Peroxidase-catalyzed Metabolism of Etoposide (VP-16-213) and Covalent Binding of Reactive Intermediates to Cellular Macromolecules

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ABSTRACT

The horseradish peroxidase- and prostaglandin synthetase-catalyzed oxidative metabolism of the highly active anticancer drug, etoposide (VP-16-213), has been studied in vitro. This oxidation of VP-16 resulted in the formation of VP-16 quinone, an aromatic VP-16 derivative and the corresponding aromatic VP-16 quinone. This oxidative metabolism of VP-16 also resulted in the formation of reactive species that covalently bound to exogenously added DNA and heat-inactivated microsomal proteins. The peroxidase-catalyzed binding was time dependent and required the presence of cofactors (hydrogen peroxide or arachidonic acid). The prostaglandin synthetase/arachidonic acid-catalyzed metabolism and binding of VP-16 were inhibited by indomethacin, an inhibitor of the cyclooxygenase, and were shown to involve the peroxidative arm of prostaglandin synthetase. Our studies show that the protein covalent binding species were formed as a result of O-demethylation of the drug as shown by the loss of specifically labeled (O-14CH3) radioactivity from O-methoxy group and by incubating proteins with VP-16 quinones. In contrast, the covalent binding intermediates for DNA appeared to be different and VP-16-derived quinone methides are suggested as DNA binding species. Co-oxidation of VP-16 and the related drug, VM-26, during prostaglandin biosynthesis may be an important pathway for the metabolism of these agents and may play a role in their cytotoxic properties.

INTRODUCTION

VP-16 (Fig. 1), a semisynthetic derivative of podophyllotoxin, is clinically active in the treatment of a number of human neoplasms including small cell lung cancer, testicular tumors, and malignant lymphomas (1, 2). Although the precise mechanism by which VP-16 induces cytotoxicity is not known, evidence suggests that DNA damage produced by VP-16 may relate to its cytotoxicity. VP-16 has been shown to cause both single- and double-strand DNA breaks in tumor cells and in isolated tumor cell nuclei (3–8). Loike and Horwitz (3) have shown that both the presence of cellular components and the free 4'-OH in the E-ring of VP-16 are essential for the DNA damage to occur. Topoisomerase II has been implicated in this DNA damage. Recent studies reported from our laboratory have shown that VP-16 undergoes cytochrome P-450-dependent metabolism which results in O-demethylation of the drug (9). The microsomal metabolism of VP-16 also resulted in the formation of reactive intermediates that covalently bound to both proteins and to DNA (9–11).

In a preliminary communication, we have recently reported that the O-demethylation of VP-16 and the related antitumor agent, VM-26, can be catalyzed by HRP and PES (12). In this report we have further examined the peroxidase-dependent metabolism of VP-16 and show that the peroxidative oxidation of VP-16 is associated with the formation of reactive intermediates that covalently bound to exogenously added DNA and proteins.

MATERIALS AND METHODS

VP-16 and [O-14CH3]-VP-16, single labeled in one of the two equivalent carbon atoms of the methoxy groups (10.8 mCi/mmol), synthesized by Dr. J. Swig, were kindly provided by Bristol-Myers Pharmaceuticals, Syracuse, NY. [3H]-VP-16 labeled in the aromatic rings only (400 mCi/mmol; >99% pure, reverse-phase HPLC) was obtained from Moravek Biochemicals, Inc., Brea, CA. HRP (type VI. Reinheitzahl = 3.2), polyethylene glycol (M, 200), calf thymus DNA (highly polymerized type I), phenobarbital, sodium salt, reduced glutathione, and indomethacin were purchased from Sigma Chemical Company, St. Louis, MO. Purified PES from ram seminal vesicles was obtained from Oxford Biochemical Research, Inc., MI. AA was purchased from Nu-check Prep, Inc., Elysiann, MN, and was stored in the dark at −70°C. VP-16-Q (Fig. 1, m.p. 241–243°C with decomposition) was synthesized from VP-16. 4'-O-methyl derivative (m.p. 153–155°C) of VP-16 was prepared by diazomethane reaction of VP-16. The purity and structures of these derivatives were confirmed by thin layer chromatography (Merck silica gel-60 using ether:acetone 3:1 as the solvent; the Rf values were 0.35 for VP-16, 0.25 for VP-16-Q, and 0.5 for O-methyl derivative, respectively). HPLC, NMR, and mass spectral analyses. The mass spectral analysis was carried out as described previously (11).

Hepatic microsomes were prepared from phenobarbital-induced male mice as previously described (13). The denatured microsomal proteins were prepared by heating at 80°C for 20 min. The protein concentration was determined according to the method of Lowry et al. (14) with bovine serum albumin as a standard.

Incubation Conditions. All incubations were carried out in 150 mM KCl-50 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM diethylenetriaminepentaacetic acid at 37°C. The incubation mixtures contained HRP or PES and VP-16 dissolved in polyethylene glycol (final volume of 0.5% for 25 μm and 0.5% for 250 μm VP-16). The mixture was then warmed to 37°C for 2 min and the reaction was initiated by adding 250 μM H2O2 or AA (dissolved in ethanol, final volume of 0.15%). Reactions under reduced oxygen concentrations were similarly carried out except that the reaction mixture was bubbled with nitrogen for 10–15 min before adding H2O2 or AA, and the mixtures were sealed under nitrogen. The incubation mixtures were continuously shaken, and the reactions were terminated by adding chloroform (for HPLC analysis), water-saturated phenol-chloroform (for DNA binding studies), or TCA (for protein binding studies).

HPLC Analysis. The HPLC analysis was carried out as previously described (11, 12). Briefly, 1 ml of the reaction mixture was extracted with 5 ml of chloroform and the chloroform layer was separated by centrifugation. The aqueous layer was reextracted with 3 ml of chloro-
form and the combined organic layers were evaporated under nitrogen. The residue was dissolved in methanol and injected onto a Bondapak phenyl column using 60% methanol-40% water (v/v) as the mobile phase at a flow rate of 1 ml/min. A LKB detector was used to monitor the absorbance at 277 nm.

Covalent Binding. The covalent binding of VP-16 or its metabolite(s) to calf thymus DNA (0.6 mg/ml) or to heat-inactivated microsomal proteins (1 mg/ml) was carried out in an incubation mixture containing 3H-VP-16 (0.25 µCi/ml) dissolved in methanol (final volume 0.025%). For the DNA binding studies, the reaction mixture was extracted with an equal volume of water-saturated phenol:chloroform (1:1) for 15 min as described previously (15) and the stable drug DNA complexes were isolated by centrifugation at 8,000 x g for 20 min. The clear supernatant was carefully removed and precipitated in 4 volumes of absolute ethanol. The ethanol precipitate was cooled to -20°C and the precipitated DNA was collected, washed with methanol (3 x 25 ml), dried with nitrogen, dissolved in the Tris-KCl buffer, and reprecipitated with ethanol. The drug-DNA complex was isolated as above and was dissolved in the buffer. The bound radioactive drug was quantitated with a Searle 6880 liquid scintillation counter (Mark III) and appropriate quenching corrections were made. The nucleotide concentration in the samples was determined spectrophotometrically at 260 nm (16). The binding ratio is defined here as the molar ratio of drug bound to mononucleotide unit.

For protein binding studies, the proteins were precipitated on ice by adding an equal volume of 20% TCA. The precipitated proteins were collected by centrifugation and washed with 20 ml of 2% TCA. Under these conditions, HRP remained in solution. The protein pellet was further washed with methanol (3 x 25 ml), dissolved in 5 ml of 1% sodium dodecyl sulfate, and reprecipitated with TCA. Following additional washings with methanol (3 x 25 ml), the protein pellet was dried with nitrogen and dissolved in 1 N sodium hydroxide. The bound drug and protein concentrations were quantitated. Under our experimental conditions of isolation of the drug-macromolecule complexes, no further radioactivity could be removed from either DNA or proteins upon additional washings.

The effects of inhibitors on the peroxidase-catalyzed metabolism and binding of VP-16 were carried out under similar conditions except that the inhibitors were preincubated with enzymes for 10 min on ice. Indomethacin was dissolved in ethanol (final volume, 0.9%). Under these experimental conditions, the vehicles (polyethylene glycol, ethanol, and methanol) had no effects on either the VI'-16 metabolism or the covalent binding.

Experiments with Dual-labeled VP-16. Because the O-demethylation of VP-16 was expected to result in preferential loss of the 14C of the labeled methoxy moiety, some of the experiments were carried out with mixtures containing both O-14CH3-VP-16 and 3H-VP-16 (0.05 µCi/ml and 0.25 µCi/ml, respectively, dissolved in methanol; final volume, 0.5%) and the cold drug. It should be mentioned that due to labeling of one of two equivalent o-methoxy groups, only a 50% decrease in 14C/3H is expected. Counting was carried out with a program (TM Analytic) which automatically compensates and corrects for the cross contribution functions such that the error in the low-energy isotope (3H) was less than 1% and there was no error in 14C counts.

RESULTS

The HPLC analysis of the incubation mixture containing VP-16 (25 µM), HRP (0.25 mg/ml), and H2O2 revealed a rapid metabolism of VP-16 (Fig. 2) such that after 2 min, more than 70% of the drug (Peak A) was metabolized (Fig. 2b) and the metabolism was complete after 15 min (Fig. 2c). While the formation of metabolites B and C decreased with time, the relative formation of metabolites D and E increased with time (Fig. 2c). At a higher drug:enzyme ratio (VP-16, 250 µM; HRP, 1 mg/ml) the metabolism of VP-16 was incomplete up to 15 min, and under these conditions metabolite B predominated (Fig. 2d). The formation of these metabolites required the presence of all three components: drug, HRP, and H2O2.

The identity of these peroxidase-catalyzed metabolites of VP-16 was determined by mass spectrometric analysis. Isolation of the metabolite B from the incubation mixtures by HPLC and the mass spectral analysis showed a molecular ion at 584 with the base peak at 396 (data not shown) and was identical to one recently published (17). The metabolite B has been identified and shown to result from the loss of four hydrogen atoms and aromatization of the C-ring of VP-16 (Fig. 1). Similarly, the metabolite D was identified as the VP-16-Q, formed from the peroxidase-mediated O-demethylation of VP-16, as previously shown (12). Isolation of the metabolite B and subsequent incubation of this compound with HRP/H2O2 formed the metab-

![Fig. 1. Structures of VP-16 and its metabolites B, D, and E.](https://example.com/fig1.png)

![Fig. 2. HPLC chromatogram obtained from the chloroform extract of an incubation containing VP-16 (25 µM), HRP (0.25 mg/ml), and H2O2 (250 µM) at 37°C at Time 0. One-fifth of the extract of 1 ml was injected into the HPLC system. b same as a, except the incubation was carried out for 2 min; c same as a, except the incubation was carried out for 15 min; d same as c above, except that it contained 250 µM VP-16 and 1 mg/ml HRP. Absorbance was measured at 0.01 absorbance units (AU), full scale.](https://example.com/fig2.png)
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olite E, suggesting that the metabolite E may be the quinone derivative of the aromatic VP-16. This was further confirmed by mass spectral analysis which showed an ion at 570 (M + 2) which resulted from the reduction of the aromatic VP-16-Q, a reaction quite common for quinones (18) and also observed with the o-quinone metabolite D (12). In order to further confirm that the ion at 570 resulted from reduction in mass spectrometry, an accurate mass measurement (5000 resolution) was also made on this ion at nominal mass 570. The accurate mass was 570.1364 ± 0.0011. The actual mass for M + 2 of aromatic VP-16-Q (C28H26O3) is 570.1372, confirming that metabolite was the aromatic VP-16-Q and the ion observed at 570 was due to the reduction of the quinone derivative.

Incubation of VP-16 (25 μM) with PES (600 units/ml and 5000 units/ml) in the presence of AA also resulted in the metabolism of VP-16 and up to 80% of the drug was metabolized with higher PES concentration in 15 min. The resulting metabolic pattern was very similar to that catalyzed by HRP; however, under these conditions, VP-16-Q was the major metabolite formed as reported previously (12).

In order to better define the peroxidase-dependent metabolism of VP-16, a mixture containing 14C-VP-16 and 3H-VP-16 (initial ratio of 14C:3H = 0.2) was incubated with HRP/H2O2 and the metabolites were separated by HPLC. The metabolites D and E were collected under the conditions described in Fig. 2c and metabolite B was collected as described in Fig. 2d. Analysis of the radioactive distribution patterns showed 14C:H ratio of 0.3 for B, 0.1 for D, and 0.14 for E, respectively. These ratios suggest that tritium was lost during the formation of B, 14C was lost during the formation of D, and both tritium and 14C were lost during the formation of E. These results are consistent with the proposed structures (Fig. 1) for these metabolites of VP-16. Because O-demethylation of VP-16 is likely to result in the formation of water-soluble products (either methanol or formaldehyde), the radioactive distribution of 14C:H was examined in the aqueous and the organic phases following the HRP-catalyzed oxidation of VP-16. As shown in Table 1, the HRP-dependent metabolism of VP-16 resulted in a greater proportion of 14C in the aqueous phase than tritium (28% versus 16% of the total radioactivity). A major portion (41%) of the total 14C-VP-16-derived radioactivity was present as low molecular weight compound(s) because 14C radioactivity was easily evaporated with nitrogen compared to the total tritium (7%). Furthermore, all of the evaporable tritium was present in the aqueous phase, indicating that irritated water (H2O) was formed during the formation of the aromatic 16 metabolite. The evaporable 14C-derived radioactivity was present in both the organic and the aqueous phases (Table 1), suggesting that either methanol or formaldehyde was formed during peroxidase-catalyzed oxidation of VP-16. A similar radioactive distribution was also observed when the metabolism of VP-16 was catalyzed by PES in the presence of AA (Table 1). However, the amounts of water soluble and of the evaporable compounds formed were much smaller than those formed by HRP oxidation of VP-16 (Table 1). This is probably due to the incomplete metabolism of VP-16 by PES (600 units/ml) as indicated by HPLC analysis.

The peroxidative metabolism of the 3H-VP-16 resulted in irreversible binding of the radiolabel to exogenously added calf thymus DNA and to heat-inactivated proteins (Table 2). The binding required the presence of both the enzyme and the cofactor (H2O2 or AA). The covalent binding to DNA and proteins in the presence of PES and AA (Table 2) was significantly inhibited by indomethacin, an inhibitor of the cyclooxygenase system (19). Furthermore, PES fortified with AA was ineffective under anaerobic conditions in catalyzing this covalent binding of VP-16-derived reactive intermediates to DNA and proteins. In contrast, when H2O2 was substituted for AA, a significant binding of the drug was detected (Table 2) under both aerobic and anaerobic conditions. PES/AA-catalyzed covalent binding to DNA and proteins was considerably lower than that catalyzed by the HRP/H2O2 system probably due to incomplete metabolism of VP-16.

The binding of VP-16 to DNA catalyzed by HRP was time dependent as shown in Fig. 3. The stable binding was linear up to 5 min and the maximum binding was obtained in 15 min where 2.7% of the total radioactivity became bound to DNA. After 15 min of incubation with HRP/H2O2, 29% of the radioactivity became bound to proteins.

In order to characterize and identify the reactive species that bound DNA and proteins, the HRP-dependent binding was carried out with a mixture of 14C- and 3H-labeled drug (initial ratio of 14C:3H = 0.2) and the resulting ratio of 14C:H in the stable DNA:drug and protein:drug complexes was determined. Following incubation under the conditions described in Fig. 2c, the ratio of the radioactive label bound to DNA was identical to the initial ratio (0.2). However, under identical conditions the drug:protein binding ratio was significantly lower (0.12), indicating a preferential loss of 14C from the reactive intermediate(s) that bound to proteins. This observation suggested that O-demethylation product(s) bound to proteins but not to DNA.

### Table 1 Distribution of drug-derived radioactivity in aqueous and organic phases following peroxidase metabolism of VP-16 in the presence of H2O2 or AA (250 μM)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Phase</th>
<th>3H (% of the total)</th>
<th>14C (% of the total)</th>
</tr>
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<tbody>
<tr>
<td>HRP/H2O2</td>
<td>Water</td>
<td>16.5 ± 2.1*</td>
<td>9.0 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>76.3 ± 4.3</td>
<td>52.2 ± 3.9</td>
</tr>
<tr>
<td>PES/AA</td>
<td>Water</td>
<td>4.2 ± 0.2</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>92.9 ± 9.4</td>
<td>93.5 ± 9.0</td>
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*Mean ± SD of at least 40 separate experiments.

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</tr>
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</table>

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### Table 2 Covalent binding of VP-16-derived reactive species to calf thymus DNA and heat-inactivated microsomal proteins during peroxidase oxidation of VP-16

<table>
<thead>
<tr>
<th>Conditions</th>
<th>DNA binding ratio × 10⁶</th>
<th>Protein binding (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRP</td>
<td>3.76 ± 1.56</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>H2O2</td>
<td>3.78 ± 0.7</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>HRP/H2O2</td>
<td>396.3 ± 62.2</td>
<td>7.23 ± 1.0</td>
</tr>
<tr>
<td>PES</td>
<td>26.1 ± 14.8</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>AA</td>
<td>4.05 ± 1.5</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>PES/AA</td>
<td>103.6 ± 21.9</td>
<td>2.0 ± 0.34</td>
</tr>
<tr>
<td>PES/AA + indomethacin</td>
<td>21.0 ± 9.7</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>PES/AA + nitrogen</td>
<td>21.5 ± 10.9</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td>PES/H2O2 + nitrogen</td>
<td>124.6 ± 28.7</td>
<td>1.76 ± 0.34</td>
</tr>
<tr>
<td>PES/AA + nitrogen</td>
<td>131.1 ± 40.5</td>
<td>1.5 ± 0.22</td>
</tr>
</tbody>
</table>

* Molar ratio of the drug:mononucleotide unit.

† Significantly higher than HRP alone (P < 0.001).

‡ Significantly higher than PES or AA alone (P < 0.001).

§ Significantly lower than PES/AA (P < 0.01).

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While the metabolism of VP-16 has not been completely elucidated, the current literature shows that VP-16 is metabolized to a hydroxyacid derivative (20, 21), which is formed as a result of hydrolysis of the trans-γ-lactone ring. In addition, cis-picro-lactone isomer (21) and a glucuronic acid conjugate (22) have been identified. Recently, we have shown that VP-16 also undergoes cytochrome P-450-dependent O-demethylation to form the 3',4'-dihydroxy derivative of VP-16, and VP-16-Q may form by oxidation of the dihydroxy derivative (10, 11). Studies described here show that both horseradish peroxidase and prostaglandin synthetase catalyze the metabolism of VP-16 which resulted in the formation of a number of metabolites. Using HPLC and mass spectrometric analysis, we have identified some of these metabolites (VP-16-Q, an aromatized VP-16-derivative, and aromatic VP-16-Q; Fig. 1). The formation of VP-16-Q from VP-16 requires O-demethylation of the drug, which is normally catalyzed by cytochrome P-450. The peroxidative O-demethylation of antitumor drugs is not very well documented and only a few examples are known. In addition to our work on O-demethylation of VP-16 and VM-26 (12), Meunier and Meunier (23) have recently reported that 9-methoxyellipticine derivatives are also O-demethylated by HRP/H\textsubscript{2}O\textsubscript{2}.

The O-demethylation of VP-16 appears to proceed through the intermediacy of the phenoxy radical which is formed by one-electron oxidation of the drug (Fig. 5), as previously reported. This was confirmed by incubating the 4'-O-methylated analog of VP-16 with HRP/H\textsubscript{2}O\textsubscript{2} and analyzing the mixture by HPLC. Under the conditions where VP-16-Q was easily detectable, no peak corresponding to the retention time of the quinone was present. Because the formation of the phenoxy radical intermediate requires the presence of a free 4'-OH (10, 24), and no free radical was detected from 4'-O-methyl analog and because no VP-16-Q was formed from the 4'-O-methylated derivative, it is reasonable to conclude that the formation of the phenoxy radical of VP-16 was essential for VP-16-Q formation. In contrast to cytochrome P-450-dependent O-dealkylation of VP-16, which is known to form formaldehyde as one of the end products (10), the peroxidative O-demethylation of VP-16 appears to form methanol as the end product since no formaldehyde was detected by Nash reagent during HRP catalysis of VP-16.

The mechanism for the formation of the aromatic VP-16 appears to depend upon the phenoxy radical formation. This is supported by the observation that the incubation of the 4'-O-methyl derivative with HRP/H\textsubscript{2}O\textsubscript{2} did not form the aromatic VP-16. A proposed mechanism for this product from VP-16 is presented in Fig. 5. In this proposal the phenoxy radical which is formed by one-electron oxidation of the drug (Fig. 5), as previously reported. This was confirmed by incubating the 4'-O-methylated analog of VP-16 with HRP/H\textsubscript{2}O\textsubscript{2} and analyzing the mixture by HPLC. Under the conditions where VP-16-Q was easily detectable, no peak corresponding to the retention time of the quinone was present. Because the formation of the phenoxy radical intermediate requires the presence of a free 4'-OH (10, 24), and no free radical was detected from 4'-O-methyl analog and because no VP-16-Q was formed from the 4'-O-methylated derivative, it is reasonable to conclude that the formation of the phenoxy radical of VP-16 was essential for VP-16-Q formation. In contrast to cytochrome P-450-dependent O-dealkylation of VP-16, which is known to form formaldehyde as one of the end products (10), the peroxidative O-demethylation of VP-16 appears to form methanol as the end product since no formaldehyde was detected by Nash reagent during HRP catalysis of VP-16.

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The peroxidative metabolism of VP-16 also resulted in the formation of alkylating species that became irreversibly bound to both proteins and DNA. We had earlier proposed that O-demethylation products of VP-16 alkylate both DNA and proteins (9). However, current experiments carried out with dual label did not show a preferential loss of 14C from O-\textsuperscript{14}CH\textsubscript{3}-VP-16 during the formation of alkylating species that bound to DNA, indicating that the O-demethylation products are not the predominant DNA binding species. This was further confirmed
by directly incubating DNA with O-demethylated products of VP-16. The precise chemical nature of the reactive species is not known at this time; however, we propose VP-16-derived quinone methides (either II or IV; Fig. 5) as DNA alkylating species since quinone methides are positively charged and thus are expected to react with nucleophilic sites on the DNA. It should be noted that the quinone methide formed from Adriamycin has been reported to alkylate DNA in biological systems (25, 26).

In contrast to DNA binding species, O-demethylated products of VP-16 bound to proteins. This was shown by isolating the choroform-soluble products after the peroxidase-catalyzed metabolism of VP-16 and incubating these stable but reactive compounds (mostly quinones) with proteins. Under these conditions, one-third of the radioactivity was irreversibly bound to proteins. Furthermore, when dual-labeled VP-16 was used during peroxidase-mediated binding of the drug to proteins, the $^{14}$C radioactivity was preferentially lost, indicating binding of O-demethylated products of the drug. In addition, when the peroxidase-catalyzed metabolism of VP-16 was carried out in the presence of proteins and the mixtures were analyzed by HPLC, a significant decrease in the concentration of the quinones was observed, indicating irreversible binding of the quinones. Authentic VP-16-Q bound to proteins nonenzymatically and this binding appears to be mediated by sulfhydryl groups because reduced glutathione also irreversibly bound to VP-16-Q. Furthermore, we have shown that the addition of reduced glutathione inhibited the covalent binding of VP-16-derived reactive species to proteins during microsomal catalyzed metabolism of VP-16 (11).

The metabolism of VP-16 with the PES system was less extensive than HRP/H$_2$O$_2$. Nevertheless, metabolism with both systems followed similar patterns and in both cases phenoxy radicals, metabolite B, VP-16-Q, and low-molecular-weight compounds were detected. Furthermore, in both cases, VP-16 metabolism was associated with covalent binding to DNA and proteins. The PES-catalyzed metabolism and binding were inhibited by indomethacin, an inhibitor of cyclooxygenase (19), and by anaerobic conditions, indicating that the dioxygenation of AA to cyclic hydroperoxyendoperoxide, PGG$_2$, was necessary. The peroxidative arm of the PES appeared to catalyze the metabolism and binding of the drug because peroxides were able to catalyze this metabolism and binding under anaerobic conditions.

It has been proposed by some investigators that topoisomerase II is a likely intracellular target for DNA strand-breaking effects of VP-16 and other antitumor drugs and that this DNA-damaging effect is related to the cytotoxicity of the drugs (5–8, 27, 28). The biological significance of the peroxidative activation of VP-16 and the role of the covalent binding in its cytotoxicity are not clear at this time. However, it is possible that the reactive intermediates formed during the peroxidative metabo-
lism of VP-16 may possess their own cytotoxic properties. In this respect, our preliminary results indicate that VP-16-Q and the related VM-26-Q are highly active against L1210 cells in vivo (29). Furthermore, studies of Van Maanen et al. (30) have shown that biologically active 8 x 174 DNA is inactivated by VP-16-Q but not by VP-16 itself. These observations clearly suggest that metabolically activated form(s) of VP-16 may participate in its antitumor activity. Since peroxidases are present in almost all mammalian cell types (31), the peroxidative activation of VP-16 and the related drug, VM-26, may provide a complementary metabolic pathway with the cytochrome P-450 monoxygenase system (9-11) for the formation of the reactive intermediates. In this regard, a wide variety of xenobiotics including ellipticine and procarbazine have been shown to be activated by peroxidases (22, 32-34). Work is currently in progress to assess the role of the peroxidative activation of VP-16 and VM-26 in their biological and pharmacological activity.

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