ABSTRACT

Trenimon belongs to a class of aziridinylbenzoquinone anticancer drugs that cross the blood-brain barrier. In this study we have investigated the molecular mechanisms for trenimon-induced toxicity in aerobic versus hypoxic conditions with the use of freshly isolated rat hepatocytes. The following evidence suggests the mechanisms for trenimon detoxification involves reduction by DT-diaphorase, while the cytotoxic mechanism involves macromolecule alkylation under hypoxic conditions as well as oxidative stress under aerobic conditions. (a) Hepatocyte cytotoxicity induced by trenimon (250 μM) under aerobic conditions ensued following an initial induction of cytochrome P-450-dependent respiration and partial oxidation of glutathione to oxidized glutathione. Trenimon reduction to the hydroquinone by the hepatocytes was rapid. Inhibition of hepatocyte DT-diaphorase by dicumarol increased trenimon-induced cytotoxicity by approximately 10-fold, and markedly inhibited hydroquinone formation. Furthermore, both cytochrome P-450-dependent respiration and oxidized glutathione formation were markedly increased, resulting in depletion of oxygen in the media. Trenimon reduction to the hydroquinone then occurred. This suggests that DT-diaphorase in normal hepatocytes prevents the formation of the semiquinone that causes cytochrome P-450 alkylation and oxidative stress. (b) Hepatocyte cytotoxicity induced by trenimon (350 μM) under hypoxic conditions ensued following glutathione depletion without oxidized glutathione formation. Inhibition of hepatocyte DT-diaphorase by dicumarol under hypoxic conditions increased trenimon-induced cytotoxicity by approximately 3.5-fold and increased semiquinone radical levels 2-fold without affecting its reduction rate. This suggests that the cytotoxic mechanism involves protein alkylation by semiquinone radicals formed by reductases catalyzing a one-electron reduction of trenimon.

INTRODUCTION

Trenimon (2,3,5-tris-ethylenimino-1,4-benzoquinone) was one of the first aziridinylbenzoquinone anticancer drugs synthesized by Gauss (1) in 1958 at the Bayer scientific laboratories in Leverkusen, Germany. Trenimon was highly effective against a broad range of experimental tumors in rats and mice (2) as well as human cancers, particularly in the treatment of hemoblastoses and local tumors (3). However, because of its toxicity the use of trenimon in chemotherapy was discontinued.

Aziridinylbenzoquinone anticancer drugs are thought to be activated to alkylating agents upon reduction of the quinone moiety. Enzymatic reduction of quinones may occur either via a direct two-electron addition by NAD(P)H:quinone acceptor oxidoreductase (EC 1.6.99.2), commonly referred to as DT-diaphorase, or via one-electron reduction by enzymes such as NADPH-cytochrome P-450 reductase (4, 5). It is thought that the reduction of the quinone to the one-electron reduction product, semiquinone, or the two-electron reduction product, hydroquinone, facilitates the protonation of the aziridine ring (6), leading to the formation of the aziridinium ion, the required species for alklylation (7, 8). Previously we have shown that trenimon overcomes the drug resistance of the quinone-resist-

MATERIALS AND METHODS

Chemicals. Trypan blue, GSH, GSSG, sodium azide, fluoro-2,4-dinitrobenzene, dicumarol, menadione, 2,6-dichlorophenol-indophenol, iodoacetic acid, and H2O2 (as a 30% solution) were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase (from Clostridium histoliticum) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid were purchased from Boehringer-Mannheim (Montreal, Canada). Trenimon and dixoquione were gifts from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Other chemicals were of the highest grade available commercially.

Animals. Male Sprague Dawley rats (body weight, 200 to 250 g) fed a standard chow diet and tap water ad libitum were used to prepare hepatocytes.

Hepatocyte Isolation and Incubation. Rat hepatocytes were prepared as previously described by Moldeus et al. (16) by collagenase perfusion of the liver. The viability of the cells were assessed by their ability to exclude trypan blue, 0.2% (w/v) final concentration, using a Neubauer chamber, by light microscope. The final viability of the hepatocytes was >85%.

The cells (1 × 10^6 cells/ml) were preincubated at 37°C in Krebs-
Henseleit buffer (final volume, 10 ml) containing 12.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid under an atmosphere of 10% O2/5% CO2/85% N2, for 30 min in continuously rotating 50-ml round bottomed flasks. For experiments performed under hypoxic conditions, the cells were incubated under an atmosphere of 95% N2 and 5% CO2 following preincubation under aerobic conditions. The oxygen concentration was near zero 30 min after the switch to the hypoxic atmosphere and at this time the experiments were started. Trenimon was dissolved in dimethyl sulfoxide and added in a final concentration of 0.5% (v/v) dimethyl sulfoxide alone.

Assays. Total GSH and GSSG in the hepatocyte incubation mixture were measured in deproteinized samples (5% metaphosphoric acid) after derivatization with iodoacetic acid and fluoro-2-4-dinitrobenzene, by high-pressure liquid chromatography, using a Bondapak NH2 column (Waters Associates, Milford, MA) (17). GSH and GSSG were used as external standards. A Waters Model 600A solvent delivery system, equipped with a Model 660 solvent programmer, a WISP 710A automatic injector, and a data module, was used for analysis.

Oxygen consumption was measured by a Clark-type electrode (Model 5300; Yellow Spring Instrument Co., Inc.) in a 2-ml chamber. When monitoring oxygen consumption in hepatocytes the incubation chamber was maintained at 37°C, whereas room temperature was used for experiments with microsomes. KCN (2 mM, neutralized with HCl) was added to hepatocytes to inhibit mitochondrial respiration.

Subcellular Preparations. Liver microsomes were obtained as described by Ernster et al. (18). Incubations with microsomes were performed in 0.1 mM Tris-HCl, pH 7.4.

Spectrophotometric Analysis of Trenimon Metabolism. The reduction of trenimon by hepatocytes was followed spectrophotometrically (at 335 nm) in a 3-ml cuvette containing 1 x 10^6 cells/ml in Krebs-Henseleit incubation buffer at 37°C, using an Amino DW2000 spectrophotometer fitted with a stirrer. Anaerobic reactions were carried out in a Thunberg-type cuvette with hepatocytes that had been under a continuous flow of 95% N2/5% CO2 for at least 45 min.

DT-Diaphorase Purification. Rat hepatic DT-diaphorase (EC 1.6.99.2) was purified from the cytosolic fraction by using cibacron blue affinity chromatography as described by Sharkis et al. (19), yielding an enzyme with a specific activity of 320 nmol/min/μg protein. This reducecata activity was completely inhibited by dicumarol.

DT-Diaphorase Activity. DT-diaphorase activity was measured as described by Ernster (4) at room temperature with NADH as the electron donor and DCPIP as electron acceptor. The standard reaction mixture consisted of 50 mM Tris-HCl, pH 7.5, 0.15 mg bovine serum albumin/ml, 0.2 mM NADH, and 0.1 mM DCPIP. DCPIP reduction was measured at 600 nm with a DW 2000 spectrophotometer. Reduction of other orthoquinones by DT-diaphorase was measured as described above with DCPIP by monitoring the disappearance of the substrate spectrophotometrically. The nonenzymatic reduction of trenimon by NADH was insignificant.

ESR Studies. Samples for ESR measurements were prepared by the addition of glutathione or ascorbate (1-2 mM) to trenimon (1-5 mM) in 0.1 M borax buffer, pH 7.4, previously bubbled with nitrogen for 10 min. Hepatocyte samples were prepared in a similar manner by gently bubbling the cells (3-5 x 10^6 cells/ml) with nitrogen for 10 min prior to trenimon addition. The sample was aspirated into a flat cell and ESR spectra were recorded (20). The spectra were scanned at least three times (usually 8-min scans). The ESR measurements were performed in an aqueous flat cell (8 mm wide), using a dual cavity CTE model at room temperature with a Varian E-6 ESR spectrometer.

Statistical significance of differences between treatment groups in these studies were determined by the Student t test. The minimal level of significance chosen was P < 0.05.

RESULTS

Role of DT-Diaphorase in Trenimon-Induced Hepatocyte Cytotoxicity under Aerobic versus Hypoxic Environments. Rat hepatocytes were treated with various doses of trenimon under aerobic conditions for a period of 3 h to determine the concentration of the drug required for cell death as determined by trypan blue exclusion. Table 1 shows that when the concentration of the drug was below 250 μM it was not toxic to cells, but at drug concentrations of 250 and 350 μM, cell death after 3 h of incubation was 78 and 100%, respectively (P < 0.001).

Aziridinylbenzoquinone antimutagen drugs have been reported to act as bioreductive alkylating agents, and hence are thought to be selectively more toxic to hypoxic cells (6). Isolated hepatocytes remain viable for up to 5 h under hypoxic conditions (95% N2/5% CO2). Toxicity studies showed that trenimon was less effective at inducing cell death under hypoxic conditions than under aerobic conditions (Table 1). A dose of 350 μM trenimon was required under hypoxic conditions to cause the same degree of cytotoxicity as 250 μM trenimon under aerobic conditions.

To determine whether the cytotoxic mechanism under aerobic conditions was different from that under hypoxic conditions, the consequences of inactivating DT-diaphorase was determined. DT-diaphorase, known to catalyze a two-electron reduction of quinones, has been implicated in the metabolism of various antitumor quinone drugs (4, 21). In order to explore the role of DT-diaphorase in trenimon-induced cytotoxicity, the cells were incubated in the presence of dicumarol, an inhibitor of DT-diaphorase. As shown in Table 1, under aerobic conditions hepatocytes were approximately 10-fold more sensitive to trenimon in the presence of dicumarol (a dose of 25 μM killed 85% of the cells in the presence of dicumarol (P < 0.001)). If cellular catalase was inactivated with azide, cell death occurred even more rapidly, indicating that H2O2 formation contributes to cytotoxicity. Under hypoxic conditions the addition of dicumarol also enhanced trenimon-induced cytotoxicity (Table 1). Under these conditions, 100 μM trenimon caused 100% cell death (the same was achieved with a dose of 350 μM in the absence of dicumarol). Lower concentrations of trenimon were not toxic. Inactivation of catalase, however, did not further affect their susceptibility to trenimon (data not shown).

Effect on GSH Status. Incubation of isolated hepatocytes

Table 1 Trenimon-induced hepatocyte cytotoxicity under aerobic and hypoxic environments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytotoxicity (% of trypan blue uptake) in following times (min)</th>
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<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Control cells</td>
<td></td>
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<tr>
<td>+trenimon, 350 μM</td>
<td>14 ± 2</td>
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<tr>
<td>+trenimon, 250 μM</td>
<td>22 ± 2</td>
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<tr>
<td>+trenimon, 100 μM</td>
<td>22 ± 2</td>
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<tr>
<td>+trenimon, 25 μM + dicumarol + azide</td>
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<td>+trenimon, 25 μM + dicumarol</td>
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<td>+trenimon, 100 μM</td>
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<td>22 ± 3</td>
</tr>
<tr>
<td>+trenimon, 100 μM + dicumarol</td>
<td>17 ± 3</td>
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* Significantly different from control, P < 0.001.

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MECHANISMS OF TRENIMON-INDUCED TOXICITY

Under aerobic conditions GSH depletion occurred rapidly within the first 10 min after trenimon addition, followed by a recovery of some GSH by the 30-min incubation period. GSSG formation was also the highest within the first 10 min of incubation followed by a slow decrease thereafter. Approximately 50% of the initial GSH depletion was attributed to GSSG formation (Fig. 1). Inactivation of DT-diaphorase markedly increased GSH depletion and GSSG formation was now nearly stoichiometric (Fig. 3). In contrast, under hypoxic conditions, GSH depletion occurred without GSSG formation. GSH depletion was also markedly increased by DT-diaphorase inactivation (results not shown).

Reduction of Trenimon by Isolated Rat Hepatocytes. The reductive metabolism of the drug during incubation with hepatocytes was determined from the loss of 335 nm absorbance. Typical spectra recorded under both aerobic and hypoxic environments are shown in Fig. 4A, Curve a and Fig. 4B, Curve a, respectively. Reduction of trenimon by hepatocytes occurred rapidly in both aerobic and hypoxic atmospheres. Trenimon reduction under hypoxic conditions was slightly faster (Fig. 4B). Addition of either dithiothreitol or sodium borohydride to

Fig. 1. GSH depletion (A) and GSSG formation (B) induced by trenimon in isolated hepatocytes under an aerobic atmosphere. Hepatocytes, 10^6 cells/ml, were incubated alone (○), and with 350 μM (●), 250 μM (■), and 100 μM (▲) trenimon. At various times, samples of the cell suspension were removed and total GSH and GSSG levels were determined by high-performance liquid chromatography analysis, as described in “Materials and Methods.” Three separate experiments were carried out. Points, mean; bars, SE.

Fig. 2. GSH depletion induced by trenimon in isolated hepatocytes under a hypoxic atmosphere. Hepatocytes, 10^6 cells/ml, were incubated alone (○), and with 350 μM (●), 250 μM (■), and 100 μM (▲) trenimon. Three separate experiments were carried out. Points, mean; bars, SE.

with increasing concentrations of trenimon under both aerobic and hypoxic conditions resulted in an immediate dose-dependent decrease in intracellular GSH (Figs. 1A and 2). Complete GSH depletion occurred only with toxic doses of trenimon.
MECHANISMS OF TRENIMON-INDUCED TOXICITY

Fig. 4. Reduction of trenimon in hepatocytes under aerobic (A) and hypoxic (B) atmospheres and cyanide-resistant respiration (C). Hepatocytes, 10⁶ cells/ml, were incubated with 50 μM trenimon in the absence (Curve a) and presence (Curve b) of 20 μM dicumarol as indicated in the figure. Both reduction of trenimon to the hydroquinone and cyanide-resistant respiration were monitored as described in "Materials and Methods." The spectra represent one experiment typical of three.

Trenimon under aerobic conditions has been shown to reduce trenimon to the hydroquinone in which the 335 nm absorbance ($E = 12,000$) disappears and a small band at 300 nm ($E = 4,500$) appears. The hydroquinone slowly autoxidizes back to the quinone with time (8). UV spectrophotometry was used to show that the product formed had the same retention time and UV spectra (8) as the hydroquinone (results not shown). Furthermore, under aerobic conditions, sodium borohydride (100 μM) but not ascorbate (1 mM) reduced trenimon at a rate similar to that shown in Fig. 4A, Curve a (results not shown).

Since DT-diaphorase plays a role in trenimon-induced cytotoxicity, we investigated the role of the DT-diaphorase inhibitor, dicumarol, on trenimon reduction by hepatocytes. Under aerobic conditions the presence of dicumarol delayed any two-electron reduction of trenimon for 5 min (Fig. 4A, Curve b). As shown in Fig. 4B, Curve b, 20 μM dicumarol, however, had little effect on the reduction of trenimon by hepatocytes in hypoxic conditions. This suggests that reducing systems other than DT-diaphorase are also able to reduce trenimon in hepatocytes.

Oxygen electrode experiments were then used to determine whether trenimon had also been reduced by various one-electron reductases to the semiquinone radical which had reacted with O₂ to reform the parent quinone. If such a futile redox cycle occurred it could be expected that hypoxia could result. When cyanide-resistant respiration by hepatocytes was monitored (Fig. 4C, Curve a), the addition of trenimon to hepatocytes caused an initial consumption of oxygen which quickly leveled off. However, in the presence of dicumarol (Fig. 4C, Curve b), cyanide-resistant respiration was markedly increased and approximately 90% of the oxygen in solution was consumed in 5 min. Hence, in a little over 5 min, the incubation system became hypoxic, enabling trenimon to be rapidly reduced to the hydroquinone, as shown in Fig. 4A, Curve b.

Interestingly, the initial rapid reductive metabolism of higher concentrations of trenimon (100 μM) by the cells leveled off to a much slower steady decrease (Fig. 5). This pattern could actually be further enhanced by performing the experiment with older hepatocytes which had been incubated for 2 h (data not shown).
shown). Since our results demonstrate that DT-diaphorase plays a major role in catalyzing the reduction of trenimon by hepatocytes, we investigated the possibility that the supply of NADH or NADPH in the cell, needed as cofactors for DT-diaphorase, was limiting in hepatocytes which had been preincubated for 2 h. It is known that the addition of ethanol to hepatocytes and perfused rat liver causes a rapid increase in NADH levels due to the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase (22), and we have recently shown that ethanol markedly increases reduction of nitrosodimethylaniline in isolated hepatocytes (23). As shown in Fig. 5, reduction of trenimon by hepatocytes in the presence of ethanol (10 mM) not only occurred at a faster rate, but also resulted in the complete reduction of the drug. This was further demonstrated by treating the cells with ethanol 4 min after the addition of trenimon. The reduction rate increased rapidly, again suggesting that increased NADH increases trenimon reduction by isolated hepatocytes. Furthermore, in the presence of 10 mM ethanol, the hepatocytes were more resistant to trenimon-induced cytotoxicity (cell death after a 3-h incubation of hepatocytes with 250 μM trenimon was 45 ± 4% (SE) in the presence of ethanol versus 100% with trenimon alone; results not shown).

Reductive Metabolism of Trenimon by Rat Liver Microsomes versus DT-Diaphorase. In order to evaluate the ability of trenimon to undergo one-electron reduction and oxygen activation, oxygen consumption was measured when trenimon was reduced by NADPH catalyzed by rat liver microsomes. A \( V_{\text{max}} \) of 38 ± 4 nmol oxygen consumed/min/mg protein was obtained in the presence of trenimon. Diaziquone, which has been shown to undergo one-electron reduction and oxygen activation in the presence of microsomes and NADPH had a \( V_{\text{max}} \) of 27 ± 4 nmol oxygen consumed/min/mg protein. These results demonstrate that microsomal NADPH reductase causes redox cycling resulting in oxygen activation.

Finally, to prove that trenimon is a substrate for DT-diaphorase, DT-diaphorase was isolated from rat liver cytosol to evaluate its specificity for trenimon. Trenimon was efficiently reduced to the hydroquinone in the presence of the enzyme with a \( V_{\text{max}} \) of 170 ± 17 μmol/min/mg protein and a \( K_m \) of 40 ± 5 μM. No oxygen uptake occurred and trenimon did not reform following reduction, indicating that hydroquinone formed did not autoxidize (results not shown). The reduction rate of trenimon was comparable to the reduction of menadione catalyzed by DT-diaphorase, \( V_{\text{max}} \) of 220 ± 22 μmol/min/mg protein and \( K_m \) of 5 ± 2 μM, another excellent substrate for DT-diaphorase. Addition of dicumarol (10 μM) completely inhibited the reduction of both substrates by DT-diaphorase.

Detection of Semiquinone Radical by ESR. Fig. 6A shows that the semiquinone radical of trenimon was formed on addition of trenimon to hepatocytes at pH 7.4. In the absence of trenimon (Fig. 6B) or hepatocytes (Fig. 6C), the semiquinone radical was not formed. When the sample in the flat cell was deaspirated and exposed to air, the trenimon semiquinone radical immediately disappeared, but was detected again after rebubbleing the sample with nitrogen. Inactivation of hepatocyte DT-diaphorase increased the amplitude of the semiquinone radical formed by hepatocytes (approximately 2-fold).

Ascorbate reduction of trenimon at pH 10 also resulted in the formation of the semiquinone radical of trenimon (Fig. 7A). In the absence of ascorbate or trenimon, the semiquinone radical was not detected (Fig. 7, B and C). One molar equivalent of glutathione also reduced trenimon, at pH 10, to its semi-
none radical, but with 3 molar equivalents of glutathione some glutathionyl conjugates of trenimon semiquinone radical were also detected. The semiquinone radical was not detected at pH 7.4 with ascorbate or glutathione, probably due to a slower rate of formation.

DISCUSSION

Incubation of isolated rat hepatocytes under either aerobic or hypoxic conditions with trenimon caused cell death in a concentration- and time-dependent manner. Cell death was preceded by GSH depletion under either atmosphere. While GSSG formation accounted for approximately 50% of GSH depleted under aerobic conditions, no GSSG was formed under hypoxia. This suggests oxidative stress contributes to trenimon-induced cytotoxicity under aerobic but not under hypoxic conditions.

Inactivation of hepatocyte DT-diaphorase by dicumarol increased trenimon toxicity approximately 10-fold under aerobic conditions and 3-fold under hypoxic conditions, indicating that DT-diaphorase is involved in the detoxification of trenimon by hepatocytes. Results with DT-diaphorase isolated from rat liver cytosol confirms that trenimon is a much better substrate for DT-diaphorase compared to that reported for diaziquone (21). Under aerobic conditions oxidative stress was markedly enhanced in DT-diaphorase-inactivated hepatocytes compared to normal hepatocytes, as shown by a marked increase in GSH oxidation and cytochrome-dihydroporin and increased cytoxicity when catalase was inactivated. No oxidative stress occurred under hypoxic conditions but GSH depletion was markedly increased, which could indicate that GSH-conjugating metabolites are formed when trenimon is reduced. Presumably trenimon reduction to the hydroquinone assists the opening of the aziridine ring, thereby liberating the alkyllating aziridinium ion. In vitro experiments show that GSH reacts very slowly with trenimon even in the presence of cytosol (results not shown), indicating that the unsubstituted C5 position of the benzoquinone ring is sterically hindered for GSH conjugation (24).

The metabolism of the drug by hepatocytes under both aerobic and hypoxic conditions occurred rapidly. This was shown to involve the rapid reduction of the quinone. However, if DT-diaphorase was inhibited, the ability of the cells to reduce trenimon to its hydroquinone derivative was prevented under aerobic conditions. Reduction of trenimon to the hydroquinone occurred only when the oxygen in the media was depleted as a result of redox cycling mediated oxygen activation. This was shown by monitoring cytochrome oxidase activity induced by trenimon in hepatocytes. Incubation of hepatocytes with trenimon under aerobic conditions caused an initial stimulation of cytochrome oxidase which quickly subsided. In contrast, with DT-diaphorase-inactivated cells, trenimon induced a rapid and continuous oxygen consumption which caused the cells to rapidly become anaerobic, and only then was trenimon reduced to the hydroquinone. Under hypoxic conditions, exposure of cells to dicumarol had little effect on the reduction rate of trenimon. The ability for DT-diaphorase to catalyze the reduction of trenimon in hepatocytes was shown further by the effect of ethanol on cells. The reduction rate of trenimon was increased in the presence of ethanol, this is presumably due to an increase in NADH levels which is normally low in isolated hepatocytes.

Evidence that intracellular reductases also catalyze the one-electron reduction of trenimon comes from the cyanide-resistant respiration induced by trenimon with hepatocytes and/or by rat liver microsomes and NADPH. Direct evidence for the formation of the semiquinone radical in hepatocytes comes from the ESR detection of the semiquinone radical. The spectrum (Fig. 6A) has seven broad and slightly overmodulated lines. A very similar spectrum was also recorded when trenimon was reduced by ascorbate (Fig. 7A), or glutathione (data not shown). We could not obtain a well-resolved spectrum in order to measure the hyperfine-coupling constants. The fact that the amplitude of the semiquinone radical was stronger (approximately 2-fold) in the presence of dicumarol suggests that a competition exists between the one-electron reductases and two-electron reduction diaphorase pathways in hepatocytes for the reduction of trenimon. Inhibition of the two-electron reduction pathway enhances the one-electron reduction pathway.

A proposed molecular mechanism for the metabolism of trenimon in isolated hepatocytes leading to cell death is summarized in Fig. 8. Reduction of the quinone to the hydroquinone occurs both under aerobic and hypoxic conditions. Reduction occurs via a direct two-electron addition and via successive one-electron additions. Under hypoxic conditions both reduction routes appear to cause the formation of the hydroquinone derivative of the drug. In the presence of oxygen, however, the one-electron product, a semiquinone radical, redox cycles back to the parent quinone and produces reactive oxygen species. Hepatocytes are well equipped with defense mechanisms against reactive oxygen species. Because trenimon depletes GSH through arylation, this renders the cells more susceptible to oxidative stress. This pathway predominates when DT-diaphorase is inhibited, whereas when DT-diaphorase is active, trenimon is rapidly reduced to its hydroquinone which may lead to the opening of the aziridine ring and alkylation, causing cell damage. However, the fact that inhibiting DT-diaphorase increases hepatocyte susceptibility under hypoxic conditions, suggests that the semiquinone is the ultimate cytotoxic species.

Recent work in our laboratory comparing toxicity of trenimon in two murine lymphoblastic cell lines, L5178Y and L5178Y/HBM10 (L5178Y/HBM10 cell line has 24x higher DT-diaphorase activity), showed trenimon to be more toxic to the cells containing higher DT-diaphorase activity and furthermore, inactivation of DT-diaphorase prevented the enhanced toxicity (9). The contradiction of DT-diaphorase apparently being a detoxifying enzyme for trenimon in the isolated hepatocytes as opposed to a toxicity-enhancing enzyme in the L5178Y murine lymphoblastic cells (9) could indicate that the...
semiquinone, by alkylating proteins or causing oxidative stress, damages the plasma membrane of the hepatocyte, whereas the hydroquinone alkylates DNA which prevents L5178Y/HBM10 cell division, the criteria used to determine toxicity of these cells.

Siegel et al. (21) also reported that diaziquone, another aziridinylbenzoquinone drug, was more toxic to HT-29 human colon carcinoma cells than BE cells with lower DT-diaphorase activity and inactivation of DT-diaphorase prevented cytotoxicity. Begleiter et al. (25) also found that mitomycin C was more toxic to L5178Y/HBM 10 cells, probably because of their much higher DT-diaphorase activity, as inactivation of DT-diaphorase prevented mitomycin C cytotoxicity.

These differences on the role played by DT-diaphorase in enhancing the toxicity of aziridinylbenzoquinone alkylating anticancer agent toward dividing tumor cells and decreasing toxicity in nondividing normal cells should encourage the development of reductive alkylating anticancer agents which are better substrates of DT-diaphorase agents which are applicable to DT-diaphorase-rich tumors without causing tissue toxicity.

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Modulation of Trenimon-induced Cytotoxicity by DT-Diaphorase in Isolated Rat Hepatocytes under Aerobic versus Hypoxic Conditions

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