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FREE RADICAL METABOLISM OF VP-16 AND INHIBITION OF ANTHRACYCLINE-INDUCED LIPID PEROXIDATION

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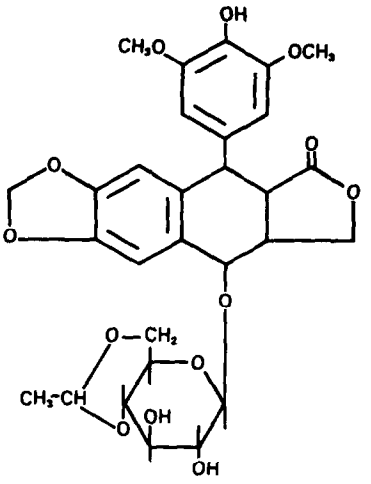
VP-16-213 (NSC 141540, Figure 1), a semisynthetic derivative of podophyllotoxin, is an effective single agent against small cell carcinoma of lung (1,2). While the precise molecular mechanism of action of VP-16 is not known, VP-16 has been shown to induce single-stranded breaks in DNA in HeLa cells (3) and to inhibit incorporation of nucleic acid precursors (4). In addition, several recent preliminary reports have suggested that a radical metabolite may be involved in the irreversible binding of VP-16 to microsomal proteins and in DNA damage (5,6).

VP-16 is being used in combination with other chemotherapeutic agents, including adriamycin, cis-platinum and cyclophosphamide, resulting in decreased toxicity (7). Adriamycin is an anthracycline antibiotic which undergoes reduction-oxidation and produces reactive oxygen radicals ($\dot{O}_2^-$, $OH^\bullet/H_2O_2$) which induce microsomal lipid peroxidation (8). Lipid peroxidation has been implicated in adriamycin-induced cardiotoxicity (9). Because of the structural similarity of VP-16 with butylated hydroxytoluene (BHT), an antioxidant, it was of interest to examine the effects of VP-16 on microsomal lipid peroxidation. The results of this communication demonstrate that VP-16 inhibits daunomycin-induced peroxidation and that it forms a stable oxygen-centered free radical during peroxidative activation.

MATERIALS AND METHODS

VP-16-213 (NSC 141540) and daunomycin (NSC 82510) were obtained from the Drug Development Branch, National Cancer Institute, NIH, Bethesda, MD. Horseradish peroxidase (HRP, type VII, RZ=3.0), thiobarbituric acid (TBA), NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Co., St. Louis, MO.

Mice hepatic microsomal proteins were prepared from CDF_1 male mice according to a previously published method (10). Food and water were provided ad libitum. Peroxidation of lipids was measured by the thiobarbituric acid assay as previously described (11). Electron spin resonance studies for the detection of free radical



VP-16
NSC-141540

Figure 1. Structure of VP-16.

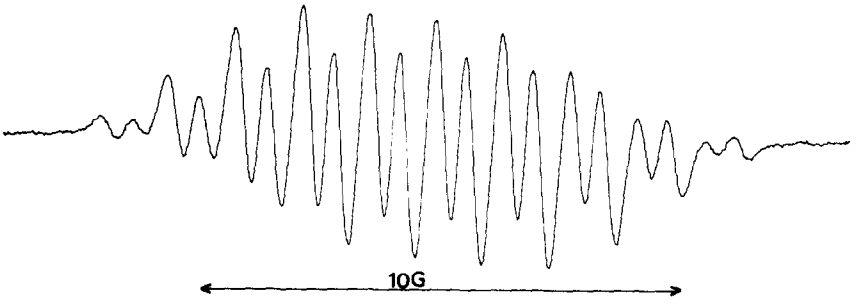


Figure 2. Electron spin resonance spectrum obtained during incubation of VP-16 (1 mM) with horseradish peroxidase (1 mg/ml) in the presence of H_2O_2 (400 μM). The ESR settings: field = 3392G; field scan = 20G; modulation frequency = 100KHz; modulation amplitude = 0.16 G; nominal power = 20mW; receiver gain = 2.0×10^4; time constant = 0.128 seconds and the scan time was 8 minutes.

intermediates were carried out on a Varian E-104 spectrometer at room temperature (22°). VP-16 (1-2 mM) was dissolved in dimethyl sulfoxide (DMSO) (1-10% final volume), and HRP (1 mg/ml) was added. The reaction was initiated by adding H_2O_2 (400 μM) and recording the spectrum. All experiments used fresh microsomes (less than 4 hr old) that were stored on ice.

RESULTS AND DISCUSSION

Recent preliminary reports by Maanen et al. (5) and Wozniak et al. (6) have hypothesized that VP-16 forms a radical intermediate. Horseradish peroxidase has been previously utilized as a model enzyme to evaluate the ability of a chemical to be metabolized to a radical intermediate (see 12). Figure 2 illustrates the ESR spectrum resulting from the incubation of VP-16 with horseradish peroxidase in the presence of H_2O_2. This multi-line VP-16 radical spectrum has been interpreted to indicate the formation of a stable oxygen-centered radical which arises from the interaction of various nucleides and possible conformations in solution. (A more detailed analysis of the interpretation of this spectrum will be published elsewhere.) The formation of the VP-16 radical depended on all three components: horseradish peroxidase, H_2O_2 and VP-16.

Since VP-16 is structurally similar to BHT, the effect of VP-16 on both basal and daunomycin augmented mouse liver microsomal lipid peroxidation was investigated. Quinone containing drugs, such as daunomycin, stimulate formation of reactive oxygen radicals ($\dot{O}_2^-$, $OH^\bullet$, H_2O_2) which initiate the peroxidation of microsomal lipids (8,11). The data in Table 1 show that incubation of daunomycin with mouse hepatic microsomes and an NADPH-generating system induced a 4-fold increase in malondialdehyde. The addition of VP-16 inhibited both the control and the daunomycin-augmented lipid peroxidation in a concentration-dependent manner (Table 1).

Table 1. Effects of VP-16 on control and daunomycin-stimulated mouse liver microsomal lipid peroxidation*

Addition to mouse liver microsomes	Malondialdehyde Equivalents (nmoles/mg protein/60 min)	
	Control	Daunomycin (100 μM)
NADPH-generating system (GS)	23.9 ± 4.0	77.8 ± 8.2
DMSO + GS	21.4 ± 0.2	79.8 ± 8.7
VP-16 + GS (10^{-4} M)	5.4 ± 0.4†	2.0 ± 0.1‡
(5×10^{-5} M)	9.4 ± 1.2†	8.3 ± 4.1‡
(10^{-5}M)	23.3 ± 3.7	80.8 ± 10.0

* Lipid peroxidation was measured by the thiobarbituric acid assay and the values represent mean ± S.D., N = 4. VP-16 was dissolved in DMSO and added to incubation mixtures. The final DMSO concentration was 0.5%.

† Significantly different ($P < 0.01$) from control.

‡ Significantly different ($P < 0.01$) from daunomycin.

The inhibition of the daunomycin-induced lipid peroxidation by VP-16 may result from either (a) the decrease or inhibition of daunomycin metabolism to its semiquinone free radical or (b) modification or quenching of the reaction of $O_2^{\cdot-}$, $OH^{\cdot}$ /H_2O_2 with lipids. Anaerobic incubation of daunomycin with microsomal protein and NADPH resulted in the formation of the ESR detectable semiquinone radical; however, the addition of VP-16 (1 mM) affected neither the rate nor the intensity of this radical formation. In addition, VP-16 does not inhibit the progressive immobilization of the semiquinone signal (13,14). These observations suggest then that, under our experimental conditions, VP-16 does not inhibit the formation of the daunomycin semiquinone free radical. Reactive hydroxyl radical has been implicated in microsomal lipid peroxidation (11). Since formation of the hydroxyl radical is H_2O_2 dependent and since the horseradish peroxidase catalyzed metabolism of VP16 is H_2O_2-dependent, this suggests that the inhibition of microsomal lipid peroxidation by VP-16 may result from the alternative utilization of H_2O_2 during the metabolism of VP-16, possibly through cytochrome P450 acting as a peroxidase (15,16). Alternatively, the direct interaction of VP-16 with $OH^{\cdot}$ could yield the VP-16 radical, a reaction which would account for the inhibition of microsomal lipid peroxidation. Work is in progress to further discriminate between these two possibilities and to further characterize the microsomal-catalyzed bioactivation of VP-16 to a radical intermediate and the relationship of this activity to the cellular action of VP-16. Moreover, if the cytotoxic action of VP-16 is demonstrated to be dependent on the formation of a VP-16 radical and if this reaction is accelerated by redox cycling chemicals, this would provide a biochemical rationale for the concurrent administering of redox cycling anti-neoplastic agent with VP-16.

REFERENCES

1. M. Rozencweig, D.D. Van Hoff, J.E. Henney and F.M. Muggia, Cancer, N.Y.40, 334 (1977).
2. A.M. Arnold, Cancer Chemother. Pharmac. 3, 71 (1979).
3. J.D. Loike and S.B. Horwitz, Biochemistry 15, 5443 (1976).
4. A. Grieder, R. Maurer and H. Stähelin, Cancer Res. 34, 1788 (1974).
5. J.M.S.V. Maanen, J.J.M. Holthuis, F. Gobas, J. deVries, W.J.V. Oort, P. Emmelot and H.M. Pinedo, Proc. Am. Assoc. Cancer Res. 24, 319 (1983).
6. A. J. Wozniak, K.R. Hande and W.E. Ross, Proc. Am. Assoc. Cancer Res. 24, 319 (1983)
7. J. Estape, A. Milla, A. Agusti, J.S. Lloret, A. Palacin and E. Soriano, Cancer N.Y. 43, 72 (1979).
8. J. Goodman and P. Hochstein, Biochem. biophys. Res. Commun. 77, 797 (1977).
9. C.E. Myers, W.P. McGuire, R.H. Liss, I. Ifrim, K. Grotzinger and R.C. Young, Science 197, 165 (1977).
10. B.K. Sinha and M.G. Cox, Molec. Pharmacol. 17, 432 (1980).
11. E.G. Mimnaugh, M.A. Trush and T.E. Gram, Biochem. Pharmac. 30, 2797 (1981).
12. B.K. Sinha, J. Biol. Chem. 258, 796 (1983).
13. B. Kalyanaraman, E. Perez-Reyes and R.P. Mason, Biochim. biophys. Acta 630, 119 (1980).
14. B.K. Sinha and J.L. Gregory, Biochem. Pharmac. 30, 2626 (1981).
15. A.D. Rahimtula and P.J. O'Brien, Biochem. biophys. Res. Commun. 62, 268 (1975).
16. G.D. Nordblom, R.E. White and M.J. Coon, Archs Biochem. Biophys. 175, 524 (1976).