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Drug-resistant human lung cancer cells are more sensitive to selenium cytotoxicity
Effects on thioredoxin reductase and glutathione reductase

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Abstract

The human U-1285 and GLC_4 cell lines, both derived from small cell carcinoma of the lung, are present in doxorubicin-sensitive (U-1285 and GLC_4) and doxorubicin-resistant MRP-expressing (U-1285dox and GLC_4/ADR) variants. These sublines were examined here with respect to their susceptibilities to the toxic effects of selenite and compared to the toxic effects of selenite on the promyelocytic leukemia cell line HL-60 and its doxorubicin-resistant P-glycoprotein expressing variant. The drug-resistant U-1285dox and GLC_4/ADR sublines proved to be 3- and 4-fold, respectively, more sensitive to the cytotoxicity of selenite than the drug-sensitive U-1285 and GLC_4 sublines, whereas no difference was observed between the HL-60 sublines. The presence of doxorubicin at a concentration equal to the IC_{10} did not significantly potentiate the toxic effects of selenite. The presence of selenite did not significantly affect the expression of the multi-drug resistant proteins (MRP1, LRP and topoisomerase IIα) in the drug-resistant cells. The activities of thioredoxin reductase (TrxR) were higher (50 and 25%, respectively) in the drug resistant cell sublines U-1285dox and GLC_4/ADR compared to the drug-sensitive parental lines. The activity of glutathione reductase (GR) was essentially the same in the drug-sensitive and -resistant cell lines. Exposure to selenite resulted in a 4-fold increase in both TrxR and GR activities in U-1285 cells, an effect, which was less pronounced in the presence of doxorubicin. Under similar conditions the increase in the TrxR activity in the resistant U-1285dox cell line, was only 30% and the activity of GR was unaltered. Different responses in the activity of the key enzymes in selenium metabolism are one possible mechanism explaining the differential cytotoxicity of selenium in these cells.

Keywords: Selenium; Thioredoxin reductase; Glutathione reductase; Cancer; Drug resistance; Doxorubicin

1. Introduction

Multi-drug resistance (MDR) is a serious clinical problem which often severely limits the effectiveness of cancer chemotherapy. A number of mechanisms have been postulated to give rise to drug resistance, of which the most extensively studied is the so-called ''classical'' MDR. This phenotype is associated with overexpression of a 170 kDa transmembrane glycoprotein (P-gp) [1] which functions as a drug efflux pump, thereby causing a maintenance of tolerable intracellular levels of the cytotoxic drugs. P-gp belongs to the ATP-binding cassette (ABC) of transport proteins, which also include the multi-drug resistance associated protein 1 (MRP1) [2–4]. MRP1 is expressed ubiquitously in human tissues [5] and its predominant substrates are in particular glutathione conjugates [6]. The characterization of the major vault protein/lung resistance-related protein (LRP) [7] which has been associated with nuclear-cytoplasmic transport of cytostatic drugs, indicates the existence of additional transport systems.

Down regulation of topoisomerases can also be a defense against topoisomerase interacting drugs [8]. Other types of cellular defense systems are also likely to make

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Abbreviations: MDR, multi-drug resistance; MRP, multi-drug resistant protein; TrxR, thioredoxin reductase; Trx, thioredoxin; GR, glutathione reductase; GSH, glutathione; PBS, phosphate buffered saline.

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important contributions to the resistant phenotype, i.e. defenses against oxidative stress and the processes, which maintain a reducing environment intracellularly. Two important enzymes in this context are TrxR and GR, which both belong to the same family of pyridine nucleotide oxidoreductases and demonstrate structural similarities [9].

The thioredoxin system is comprised of NADPH, Trx and TrxR and acts efficiently to reduce protein disulfide bonds in general, thereby serving several important functions, especially in connections with maintenance of a reducing environment within the cell [10,11]. Mammalian TrxRs are homodimeric flavoenzymes exhibiting structural and functional similarities to GR and lipoamide dehydrogenase [9]. In contrast to the other members of this relatively conserved family of enzymes, mammalian TrxRs demonstrate a broad substrate specificity, reducing Trx not only from the same, but also from many other species, as well as reducing a wide variety of low molecular weight substances such as hydroperoxides and selenium compounds [10–12]. This broad substrate specificity can be explained by the presence of a penultimate C-terminal selenocysteine residue in each 58 kDa subunit of these enzymes [13–15].

GR is a homodimeric enzyme mainly found in the cytosol regenerating GSH with NADPH as cofactor. Besides this, GR is also very important in the selenium metabolism, e.g. it reduces selenodiglutathione and thereby selenide is formed, a reaction also performed by TrxR [16,17].

The biological effects of selenium are highly concentration-dependent. At lower concentrations, selenium compounds stimulate cell growth and induce the synthesis of selenoproteins. Moderate to high concentrations of selenium compounds are known to inhibit cell growth [18–20] and to prevent chemically-induced carcinogenesis [21,22]. Furthermore, in certain earlier studies selenium was shown to inhibit the growth of drug-resistant cells [23], as well as to prevent the development of drug resistance [24]. Certain selenium compounds are strongly redox active and act as potent prooxidants, oxidizing intracellular thiols non-stoichiometrically, thereby leading to excessive oxidative stress [25,26], oxygen consumption and apoptosis [27,28]. At higher concentrations selenium is extremely toxic and may cause necrosis and tissue destruction.

The purpose of the present study was to investigate if selenium has a potential for treatment of multi-drug resistant cancer by differences in the toxic effects of selenium on drug-sensitive and -resistant cell lines. These effects were also compared to a human promyelocytic leukemia cell line present in a drug-sensitive variant and a resistant P-gp overexpressing subline. Possible explanations for such differences were examined by investigating the expression of MDR proteins and the activities of TrxR and GR, known to play essential roles in selenium metabolism.

2. Materials and methods

2.1. Chemicals and drugs

Trx from *Escherichia coli* was purchased from Promega. Bovine TrxR (used for preparing standard curves) was obtained from IMCO. Sodium selenite, yeast glutathione reductase (used for preparing standard curves), seleno-D,L-cystine and all chemicals employed for enzyme assays were procured from Sigma. Doxorubicin (Adriamycin®) was purchased from Amersham Biosciences. RPMI 1640 and PBS Dulbecco's without calcium and magnesium, without sodium bicarbonate was from GIBCO BRL, Invitrogen.

2.2. Cell lines

The parental human lung carcinoma U-1285 cell line [29] and its doxorubicin-resistant subline U-1285dox,[2] which was selected to tolerate 1.6 μM doxorubicin, were kindly provided by Prof. Jonas Bergh at Radiumhemmet, Karolinska Hospital, Stockholm, Sweden. The drug-resistant variant of this cell line has an overexpression of MRP1 (see footnote 1).

The human small cell lung carcinoma cell line GLC_4 and its doxorubicin-selected subline GLC_4/ADR, tolerating 1.18 μM doxorubicin, were kindly provided by de Vries, Division of Medical Oncology, Department of Internal Medicine, at the University Hospital in Groningen, The Netherlands. The drug-resistant variant of this cell line has decreased topoisomerase IIα expression, overexpression of MRP1 [30] and LRP [31].

The human promyelocytic leukemia cell line HL-60, which does not express P-gp, and its doxorubicin-selected multi-drug resistant variant which overexpresses P-gp, HL-60-R, were previously described [32].

Cells were grown in suspension in RPMI 1640 medium containing Glutamax-I and 25 mM HEPES supplemented with 10% (v/v) fetal calf serum, at 37°, in a humidified atmosphere containing 5% CO_2. In order to eliminate revertants, the drug-resistant sublines were cultured in the presence of doxorubicin once every fourth week. All cells were maintained in drug-free medium for 2–4 weeks prior to initiation of the experiments. All cultures were free of mycoplasma.

2.3. Assay of cytotoxicity

Drug-sensitivity was determined employing growth inhibitory test to obtain IC_{50} values, as described elsewhere [33]. In each experiment, such determinations were performed in duplicate and compared to the growth of unexposed cells in quadruplicate, 2 mL cell suspensions

[2] Jönsson K, *et al*. Doxorubicin-resistant, MRP1 expressing U-1285 cells are sensitive to idarubicin, submitted for publication.

(0.5×10^5 cells/mL) were continuously incubated and cultured with increasing concentrations of selenite (0.1–100 μM) or selenocystine (0.1–50 μM) for 4 days. For determination of drug cytotoxicity at the end of the culture period, the cells were extracted in 1.25% TCA and the ATP levels in these extracts determined utilizing an automated bioluminiscent assay [34] and each sample being compared individually to an internal ATP standard. Drug cytotoxicity was then expressed as the ratio between the ATP contents in drug-exposed compared to unexposed cells. For determination of IC_{50} values, at least four such experiments measuring cellular ATP levels of treated cells was performed and divided it by the ATP levels of non-treated cells cultured in media with PBS. The concentration representing 50% inhibition was determined as the drug concentration that resulted in a 50% reduction of cellular ATP levels (indirect cell viability). The inhibitory effect of the compound at each concentration was expressed as a percentage and the value representing 50% inhibition was estimated by interpolation.

U-1285 and U-1285dox, GLC_4 and GLC_4/ADR, HL-60 and HL-60-R cells were also exposed to doxorubicin at IC_{10} (see footnote 1; [32] in a manner analogous to the determination of IC_{50} already described) together with selenite or selenocystine (for U-1285 and U-1285dox cell lines only).

2.4. Preparation of cell extracts

For biochemical determinations cells were cultured in the absence or presence of selenite in a concentration representing the estimated IC_{50} for the U-1285 and U-1285dox cells (30 and 7 μM, respectively) and the estimated IC_{50} for the HL-60 and HL-60-R cells (3 and 3 μM, respectively) and below IC_{50} for the GLC_4 and GLC_4/ADR cells (4 and 2.5 μM, respectively), and/or in the absence or presence of doxorubicin at IC_{10} for the drug-sensitive and -resistant cells (0.02 and 1.6 μM, respectively). The cells were harvested after 4 days, washed twice with PBS and resuspended in 1 mL PBS. Cells were lysed by freezing and thawing three times in liquid nitrogen and 37° waterbath, respectively. The cells were then homogenized for 30 s at 4°. The homogenates thus obtained were subsequently centrifuged at 25,200 rcf for 7 min and the resulting supernatants were used for biochemical measurements. Protein was quantitated employing the Biuret procedure [35].

2.5. Enzyme assays

The activities of TrxR and GR were assayed in the presence of insulin, essentially as described by Holmgren and Björnstedt [11], and GSSG, respectively. Each sample was incubated for 20 min at 37° in 80 mM HEPES, pH 7.5, 0.9 mg NADPH/mL and 6 mM EDTA. For measuring TrxR activity, 2 mg insulin/mL and 10 μM *E. coli* Trx were also present; while for determining GR activity, 1 mM GSSG was employed. The reactions were terminated by the addition of 0.4 mg/mL DTNB/6 M guanidine–HCl in 0.2 M Tris–HCl (pH 8.0). The absorbance at 412 nm was measured and the absorbance of a reference cuvette, containing everything except Trx or GSSG, respectively, was subtracted from the values of the corresponding sample. Standard curves obtained using purified calf thymus TrxR or yeast GR, were used to calculate the amount of TrxR or GR, respectively, in the cell extracts.

2.6. Western blot analysis

For Western blot analysis GLC_4 and GLC_4/ADR cells were cultured with or without addition of selenite and/or doxorubicin in the same concentrations as used for the biochemical measurements. For analysis of topoisomerase IIα, nuclei were isolated using a method described elsewhere [32]. For analysis of MRP1, plasma membranes and endoplasmic reticulum membranes from the cells were isolated using a method described elsewhere [32]. For Western blot analysis of LRP, cell extracts isolated as already described, were used. Protein concentrations were determined according to Lowry *et al.* [36]. Electrophoresis of 25 μg protein (MRP1) or 50 μg protein (LRP and topoisomerase IIα) aliquots were carried out in a 6% SDS-polyacrylamide mini-gel 100 V for 1.5 hr and protein were transferred to a PVDF membrane (Bio-Rad). Filters were blocked in Tris–saline buffer containing 10% non-fat milk. MRP1-protein was detected by staining with the monoclonal antibody MRPm6 (Research Diagnostic Inc.) at 1/100 dilution. Topoisomerase IIα was detected by staining with the monoclonal antibody topoisomerase IIα (Topogen) diluted 1/1000A. LRP-protein was detected by staining with the monoclonal antibody LRP56 (Neo-Markers) diluted 1/200. A rabbit anti-mouse IgG1 (Santa Cruz Biotechnology) secondary antibody diluted 1/5000 together with chemiluminiscence plus (NEN) were used for visualization and the membranes were exposed to Hyperfilm ECL (Amersham).

3. Results

3.1. Relative toxicity of selenite in the drug-sensitive and -resistant cell sublines

U-1285, U-1285dox, GLC_4, GLC_4/ADR, HL-60 and HL-60-R cells were exposed to medium containing 0.1–100 μM selenite for 4 days and inhibition of growth was monitored employing a bioluminescence assay for ATP. The growth of the U-1285dox cell subline was inhibited by concentrations of selenite which were approximately one-third of those required to inhibit growth of the parental cell line to the same extent (Fig. 1A). The GLC_4/ADR cells showed a similar response after selenite exposure, only one-fourth of the concentration required to reach IC_{50} than

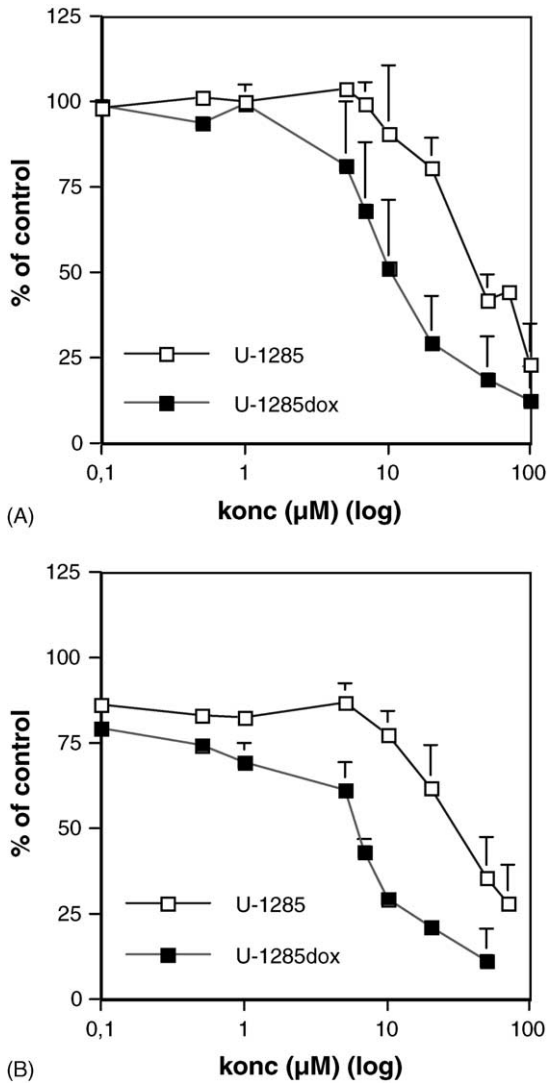

Fig. 1. Growth inhibition by (A) selenite in the drug-sensitive cell line U-1285 (□) and the drug-resistant cell line U-1285dox (■) and (B) in combination with doxorubicin (at extrapolated IC_{10} concentration). Each point are representatives of four to five independent experiments; points given represents the mean, bars show ±SD. The IC_{50} value of U-1285dox was compared to the IC_{50} value of U-1285, $P < 0.05$ and $P < 0.001$, respectively.

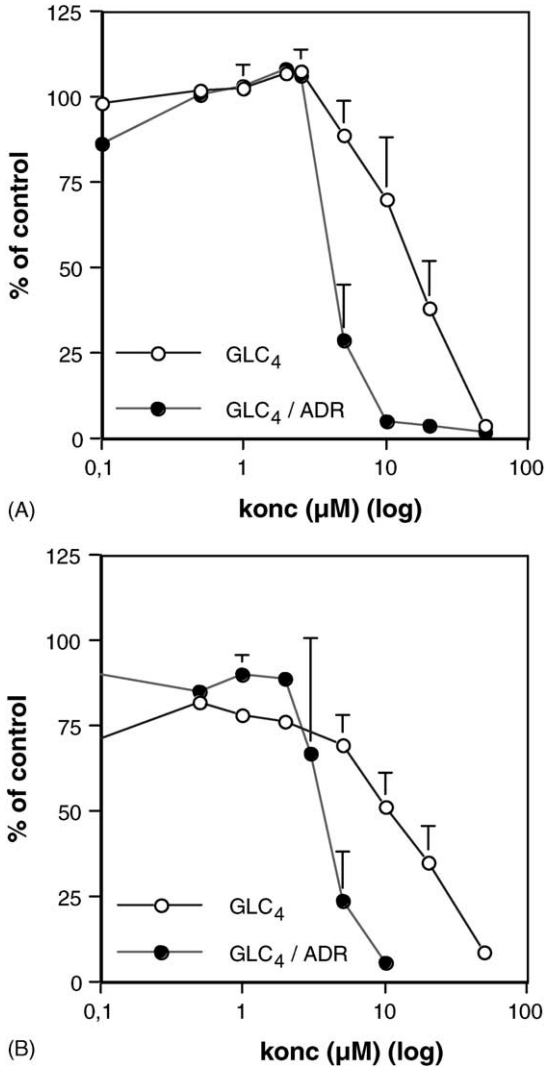

Fig. 2. Growth inhibition by (A) selenite in the drug-sensitive cell line GLC_4 (○) and the drug-resistant cell line GLC_4/ADR (●) and (B) in combination with doxorubicin (at extrapolated IC_{10} concentration). Each point are representatives of three to four independent experiments; points given represents the mean, bars show ±SD. The IC_{50} value of GLC_4/ADR was compared to the IC_{50} value of GLC_4, $P < 0.001$ and $P < 0.01$, respectively.

needed in the parental cell line (4 µM compared to 14 µM) (Fig. 2A). The effect of doxorubicin in combination with selenite on cell growth was investigated (Figs. 1B and 2B). The concentration of doxorubicin used was the extrapolated IC_{10} but had a slightly higher toxic effect. In the presence of doxorubicin the U-1285dox subline was approximately 4-fold more sensitive to selenite than was the parental subline (Fig. 1B). For this reason, growth in the presence of a selenite concentration representing the estimated IC_{50} for each subline, i.e. 7 µM for U-1285dox and 30 µM for U-1285 cells, was employed to examine possible effects on enzyme activities.

For GLC_4 and GLC_4/ADR the addition of doxorubicin lowered the IC_{50} for the combination with selenite mutually equal (Fig. 2B) so the relation between the two cell sublines did not alter. However, HL-60 cells exposed to the combination of doxorubicin and selenite showed only a small difference in cytotoxicity compared to the P-gp expressing HL-60-R cells (Fig. 3).

Selenocystine had a similar effect but the differences in cytotoxic response were less pronounced compared to selenite. The IC_{50} values for selenocystine were for U-1285 11 µM and for U-1285dox 8 µM and in the combination with doxorubicin 11 and 6 µM, respectively.

3.2. Western blot analysis

Western blot analysis of GLC_4 and GLC_4/ADR showed overexpression of MRP1 and LRP and a down regulation of topoisomerase IIα only in the drug-resistant cells as

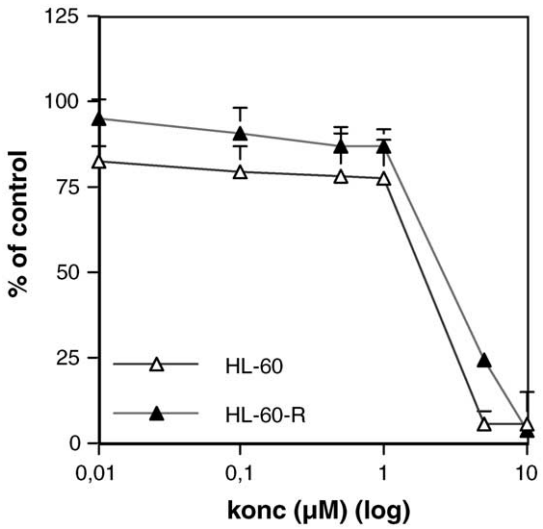

Fig. 3. Growth inhibition by selenite in the presence of doxorubicin (IC_{10}) in the drug-sensitive cell line HL-60 (△) and the drug-resistant cell line HL-60-R (▲). Each point are representatives of three independent experiments; points given represents the mean, bars show ±SD.

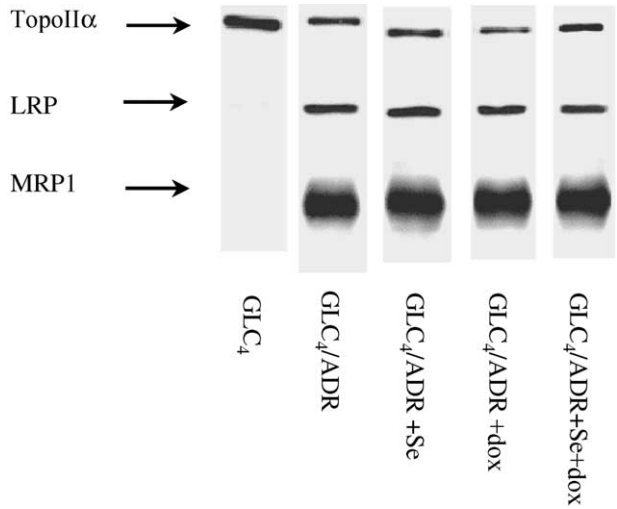

Fig. 4. Western blot analysis of topoisomerase IIα (TopoIIα), LRP and MRP1 in drug-sensitive GLC_4 cells and in drug-resistant GLC_4/ADR cells cultured without or with addition of selenite (+Se) and/or doxorubicin (+dox).

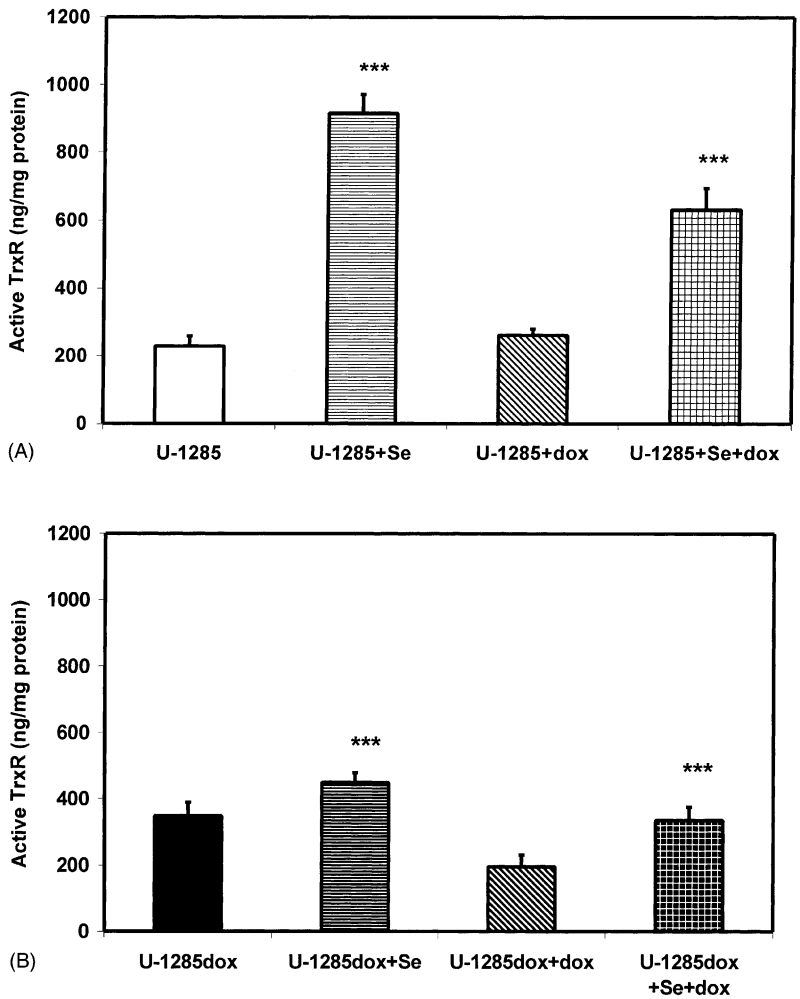

Fig. 5. TrxR in the drug-sensitive U-1285 (A) and the drug-resistant U-1285dox (B) cell lines in the absence and presence of selenite (+Se), doxorubicin (+dox) or both (Se + dox). The values given are means of six repeat assays performed on cell extracts from three independent preparations, bars show ±SD. (A) (***) $P < 0.001$ compared to the U-1285 and U-1285 + dox value, respectively, (B) (***) $P < 0.001$ compared to the U-1285dox and the U-1285dox + dox value, respectively.

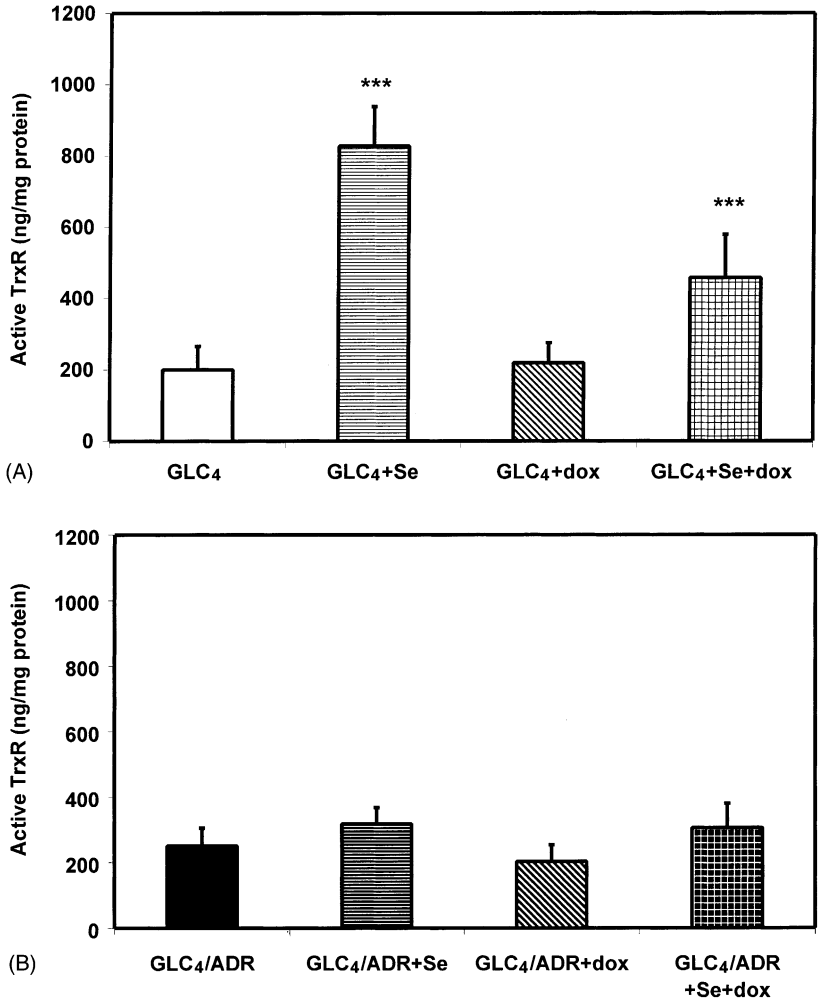

Fig. 6. TrxR in the drug-sensitive GLC_4 (A) and the drug-resistant GLC_4/ADR (B) cell lines in the absence and presence of selenite (+Se), doxorubicin (+dox) or both (Se + dox). The values given are means of six repeat assays performed on cell extracts from three independent preparations, bars show ±SD. (***) $P < 0.001$ compared to the GLC_4 and GLC_4 + dox value, respectively.

expected. There were no significant changes in these expressions as detected by Western blot when the cells were exposed to selenite and/or doxorubicin (Fig. 4).

3.3. Thioredoxin reductase activity

The basal level of active TrxR in the resistant U-1285dox cell subline (350 ng/mg protein) was significantly higher than in the drug-sensitive U-1285 cell line (230 ng/mg protein) (Fig. 5). After addition of selenite (IC_{50}, 30 μM) to the drug-sensitive cells the activity was 4-fold increased to 920 ng/mg protein, whereas in the presence of doxorubicin as well, TrxR activity was increased less than 3-fold to 630 ng/mg protein. Doxorubicin alone did not affect the activity significantly (260 ng/mg protein) (Fig. 5A).

In the case of the drug-resistant U-1285dox cell subline, exposure to selenite (IC_{50}, 7 μM) resulted in only a 30% increase in TrxR activity to 450 ng/mg protein; while addition of both selenite and doxorubicin caused virtually no change in this activity (340 ng/mg protein) compared to unexposed cells (Fig. 5B). However, addition of doxorubicin alone decreased the level of active enzyme to 200 ng/mg protein. Titration revealed that the greatest relative increase in TrxR activity was obtained upon exposure to 1 μM selenite in the case of both sublines (data not shown).[3] The activity of TrxR in the GLC_4 and GLC_4/ADR cells at selenite concentrations below IC_{50} (4 and 2.5 μM, respectively) followed the same pattern as the U-1285 and U-1285dox cells, i.e. higher activity in the resistant cell subline and failure to increase activity upon selenite exposure (Fig. 6A and B).

In the HL-60 and HL-60-R cells the TrxR activity were 250 and 260 ng/mg total protein and after selenite exposure the activity increased only marginally, to 270 and 275 ng/mg total protein, respectively.

[3] Jönsson K, Björkhem-Bergman L, Hossain A, Söderberg A, Eriksson LC, Paul C, Rosén A, Björnstedt M. Selenite induces apoptosis in drug-resistant cells and affects the Trx system, submitted for publication.

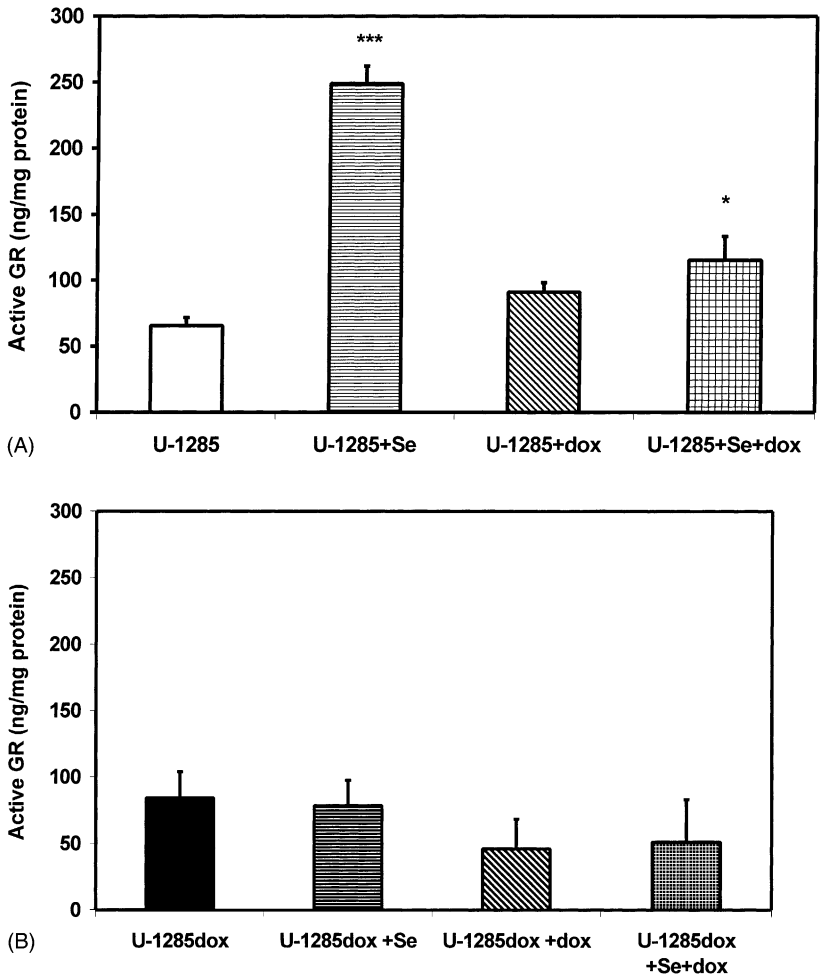

Fig. 7. Glutathione reductase in drug-sensitive U-1285 (A) and drug-resistant U-1285dox (B) cell lines in the absence and presence of selenite (+Se), doxorubicin (+dox) or both (Se + dox). The values given are means of six repeat assays performed on cell extracts from three independent preparations, bars show ±SD. (***) $P < 0.001$ compared to the U-1285 value, (*) $P < 0.05$ compared to the U-1285 + dox value.

3.4. Glutathione reductase activity

The level of active GR were in U-1285 and U-1285dox cell lines basically the same, 66 and 84 ng/mg protein, respectively (Fig. 7A and B). Upon exposure to selenite, the GR activity of the U-1285 cells increased almost 4-fold to 250 ng/mg protein in the sensitive subline (Fig. 7A); whereas the GR activity in the drug-resistant cell subline was virtually unaffected (78 ng/mg protein) (Fig. 7B). After exposure to both selenite and doxorubicin, the GR activity in the drug-sensitive cells was only doubled (120 ng/mg protein) and doxorubicin alone resulted in a 50% increase in this activity (Fig. 7A). Addition of selenite and doxoribucin or of doxoribicin alone to the drug-resistant cells caused essentially no change in GR activity (46 and 51 ng/mg protein, respectively) (Fig. 7B).

3.5. Statistical analysis

Statistical analysis was performed using Student's *t*-test (Figs. 1–7).

4. Discussion

In the present study we demonstrate that doxorubicin-resistant U-1285dox and GLC_4/ADR cells were more sensitive to the cytotoxic effects of selenite than were the parental doxorubicin-sensitive U-1285 and GLC_4 cell lines. The U-1285dox cells were also more sensitive to the organic selenium compound selenocystine compared to the parental drug-sensitive U-1285 cells, but in this case the differences between the sublines were less pronunced. In contrast, this pattern was not repeated after the combination of doxorubicin and selenium to the P-gp expressing HL-60-R and the parent drug-sensitive HL-60 cells. However, both of these cell lines were highly sensitive to selenium treatment. This implies that selenium does not affect P-gp but other putative mechanisms for cell growth and drug resistance in these cell systems. The results from our Western blot analysis indicated that selenite exposure did not change the expressions of the MDR-proteins MRP1, LRP or topoisomerase IIα, in these cells, as expected, since there were virtually no synergism between doxorubicin and selenite.

We have also explored other possible explanations for these differences in selenite sensitivity by determining the activity of two important flavoenzymes, TrxR and GR, which are known to play a key role in selenium metabolism [12,17].

The classical cytosolic TrxR is a multi-functional selenoenzyme known to be induced in a number of human tumors [37] and during carcinogenesis [38]. A recent study demonstrated that TrxR activity and the corresponding mRNA are up regulated in neoplastic nodules in rat liver [39]. The fact that both neoplastic nodules and drug-resistant cells exhibit a resistant phenotype suggests that elevated levels of TrxR are an important characteristic of such a phenotype. In an earlier study, it was also suggested that the thioredoxin system is involved in cellular responses to cytotoxic drugs [40].

Mammalian TrxRs have a relatively unique relationship to selenium, being both selenoproteins and key enzymes in selenium metabolism [10,12]. The classical cytosolic TrxR-1 reduces a wide variety of selenium compounds including selenite [41], selenodiglutathione [16] and selenocystine [12,42] thereby providing active selenium for its own synthesis and for the synthesis of other selenoproteins as well. This protein is also a peroxidase [42], contributes to the action of GSHPx [43] and plays an important role for the synthesis of DNA and participates in the regulation of protein activities by thiol redox control [44]. Consequently, TrxR is of primary interest in connection with studies concerning the mechanisms by which selenium exerts its effects on different types of cells, especially tumor cells. Since TrxR is a selenoenzyme, its activity is of course dependent on the intracellular level of selenium to a certain extent. The difference in the TrxR activities of the two cell lines studied here can not, however, be explained solely by the difference in the concentrations of selenite to which they were exposed, since TrxR expression and activity is known to most prominently increase at concentrations of approximately a few micromolars (see footnote 2; [45]). In addition, the IC_{50} correlated TrxR activity values stated here, were almost identical to the TrxR levels achieved at a selenite exposure of 10 μM to both U-1285 and U-1285dox (see footnote 2). This is also supported by our results with the GLC_4 sublines, where selenite at concentrations below IC_{50} resulted in the same fold induction and similar levels of TrxR as in the U-1285 sublines.

In the sensitive U-1285 cells, exposure to selenite resulted in a 4-fold increase in the activities of both TrxR and GR, whereas in the resistant cells, these parameters were largely unaltered. Thus, addition of selenite to the medium led to a shift in the relative activities in these cell lines, from a 50% higher TrxR activity in the drug-resistant cells to a 2-fold higher activity in the drug-sensitive cells. We suggest that the lack of increase in the TrxR and GR activities in the drug-resistant cells might explain, at least in parts, why these cells are more sensitive to the cytotoxic effects of selenite. This is supported by the finding that in the HL-60 and HL-60-R cell lines, which have similar, high sensitivity for selenite, there were no significant differences in the TrxR values after selenite exposure in both sublines. Since selenite is very reactive with thiols, a massive oxidation of thiols such as GSH and Trx is expected. An up-regulation of GR and TrxR after selenite exposure is therefore a rational response to selenite mediated oxidative stress and will lead to a survival advantage in the U-1285 and GLC_4 cell lines.

Differential toxicity by selenium treatment with the resulting differences in the activities of the two most central enzymes in selenium metabolism are quite interesting and suggest mechanisms and therapeutic possibilities to the treatment of drug-resistant tumors. Especially since most selenium concentrations employed are rather low and applicable *in vivo* in man. Furthermore, arsenic, which has chemically properties similar to those of selenium, has recently been shown to be highly effective in acute promyelocytic leukemia [46]. Studies presently ongoing in our laboratory on cancer cells from patients may reveal whether the present findings are of relevance to human cancers.

Acknowledgments

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