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VP16-213 and Podophyllotoxin

A Study on the Relationship Between Chemical Structure and Biological Activity

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Summary. *VP16-213, a semi-synthetic derivative of podophyllotoxin, is an effective antitumor agent in the treatment of a variety of leukemias and solid tumors. A comparison of the mechanism of action of VP16-213 and podophyllotoxin has revealed that although both drugs inhibit the uptake of nucleosides into HeLa cells, they exhibit other biological properties which are quite distinct. Podophyllotoxin is a potent inhibitor of microtubule assembly in vitro, while VP16-213 has no effect in this system. VP16-213 induces single stranded breaks in HeLa cell DNA, an effect which may be related to its antitumor activity. In contrast to VP16-213-treated cells, podophyllotoxin-treated cells maintain DNA integrity. Structure-activity relationship studies have identified some of the chemical sites of VP16-213 and podophyllotoxin responsible for each of their biological properties. These studies illustrate that chemical modification of podophyllotoxin can generate derivatives which possess new and unique biological properties.*

Introduction

Podophyllotoxin and many of its closely related lignans are potent inhibitors of cell mitosis and have therefore attracted considerable interest as cancer chemotherapeutic agents in man [20, 25]. The first recorded use of podophyllum and its resin as an anticancer drug may date back over 1,000 years to 900–950 A.D. According to the Leech book of BALD, an early English medical book, the roots of wild Chervil (which contain deoxypodophyllotoxin) were used in a salve for treating cancer [11]. More recent clinical studies of podophyllotoxin and its derivatives done between 1940–1969, revealed severe toxic side effects associated with these drugs, virtually eliminating their clinical usefulness in cancer therapy [25]. However, podophyllum continues to be the drug of choice in the treatment of the venereal wart *Condyloma acuminatum* [12].

The design and synthesis of new podophyllotoxin derivatives were undertaken in an attempt to produce less toxic analogues. In 1970 [45] and 1973 [46], Stähelin reported that two semi-synthetic podophyllotoxin derivatives, VP16-213 and VM26, had potential anticancer activity. VP16-213 and VM26 are presently undergoing phase III clinical trials and appear to have promise in the treatment of a variety of cancers including testicular teratoma and some types of leukemias [see 5, 23 for reviews]. In fact, VP16-213 is the most active single agent yet tested for the treatment of small cell carcinoma of the lung [10, 49].

This review will compare the biological properties of VP16-213 to those of podophyllotoxin and will assess the relationship of the chemical structure of VP16-213 to its activities. An understanding of how these drugs act at a cellular level may offer insights into the mechanisms by which VP16-213 and VM26 are able to inhibit tumor growth. Such information may facilitate the formulation of new chemotherapeutic combination strategies using VP16-213 or VM26 with other effective antitumor agents or immunotherapy protocols.

The chemical structure of VP16-213 (4′-demethyl epipodophyllotoxin ethylidene β-D glucoside) (Fig. 1) differs from that of podophyllotoxin at three positions: 1) VP16-213 contains a glucoside moiety at the C-4 carbon, 2) the C-4 position of VP16-213 has the enantiomeric configuration of podophyllotoxin, and 3) it contains a hydroxyl group, rather than a methoxy group, at the C-4′ carbon. We have found that due to these chemical modifications, VP16-213 and podophyllotoxin have different biological properties with respect to three systems: microtubule assembly in vitro, intracellular degradation of DNA in HeLa cells and nucleoside transport in HeLa cells.

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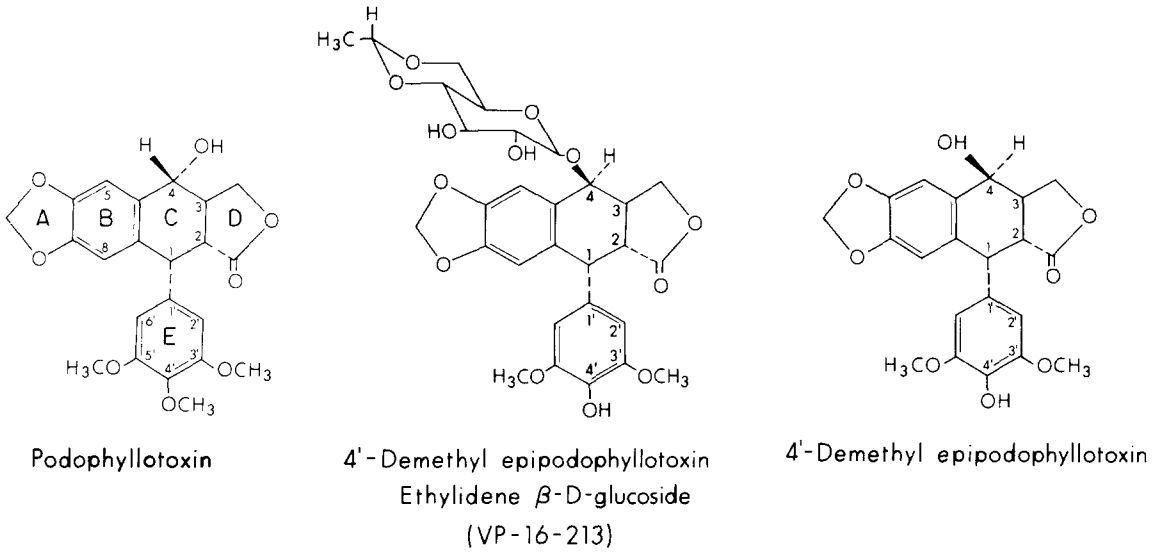


Fig. 1. Structural formulas of podophyllotoxin, VP16-213 and 4'-demethyl epipodophyllotoxin

In fact, these chemical changes have conferred on VP16-213 a mode of action distinct from that of its parent compound, podophyllotoxin.

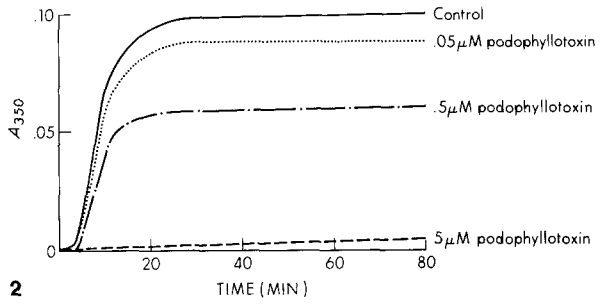
Our studies which relate the structures and activities of VP16-213 and other podophyllotoxin derivatives have led us to identify the chemical moieties responsible for various biological characteristics. The results of these studies emphasize that even slight chemical alterations can modify the uptake, distribution and metabolism of VP16-213 and can generate new mechanisms by which this drug acts. Investigations such as these illustrate the intimate relationship between chemical structures and biological properties and should facilitate effective and rational designs of new podophyllotoxin derivatives.

Effects on Microtubules

Microtubules [13] are long cylindrical polymers about 240 Å in diameter which show structural polarity and have been implicated in a variety of cellular functions including motility, mitosis, secretion and movement of surface receptors. Tubulin, the receptor for podophyllotoxin, is the major protein component of microtubules. It is a 6S dimer, composed of two subunits (α- and β-tubulin) which, under the proper conditions, can polymerize to form microtubules. In the cell cytoplasm and in the mitotic apparatus, microtubules are thought to exist in a dynamic equilibrium with their tubulin subunits [22]. This equilibrium has also been studied in vitro [6] using the microtubule assembly system originally described by Weisenberg [51]. The cytotoxic effects of podophyllotoxin in animal cells are due, in part, to its binding to tubulin dimers and subsequent arrest of cell division.

Margolis and Wilson [31] have formulated a model describing the dynamic equilibrium between tubulin and microtubules and have proposed hypothesis of how podophyllotoxin disrupts this dynamic state. They have found that microtubules at steady state preferentially assemble tubulin at one end of the microtubule and preferentially disassemble tubulin at the opposite end in a process which they refer to as "treadmilling". Thus, the dynamic equilibrium of microtubules reflects a steady state summation of these two different reactions occuring at opposite ends of the microtubule. Furthermore, they have found that podophyllotoxin blocks the steady state addition of tubulin at the assembly end but does not interfere with the disassembly at the opposite end. Based on this and other evidence, they postulate that podophyllotoxin first binds to the tubulin dimer to form tubulin-podophyllotoxin complexes. Then, at substoichiometric concentrations, podophyllotoxin freezes the assembling end of the microtubule without inhibiting the disassembly reaction occuring at the other end. There are other theories [8, 14, 47] which describe the dynamics of microtubule assembly at steady state and which offer alternative explanations on how podophyllotoxin disrupts this equilibrium; however, all investigators agree that podophyllotoxin inhibits the assembly of tubulin into microtubules.

These in vitro studies suggest a mechanism



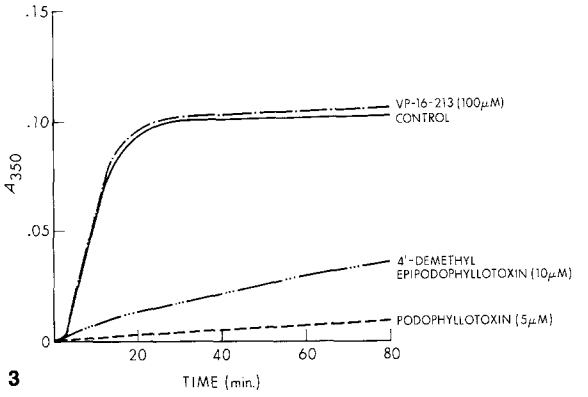


Fig. 2. Effects of different concentrations of podophyllotoxin on microtubule assembly in vitro. Tubulin was isolated from chicken brains as described [30]. Cuvettes containing Mes buffer, plus or minus different concentrations of podophyllotoxin were maintained at room temperature and shifted to 37° C only after the addition of 1.0 mg/ml of tubulin and 1 mM GTP. Data points were taken at 1 min intervals. For further information of experimental details [30]. (Reprinted with permission; copyright Cancer Research Journal)

Fig. 3. Effects of podophyllotoxin and VP16-213 on microtubule assembly. Tubulin was isolated from chicken brains as described [28]. Cuvettes containing Mes buffer, plus or minus drug were maintained at room temperature and shifted to 37° C only after the addition of 1.0 mg/ml of tubulin and 1 mM GTP. Data points were taken at 1 min intervals. For further experimental details [28]. (Reprinted with permission; copyright by American Chemical Society)

whereby podophyllotoxin arrests cells at mitosis. During cell division, spindle fibers, composed of microtubules separate the duplicated chromosomes to poles at opposite ends of the dividing cell [22, 33]. The microtubules in the spindle fibers are thought to serve as cytoskeletal structures providing a vectorial framework upon which other cellular components (microtribeculae or actin microfilaments) function as contractile elements to pull the chromosomes apart. Thus, in podophyllotoxin-treated cells, the equilibrium between polymer and tubulin dimer is disrupted, thereby destroying the cytoskeletal framework for chromosome separation and arresting cell division at the mitotic stage of the cell cycle.

We have used in vitro assembly of microtubules to study the effects of VP16-213, podophyllotoxin and its congeners. In our system [28, 30], microtubule assembly is followed by electron microscopy and by monitoring changes in turbidity as the unassembled tubulin polymerize into microtubules. Podophyllotoxin inhibits the assembly of tubulin into microtubules in a concentration-dependent fashion as measured by turbidity (Fig. 2) and electron microscopy. Podophyllotoxin concentrations as low as 5 μM completely inhibit tubulin polymerization. In contrast to podophyllotoxin, neither 100 μM VP16-213 (Fig. 3) nor VM26 [28, 30] have any effect on tubulin assembly; normal microtubules are observed by turbidity and electron microscopy. However, the nonglucoside congener [4′-demethyl epipodophyllotoxin (Fig. 1)] of both VP16-213 and VM26 inhibits tubulin assembly at 10 μM (Fig. 3). These data suggest that it is the presence of the bulky gluocoside moiety of VP16-213 and VM26 that is responsible for the inability of these drugs to inhibit microtubule assembly. Experiments performed in our own laboratory [7, 28, 30] and in that of Kelleher's [24] have shown that the C and D rings of podophyllotoxin are important chemical sites for the interaction of podophyllotoxin with tubulin. Furthermore, we have shown by NMR analysis [7] of VP16-213 that its glucoside moiety preferentially occupies a position over the D ring of the molecule and could be responsible for sterically blocking an interaction of the drug with tubulin.

A question arises as to whether cells can metabolize VP16-213 or VM26 to their non-glucoside derivative (4′-demethyl epipodophyllotoxin). The resulting metabolite could then act as an effective intracellular microtubule poison. However, Pelsor et al. [38] and Allen et al. [3] have studied some of the metabolic products of VP16-213 in man and have found no evidence that the glucoside moiety is removed. Further evidence that the glucoside moiety is not cleaved intracellularly is provided by studies from Phaire-Washington et al. [39] which suggests that VP16-213 does not affect the cytoplasmic organization of microtubules.

These experiments confirm our finding that VP16-213 is not an inhibitor of microtubule assembly and suggest that VP16-213 must be inhibiting cell proliferation by some other mechanism.

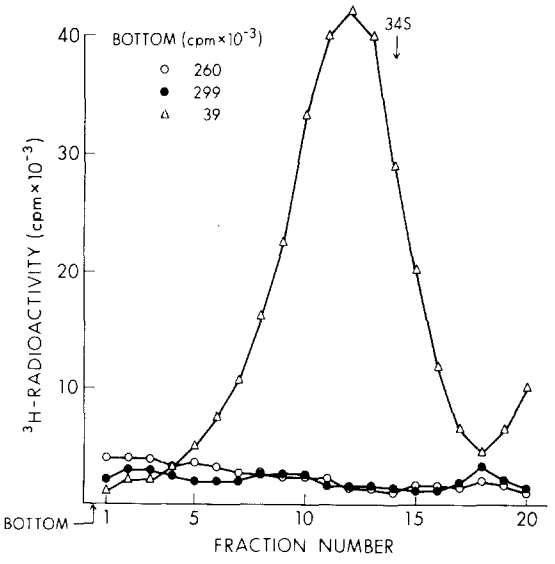


Fig. 4. Sedimentation of DNA in HeLa cells. HeLa cells, containing labeled DNA were incubated at 37° C in the presence or absence of 100 μM drug. After 60 min, the cells were analyzed by alkaline sucrose gradient centrifugation [29]. "Bottom" refers to the lower 1.5 cm of the gradient tube. No additions (●); 100 μM podophyllotoxin (○); VP16-213 (△). For further experimental details [29]. (Reprinted with permission; copyright by American Chemical Society)

Intracellular Degradation of DNA in HeLa Cells

One conclusion from cell cycle studies [18, 19, 27] done with VP16-213 and VM26 is that these drugs arrest cells in either the late S or early G_2 phase of the cell cycle, an effect seen with many drugs which inhibit DNA synthesis. Moreover, Huang et al. [21] have reported that cells treated with VP16-213 have chromosomal abberations. These studies suggest that VP16-213 may have an effect on DNA.

We chose to study VP16-213 and VM26 in HeLa cells using alkaline sucrose gradient centrifugation analysis of cellular DNA [29]. This method allows detection of single-stranded breaks in DNA which might result from an interaction of drug with DNA . When HeLa cells containing labeled DNA were treated with 100 μM podophyllotoxin, their high molecular weight DNA sedimented through alkaline sucrose gradients to the bottom of the gradient tube in a manner identical to that of control cells (Fig. 4). However, in VP16-213- or VM26-treated HeLa cells, the high molecular weight DNA is converted to lower molecular weight forms, whose size is approximately 40S. This DNA appears in the middle of the gradient (Fig. 4). The effect of VP16-213 on HeLa cell DNA degradation is dose dependent and temperature dependent and it is also reversible after the drug has been washed out of the cells for approximately 2 h. The observation that the action of VP16-213 on DNA is reversible suggests that HeLa cells can repair the DNA damage and that the drug induces single-stranded DNA breaks. Recently, Roberts et al. [43] have reported that micromolar concentrations of VM26 generate single-stranded DNA breaks in L1210 cells and that these cells can repair the VM26 induced breaks.

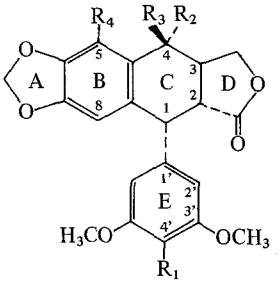
In addition, we have found that VP16-213 had no effect on the melting temperature of calf thymus DNA, on the sedimentation of either purified HeLa DNA or purified adenovirus DNA on alkaline sucrose gradients [29]. These results indicate that VP16-213 does not interact with DNA in vitro to cause cleavage but rather induces single-stranded breaks only in DNA in situ. While it is certainly possible that a VP16-213 metabolite may be acting directly with the DNA molecule to cause DNA breaks, it remains unclear how VP16-213 induces these single-stranded breaks.

The capacity of VP16-213 to induce DNA breaks is not shared by podophyllotoxin and can be explained by their structural differences. We have found that the presence of the 4′-hydroxyl group on VP16-213 alters the drug in a way that enables it to induce single-stranded DNA breaks. Structure-activity relationship studies [29] have shown that all podophyllotoxin and VP16-213 derivatives which contain a 4′-hydroxyl group induce DNA breaks whereas their respective 4′-methoxy derivatives are all inactive (Table 1). Furthermore, the presence of the glucoside moiety on VP16-213 and VM26 increases their ability to induce DNA breaks as compared to the non-glucoside derivative, 4′-demethyl epipodophyllotoxin.

Why does changing a methoxy group to a hydroxyl group result in a new biological property? In an attempt to answer this question, we have performed NMR analyses of several VP16-213 derivatives. Analysis of epipodophyllotoxin (which contains a 4′-methoxy group), 4′-demethyl epipodophyllotoxin (the non-glucoside form of VP16-213) and VP16-213 at 360 mHz reveals that there are no major steric or electronic changes which are effected by the presence of the 4′-hydroxyl group [7]. One can speculate that the 4′-OH group, because it has a greater H-bonding capacity than a methoxy group, confers a new binding specificity on VP16-213 (or its metabolite) for DNA or for its unidentified intracellular receptor.

There are numerous examples in which the addition of a hydroxyl group to a parent compound has produced a better chemotherapeutic agent. Adriamycin is the hydroxyl form of daunorubicin, and vincristine is the hydroxyl form of vinblastine. In

Table 1



Congener	R_1	R_2	R_3	R_4	% Low-mol-weight form DNA[a]
Podophyllotoxin	OCH_3	OH	H	H	5
4′-Demethyl podophyllotoxin	OH	OH	H	H	33
Epipodophyllotoxin	OCH_3	H	OH	H	3
4′-Demethyl epipodophyllotoxin	OH	H	OH	H	68
VP16-213	OH	H	Ethylidene glucoside	H	75
VM26	OH	H	Thenylidene glucoside	H	83
Deoxypodophyllotoxin	OCH_3	H	H	H	5
4′-Demethyl	OH	H	H	H	56
β-Peltatin	OCH_3	H	H	OH	5
α-Peltatin	OH	H	H	OH	40

[a] HeLa cells (4×10^5 cells/ml) containing labeled DNA were incubated at 37° C for 1 h in the presence or absence of 100 μM drug. The cells were analyzed by alkaline sucrose gradient centrifugation as described [29]. Values are expressed as the percent of radioactivity present as lower molecular-weight species, which corresponds to the DNA present in the entire gradient, except for the 1.5 cm CsCl cushion at the bottom. In untreated cells, 15% of the labeled DNA was found thorughout the gradient above the CsCl cushion. These counts were substracted from all values. For further experimental details [29]

each case, the hydroxyl form has reduced the clinical toxicity of the drug [4, 15]. Yet, unlike the case of VP16-213, there is no evidence that the addition of a hydroxyl group has conferred any new biological properties on these drugs.

Thus, chemical structures can be deceiving. Slight chemical modifications of a parent compound can alter potency, metabolism and toxicity. Indeed, they might even result in a congener whose mechanism of action differs intrinsically from that of its parent compound.

Nucleoside Transport

Although mammalian cells do not require exogenous nucleosides for growth, they possess specific plasma membrane transport components which mediate the movement of nucleosides across the plasma membrane [9, 40, 41, 52]. Current evidence [36, 41, 52] suggests that there are two mechanisms for nucleoside transport. In the presence of low concentrations of exogenous nucleosides, nucleosides are transported into cells by facilitated diffusion, a saturable, carrier-mediated process. At high concentrations, nucleosides enter the cell by a non-saturable process which might include simple diffusion. Once nucleosides have transversed the membrane by either mechanism, specific kinases phosphorylate them to their respective 5′ mono-, di- and triphosphate forms. The phosphorylation of nucleosides is not required for their transport across the plasma membrane [36, 52].

Since there is evidence that podophyllotoxin inhibits nucleoside transport [35], we studied the effects of VP16-213 on nucleoside transport in HeLa cells by following the incorporation of labeled nucleosides into the acid soluble fraction of monolayer cell cultures [28]. Within 5 min after incubating HeLa cells with either 100 μM VP16-213 or 100 μM podophyllotoxin, the uptake of thymidine, uridine, adenosine and guanine into the acid soluble fraction of the cell is inhibited by over 80%. Since these drugs reduce the acid soluble nucleoside compartment, the incorporation of these labeled nucleosides into the acid insoluble fraction (representing DNA and RNA) is also markedly inhibited. Further experiments [28] revealed that podophyllotoxin and VP16-213 only

Table 2. Effect of podophyllotoxin and VP16-213 on the uptake of nucleosides into HeLa cells[a]

Nucleoside	Drug concentration (μM)	
	Podophyllotoxin	VP16-213
Thymidine	10	25
Uridine	5	20
Adenosine	8	30
Guanosine	9	20

[a] HeLa cells grown in monolayer culture were incubated for 15 min at 37° C with either 1 μCi/ml ^{3}H-thymidine, 0.2 μCi/ml ^{14}C-uridine, 1.5 μCi/ml ^{3}H-adenosine or 1 μCi/ml ^{3}H-guanosine in the presence or absence of drug. The experimental procedures are described [28]. Results are expressed as the concentration of drug required for 50% inhibition of total uptake of nucleosides into cells

inhibit the saturable rate component of nucleoside transport, without affecting the non-saturable rate component and that these drugs do not inhibit the phosphorylation of nucleosides into nucleotides. The inhibition of nucleoside transport by VP16-213 and podophyllotoxin is concentration dependent, podophyllotoxin being a more potent inhibitor than VP16-213. The concentration of podophyllotoxin required to inhibit 50% incorporation of labeled nucleoside into the acid soluble fraction of HeLa cells is 5–10 μM, whereas 20–30 μM VP16-213 is necessary to achieve the same degree of inhibition (Table 2). Furthermore, the effects of VP16-213 and podophyllotoxin on nucleoside transport are completely reversible [28].

Since all animal cells have de novo pathways for synthesizing purines and pyrimidines, it remains unclear what, if any, cytotoxic effects result from the inhibition of nucleoside transport by either VP16-213 or podophyllotoxin. However, there are two reasons why it is unlikely that the biological properties seen with these drugs are only related to their action on nucleoside transport. First, much higher concentrations of podophyllotoxin and VP16-213 are required to inhibit nucleoside transport than are required for their other biological properties. Second, structure-activity relationship studies have revealed that there are several podophyllotoxin analogues, such as picropodophyllotoxin, VM26 and VP16-213, which do not inhibit microtubule assembly and continue to inhibit nucleoside transport. Thus, the different biological actions of VP16-213 and podophyllotoxin in cells are better explained by their divergent actions on DNA and on microtubule assembly.

The effects of VP16-213 on nucleoside transport are important with respect to combination chemotherapy in cancer. There are currently several cytotoxic nucleoside analogues, for example cytosine arabinoside, which are being tested in combination with VP16-213 [42]. Such nucleoside analogues probably utilize the known nucleoside transport carriers. It is conceivable, then, that VP16-213 which inhibits nucleoside transport might also alter the entry of antitumor nucleoside analogues into the tumor cell, thereby counteracting the effects of combination chemotherapy.

Paterson et al. [37] have reported an example of this type of drug-drug counterbalance. They showed that the nucleoside transport inhibitor, nitrobenzylthioinosine (NBMPR) protects RPMI 6410 cells from the antiproliferative effects of several cytotoxic nucleoside derivatives including 5-azacytidine, formycin, and arabinosylcytosine. NBMPR in itself is a specific inhibitor of adenosine and uridine transport in a variety of cell types, but does not arrest cell division. Moreover, it is interesting that NBMPR does not protect the cells from all nucleoside cytotoxic analogues. For example, 5-fluorouracil continues to inhibit cell division in cells incubated in the presence of NBMPR. Clearly, more research must be done to determine if VP16-213 can inhibit the uptake of cytotoxic nucleoside analogues, thereby resulting in combinations of VP16-213 with nucleoside analogues that might be counterproductive.

Future Directions in the Design of New VP16-213 and VM26 Derivatives

One approach to developing new antitumor agents is through selective chemical modifications of existing natural products. However, each chemically modified derivative must be examined using in vitro screening assays and animal models before it can enter clinical trials in man. The reason for such extensive testing is related to our inability to predict how specific chemical changes in a parent compound may influence the pharmacological properties of the derivative. Any chemical alteration of a drug might have dramatic effects on the uptake, distribution, metabolism and toxicity of the new product and at times, can even result in a product whose mechanism of action differs from that of the original drug.

Over the last 40 years, hundreds of podophyllotoxin derivatives have been tested in an attempt to find clinically effective antitumors agents. The original rationale for exploring podophyllotoxin and its analogues as antitumor agents was related to the potent capacity of podophyllotoxin to arrest cell mitosis, an effect related to its inhibition of microtubule assembly. Ironically, the two podophyllotoxin derivatives, VP16-213 and VM26, which show prom-

ise in phase III clinical trials as anticancer drugs, possess a mechanism of action distinct from that of podophyllotoxin itself. Our studies [29] suggest that the primary mechanism of action of VP16-213 and VM26 involves an inhibition of DNA synthesis which is probably due to the ability of these drugs to induce single-stranded breaks in cellular DNA. Neither VP16-213 nor VM26 inhibits microtubule assembly in vitro. However, there are reports [16, 17] that VM26 impairs the activity of the respiratory chain in cells, an effect which has also been observed with high concentrations of podophyllotoxin [34, 50]; the significance of these observations remains to be elucidated. It appears logical that at the present time future development of new analogues of VP16-213 and VM26 should focus on the DNA effect of these drugs.

The capacity of VP16-213 to induce DNA breaks is directly related to the presence of a hydroxyl group on the 4′-carbon on the E ring of the molecule. All 4′-hydroxyl derivatives have the ability to induce single stranded DNA breaks in HeLa cells, whereas derivatives with a 4′-methoxy group are inactive in this system (Table 1). These studies suggest testing other 4′-demethyl derivatives including 4′-demethyl picropodophyllotoxin and the 4′-demethyl analogues of SP-I and SP-G [26] for the induction of DNA breaks. Furthermore, the trans hydroxyl acid of VP16-213 is an important analogue to isolate and test in this system since it is thought to be the major urinary metabolite of VP16-213 [3, 38, 48]. In fact, this metabolite may even be capable of inducing breaks in purified DNA and thus may be an active metabolite of VP16-213.

A second direction for future studies is to examine several of the non-glucoside 4′-demethyl-analogues in animal screens for antitumor activity. Compounds such as 4′-demethyl epipodophyllotoxin, 4′-demethyl deoxypodophyllotoxin, 4′-demethyl podophyllotoxin, and α-peltatin possess two mechanisms of action. They inhibit microtubule assembly and induce breaks in cellular DNA. Since these analogues have their own built-in "drug combination" of podophyllotoxin and VP16-213, they may prove to be effective antitumor agents in man.

The most potent inducers of DNA breaks among the 4′-demethyl derivatives are the two glucoside analogues, VP16-213 and VM26. However, it remains unclear what role these glucoside moieties have in the clinical application or mechanism of action of these drugs. Studies by Allen [1] and Allen and Creaven [2] have shown that the small chemical differences in the sugar groups between VP16-213 and VM26 have conferred different uptake and metabolic characteristics of these drugs. Furthermore, our observations (Table 1) that VP16-213 is a more potent inducer of DNA breaks than 4′-demethyl epipodophyllotoxin (the non-glucoside form) suggests that the glucoside moiety influences either the cellular uptake, metabolism or intracellular binding of VP16-213 to its "receptor(s)". Therefore, future studies should compare the pharmacokinetic properties of VP16-213, VM26 with 4′-demethyl epipodophyllotoxin to understand the role of the glucoside groups. It might even be advantageous to develop a glucoside derivative of 4′-demethyl epipodophyllotoxin containing a labile glucoside linkage which tumor cells could enzymatically cleave with one of their glucosidases. Such a derivative might have the "proper" pharmacokinetic properties of VP16-213 coupled with the dual cytotoxic mechanisms of 4′-demethyl epipodophyllotoxin to be an effective antitumor agent.

The similarities between colchicine and podophyllotoxin with respect to their inhibitory action on microtubule assembly has stimulated several investigators [24, 32] to speculate on common chemical sites in these two structures. One site of chemical similarity between colchicine and podophyllotoxin is the tri-methoxy saturated A ring of colchicine and the E ring of podophyllotoxin. In fact, Rosner et al. [44] have recently tested a series of colchicine derivatives and have found that 2-demethyl and 3-demethyl colchicine have high in vitro cytotoxicity, low inhibitory effect on microtubule assembly and low animal toxicity. These demethyl colchicine derivatives might be analogous to the 4′-demethyl epipodophyllotoxin derivatives and induce single-stranded DNA breaks. Future research with these colchicine derivatives is therefore warranted.

Conclusions

We have shown that two semi-synthetic derivatives of podophyllotoxin, VP16-213 and VM26, have different biological properties from their parent compound, podophyllotoxin. Specifically, VP16-213 and VM26 induce single-stranded breaks in DNA, a characteristic which may be related to their antitumor activities and is not observed with podophyllotoxin. Furthermore, VP16-213 and VM26 do not inhibit microtubule assembly whereas podophyllotoxin is an effective inhibitor. Structure-activity relationship studies have identified several of the chemical sites on VP16-213 and podophyllotoxin which are responsible for their varied biological properties. Future studies on the molecular mechanism by which VP16-213 and VM26 induce DNA breaks and on the identification of the chemical sites on the drug responsible for each

of its biological properties should facilitate the development of more effective derivatives of VP16-213 and enhance our understanding of rational drug design.

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References

1. Allen LM (1978) Comparison of uptake and binding of two epipodophyllotoxin glucopyranosides, 4′-demethyl epipodophyllotoxin thenylidene-β-D-glucoside and 4′-demethyl epipodophyllotoxin ethylidene-β-D-glucoside, in the L1210 leukemia cell. Cancer Res 38: 2549
2. Allen LM, Creaven PJ (1975) Comparison of the human pharmacokinetics of VM26 and VP16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11: 697
3. Allen LM, Marks C, Creaven J (1976) 4′-Demethyl-epipodophyllic acid-9-(4-6,-o-ethylidene-β-D-glucopyranoside). The major urinary metabolite of VP16-213 in man. Proc Am Assoc Cancer Res 17: 6
4. Arcamone F (1981) Doxurubicin anticancer antibiotics. Academic Press, New York
5. Arnold AM (1979) Podophyllotoxin derivative VP16-213. Cancer Chemother Pharmacol 3: 71
6. Borisy GG, Marcum JM, Olmsted JB, Murphy DB, Johnson KA (1975) Purification of tubulin and associated high molecular weight proteins from porcine brain and characterization of microtubule assembly in vitro. Ann NY Acad Sci 253: 107
7. Brewer CF, Loike JD, Horwitz SB, Sternlicht H, Gensler WJ (1979) Conformational analysis of podophyllotoxin and its congeners. Structure-activity relationship in microtubule assembly. J Med Chem 22: 215
8. Caplow M, Zeeberg B (1981) Incorporation of radioactive tubulin into microtubules at steady state. Experimental and theoretical analysis of the effect of podophyllotoxin. J Biol Chem 256: 5608
9. Cass CE, Paterson ARP (1972) Mediated transport of nucleosides in human erythrocytes. Kinetics of exchange diffusion of uridine and thymidine and specificity towards pyrimidine nucleosides as permeants. J Biol Chem 247: 3314
10. Cavalli F, Sonntag RW, Jungi F, Senn HJ, Brunner KW (1978) VP16-213 monotherapy for remission induction of small cell lung cancer: a randomized trial using three dosage schedules. Cancer Treat Rep 62: 473
11. Cockayne TO (1961) Leech book of BALD: In: Leechdom, Wortcunnings and Starcraft of Early England, vol II. The Holland Press, London, p 313
12. Corey L (1980) Warts and molluscum contagiosum: In: Isselbacher KJ, Adams RD, Braunwald E, Petersdorf RG, Wilson JD (eds), Harrison's principles of internal medicine, 9th ed. McGraw Hill Book Co., New York, p 859
13. DeBrabander M, DeMey J (ed) (1980) Microtubules and microtubule inhibitors. North Holland, Amsterdam
14. Deery WJ, Weisenberg RC (1981) Kinetic and steady-state analysis of microtubules in the presence of colchicine. Biochemistry 20: 2316
15. Gerzon K (1980) Dimeric catharanthus alkaloids: In: Cassady JM, Douros JD (eds), Anticancer agents based on natural product models. Academic Press, New York, p 271
16. Gosalvez M, Perez-Garcia J, Lopez M (1972) Inhibition of NADH-linked respiration with the anticancer agent 4′-demethylepipodophyllotoxin thenylidene glucoside (VM26). Eur J Cancer 8: 471
17. Gotzos V, Capelli-Gotzos B, Despond JM (1979) A quantitative microdensitometric and autoradiographic study of the effect of 4′-demethylepipodophyllotoxin-β-D-thenylidene glucoside (VM26) on the cell cycle of cultured fibroblasts. Histochemistry J 11: 691
18. Grieder A, Maurer R, Stähelin H (1974) Effect of an epipodophyllotoxin derivative (VP16-213) on macromolecular synthesis and mitosis in mastocytoma cells in vitro. Cancer Res 34: 1788
19. Grieder A, Maurer R, Stähelin H (1977) Comparative study of the early effects of epipodophyllotoxin derivatives and other cytostatic agents in mastocytoma cultures. Cancer Res 37: 2998
20. Hartwell JL, Shear MJ (1947) Chemotherapy of cancer: classes of compounds under investigation and active components of podophyllin. Cancer Res 7: 716
21. Huang CC, Hou Y, Wang JJ (1973) Effects of a new antitumour agent, epipodophyllotoxin, on growth and chromosomes in human haemopoietic cell lines. Cancer Res 33: 3123
22. Inoué S, Sato H (1967) Cell motility by labile association of molecules: The nature of mitotic spindle fibers and their role in chromosome movement. J Gen Physiol (Suppl) 50: 259
23. Issell BF, Crooke ST (1979) Etoposide (VP16-213). Cancer Treat Rev 6: 107
24. Kelleher JK (1977) Tubulin binding affinities of podophyllotoxin and colchicine analogues. Mol Pharmacol 13: 232
25. Kelly MG, Hartwell JL (1954) The biological effects and the chemical composition of podophyllin: a review. J Natl Cancer Inst 14: 967
26. Kovarik J, Svec F (1968) The effect of podophyllin cytostatics SP-I and SP-G on the proliferation and respiration of HeLa cells in vitro. Neoplasma 15: 6
27. Krishan A, Paika K, Frei E III (1975) Cytofluorometric studies on the action of podophyllotoxin and epipodophyllotoxins (VM26, VP16-213) on the cell cycle traverse of human lymphoblasts. J Cell Biol 66: 521
28. Loike JD, Horwitz SB (1976) Effects of podophyllotoxin and VP16-213 on microtubule assembly in vitro and nucleoside transport in HeLa cells. Biochemistry 15: 5435
29. Loike JD, Horwitz SB (1976) Effect of VP16-213 on the intracellular degradation of DNA in HeLa cells. Biochemistry 15: 5443
30. Loike JD, Brewer CF, Sternlicht H, Gensler WJ, Horwitz SB (1978) Structure-activity study of the inhibition of microtubule assembly in vitro by podophyllotoxin and its congeners. Cancer Res 38: 2688
31. Margolis RL, Wilson L (1978) Opposite end assembly and disassembly of microtubules of steady state in vitro. Cell 13: 1
32. Margulis TN (1974) Structure of the mitotic spindle inhibitor colcemid N-desacetyl-N-methylcolchicine. J Am Chem Soc 96: 899
33. McIntosh JR (1981) Microtubule polarity and interaction in mitotic spindle function: In: Schweiger HG (ed), International Symposium Cell Biology. Springer, Berlin Heidelberg New York p 359
34. Miller ZB, Davison C, Smith PK (1949) The effect of podophyllotoxin on tissue metabolism and enzyme systems. J Exp Med 90: 524

35. Mizel SB, Wilson L (1972) Nucleoside transport in mammalian cells. Inhibition by colchicine. Biochemistry 11: 2573
36. Paterson ARP, Naik SR, Cass CE (1977) Inhibition of uridine uptake in HeLa cells by nitrobenzylthioinosine and related compounds. Mol Pharmacol 13: 1014
37. Paterson ARP, Yang SE, Lau EY, Cass CE (1979) Low specificity of the nucleoside transport mechanism of RPMI 6410 cells. Mol Pharmacol 16: 900
38. Pelsor FR, Allen LM, Creaven PJ (1978) Multicompartment pharmacokinetic model of 4′-demethylepipodophyllotoxin 9-(4,6-o-ethylidene-β-D-glucopyranoside) in humans. J Pharm Sci 67: 1106
39. Phaire-Washington L, Silverstein SC, Wang E (1980) Phorbol myristate actetate stimulates microtubule and 10 nm filament extension and lysosome redistribution in mouse macrophages. J Cell Biol 86: 641
40. Plagemann PGW, Richey DP (1974) Transport of nucleosides, nucleic acid bases, choline, and glucose by animal cells in culture. Biochem Biophys Acta 344: 263
41. Plagemann PGW, Marz R, Wohlheuter RM (1978) Uridine transport in Novikoff rat hepatoma cells and other cell lines and its relationship to uridine phosphorylation. J Cell Physiol 97: 49
42. Rivera G, Aur RJ, Dahl GV, Pratt CB, Wood A, Avery TL (1980) Combined VM26 and cytosine arabinoside in treatment of refractory childhood lympholytic leukemia. Cancer 45: 1284
43. Roberts D, Hilliard S, Peck C (1980) Sedimentation of DNA from L1210 cells after treatment with 4′-demethylepipodophyllotoxin-9-(4,6-0-2-thenylidene-β-D-glucopyranoside) or 1-β-D arabinofuranosylcytosine or both drugs. Cancer Res 40: 4225
44. Rösner M, Capraro HG, Jacobson AE, Atwell L, Brossi A, Iorio MA, Williams TH, Sik RH, Chignell CF (1981) Biological effects of modified colchicines. Improved preparation of 2-demethylcolchicine, 3-demethylcolchicine and (+)-colchicine and reassignment of the position of the double bond in dehydro-7-deacetamidocolchicine. J Med Chem 24: 257
45. Stähelin H (1970) 4′-Demethyl-epipodophyllotoxin thenylidene glucoside (VM26), a podophyllum compound with a new mechanism of action. Eur J Cancer 6: 303
46. Stähelin H (1973) Activity of a new glycosidic lignan derivative (VP16-213) related to podophyllotoxin in experimental tumours. Eur J Cancer 9: 215
47. Sternlicht H, Ringel I (1979) Colchicine inhibition of microtubule assembly via copolymer formation. J Biol Chem 254: 10540
48. Shife RJ, Jardine I (1980) Analysis of the anticancer drugs VP16-213 and VM26 and their metabolites by high-performance liquid chromatography. J Chromatogr 182: 211
49. Tucker RD, Ferguson A, Van Wyk C, Sealy R, Hewitson R, Levin W, Rad RF (1978) Chemotherapy of small cell carcinoma of the lung with VP16-213. Cancer 41: 1710
50. Waravderkar VS, Domingue A, Leiter J (1952) Effect of podophyllotoxin, α-peltatin and β-peltatin on the cytochrome oxidase activity of sarcoma 37. J Natl Cancer Inst 13: 393
51. Weisenberg RC (1972) Microtubule formation *in vitro* in solutions containing low calcium concentrations. Science 177: 1104
52. Wohlheuter RM, Marz R, Plagemann PGW (1979) Thymidine transport in cultured mammalian cells. Kinetic analysis, temperature dependence and specificity of the transport system. Biochim Biophys Acta 553: 262

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