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Free Radicals Induced by Adriamycin-Sensitive and Adriamycin-Resistant Cells: A Spin-Trapping Study†

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ABSTRACT: The radicals generated by adriamycin-sensitive (CHO-AB) and adriamycin-resistant (CHO-C5) Chinese hamster ovary cells as well as by adriamycin-sensitive and -resistant human breast cancer cells (MCF7-WT and MCF7-ADR) have been studied with spin-trapping and ESR spectroscopy. During anoxic exposure to adriamycin (ADR) both pairs of cell lines produced the broad ESR singlet characteristic of ADR semiquinone ($AQ^{\bullet}$). By use of tris(oxalato)chromate (CrOx) as an extracellular line-broadening agent, the distribution of $AQ^{\bullet}$ between the intra- and extracellular compartments was studied. For cell densities of $(1-3) \times 10^7$ cells/mL, CrOx eliminated most, though not all, of the ESR signal, indicating that the $AQ^{\bullet}$ radicals freely diffuse and partition between the intra- and extracellular compartments proportionally to their respective volumes. Similar behavior was exhibited by all four cell lines studied. Upon introduction of oxygen to anoxic cells in the presence of the spin trap 5,5-dimethylpyrroline *N*-oxide (DMPO), the $AQ^{\bullet}$ signal was replaced by that of the DMPO–OH spin adduct. Metal chelators such as desferrioxamine had no effect on DMPO–OH or $AQ^{\bullet}$ formation. Superoxide dismutase, not catalase, totally eliminated the ESR signal, indicating that DMPO–OH produced by ADR-treated cells originates from superoxide rather than from $^{\bullet}OH$ produced from H_2O_2. In the presence of CrOx, the DMPO–OH signal was not distinguishable from the background noise, thus excluding any contribution to the signal by intracellular spin adducts. Without excluding a possible role for intracellular $O_2^{\bullet-}$ and $^{\bullet}OH$ radicals, the present results show that $AQ^{\bullet}$ radicals partition predominantly outside the cell and react there with oxygen to yield extracellular superoxide. Since ADR-resistant and -sensitive cell lines generated comparable levels of the radicals, there is little support for the assumption that ESR-observable oxygen-derived radicals play a role in ADR antitumor activity.

The anthracycline antibiotics, which include adriamycin (ADR),[1] possess significant therapeutic activity against most hematologic, lung, breast, and ovarian malignancies; however, their clinical use is restricted by cumulative dose-dependent cardiotoxicity. Were the chemical and biological mechanisms of action of ADR more thoroughly defined, strategies could be instituted to improve the therapeutic index. Although ADR has been found to have multiple cellular targets, the cardiotoxicity and antitumor effects have been suggested to occur through different biochemical mechanisms. The proposed cardiotoxic mechanisms include production of ADR-mediated

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[1] Abbreviations: ESR, electron spin resonance; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DTPA, diethylenetriaminepentaacetic acid; DFO, desferrioxamine; CrOx, tris(oxalato)chromate; CHO, Chinese hamster ovary cells; MCF7, human breast carcinoma cells; ADR, adriamycin; $AQ^{\bullet}$, adriamycin semiquinone free radical; GSH, glutathione; PBS, phosphate buffered saline.

free radical species capable of damaging DNA or impairing membrane organization and function (Goodman & Hochstein, 1977; Gianni et al., 1983). ADR itself is thought not be cardiotoxic; rather, toxicity reportedly requires initial metabolic reduction of ADR to a semiquinone free radical (AQ•) which then readily reduces oxygen to superoxide (Bates & Winterbourn, 1982; Nohl & Jordan, 1983; Pietronigro et al., 1979; Berlin & Haseltine, 1981). The AQ• and the oxygen-derived active species •OH, $O_2^{\bullet-}$, and H_2O_2 have been implicated in ADR-induced DNA strand scission (Lown et al., 1977), DNA–protein cross-linkage (Scheulen et al., 1982; Sinha & Chignell, 1979), and lipid peroxidation (Myers et al., 1977; Mimanugh et al., 1981, 1983; Gutteridge, 1984).

Identifying, quantifying, and ultimately attempting to correlate ADR-induced intracellular, active species with various parameters of biological damage have been the object of many studies. Electron spin resonance (ESR) spectroscopy is commonly used for studying AQ•, whereas spin-trapping techniques are often required to detect oxygen-derived radicals which, unlike AQ•, cannot be directly observed (Sato et al., 1977; Thayer, 1977; Kalyanaraman et al., 1980; Lown et al., 1978; Gutierrez et al., 1983; Lown, 1983). There are suggestions in the literature that ADR can inflict cellular damage without entering the cell (Tritton & Yee, 1982), but most evidence implicates DNA as the critical biologic target. If, as suspected, production of •OH and $O_2^{\bullet-}$ by AQ• is the final common pathway, then because of the exceedingly high reactivity of •OH or the limited membrane permeability of $O_2^{\bullet-}$ radical, AQ• must activate oxygen inside the cell. Unfortunately in most ESR studies ADR-induced free radicals have been investigated in cell-free systems, and few attempts have been made to spin trap and detect radicals produced by ADR-treated cells (Bannister & Thornalley, 1983; Sinha et al., 1987a,b).

Recently, it has been suggested that not only the formation of active oxygen species but the rate at which they are produced may also be a fundamental component of the antitumor effect of ADR (Doroshow, 1986). This conclusion was supported by findings obtained with ESR spin-trapping techniques showing a dramatic difference in •OH radical production between ADR-sensitive (MCF7-WT) and resistant (MCF7-ADR) human tumor cells (Sinha et al., 1987a,b). It is well recognized that the ESR spin-trapping technique is a powerful tool; however, when this methodology is applied to cellular systems, there are several potential drawbacks which could lead to erroneous conclusions: (a) the trapping efficiency of intracellular radicals by scavengers (including spin traps) might decrease due to relatively high concentrations of cellular components which compete for the radicals; (b) •OH radicals which are produced *site specifically* and preferentially react with the cellular components, rather than the scavenger (spin trap), may remain undetectable by spin trapping; (c) •OH spin adducts, which otherwise are fairly stable, may not survive detection because of rapid destruction by the cell (Samuni et al., 1986, 1989a); (d) $O_2^{\bullet-}$ reacts with DMPO spin adducts of both oxygen- and carbon-centered radicals (Samuni et al., 1989b) (eq 1), and under high superoxide flux DMPO spin

(1)

adducts are subjected to fast decay (Samuni et al., 1988). Consequently a lower level of the spin adducts does not necessarily imply a lower level of precursor radicals. Furthermore, caution is necessary in interpreting the origin of observed spin adducts when the intracellular volume is but a miniscule proportion of the total sample volume. The detection of intracellular radicals in such a system implies either a long-lived and accumulating radical like the paraquat radical or an almost *inconceivable* partition ratio of free radicals between the extra- and intracellular compartments. With this knowledge, we have used ESR and spin trapping to compare the production of AQ• and active oxygen species in two pairs of parental (ADR sensitive) and mutant (drug resistant) cell lines. During anoxic exposure both ADR-sensitive and ADR-resistant cell lines produced comparable AQ• concentrations. With all cells studied the radicals predominantly partitioned in the extracellular volume. Similarly, in an aerobic environment DMPO–OH formation was observed in all cell lines and was fully inhibitable by extracellular SOD.

Experimental Procedures

Chemicals. Desferrioxamine (desferal) was a gift from Ciba Geigy; superoxide dismutase (SOD) and adriamycin were obtained from Sigma; catalase was purchased from Boehringer-Mannheim; 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) was purchased from Aldrich Chemical Co.; tris(oxalato)-chromate was purchased from Pfaltz and Bauer Inc. DMPO was purified by vacuum distillation and checked for the absence of ESR-observable contamination. All other chemicals were prepared and used without further purification. Distilled–deionized water was used throughout, and unless otherwise stated, the experiments were conducted at room temperature. To prepare inactive SOD and catalase, the enzymes were autoclaved for 60 min, checked for enzymatic activity and protein content, and kept at 4 °C.

Cell Culture. Chinese hamster ovary cell lines were grown in F12 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Both the parental line (CHO-AB) and the pleiotropic drug-resistant line (CHO-C5) were kindly supplied by Dr. V. Ling. The methods of inducing multidrug resistance have been previously described (Ling et al., 1983). Human breast cancer cells (kindly supplied by Dr. K. Cowan) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. The parental cell line (MCF7-WT) and the isolation of the ADR-resistant clone (MCF7-ADR) were previously described (Batist et al., 1986). Cell survival after 1-h exposure to ADR was assayed by the clonogenic assay as previously described (Puck & Marcus, 1956). For ESR studies cells were harvested from exponentially growing stock cultures by trypsinization, washed in phosphate-buffered saline (PBS), resuspended at a concentration of 10^8/mL in PBS, and stored either at room or at ice temperature until used.

Glutathione (GSH) Assay. Exponentially growing cultures of all cell lines were assayed for GSH. In brief, cells were collected for GSH assay, rinsed with cold phosphate-buffered saline, pelleted by centrifugation, and immediately treated with cold 0.6% sulfosalicylic acid. The total GSH level (GSH + glutathione disulfide) was determined according to the cyclic–reductase method (Tietze, 1969) wherein the absorbance of reduced 5,5′-dithiobis(2-nitrobenzoic acid) was followed at 412 nm and recorded after 3 min.

Electron Spin Resonance Measurements. Samples, 75–100 μL, of cell suspensions were drawn by a syringe into a gas-permeable, 800 μm inner diameter, capillary Teflon tubing. This permeable capillary allowed specific oxygen levels to be maintained throughout the experiments. Each capillary was inserted into a 2.5 mm i.d., quartz, ESR tube (open at both ends) and then placed within the ESR cavity. Gases of desired compositions were flowed around the sample within the spectrometer cavity. ESR spectra were recorded on a Varian

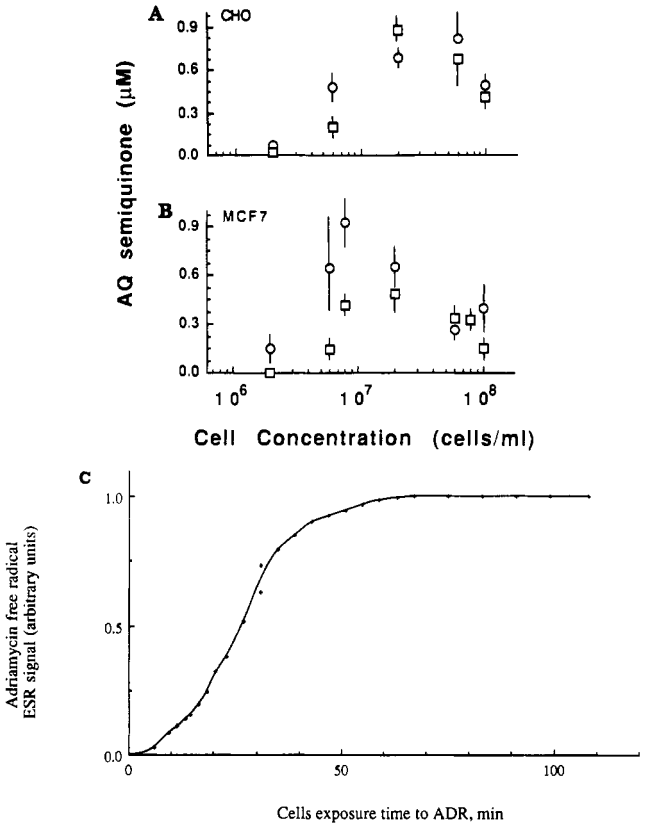


FIGURE 1: Adriamycin semiquinone concentration dependence on cell concentration and on time. The intensity of the ESR signal of adriamycin semiquinone radical (AQ•) formed upon 2-h anaerobic incubation of 1 mM adriamycin (ADR) in PBS/10 mM HEPES/0.1% glucose at room temperature with ADR-sensitive (circles) and ADR-resistant (squares) cells. (A) CHO-AB and CHO-C5 cells; (B) MCF7-WT and MCF7-ADR human breast cancer cells. (C) Time dependence of ESR signal intensity of AQ• accumulating upon anoxic exposure of 10^7 CHO-C5 cells/mL to 1.6 mM ADR.

E4 (or E9) X-band spectrometer, with a field set at 3357 G, modulation frequency of 100 kHz, modulation amplitude of 1 G, and nonsaturating microwave power. Radical concentrations were standardized against a known concentration of stable nitroxide spin-label.

Results

AQ• Production by ADR-Resistant and -Sensitive Cells. Since ADR resistance has been ascribed to lower concentrations of intracellular drug, the metabolic reduction of ADR to its semiquinone by ADR-sensitive and -resistant cells was compared. Scanning for ESR spectra of AQ• induced by parental CHO-AB cells exposed to 1 mM adriamycin, in PBS supplemented with 10 mM HEPES buffer and 0.1% glucose under anoxic conditions, showed the previously described singlet characteristic of ADR semiquinone radical, which subsequently became asymmetric with the appearance of the 7-deoxyadriamycinone semiquinone (Kalyanaraman et al., 1980; Schreiber et al., 1987). The AQ• concentration increased with time and was unaffected by SOD or metal chelators such as DFO. ADR-resistant CHO-C5 cells and both human breast cancer cell lines MCF7-WT and MCF7-ADR also produced the AQ• signal when exposed to ADR. The ESR signals accumulated during a 2-h incubation of ADR with varying cell densities of CHO-AB and CHO-C5 were measured and are shown in Figure 1A. The results of similar experiments obtained with MCF7-WT and MCF7-ADR cell lines are shown in Figure 1B. For all cell lines studied, the signal intensity of AQ• depended on cell concentration. Below

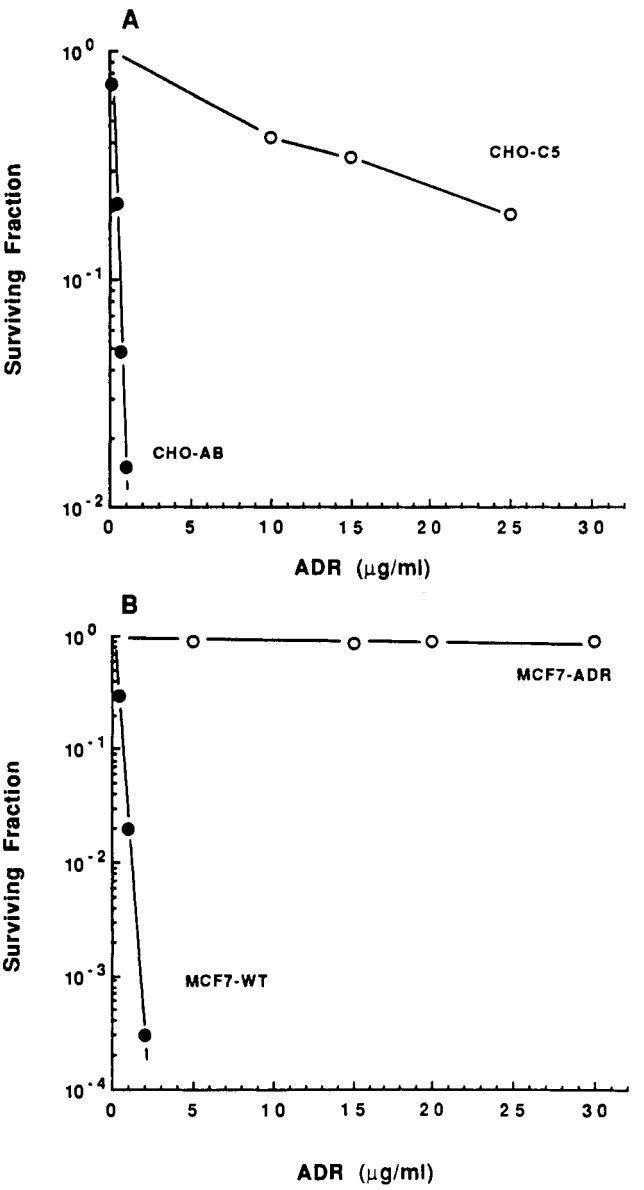


FIGURE 2: Dose–response survival curves of ADR-sensitive and -resistant CHO and human MCF7 cells. Dose dependence of cell surviving fraction following 1-h exposure of monolayered log-phase CHO and human breast cancer cells in full medium at 37 °C to various ADR concentrations. (A) CHO-AB (solid circles); CHO-C5 (open circles). (B) MCF7-WT (solid circles); MCF7-ADR (open circles).

6×10^7 cells/mL the ADR-sensitive cells (CHO-AB and MCF7-WT) gave rise to a slightly larger AQ• signal than did the respective resistant cells. However, the concentration of AQ• generated by all four cell lines exhibited a similar increase with increasing cell concentration up to 2×10^7 cells/mL and then decreased at higher cell density. All four cell lines exhibited similar kinetics of radical accumulation, and the results of a typical experiment carried out with CHO-C5 cells are illustrated in Figure 1C.

The lack of a significant difference in radical generation by ADR-resistant and -sensitive cells could result if the resistant cell lines had reverted back to ADR sensitivity. Retention of the adriamycin-resistant phenotype compared with the parental cells was confirmed before and after the completion of the study for both CHO-C5 and MCR-7 ADR (Figure 2).

Effect of Extracellular Line-Broadening Agents on AQ• ESR Signal. To determine the degree that AQ• partitioned outside the cell, 25–50 (final concentration) mM line-broad-

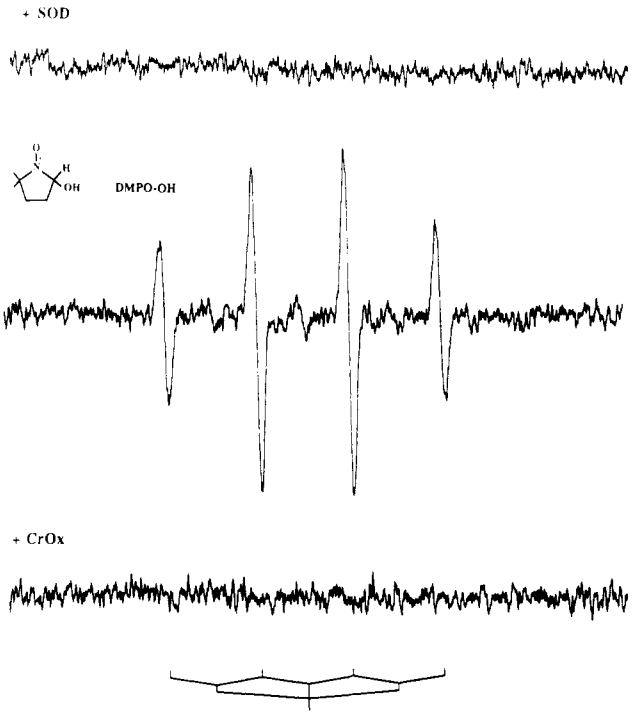


FIGURE 3: Effect of extracellular SOD and CrOx on DMPO–OH ESR signal. Adriamycin-resistant human breast cancer cells (MCF7-ADR) were incubated in PBS/10 mM HEPES/0.1% glucose at room temperature (5×10^7 cells/mL) for 1 h with 1 mM ADR, 0.1 mM DFO, and 0.1 M DMPO, placed in a gas-permeable capillary under air flow, and scanned for ESR spectra. Upper trace: with 14 units/mL superoxide dismutase added before incubation. Middle trace: control, no additives. Lower trace: with 45 mM CrOx added at the end of the incubation.

ening agent tris(oxalato)chromate (CrOx), which is completely excluded from the intracellular space (Lai et al., 1987), was anaerobically added to a CHO-AB cell suspension following 2-h incubation with 1 mM ADR, and the residual AQ• signal was monitored. To avoid prolonged cell exposure to the line-broadening agent, the CrOx was introduced anaerobically only at the end of the incubation, and the spectra were immediately recorded. The signal intensity of AQ• was greatly diminished but not completely abolished. With 1.6×10^7 cells/mL the AQ• signal intensity decreased by 95%. Similar effects of CrOx were observed when used with CHO-C5, MCF7-ADR, and MCF7-WT, indicating that AQ• was predominantly partitioned *outside* the cells. Given that the CHO cell volume is 1200 μm^3 (2400 μm^3 for MCF7-WT), then the volume fraction occupied by cells is about 2%, implying that the radicals distribute between the intra- end extracellular compartments roughly in proportion to their respective volumes.

Spin Trapping of Oxygen-Derived Radicals. When oxygen was introduced to cells that were anoxically incubated with ADR and the spin-trap DMPO (the spin trap itself had no effect on cell viability as confirmed clonogenically), the AQ• signal rapidly disappeared. Concomitant with the disappearance of the AQ• signal was the emergence of a four-line signal having hyperfine coupling constants of $a_N = 14.9$ G and $a_H = 14.9$ G characteristic of the DMPO–OH spin adduct (Finkelstein et al., 1982). An illustrative spectrum obtained with MCF7-ADR cells is shown in Figure 3 (middle trace). Since DMPO–OH can result from oxygen-derived radicals other than •OH, i.e., the decomposition of DMPO–OOH that results from $O_2^{\bullet-}$ addition (Finkelstein et al., 1982), appearance of the DMPO–OH signal does not prove the presence of genuine •OH radicals. The origin of the DMPO–OH spin

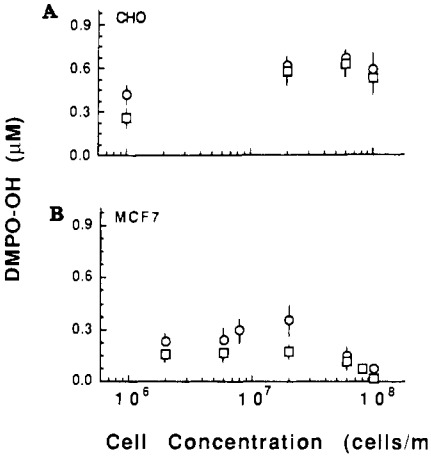


FIGURE 4: Cell concentration effect on the ESR signal intensity of DMPO–OH spin adduct. The intensity of the ESR signal of DMPO–OH spin adduct formed upon 2-h aerobic incubation of 1 mM adriamycin (ADR) in PBS/10 mM HEPES/0.1% glucose at room temperature with ADR-sensitive (circles) and ADR-resistant (squares) cells in the presence of 0.1 M DMPO spin trap. (A) CHO-AB and CHO-C5 cells; (B) MCF7-WT and MCF7-ADR human breast cancer cells.

adduct was checked with superoxide dismutase (SOD); 14 units/mL SOD entirely blocked the appearance of DMPO–OH, as seen in Figure 3 (upper trace), whereas inactive SOD had no effect. Since SOD does not penetrate into the cell, the loss of the DMPO–OH signal indicates that extracellular $O_2^{\bullet-}$ is exclusively responsible for the observed spin adduct. Since •OH radical can be generated through a Fenton reaction by adventitious transition metal ions, 0.1 mM DFO was added to the system. The addition of DFO had no effect on the DMPO–OH signal intensity. Moreover, 350 units/mL catalase did not affect the DMPO–OH signal, thus corroborating the conclusion that no authentic •OH radicals, formed through the Fenton reaction, give rise to the DMPO–OH signal.

Had the detected radicals been intracellular, it would be anticipated that a larger signal would result when higher cell concentrations were used. Therefore, if the observed DMPO–OH signal resulted from an intracellular site as previously reported (Sinha et al., 1987a,b), then DMPO–OH signal intensity would increase with increasing cell concentration. Such was not the case, as DMPO–OH signal did not increase with cell concentrations greater than 2×10^7 cells/mL (Figure 4).

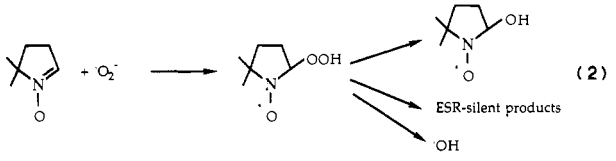
Since superoxide dismutase does not cross the cell membrane, the complete elimination of DMPO–OH signal brought about by externally added SOD would not be anticipated if $O_2^{\bullet-}$ radicals were distributed intracellularly. Likewise, since the line-broadening agent CrOx does not enter the cell, if the demonstrable DMPO–OH signal was inside the cell, it would not be expected to disappear upon CrOx addition. CHO-AB cells were aerobically incubated with ADR and DMPO. Following the development of the DMPO–OH signal, CrOx was added, and the sample was scanned for ESR spectrum. Forty-five mM CrOx to 10^6–10^8 cells/mL (with all cell lines studied) made the DMPO–OH signal invisible. The results of a typical experiment, carried out with MCF7-ADR cells, are illustrated in Figure 3 (middle and lower traces). Contrary to previous reports in which DMPO–OH is stated to be observable both inside and outside of the cell (Sinha et al., 1987a,b), DMPO–OH is detectable exclusively outside the cells.

Cellular Reducing Enzymes and Reductants. The GSH levels of the two different cell types were compared. The GSH concentration for the wild-type MCF-7 was two times that of

the adriamycin-resistant cell line (98.5 ± 3.5 vs 40.9 ± 1.9 nmol/mg of protein), and for the CHO cells the wild type had only half the GSH level of the pleiotropic multidrug-resistant cell line (13.9 ± 5.7 vs 26.0 ± 5.2 nmol/mg of protein). When the levels of GSH for the two different sets of cells are compared, in one set the resistant cells have more GSH, and in the other set the resistant cells have less GSH; it is apparent that resistance to adriamycin is not associated with absolute GSH levels. For the MCF-7 cells, the H_2O_2-related detoxifying enzymes apparently correlate with adriamycin resistance. That is, GSH *S*-transferase activity is markedly elevated in the resistant cells (40-fold), GSH peroxidase activity is 5-fold higher, but GSH reductase activity is the same (Batist et al., 1986). However, when the same enzymes are looked at for the CHO cells, the GSH reductase and GSH peroxidase activities are the same and only the GSH transferase is elevated by a mere factor of 2 (Mitchell et al., 1988). Such differences in activity in the two pairs do not indicate that GSH-related detoxification of H_2O_2 is responsible for the 100–500-fold differences in ADR resistances seen in Figure 2.

Discussion

Reactive Intermediates. It has long been known that both DMPO–OH and "artifactual" •OH radicals are formed upon decay of DMPO–OOH (Finkelstein et al., 1982):



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Therefore, the reported ESR detection of DMPO–OH induced by MCF7-WT does not necessarily mean that primary •OH radicals are indeed being formed by ADR-treated cells. On the contrary, the present results, which demonstrate the failure of catalase to affect DMPO–OH and the total elimination of DMPO–OH signal by SOD, in both pairs of CHO and human cell lines, strongly suggest that $O_2^{\bullet-}$ rather than •OH radical is responsible for the observed DMPO–OH spectra.

Distribution of the Spin Adduct. Since CrOx does not enter into the cell over the time course of the experiment (Lai et al., 1987), it can be used to define whether an observed ESR signal results from intracellular or extracellular sites. In the present study when CrOx was added to cells preexposed to ADR and DMPO, the ESR signal of DMPO–OH became totally invisible. The elimination of the signal by the extracellular line-broadening agent substantiates the conclusion that the observed spin adduct from ADR-treated cells, under air, exists exclusively in the extracellular compartment. The present results contrast with a previous study, in which it was reported that CrOx did not abolish the DMPO–OH signal produced by ADR-treated MCF7-WT cells but rather only partially reduced it (Sinha et al., 1987b). However, on the basis of a MCF7-WT cell having a volume of 2400 μm^3, 2.5 × 10^6 cells/mL would occupy only 0.5% of the total sample volume. If 35–40% of the observed DMPO–OH indeed originates from intracellular sites (Sinha et al., 1987b), then the DMPO–OH would have to partition 100–200-fold more into the intra- than into the extracellular compartment. Such a "concentrating factor" seems highly unlikely, particularly in view of the cell-facilitated destruction of intracellular DMPO spin adducts (Samuni et al., 1986, 1989a).

Site of Radical Trapping. The total elimination of DMPO–OH signal by CrOx does not exclude, however, the possibility that radicals are spin trapped by DMPO inside the cell and later diffuse outside. Yet, such a possibility is ruled out by noting that exogenously added SOD prevents the appearance of DMPO–OH. Such results, therefore, support the conclusion that spin trapping by DMPO of superoxide generated from an interaction between oxygen and AQ• occurs *outside* the cell. This conclusion is valid whether AQ• is produced inside the cell or at the cell membrane via one-electron reduction or outside the cell by comproportionation (Kalyanaraman et al., 1980; Powis, 1989), and it agrees with previous reports that AQ• readily crosses the cell membrane and predominantly accumulates outside the cell (Sinha et al., 1987b).

Site of Radical Formation. The trapping of free radicals outside the cell does not prove conclusively that they are exclusively formed on the outside. Since intracellular •OH because of its extreme reactivity would not survive diffusing outside the cell and since the DMPO–OH spin adduct is rapidly destroyed by the cell (Samuni et al., 1986, 1989a), no signal due to intracellular species would be expected or is detectable. Therefore, the present results, which demonstrate conclusively an extracellular origin of the observable spin adduct, do not imply that •OH radicals are not formed also within ADR-treated cells, nor do they exclude intracellular production of superoxide. However they do imply that (a) cells are instrumental in ADR reduction, (b) AQ• freely diffuses between the intra- and extracellular compartments, (c) most $O_2^{\bullet-}$ are formed extracellularly, (d) the observed DMPO–OH derives from $O_2^{\bullet-}$, and (e) the $O_2^{\bullet-}$ radicals are trapped by DMPO outside the cell.

Comparison between ADR-Sensitive and ADR-Resistant Cells. Contrary to recent findings (Sinha et al., 1987a,b), the present results do not reveal any significant difference between ADR-resistant and wild-type cells in their abilities to produce semiquinone free radicals (Figure 1). This conclusion is based on results obtained with both human and CHO cells. Despite the huge differences in their sensitivity to ADR (ADR-induced killing rates of MCF7-ADR and MCF7-WT differ by more than 500-fold), the four cell lines examined gave rise to comparable levels of AQ• radicals. The conclusion is substantiated by comparing the intensities of the DMPO–OH signal (Figure 4). Moreover, the ADR-resistant and -sensitive pairs of cell lines (both human and CHO) did not differ in their GSH levels. Nor could a correlation be found between ADR sensitivity and the level of cellular-reductive enzymes. These results do not support the previously presumed correlation (Sinha et al., 1987) between oxy radical production and drug antitumor activity.

In conclusion, the present results verify previous reports concluding that cellular reduction of ADR generates AQ• radicals, which are capable of diffusing across the cell membrane and reducing oxygen to yield extracellular $O_2^{\bullet-}$, which in turn can be intercepted by DMPO. The observed DMPO–OH signal results from DMPO–OOH decomposition rather than from trapping of genuine hydroxyl radicals. Such findings neither prove nor disprove ADR-induced formation of intracellular free radicals but do demonstrate that even with DMPO the feasibility of spin trapping and detecting intracellular •OH is very slim.

Since no significant difference between radical production by cells sensitive or resistant to ADR is found, no support is lent, considering the vast difference in biological response between these cells, to the assumption that ADR antitumor activity is affected by radical production rate. Therefore, neither our findings nor previous ones (Meijer et al., 1987; Keizer et al., 1988; Cervantes et al., 1988) corroborate the

proposition that ADR-induced oxygen-derived radicals, and particularly •OH radicals, play a role in ADR antitumor activity. We realize that the mechanism by which ADR exerts its antitumor activity is still unknown and may differ from that responsible for cardiotoxicity and, potentially, that difference may allow a strategy to improve ADR therapeutic index.

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