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Short Communication

Capability of Adriamycin and Busulfan to Induce Adaptive Response *in Vitro*

ELŻBIETA L. ANUSZEWSKA and JADWIGA H. KOZIOROWSKA

Drug Institute, Chełmska 30/34, 00-725 Warsaw, Poland

Abstract. The capability to induce an adaptive response by low doses of busulfan (BS) or adriamycin (ADR) was studied in two kinds of mammalian cells with acquired or inherent resistance to ADR (ME18/R and V_3) cultured *in vitro*. The results indicate the presence of an adaptive response to ADR in both kinds of used cells pretreated with a low priming dose of ADR. In the same kind of cells no adaptive response to BS after priming with a low dose of this drug was found.

Key words: adriamycin; busulfan; adaptive response.

Introduction

Since the discovery of the adaptive response in *Escherichia coli*[9], many experiments were devoted to tracing a similar phenomenon in eucariotic cells. *In vitro* it has been shown that chronic treatment of mammalian cells with non-toxic doses of alkylating agents renders the cells resistant to killing upon further treatment with toxic doses of these agents[12, 18]. Single incubation of V79 Chinese hamster cells with a subtoxic dose of N-methyl-N'-nitro-N-nitrosoquanidine (MNNG) or methyl methanesulphonate (MMS) modifies the cell sensitivity towards these agents[24].

Adaptation to oxidative damage has also been reported in both bacteria[9, 13, 22] and cultured mammalian cells[8, 10, 15, 25].

The present paper describes experiments on the effects in cells with acquired (ME18/R) or inherent (V_3) resistance to adriamycin (ADR)[2], cultured *in vitro* when they are "primed" with ADR or busulfan (BS) before treatment with a challenge doses of these drugs. Busulfan is a bifunctional alkylating agent used in the clinical treatment of chronic myeloid leukaemia[5, 19]. Adriamycin is one of the widely used chemotherapeutic agents in cancer treatment. From a biochemical viewpoint ADR is a "redox cycling drug" [11, 16, 20].

The appearance of an adaptive response during the treatments with cytostatics cannot be excluded. Therefore, according to the drug and its properties, adaptation could occur for survival and for mutagenesis thus modifying the efficiency of the treatment or the appearance of iatrogenic diseases.

Materials and Methods

Chemicals. ADR was obtained from Farmitalia (Milan, Italy), BS and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) from Sigma (St. Luis USA), 199 medium and minimum Eagle's medium 1959 (MEM) from the Institute of Immunology and Experimental Therapy (Wrocław, Poland), fetal calf serum from Bioproduct (Budapest, Hungary).

Cell cultures. African Green monkey kidney cells (V_3) were grown in 199 medium supplemented with 8% fetal calf serum and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Human melanoma ADR-resistant sublines (ME18/R) were grown in MEM supplemented with 10% fetal calf serum and antibiotics as described above.

Cells were grown in Nunclon flasks in a humidified atmosphere at 37^0C in 5% CO_2. ME18 and V_3 cells were a gift from the Cancer Center-Institute of Oncology (Warsaw, Poland).

Production of ADR-resistant cells. The method described by HERMAN et al.[14] and modified by ANUSZEWSKA et al.[3] was used. Cells in exponential growth were exposed to ADR (5 μg/ml) for 1 h. After treatment, the drug was removed and the cultures washed with PBS. These treated cells were then trypsinized and replated in 100 mm plastic Petri dishes containing 15 ml of medium. These dishes were then incubated at 37^0C and 5% CO_2 atmosphere for 7 days. Surviving colonies, designated as R cells, were then trypsinized and replated into plastic flasks. Thereafter, these R cells were routinely maintained in ADR (0.02 μg/ml) in addition to standard medium. This procedure was used because it was shown that reversion to sensitivity in R cells occurred after approximately 90 days if the latter cells were maintained in growth medium without the drug. Chronic treatment of ME18/R cells with ADR caused a transient lengthening of the doubling times in the first weeks of passaging. The experiments described below were performed after 2 months of passaging. At this time, the doubling time in ME18/R cells did not differ significantly from those of parental cells.

Cytotoxicity studies (MTT assay). MTT assay was performed as described by MOSMANN[21]. The suspension of cells was diluted, usually to 5×10^4 cells/ml in medium and 100 μl of this suspension was placed into individual wells on a 96-well multiplate for 24 h in a humidified atmosphere at 37^0C in 5% CO_2. ADR or BS dissolved in water at "priming" doses was then added in a volume of 100 μl at double strength drug dilution and plates were incubated for 1 h. The wells containing medium without drugs were used for the control of cell viability. Cells were then continuously exposed to ADR or BS at the challenge dose for 24 h. After exposure the plates were inverted to remove the medium and 100 μl of a 5 mg/ml MTT solution in PBS was added to each well and plates were incubated for another 4 h. The plates were then inverted again to remove the unconverted MTT and the formazan crystals were left at the bottom of the wells. These crystals were dissolved in 100 μl of dimethylsulphoxide by agitating on a plate shaker for 5 min and absorbance at 500 nm was measured (spectrophotometer Multiscan Plus). The effects of drug treatment were determined by calculating the absorbance of the test wells as a percentage of that of the control wells. In all experiments, 8 replicate wells were used at each point.

The same manipulations except the pretreatment with a low dose of ADR or a low dose of BS were carried out with the cells receiving the challenge doses alone.

Results

The cells employed in this study were exposed to low priming doses of ADR (0.05 μg/ml) or BS (0.1 μg/ml). The surviving of ME18/R and V_3 cells after priming doses of both drugs were on the level of untreated cultures. Primed and unprimed cells were then treated with challenge doses of ADR (0.5 μg/ml and 5.0 μg/ml) or BS (25 μg/ml, 50 μg/ml and 75 μg/ml). Both, ME18/R and V_3 ADR primed cells demonstrated significant survival enhancements. After treatment with ADR at priming dose survival of ME18/R cells increased from 25 to 32% and V_3 cells from 16 to 39% over their unprimed counterparts following a single high dose treatment of the drug (Table 1).

Table 1. Survival responses in cells primed with adriamycin at the concentration 0.05 μg/ml

Cells	Challenge dose (μg/ml)	Percent of survival cells	
		primed	unprimed
ME18/R	0	100.0±3.0	100.0±2.1
	0.5	90.7±3.2	65.8±4.2
	5.0	75.8±2.9	43.6±3.6
V_3	0	100.0±4.1	100.0±6.3
	0.5	98.8±7.3	82.4±2.4
	5.0	95.3±6.8	56.7±4.8

24 h old cultures were exposed to adriamycin at prime dose for 1 h or received no low dose exposure (unprimed cells). Cells were then challenged with a single high dose of the drug and cell survival was assessed after 24 h as indicated under Materials and Methods.

In contrast, cells treated with BS did not demonstrate adaptive response and an apparent decrease in survival of both kinds of primed cells was observed. The survival of primed cells was lower than that of high dose BS treated cells which were not given a low dose priming exposure (Table 2).

An adaptive survival response to ionizing radiation and to certain groups of chemicals seems to be dependent on the genetic features of the cells[24]. Our data indicate that both kinds of the cells employed in the present study express the capability to adapt to ADR.

Both kinds of cells pretreated with low dose of BS did not express an adaptive response but rather a synergic response with challenge doses.

Table 2. Survival responses in cells primed with busulfan at the concentration 0.1 μg/ml

Cells	Challenge dose (μg/ml)	Percent of survival cells	
		primed	unprimed
ME18/R	0	100.0±1.2	100.0±6.7
	25	7.1±3.5	81.3±1.1
	50	2.8±1.2	75.5±3.1
	75	1.9±0.5	68.2±5.2
V_3	0	98.3±7.1	100.0±1.1
	25	6.5±4.8	92.3±2.8
	50	4.2±3.8	85.4±3.3
	75	5.4±2.1	61.1±4.7

24 h old cultures were exposed to busulfan at prime dose for 1 h. Cells were then challenged with a single high dose of the drug and survival was assessed sfter 24 h as described under Materials and Methods.

Discussion

Adriamycin is a common antineoplastic drug used in the therapy of many forms of cancer including breast cancer, acute leukemia, sarcomas and lymphomas.

Its mechanism of action is probably complex. Adriamycin inhibits synthesis of DNA and RNA, gives a rise in protein-associated breaks and crosslinks in DNA, induces mutation, sister-chromatid exchange and chromosome aberrations[17]. We have shown previously that the cells employed now could be adapted to high doses of ADR by priming with low dose of H_2O_2[2, 3]. In the present study we have shown that the adaptive response to ADR can be induced by low doses of ADR also. It is tempting to suppose that the effects observed now are the consequence of the action of H_2O_2 or oxygen radicals generated by ADR.

Busulfan is a four-carbon chain member of the homologous of dialkanesulfonic acid esters. Busulfan produces crosslinks in DNA through the four-carbon bridge by displacement of the first one and then the other methylsulfonate group[4]. BS is a weakly bifunctional alkylating agent[1]. A failure to induce adaptive response was also demonstrated for MMS[24]. A large body of evidence indicates that adaptive response can occur *in vivo*[6, 7, 23, 26]. In our study both kinds of cells pretreated with low doses of BS did not express an adaptive response. Our results show the existence of differences between individual cytostatics. The knowledge of the possibility of such differences to occur can be useful in evaluating the efficiency of cancer therapy.

This study provides new data on the effects produced by two antineoplastic agents but does not explain the cause of the differences in their behavior. It is hoped that by studying these systems the differences in the capability of ADR and BS to establish adaptive response might be understood.

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