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DNA repair of adriamycin induced damage in mouse and human cells differing in catalase activity★✪

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Catalase is known to counteract cytotoxic effect of adriamycin, used as an anti-neoplastic drug. In cells with low catalase activity no repair of adriamycin induced lesions was observed up to 48 h post treatment. In cells with high catalase activity after 48 h the repair was either complete or partial depending on the human or mouse cell type used.

Adriamycin, an anthracycline antibiotic is one of the most effective anticancer agents but application of chemotherapy is severely limited by its cytotoxicity. The mechanism of adriamycin cytotoxicity appears to be linked to formation of semiquinone free radical. In the presence of molecular oxygen, the semiquinone rapidly reduces the oxygen to superoxide that is converted to hydrogen peroxide (H_2O_2) [1].

Catalase is a major antioxidant enzyme involved in detoxification of hydrogen peroxide. Lee *et al.* [2] have demonstrated that catalase offers significant protection against adriamycin induced effects, and suggested that H_2O_2 might play a major role in mediating the toxic effects of adriamycin *in vitro*.

Our previous study also revealed a possible association between the levels of catalase and the cytotoxic response to the action of adriamycin [3]. Thus, we have expected that the cells differing in catalase activity treated with adriamycin may give diversified response with respect to DNA damage and repair.

In the current work, we quantified catalase activity in several human cell lines isolated from various xeroderma pigmentosum patients and normal donors and in primary cell cultures from four inbred strains of mice, to choose two pairs of cells with the greatest dif-

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Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

ferences in catalase activity. Single-strand breaks in DNA and repair of these lesions in those cells following exposure to adriamycin were studied by alkaline elution method at 0, 24 and 48 h after treatment.

MATERIALS AND METHODS

Chemicals. Minimum Eagle's medium 1959 (MEM) and phosphate-buffered saline (PBS) from IITD (Wrocław, Poland); fetal calf serum from Bioproduct (Budapest, Hungary); lauryl sulfate (sodium salt, L-4509) from Sigma; phosphate buffer (40 ml 0.07 M KH_2PO_4 + 60 ml 0.07 M Na_2HPO_4), pH 7.00.

Human cells. Normal human fibroblasts and xeroderma pigmentosum cells were obtained from the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852, U.S.A.).

Mouse embryonic cells. Primary cell cultures from mouse embryos (on day 19th of gestation) were prepared by a conventional method as described previously [4]. Cells at early passages (2nd to 3rd) were used in all experiments. Mouse inbred strains were obtained from Cancer Center – Institute of Oncology, Dept. Laboratory Animal Genetics and Breeding (Warsaw, Poland). Cell cultures were grown in MEM, supplemented with 10% fetal calf serum and antibiotics (100 u/ml of penicillin and 100 μg/ml of streptomycin).

Cell extracts. The cell cultures, cultivated at 37°C and 5% CO_2 atmosphere for 48 h, were used to determine the catalase activity. Cells were scraped off, resuspended in phosphate buffer, pH 7.00, and sonicated. The enzyme activity and protein concentration measurements were carried out on the homogenates [5, 6]. The protein contents were determined as described by Schacterle & Pollack [7].

Spectrophotometric determination of catalase activity. The decomposition of H_2O_2, catalyzed by catalase was followed by ultraviolet spectroscopy at 240 nm. Catalase specific activity at 25°C was defined in terms of micromoles of H_2O_2 consumed per minute per milligram of the protein sample [5, 6].

Cytotoxicity studies (MTT assay). The MTT assay was performed as described by Anuszewska *et al.* [3]. In this work cells were exposed to adriamycin at the concentration range 0.005–5.0 μg/ml.

Alkaline elution assay. Alkaline elution was performed as described by Kohn [8] and modified by Anuszewska & Gruber [9]. DNA was labelled by adding [^{14}C]thymidine after 24 h of cell growth. Radioactive medium was removed after 48 h and then the cells were exposed to adriamycin at the various concentrations applied for 1 h at 37°C. Single-stranded DNA was eluted from the filter in the dark by passing of 20 mM Na_2EDTA (pH 12.5) through the filter with the use of the peristaltic pump (about 0.05 ml/min) and 2 ml fractions were collected. Radioactivity in fractions was measured using liquid scintillation counter and results were expressed as percentage of the total radioactivity recovered.

RESULTS AND DISCUSSION

On the basis of determinations of catalase activity in several human cell lines and primary cultures of mouse embryo cells, two pairs of cells considerably different in catalase activity were chosen as presented in Table 1. Sensitivity of these cells to adriamycin was defined on the basis of IC_{50} values obtained for each kind of cells in the MTT assay (see Table 2). Repair of DNA single strand breaks was studied at adriamycin concentrations both above and below IC_{50} values. The concentration of adriamycin of 0.5 μg/ml was chosen as optimal for comparing the effects of treatment of the cells.

DNA single strand breaks and repair in cells, following exposure to that drug were observed by the alkaline elution method. As shown in Fig. 1, DNA lesions in normal human fibroblasts CRL 2088 were in part repaired within 24 h after drug removal and completely re-

Table 1. Catalase activity in human and mouse cells.

The pairs of cells were chosen on the basis of the highest difference in catalase activity. Values represent the mean ± S.D. from 3–6 independent determinations).

	Human cells		
	CRL 2088	CRL 1307	
Catalase (u/mg protein)	Normal	Xeroderma pigmentosum	Ratio
	72.4 ± 4.0	20.6 ± 0.7	3.5
	Mouse cells		
	AKR	BALB/c	Ratio
Catalase (u/mg protein)	71.3 ± 6.1	32.0 ± 1.3	2.2

Table 2. IC_{50} values for adriamycin in chosen human and mouse cells determined by the MTT assay.

Cells were exposed to adriamycin at the concentration range 0.005–5.0 μg/ml. Values represent the mean from three separate experiments.

Cells	IC_{50} (μg/ml)
CRL-2088	0.25
CRL-1307	0.25
AKR	0.5
BALB/c	0.05

paired within 48 h at adriamycin concentration of 0.5 μg/ml. The elution pattern after treatment at 0.25 μg/ml was similar to that at 0.5 μg/ml. This is in agreement with our earlier observation with human cells treated with adriamycin [9]. At lower concentration of drug no significant differences in elution patterns between normal and xeroderma pigmentosum fibroblasts were observed. No repair of single strand breaks was observed in xeroderma pigmentosum CRL 1307 cells up to 48 h at any of the adriamycin concentrations tested.

As far as the mouse cells are concerned (see Fig. 2) our studies revealed incomplete repair till 48 h after treatment with adriamycin (0.5 μg/ml) in AKR cells and lack of any repair in BALB/c cells up to 48 h at any tested concentration of this drug. At lower concentrations of adriamycin no significant differences were observed between AKR and BALB/c cells.

Xeroderma pigmentosum is an autosomal recessive genetic disease. It is clinically characterized by an extreme sunlight sensitivity and a high incidence of skin cancer [10]. Several observations reported in the literature suggest that the antioxidant status of xeroderma pigmentosum may by altered in com-

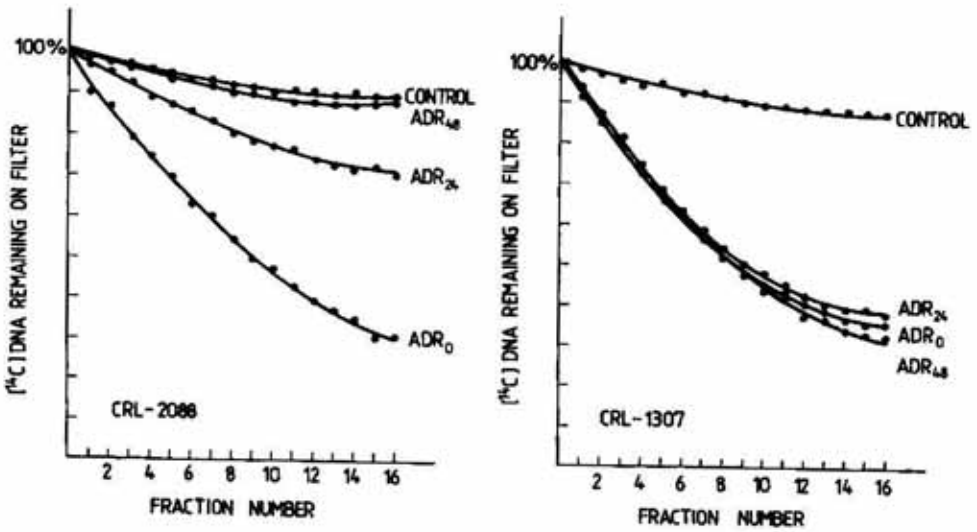

Figure 1. Representative alkaline elution profiles of CRL-2088 (a) and CRL-1307 (b) cells at various time intervals (0, 24, 48 h) after adriamycin treatment (0.5 μg/ml for 1 h).

Results are expressed as percentage of the total radioactivity recovered.

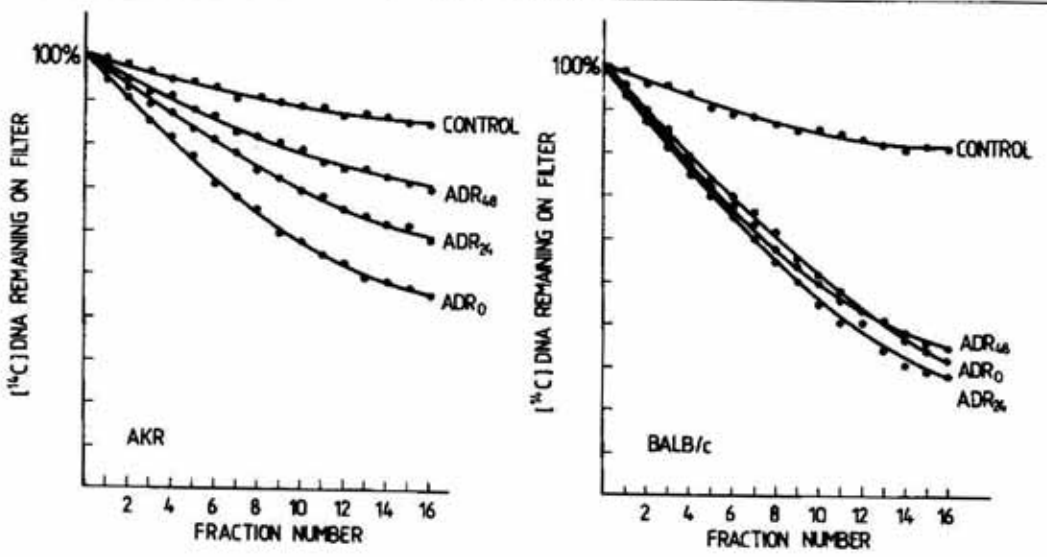

Figure 2. Representative alkaline elution profiles of AKR (a) and BALB/c (b) cells at various time intervals (0, 24, 48 h) after adriamycin treatment (0.5 μg/ml for 1 h).

Results are expressed as percentage of the total radioactivity recovered.

parison to normal cells [11–13]. Also female BALB/c mice are rather sensitive to the induction of mammary cancer after whole-body irradiation [14].

If the mechanism of action of adriamycin is in part *via* free radicals i.e. similar to the mechanism of action of UV radiation, there should be difference in cytotoxic effects and repair ability in cells differing in catalase activity.

The repair of adriamycin induced DNA breaks was strongly reduced in xeroderma pigmentosum and BALB/c cells in comparison to normal human and AKR cells, respectively (Figs. 1 and 2). This repair correlates with the catalase activity in cells (Table 1) suggesting that catalase plays an important role in protecting DNA against the cytotoxic action of adriamycin. However, there are no differences in sensitivity of normal human and xeroderma pigmentosum cells to adriamycin (Table 2). This suggests that removal of breaks after adriamycin treatment is not necessary for survival of xeroderma pigmentosum cells [15] and that other defence mechanisms are also involved. In contrast, in mouse cells a correlation between cytotoxicity, repair and catalase activity does exist.

The lower repair ability observed in AKR cells than in normal human cells (Fig. 1 *vs* Fig. 2) is in accordance with the observations by Yagi [16] who compared DNA repair capacity in mouse and human cells after UV irradiation.

From these results it is not possible to draw definitive conclusions concerning the importance of catalase in adriamycin induced breaks repair and counteracting the cytotoxicity of this drug. Further investigations in the same experimental conditions with the use of D-mannitol, thiourea, catalase and superoxide dismutase are in progress.

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