells in 0.2 ml medium 199 into (CBA × C57BL/6)F_1 hybrid mice aged 2-3 months. Resistance to DX in ELD cells was induced in a system in vivo by injecting therapeutic doses of DX in accordance with the scheme described previously [1]. To maintain the resistance of the strains to DX and to a combination of FP + DX, the animals were given a single injection either of the cytostatic alone or of a combination of it with FP 24 h after transplantation of the tumor.

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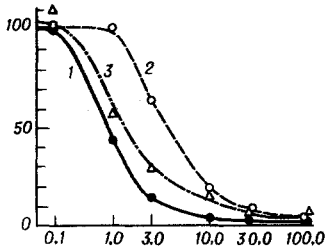

Fig. 1

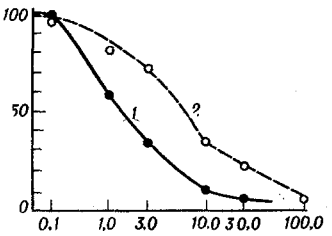

Fig. 2

Fig. 1. Effect of doxorubicin on ^{3}H-uridine incorporation into leukemia P 388, P 388/DX, and P 388/FP + DX cells. Abscissa, concentration of DX (in μg/ml); ordinate, percentage incorporation of ^{3}H-uridine into cells compared with control. Curve 1) P 388, 2) P 388/DX, 3) P 388/FP + DX. Mean results of three measurements are shown.

Fig. 2. Effect of doxorubicin on incorporation of ^{3}H-uridine into ascites cells ELD and ELD/DX. Curve 1) ELD, 2) ELD/DX. Remainder of legend as to Fig. 1.

Activity of cytosolic GT of the tumor cells was determined by the method described previously [7]. Chlorodinitrobenzene was used as the substrate for GT. The kinetics of formation of glutathione—chlorodinitrobenzene was recorded on a "Cary-219" spectrophotometer (USA) at a wavelength of 340 nm, with coefficient of molar extinction ε = 9.6 mM^{-1}.

The level of resistance of the cells was determined by measuring incorporation of ^{3}H-uridine by the short-term incubation test [10]. A cell suspension (500,000 cells/ml) was incubated with different concentrations of DX for 3 h at 37°C. ^{3}H-uridine was added 2 h after the beginning of incubation. Aliquots of the cell suspensions were transferred to cellulose filters, the acid-soluble fraction was washed off, and the incorporated label was determined with a scintillation counter. The concentration of DX inhibiting incorporation of the label by 50% (IC_{50}) was determined graphically.

The protein concentration was determined by Bradford's method [6].

The results were subjected to statistical analysis by the Fisher—Student test. Differences were considered to be significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

The results of a study of the effect of DX in different concentrations on incorporation of ^{3}H-uridine into leukemia P 388, P 388/DX, and P 388/FP + DX cells are given in Fig. 1. An increase in IC_{50} will be observed from 0.9 μg/ml for sensitive cells to 5.3 μg/ml for leukemia P 388/DX cells and to 1.8 μg/ml for leukemia P 388/FP + DX cells. This change in IC_{50} corresponds to a sixfold increase in the level of resistance of the leukemia P 388/DX cells compared with leukemia P 388 cells (the ratio of IC_{50} for the resistant cells to IC_{50} for the sensitive cells).

Similar results showing the effect of different concentrations of DX on ^{3}H-uridine concentration into Erhlich's ascites tumor cells sensitive to DX, and with induced resistance to the preparation are given in Fig. 2. In this case also the curve was shifted to the right, with an increase in IC_{50} from 1.7 μg/ml for ELD cells to 7.6 μg/ml for ELD/DX cells, in agreement with elevation of the level of resistance by 4.5 times for ELD/DX cells compared with ELD cells. Such a low level of resistance of leukemia P 388/DX and ELD/DX cells is probably characteristic of tumors whose resistance was induced in a system in vivo, but at the same time, it is sufficient to produce resistance of the tumor to chemotherapy [1].

Table 1 gives the results of measurement of the level of GT activity in the cells tested. An increase in enzyme activity was demonstrated in resistant leukemia P 388 DX and P 388/FP + DX cells by 3.5 and 4.4 times, respectively, compared with sensitive leukemia P 388 cells. However, induction of activity of these enzymes was not observed in another resistant cell line (ELD/DX) compared with sensitive ELD cells.

TABLE 1. Activity of Glutathione-S-Transferases in Sensitive and Resistant Cells of Leukemia P 388 and Erhlich's Ascites Tumor (M ± m)

Type of cells	Glutathione-S-transferase activity, mmoles/mg protein·min	Ratio of activity of enzymes in resistant and sensitive cells
P 388	0,48±0,04	1
P 388/P 388/DX	1,67±0,19	3,5*
P 388/FP + DX	2,12±0,02	4,4*
ELD	0,77±0,15	1
ELD/DX	0,85±0,15	1,1

Legend. Values for which $p < 0.05$ between GT activity in resistant and sensitive cells are indicated by an asterisk.

Thus the level of GT activity could be determined in tumor cells of all five strains described. Of the three resistant strains, in two cases a raised level of GT activity was found. However, the level of enzyme activity in ELD/DX cells did not differ from that in the sensitive ELD cells. Similar results were obtained in an in vitro study of human leukemia cells resistant to DX and colchicine. For instance, a 70-fold increase in GT activity was observed in resistant cells of line MCF-7/C 10, compared with the sensitive cell line MCF-7. Meanwhile, other sensitive cells (K562/ADM, 2780AD, CEM-VLB100) had the same GT activity as cells of the corresponding sensitive lines [11]. From our point of view, our results in which the development of the resistance of the cells was not accompanied by any change in activity of the test enzyme system, are particularly interesting, for they lead to the conclusion that there is no "rigid" dependence between induction of resistance to DX and GT activity in vivo.

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