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Studies on Adaptation to Adriamycin in Cells Pretreated with Hydrogen Peroxide

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ABSTRACT. Various investigations have reported the occurrence in bacterial and mammalian cells of an adaptive response to the toxic effects of oxidants or agents that cause oxidation via redox reactions. In our previous study, it was shown that several cell lines pretreated with a low dose of hydrogen peroxide (H_2O_2) exhibited an adaptive response to subsequent high doses of adriamycin (ADR), whereas other cell lines did not. Based on the observation that the cell lines utilized differed in their sensitivity towards adriamycin, we undertook the present investigation with the goal of evaluating possible relationships between the levels of antioxidant enzymes and sensitivity towards adriamycin. Another aim was to determine relationships between the inducibility of these enzymes and the occurrence of adaptation. We utilized African Green monkey kidney (V_3), human embryo (CLV98), human melanoma (ME18), and Chinese hamster ovary (CHO) cell lines and experimentally developed adriamycin-resistant human melanoma ($ME18/R^N$) and Chinese hamster ovary (CHO/R^N) cell sublines. Cytotoxicity was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and trypan blue exclusion. The levels of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were determined in the same kind of experiment as that revealing the occurrence of adaptation. The rank order established for catalase activities was similar to that for sensitivity towards adriamycin. Aberrant increases in the tested enzymes were demonstrated in experimental groups of all kinds of cells. We conclude that in our cell systems catalase is a major determinant of adriamycin resistance. Whether the occurrence of the adaptive response under study is dependent on the contribution of catalase, itself dependent on the degree of resistance to the drug, is discussed. BIOCHEM PHARMACOL **54**;5:597–603, 1997.

KEY WORDS. adriamycin; adaptation; antioxidant enzymes

Pretreatment with low doses of hydrogen peroxide (H_2O_2)† or with superoxide generating agents renders bacteria more resistant to oxidative damage that normally would be lethal [1–3]. Attempts have been made to trace a similar phenomenon in mammalian cells. Spitz *et al.* [4] demonstrated that mammalian cells treated with a bolus of H_2O_2 undergo a transient adaptive response that results in protection from a subsequent challenge with the oxidant. According to Laval [5], pretreatment with nontoxic doses of H_2O_2 or xanthine-xanthine oxidase reduces the lethal effect of gamma rays in both Chinese hamster ovary cells (CHO) and rat hepatoma (H_4) cell lines. In a recent study, Wiese *et al.* [6] reported a transient adaptation to the oxidative stress of H_2O_2 exposure in five mammalian cell lines.

In bacterial cells, pretreatment with H_2O_2 seems to be related to the induction of catalase [2]. A moderate increase (about twofold) of superoxide dismutase was detected in CHO cells pretreated with xanthine-xanthine oxidase [5]. Wiese *et al.* [6] found that HA-1 cells (a defined CHO subclone) preexposed to relatively low "priming" treatment with H_2O_2 are able to survive a subsequent challenge without increasing their overt antioxidant activity.

Recent work performed in our laboratory has demonstrated that some cell lines exposed to nontoxic doses of H_2O_2 became less sensitive to the cytotoxic effect induced by a high dose of adriamycin (ADR) [7], while other cell lines did not exhibit this kind of "adaptive" response. The results of this study suggest that the occurrence of this phenomenon depends in some way on inherent or acquired resistance. Exogenous superoxide dismutase, catalase, and hydroxyl radical scavengers were shown to partially protect human mammary (MCF) tumor cells from adriamycin cytotoxicity [8].

We undertook the present investigation with the goal of reexamining whether a relationship exists between the adriamycin-resistant phenotype and the capability of the cells to adaptive response. Another aim of this work was to compare the levels of catalase, superoxide dismutase, and

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† *Abbreviations:* H_2O_2, hydrogen peroxide; V_3, African Green monkey kidney cells; CLV98, human embryo cells; ME18, human melanoma cells; CHO, Chinese hamster ovary cells; $ME18/R^N$, human melanoma adriamycin-resistant subline; CHO/R^N, Chinese hamster ovary adriamycin-resistant subline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ADR, adriamycin; MEM, minimum Eagle's medium 1959; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase.

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glutathione peroxidase in different kinds of cells (sensitive and resistant) in four experimental groups: 1) untreated cells; 2) H_2O_2-pretreated cells; 3) ADR-challenged cells; and 4) H_2O_2-pretreated and ADR-challenged cells.

MATERIALS AND METHODS

Chemicals

Adriamycin was obtained from Farmitalia (Milan, Italy); hydrogen peroxide 30% from Sigma Chemical Co. (St.Louis, MO); RANSOD—the kit for the measurement of the superoxide dismutase (SOD) activity, and RANSEL—the kit for the measurement of the glutathione peroxidase activity—from Randox (Crumlin, UK); minimum Eagle's medium 1959 (MEM) and Parker's medium No. 199 from WSS (Lublin, Poland); and fetal calf serum from Bioproduct (Budapest, Hungary).

Cell Cultures

Human melanoma cells (ME18), Chinese hamster ovary cells (CHO) and human embryo cells (CLV98) were grown in MEM supplemented with 10% fetal calf serum and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin). African Green monkey kidney cells (V_3) were grown in Parker's medium supplemented with fetal calf serum and antibiotics as described above. Cells were grown in Nunclon flasks in a humidified atmosphere at 37°C in 5% CO_2. CLV98 were obtained from Serum and Vaccines Research Laboratories, Warsaw, Poland. The other cell lines were a gift from the Institute of Oncology, Warsaw, Poland. The cells were continually checked for lack of mycoplasma contamination. As determined by haemocytometer (Coulter Electronics, Inc., USA) counts of several cultures on 3 consecutive days, the doubling times for CHO cells were 19 ± 2.9 hr, for ME18 cells 23 ± 2.7 hr, for V_3 cells 25.3 ± 2.1 hr, and 24 ± 2.5 hr for CLV98 cells. CLV98 cells were employed between 9–15 passage in vitro.

Production of ADR-Resistant Cells

The adaptation of the method described by Herman *et al.* [9] was used. Cells in exponential growth were exposed to ADR (5 μg/mL) for 1 hr. 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°C 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 CHO/R or ME18/R cells with adriamycin 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 times in CHO/R and ME18/R cells did not differ significantly from those of parental cells.

Cytotoxicity of ADR

For measurement of ADR cytotoxicity, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and trypan blue exclusion were employed.

MTT Assay

MTT assay was performed as described by Mosmann [10]. The suspension of cells was diluted, usually to 5×10^5 cells/mL, in MEM and 100 μL of this suspension was placed into individual wells on a 96-well multiplate. ADR dissolved in water was then added in a volume of 100 μL at double strength drug dilution. The wells, containing MEM without ADR, were used for the control of cell viability. The plate was then incubated in a humidified atmosphere for 72 hr at 37°C in 5% CO_2. Cells were continuously exposed to ADR at the concentration range of 1 nM to 1 mM throughout this period. After the ADR exposure, the plate was inverted to remove the medium, then 100 μL of a 5 mg/mL MTT solution in PBS was added to each well and the plate was incubated for another 4 hr. The plate was 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. The effects of ADR treatment were determined by calculating the absorbance of the test wells as a percentage of that of the control wells. In all experiments, eight replicate wells were used at each point.

Trypan Blue Exclusion Test

Twenty-four hours after seeding in 25 cm^2 Nunclon flasks (ca. 150 000 cells/flask), the cultures were exposed to complete media free from ADR (control cultures) or to media containing ADR at different concentrations (1 nM to 1 mM) obtained by dissolving the drug in sterile water. Appropriate dilutions of adriamycin were added to fresh medium. After 24-hr treatment the medium was removed and the cultures were washed twice with PBS and trypsinized. Cell number and viability were determined by a haemocytometer and trypan blue exclusion using a 0.4% solution of trypan blue in PBS.

Cell suspensions (0.5 mL) were exposed to trypan blue solution (0.1 mL) for 5 min.

Cytotoxicity of H_2O_2

Hydrogen peroxide concentration was determined before each experiment by measuring the absorbance of a dilute

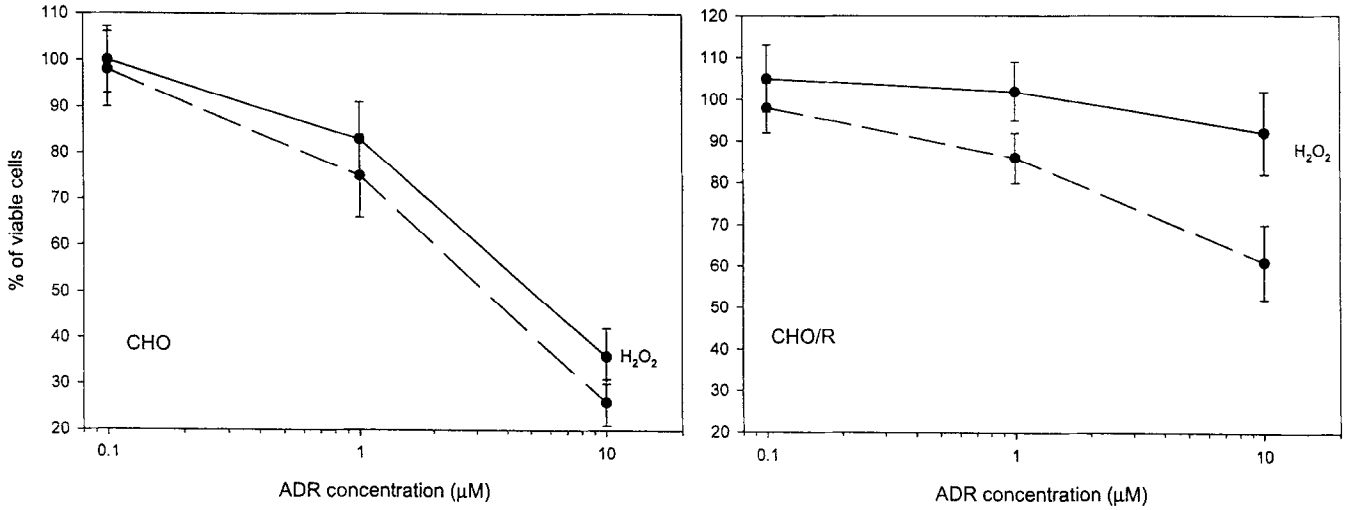

FIG. 1. Effect of H_2O_2 pretreatment on cytotoxicity of ADR towards parental cells—CHO, and resistant subline—CHO/R. Broken line represents cells treated with ADR only and permanent line represents cells pretreated with H_2O_2 and then incubated with ADR. Twenty-four-hour cultures were pretreated with H_2O_2 or fresh medium for 30 min, and then washed and incubated with increasing concentrations of ADR for 24 hr at 37°C. Cell viability was measured by the trypan blue exclusion test. The data represent the percent of viable cells compared to control (cells incubated in the absence of ADR). Each point is the mean ± SD of three independent determinations.

solution at 240 nm. The H_2O_2 solution was also filter sterilized prior to addition to cell cultures. The cytotoxicity of H_2O_2 was estimated at the concentration range of 0.01 μg/mL to 10 μg/mL, using the MTT method and trypan blue exclusion. The chosen concentration of H_2O_2, 0.07 μg/mL, was not toxic for all kinds of cells used.

Evaluation of the Effects of H_2O_2 Pretreatment on the Cytotoxicity of ADR

After 24 hr, the cultures were washed twice with PBS and pretreated with H_2O_2 at the concentration of 0.07 μg/mL. After incubation for 30 min, the medium with H_2O_2 was removed, the cultures washed with PBS and the cells exposed to ADR at different concentrations (0.1 μM to 10 μM) for 24 hr. Then, cell viability was measured by trypan blue exclusion.

Determinations of Enzyme Activities

Enzyme activities were determined in four groups of cultures according to the following experimental procedure:

1. Untreated cells (control cultures). Cell cultures grown for 24 hr were exposed to complete media for 30 min and thereafter were washed twice with PBS and incubated for the next 24 hr with appropriate medium.
2. H_2O_2-pretreated cells. Twenty-four-hour-old cultures were exposed to complete media containing 0.07 μg/mL H_2O_2 for 30 min. After treatment with H_2O_2, cells were rinsed twice with PBS and then incubated for the next 24 hr with complete media.
3. ADR-challenged cells. Twenty-four-hour-old cultures were exposed to complete media for 30 min and then washed twice with PBS. After that, cells were incubated for the next 24 hr with medium containing ADR at a concentration of 50 μM for resistant sublines and 0.5 μM for other kinds of cells.
4. H_2O_2-pretreated and ADR-challenged cells. These experiments involved pretreating 24-hr-old cultures with H_2O_2 at a concentration of 0.07 μg/mL for 30 min and then exposing them to ADR-challenge concentrations for the next 24 hr to assess possible adaptive increases in antioxidant enzyme activities.

Cell Extracts

The cell cultures, cultivated at 37°C and 5% CO_2 atmosphere for 48 hr, were used to determine enzyme activities. Cells were scraped, resuspended in phosphate buffer, pH = 7.00 and sonicated. The enzyme activity and protein concentration measurements were carried out on the homogenates. The protein content was determined according to the method described by Schacterle and Pollack [11].

Spectrophotometric Determination of Catalase (CAT) Activity

The decomposition of H_2O_2 catalyzed by CAT could be followed by ultraviolet spectroscopy due to the absorbance of H_2O_2 in this region (240 nm). CAT-specific activity was defined in terms of micromoles of H_2O_2 consumed per minute per milligram of the protein sample.

Colorimetric Determination of SOD Activity

This method employs xanthine and xanthine oxidase to generate the superoxide radicals which react with iodonitrotetrazolium violet (INT) to form a red formazan form.

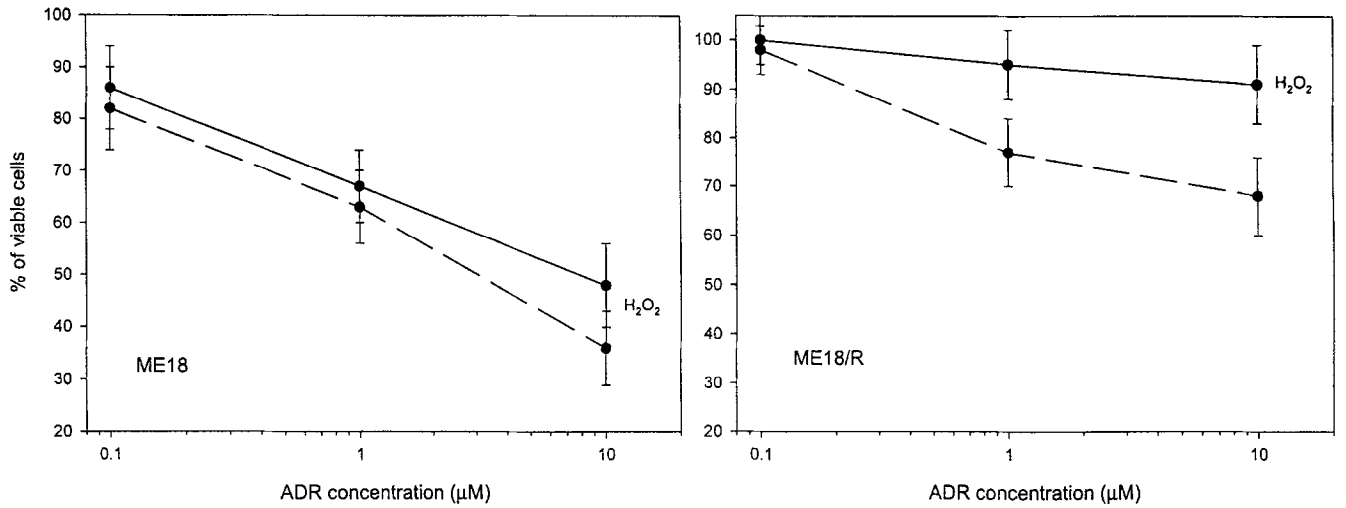

FIG. 2. Effect of H_2O_2 pretreatment on cytotoxicity of ADR towards parental cells—ME18 and resistant subline—ME18/R. Broken line represents cells treated with ADR only and permanent line represents cells pretreated with H_2O_2 and then incubated with ADR. Twenty-four-hour cultures were pretreated with H_2O_2 or fresh medium for 30 min, and then washed and incubated with increasing concentrations of ADR for 24 hr at 37°C. Cell viability was measured by the trypan blue exclusion test. The data represent the percent of viable cells compared to control (cells incubated in the absence of ADR). Each point is the mean ± SD of three independent determinations.

The SOD activity was measured by the degree of inhibition of this reaction. Units of SOD were obtained from the standard curve (percentage inhibition against $\log_{10}$-standard concentration in SOD U/mL). The final results were defined as units of SOD per milligram of the protein sample.

Spectrophotometric Determination of Glutathione Peroxidase (GPx) Activity

GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. This process could be followed by ultraviolet spectroscopy due to the absorbance of NADPH in this region (340 nm). Enzyme-specific activity was defined in terms of micromoles of NADPH oxidized per minute per milligram of the protein sample.

RESULTS

In our previous study [7], a protective effect of preexposure to H_2O_2 against ADR damage was found in V_3 cells, ME18/R, and CHO/R cells. No occurrence of adaptive

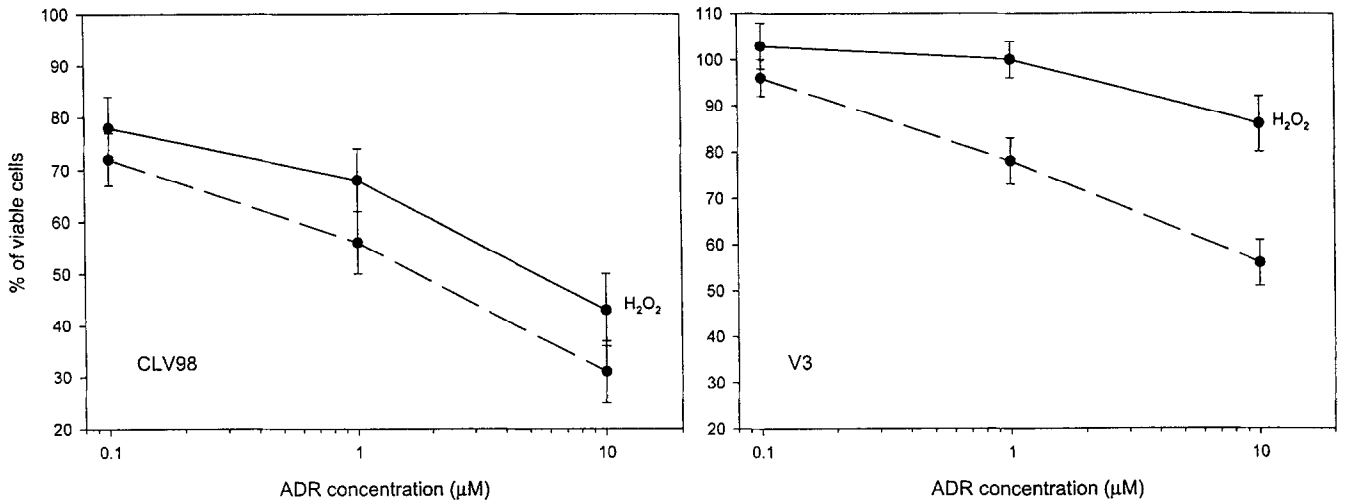

FIG. 3. Effect of H_2O_2 pretreatment on cytotoxicity of ADR towards CLV98 cells and V_3 cells. Broken line represents cells treated with ADR only and permanent line represents cells pretreated with H_2O_2 and then incubated with ADR. Twenty-four-hour cultures were pretreated with H_2O_2 or fresh medium for 30 min, and then washed and incubated with increasing concentrations of ADR for 24 hr at 37°C. Cell viability was measured by the trypan blue exclusion test. The data represent the percent of viable cells compared to control (cells incubated in the absence of ADR). Each point is the mean ± SD of three independent determinations.

TABLE 1. IC_{50} values for ADR in different cell lines

Cell lines	Trypan blue exclusion μM	MTT assay μM
ME18	2.0	0.01
CLV98	2.0	0.05
CHO	5.0	0.1
V_3	20.0	5.0
ME18/R	30.0	5.0
CHO/R	80.0	50.0

Approximate values of IC_{50} were calculated from growth inhibition studies performed as described under Materials and Methods. Before determining the cell number with trypan blue exclusion, cells were treated with ADR at different concentrations, from 1 nM to 1 mM, for 24 hr. Before determining the cell number using the MTT method, cells were continuously exposed to ADR at the concentration range of 1 nM to 1 mM for 72 hr.

response was observed in CLV98, ME18, and CHO cells. In this study, we repeated the experiments with V_3 cells, CLV98 cells, ME18, and CHO cells and performed analogous experiments with two newly developed the human melanoma adriamycin-resistant ($ME18/R^N$) and Chinese hamster ovary adriamycin-resistant (CHO/R^N) sublines. As shown in Figs. 1–3, pretreatment with H_2O_2 improved viability of ADR-treated V_3, CHO/R, and ME18/R cells but did not improve viability of CLV98, ME18 and CHO cells.

The goal of the next set of experiments was to compare the sensitivity of these cell lines to the action of ADR. ADR cytotoxicity was determined by two assays using a range of concentrations of the drug. The IC_{50} values (as shown in Table 1) determined by trypan blue exclusion after 24 hr of drug treatment were 2 to 5 μM for the CLV98, CHO and ME18 cells and 20 to 50 μM for the V_3, ME18/R, and CHO/R cells. The IC_{50} values for ADR determined by MTT assay after 72 hr of drug treatment were 0.01 μM to 0.1 μM for CLV98, CHO, and ME18 cells and 5 μM to 50 μM for V_3, ME18/R, and CHO/R cells. These apparent differences in the IC_{50} values resulted from differences in the time of cytotoxicity determinations and slight differences in the methods. Irrespective of the assay employed, the first group of cell lines was found to be 10- to 500-fold more sensitive to ADR than the second group of cell lines.

The levels of CAT, SOD and GPx were determined in four experimental groups: 1) untreated cells; 2) H_2O_2-pretreated cells; 3) ADR-treated cells; and 4) H_2O_2-pretreated and ADR-challenged cells. The results of these experiments are shown in Tables 2–4. The CAT activity was found to be higher in CHO/R^N and $ME18/R^N$ sublines compared with parental cell lines. The SOD activity in CHO/R^N cells was unchanged towards the CHO cell line. This enzyme had the highest activity in $ME18/R^N$ cells compared to all other cell lines. The GPx activity was at the same level (in CHO/R^N cells) or lower (in $ME18/R^N$ cells) compared with parental cell lines. The rank order of CAT activity values for all cell lines tested showed: $CHO/R^N > ME18/R^N > V_3 > CHO > CLV98 > ME18$ and was similar to the rank order of IC_{50} values (comp. Table 1).

As shown in the same tables, pretreatment with H_2O_2 induced a moderate increase in SOD activity exclusively in ME18 cells. GPx and CAT activities remained unchanged in all kinds of cells. Twenty-four hours after treatment with ADR, the levels of CAT were significantly increased in CLV98 cells. At this time, ME18, CLV98, and V_3 cells displayed a significantly higher SOD activity. Treatment with ADR induced a moderate increase in the activity of GPx in $ME18/R^N$, V_3, and CLV98 cells. From comparisons of the data presented in Tables 2–4, it can be seen that pretreatment with H_2O_2 and challenge with ADR caused a significant elevation in GPx activity in ME18, $ME18/R^N$,

TABLE 2. Antioxidant enzyme activities in sensitive and resistant cell lines (units/mg protein).

Enzymes	Treatment: Adaptive agent	Treatment: Challenge drug	Cells: CHO	Cells: CHO/R^N	CHO/R^N / CHO
CAT	—	—	30.9 ± 6.4	54.6 ± 0.1	1.76
units/mg	H_2O_2	—	33.3 ± 4.1	60.1 ± 1.1	
protein	—	ADR	33.2 ± 7.0	58.7 ± 5.1	
	H_2O_2	ADR	33.4 ± 4.8	57.3 ± 13.0	
SOD	—	—	2.1 ± 0.4	2.5 ± 0.3	1.19
units/mg	H_2O_2	—	2.7 ± 0.0	3.1 ± 0.3	
protein	—	ADR	2.6 ± 0.1	3.1 ± 0.1	
	H_2O_2	ADR	3.1 ± 0.1*	3.3 ± 0.2*	
GPx	—	—	67.2 ± 11.9	64.4 ± 8.4	0.96
units/mg	H_2O_2	—	84.9 ± 13.2	62.7 ± 5.4	
protein	—	ADR	75.8 ± 15.2	75.8 ± 12.5	
	H_2O_2	ADR	80.1 ± 12.3	100.8 ± 11.3*	

CHO and CHO/R^N cells were pretreated with H_2O_2 at a concentration of 0.07* μg/mL and then challenged with ADR for 24 hr at a concentration of 50 μM for resistant sublines and 0.5 μM for sensitive cells.

Enzyme activities were assayed as described in Materials and Methods. Values represent the mean ± SD from 12–23 determinations in separate cultures. Significantly different from control untreated cells: $*P < 0.1$.

TABLE 3. Antioxidant enzyme activities in sensitive and resistant cell lines (units/mg protein).

Enzymes	Treatment: Adaptive agent	Treatment: Challenge drug	Cells: ME18	Cells: ME18/R^N	ME18/R^N / ME18
CAT	—	—	9.3 ± 1.3	43.9 ± 5.3	4.72
units/mg	H_2O_2	—	8.2 ± 0.2	48.1 ± 4.7	
protein	—	ADR	11.6 ± 0.4	44.1 ± 1.8	
	H_2O_2	ADR	10.4 ± 2.1	41.8 ± 3.5	
SOD	—	—	1.7 ± 0.1	5.6 ± 1.3	3.34
units/mg	H_2O_2	—	2.6 ± 0.0*	6.2 ± 0.8	
protein	—	ADR	5.1 ± 0.4**	6.3 ± 1.2	
	H_2O_2	ADR	3.7 ± 0.2**	5.8 ± 1.1	
GPx	—	—	52.4 ± 9.2	26.9 ± 1.3	0.51
units/mg	H_2O_2	—	58.8 ± 11.4	32.6 ± 5.9	
protein	—	ADR	60.7 ± 9.5	38.1 ± 6.6*	
	H_2O_2	ADR	67.8 ± 5.1*	36.8 ± 4.1*	

ME18 and ME18/R^N cells were pretreated with H_2O_2 at a concentration of 0.07 μg/mL and then challenged with ADR for 24 hr at a concentration of 50 μM for resistant sublines and 0.5 μM for sensitive cells.

Enzyme activities were assayed as described in Materials and Methods. Values represent the mean ± SD from 9–18 determinations in separate cultures. Significantly different from control untreated cells: *$P < 0.1$; **$P < 0.01$.

V_3, and CHO/R^N cells. The activity of SOD was elevated in all kinds of cells except ME18/R^N cells. The CAT activity was augmented exclusively in CLV98 cells.

DISCUSSION

In the present work, experiments were carried out to study further the adaptive response in different cell lines conditioned with H_2O_2 and challenged with ADR. As reported previously [7], the cross adaptation observed seems to depend on intrinsic or experimentally developed resistance of tested cell lines towards ADR. According to Minotti [12] mechanism of ADR cytotoxicity appears to be linked to the formation of ADR^-, and hence, superoxide anion and H_2O_2, thereby releasing iron for generation of hydroxyl radicals. Following the considerations that some cellular antioxidant enzymes may be important both for ADR resistance [13–17] and for adaptation induced by H_2O_2 [5], we measured SOD, CAT, and GPx activities in the untreated control cultures and in three groups of experimental cultures. The measurements of the activities of these enzymes in untreated control cells revealed that these activities vary among wild-type CHO and ME18 cell lines and their resistant sublines. Different variations in the activity of antioxidant enzymes in experimentally developed ADR-resistant cell lines have been observed by other authors [13–15]. Elevated levels of CAT and glutathione-*S*-transferase were demonstrated in human small cell lung cancer cell lines that were established during clinical treatment with ADR [16]. The results presented in our

TABLE 4. Antioxidant enzyme activities (units/mg protein) in CLV98 and V_3 cells pretreated with H_2O_2 at a concentration of 0.07 μg/mL and then challenged with ADR for 24 hr at a concentration of 0.5 μM for CLV98 and 50 μM for V_3 cells.

Enzymes	Treatment: Adaptive agent	Treatment: Challenge drug	Cells: CLV98	Cells: V_3
CAT	—	—	16.3 ± 2.7	36.2 ± 7.0
units/mg	H_2O_2	—	17.8 ± 3.4	29.3 ± 6.4
protein	—	ADR	32.1 ± 0.7**	32.5 ± 6.0
	H_2O_2	ADR	32.2 ± 1.8**	32.8 ± 2.6
SOD	—	—	2.7 ± 0.1	1.1 ± 0.1
units/mg	H_2O_2	—	3.1 ± 0.5	1.4 ± 0.1
protein	—	ADR	3.7 ± 0.1	1.6 ± 0.1*
	H_2O_2	ADR	3.2 ± 0.2*	1.5 ± 0.1*
GPx	—	—	34.1 ± 3.2	47.1 ± 0.5
units/mg	H_2O_2	—	30.4 ± 2.8	57.1 ± 1.0
protein	—	ADR	43.9 ± 1.9*	76.1 ± 4.5**
	H_2O_2	ADR	38.6 ± 0.0	75.5 ± 2.7**

Enzyme activities were assayed as described in Materials and Methods. Values represent the mean ± SD from 8–16 determinations in separate cultures. Significantly different from control untreated cells: *$P < 0.1$; **$P < 0.01$.

study seem to indicate an association between developed resistance and overexpression of CAT in CHO/R^N cells and an association between developed resistance and overexpression of CAT and SOD in ME18/R^N cells.

Comparisons of enzyme activities in relatively sensitive and relatively resistant cell lines revealed a possible association between the levels of CAT and the cytotoxic response to the action of ADR and no association between the levels of the other two enzymes. Comparisons between cell lines in the three experimental groups indicate that no association between sensitivity to ADR and inducibility of the measured enzymes exists.

Oxidative injury involves responses to oxidants or agents that lead to this kind of injury via intracellular redox reactions. H_2O_2 appears to play a major role in mediating the acute effects of ADR *in vitro* [18]. From the results of our study, it is possible to conclude that in the cell lines studied differences in CAT activity play a role in cellular resistance to ADR toxicity; however, these differences do not explain why three of the six examined cell lines display an adaptive response while the remaining cell lines do not. The relative contribution of CAT in destroying the oxidant is markedly dependent on the concentration of its substrate. CAT might be of greater importance in cells that have developed resistance to the oxidant where in order to obtain significant toxicity, high concentrations have to be used [19].

The possibility is not excluded that in our study one of the factors enabling the occurrence of crossadaptation to ADR is the concentration of the drug determining both the occurrence of oxidative injury and the contribution of catalase.

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