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The effect of the two epipodophyllotoxin derivatives etoposide (VP-16) and teniposide (VM-26) on cell lines established from patients with small cell carcinoma of the lung*

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Summary. To determine whether there is any difference between the two epipodophyllotoxin derivatives etoposide and teniposide in their therapeutic effect in small cell carcinoma of the lung (SCCL), they were compared against five human SCCL cell lines in vitro. When the two were compared at equimolar concentrations teniposide was found to be 8–10 times more potent than etoposide, both with 1-h incubation and with continuous incubation in a clonogenic assay and in inducing cell cycle perturbations monitored by flow cytometry. Published pharmacokinetic data suggest that this potency difference is not accompanied by an equivalent increase in toxicity.

The concentrations used for the 1-h incubation were about 100-fold the concentrations used in the experiments with continuous incubation to obtain the same degree of cell kill for both drugs. This suggests that they should be given according to a continuous rather than an intermittent schedule.

Introduction

Much effort is devoted to the search for derivatives of well-established cytotoxic agents with either higher antitumor activity and/or different patterns of antineoplastic effect or different toxicity patterns. Selection of the best of these analogues in clinical trials is a problem, since the demonstration of minor differences in clinical trials may require a large number of patients. In view of these difficulties it is important to determine to what extent preclinical parameters are useful to detect differences in antitumor activity between analogue compounds [17]. In vitro tests have been proposed as a way of both screening potential anticancer drugs and assessing the activity of drugs against a given type of tumor. If such methods could be validated they would conceivably diminish the need for drug testing in patients [18].

In the early 1970s two structurally related semisynthetic derivatives of podophyllotoxin, teniposide and etoposide, were introduced. Although etoposide has been established as one of the most active single agents in the treatment of small cell lung cancer [14] it is still uncertain whether there is any therapeutic difference between etoposide and teniposide.

To elucidate this point the activity of the two drugs at equimolar concentrations was compared in vitro in five cell lines established from patients with small cell carcinoma of the lung (SCCL).

Materials and methods

Cell lines. The cell lines used and their source, maintenance, and monitoring have been described elsewhere [16]. Briefly, the cell lines used were NCI-H69, NCI-N592, OC-TOL, OC-ROL, OC-NYH maintained in Roswell Park Memorial Institute medium 1640 with 10% fetal calf serum and in a 7.5% CO_2 humidified atmosphere. The cell lines were free of mycoplasm contamination and had a stable DNA content.

Drugs. Teniposide (VM-26) was supplied in solution for infusion, containing 10 mg drug, 30 mg benzyl alcohol, 60 mg *N, N*-dimetylacetamide, 500 mg macrogoli ricinoleas, maleic acid to pH 5, and absolute alcohol to give a final volume of 1 ml.

Etoposide (VP-16) was supplied in solution for infusion, containing 20 mg drug, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg macrogol, and absolute alcohol to give a final volume of 1 ml. Drugs and solvents without active drugs were obtained from Bristol-Myers. 100× or 300× final concentrations were made with tissue culture medium. On the untreated control plates the cells were exposed to tissue culture medium, following observations that although the cell kill caused by the two solvents at the highest concentrations used in 1 h incubation was approximately 60% it did not differ significantly, and the highest concentrations used in continuous incubation caused no cell kill.

Clonogenic assay. Cell survival was assessed by colony formation in soft agar as described previously [16]. A single-cell suspension was exposed to one of the drugs for 1 h, washed twice, and plated in soft agar on top of a feeder layer. After solidification of the agar 1 ml medium was added to prevent drying.

For continuous incubation the cells were plated in agar with 3 times the desired final concentration to compensate for equilibration of the drug into the feeder layer and the

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added medium. The feeder layers were prepared with sheep red blood cells and mercaptoethanol [16].

The colonies were counted after 3 weeks, using a dissecting microscope. The surviving fractions were calculated by dividing the number of colonies on the treated plates with the number of colonies on the control plates. Correction for lack of proportionality is unnecessary when analogues are compared in simultaneous experiments, as the correction for nonlinearity will be identical [16].

Determination of drug-induced cell-cycle perturbations by flow-cytometric DNA analysis. The cell lines were exposed to drug for 24 h in tissue culture flasks. After centrifugation the cells were suspended in citrate buffer, frozen on ethanol with dry ice, and stored at −80 °C until analysis [21]. Before analysis, the samples were stained with propidium iodide [22]. The percentage of cells in each cell cycle phase was determined by statistical analysis of the DNA distribution [4].

Results

The in vitro chemosensitivity at equimolar concentrations of the five cell lines tested after 1 h incubation is given in Fig. 1, indicating that all five cell lines are more sensitive to teniposide than to etoposide. When the cell kill obtained with etoposide at 25 μmol/l is compared with that obtained with teniposide at 2.5 μmol/l it can be seen that four cell lines are at least 10 times more sensitive to teniposide than to etoposide. In the cell line that is most sensitive

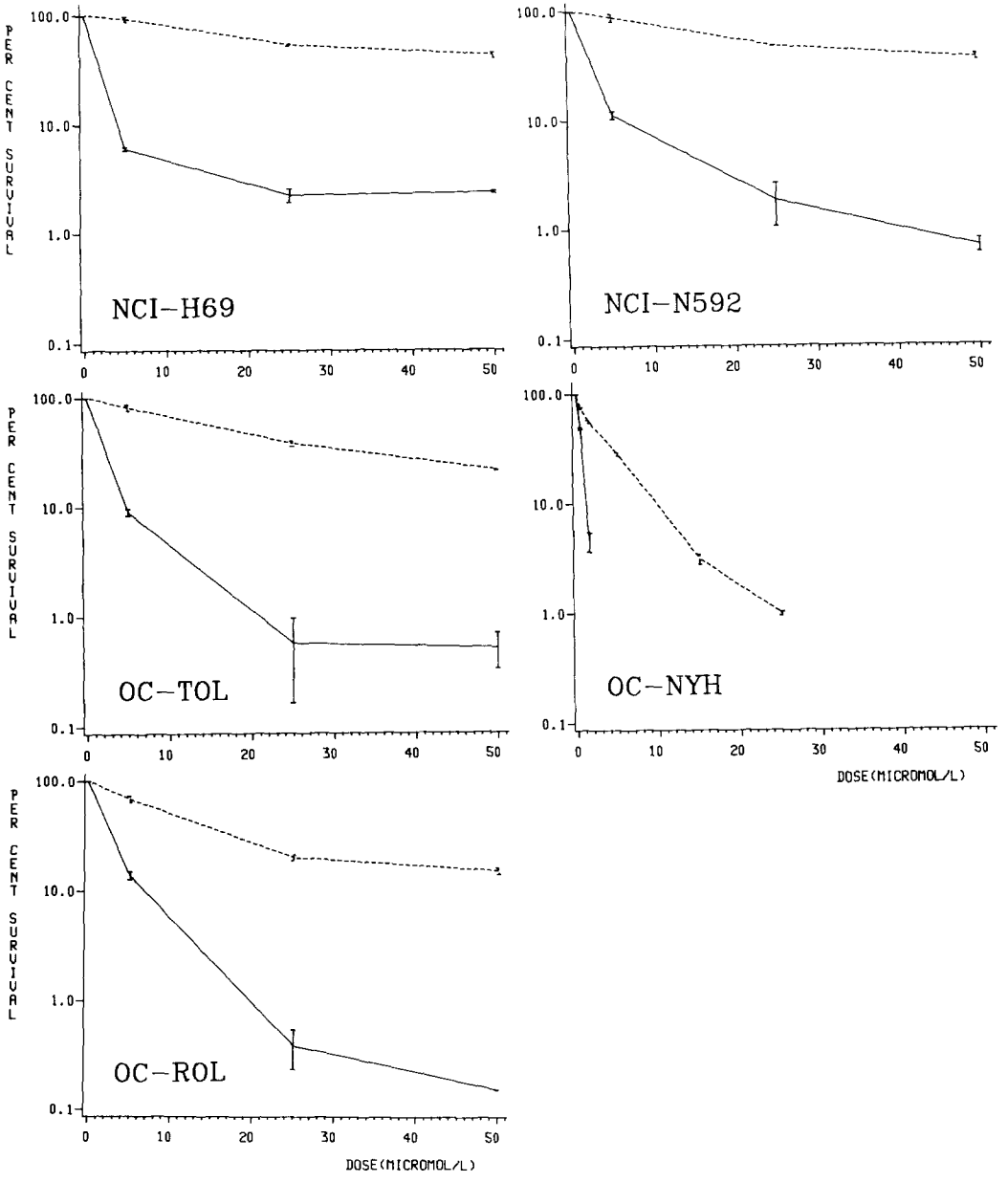

Fig. 1. Dose-survival curves for 1 h in vitro exposure to either teniposide *(solid lines)* or etoposide *(broken lines)* at equimolar concentrations. *Bars* represent 2 SEM. Each experiment was done in triplicate. Plates without colonies have been omitted

to epipodophyllotoxin derivatives in 1-h incubation (OC-NYH), teniposide is less than 10 times more potent than etoposide.

In Fig. 2, dose-response curves obtained during continuous exposure are shown. Comparison of the doses needed to achieve approximately 75%–90% cell kill shows that teniposide is about 8 times more potent than etoposide. When dose-response curves obtained after a 1-h incubation are compared with the dose-response curves obtained with continuous exposure, the doses required in the 1-h incubation experiments to obtain the same cell kill are seen to be about 100-fold the concentrations used in the experiments with continuous exposure.

In Fig. 3, histograms obtained by flow cytometry after exposure of the most sensitive (Fig. 3a) and the least sensitive (Fig. 3b) cell lines to two doses of etoposide and teniposide for 24 h are shown. It appears that both drugs cause an accumulation of cells in the late part of the S phase and in the G2 + M phase. The cell cycle perturbations are most prominent when the effect of the high concentration of teniposide on the most sensitive cell line is considered. The fact that the cell cycle perturbations encountered with the high concentration of etoposide are similar to those encountered with the low concentration of teniposide also implies that teniposide in this test system is about 8 times more potent than etoposide.

Discussion

When comparing the cytotoxic activity of teniposide with etoposide in various in vitro and in vivo tumor systems it is evident that teniposide is consistently superior to that of etoposide [2]. The drugs have been compared in clonogenic assays in the following cell lines: mouse mastocytoma (P-815), human cervix carcinoma (HeLa) [19], human lymphoblastic leukemia (CCRF-CEM) [7], human squamous cell carcinoma of the tongue (HN-1) [9], and Chinese hamster ovary cells [8]. In all these investigations teniposide has been found to be about 10 times more potent than etoposide.

The present investigation conducted in a panel of SCCL cell lines also revealed that teniposide is 8–10 times more potent than etoposide at equimolar concentrations in both 1-h and continuous incubation in a clonogenic assay and also in the induction of cell cycle perturbations. To have any clinical usefulness, such a potency difference should not, of course, be accompanied by an equivalent increase in toxicity. The toxicity of the two drugs have

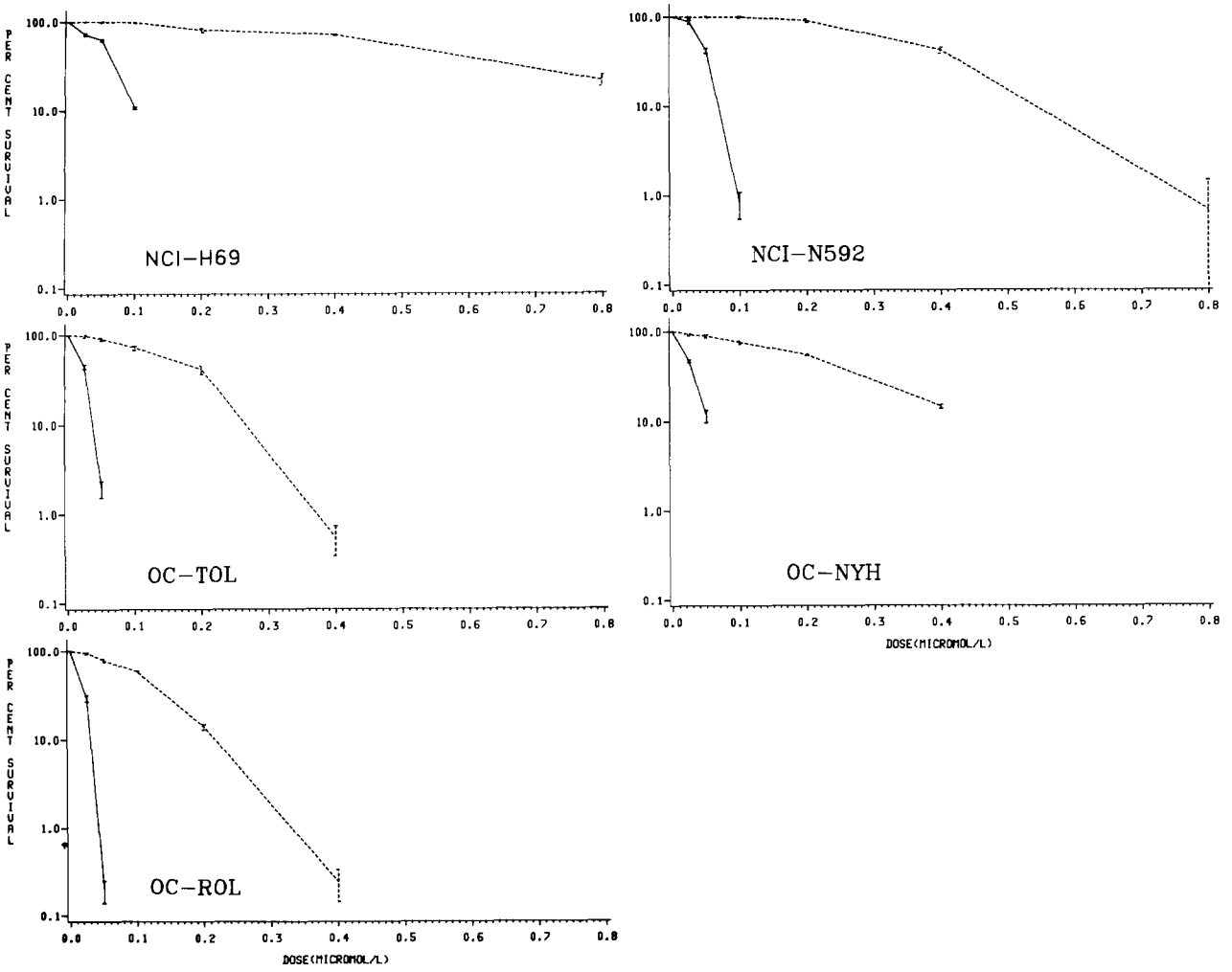

Fig. 2. Dose-survival-curves for continuous exposure to epipodophyllotoxin derivatives. Symbols as in Fig. 1

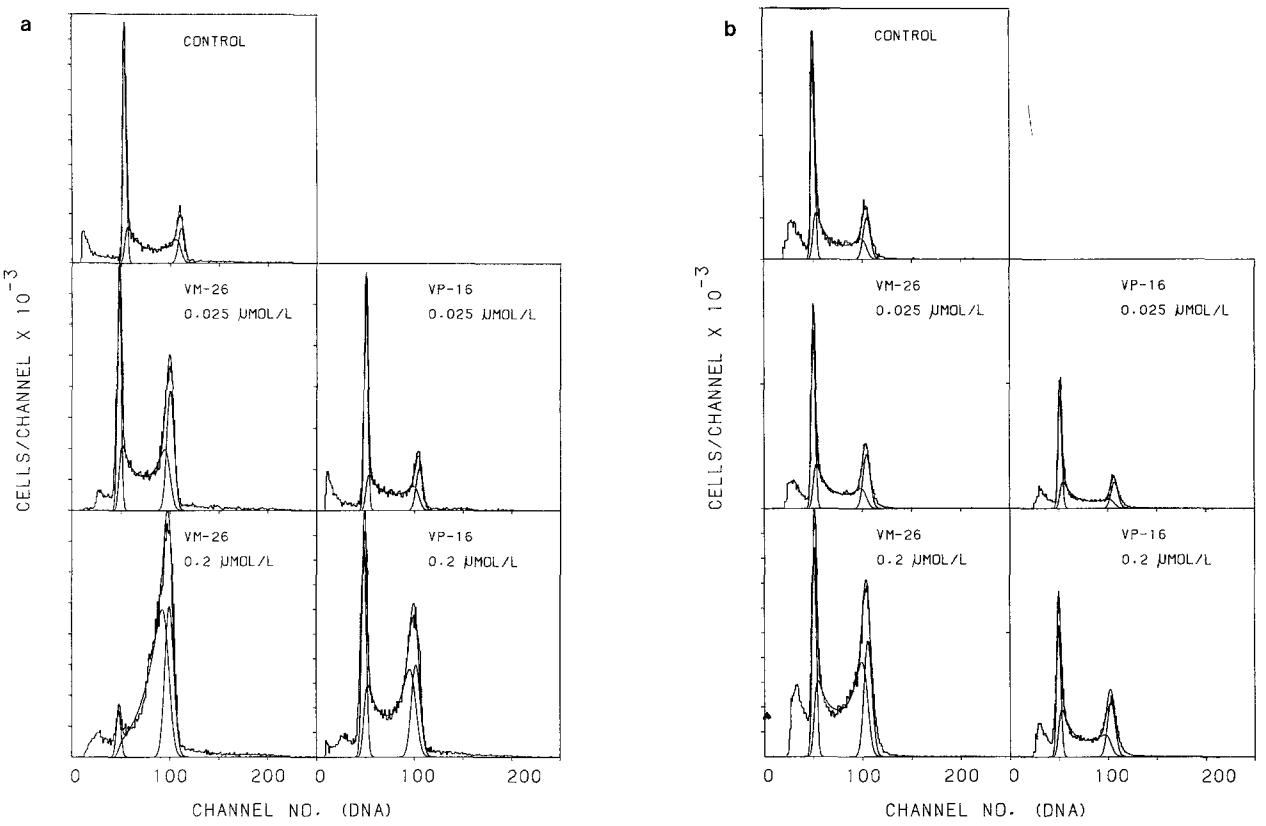

Fig. 3a, b. Flow cytometric DNA histograms obtained after exposing OC-Rol **(a)** and NCI-H69 **(b)** for 24 h to 0.025 micromolar or 0.2 micromolar etoposide or teniposide

been evaluated in clinical phase I trials. With administration once weekly a recommended dose of 70 mg/m^2 was established for teniposide [12], with the same schedule the dose of etoposide was 290 mg/m^2 [6].

The plasma clearance of etoposide is nearly 3 times that of teniposide [1], and there is also a difference in the degree of plasma protein binding of the two drugs (94% for etoposide and 99.4% for teniposide). This could account for the difference in plasma decay kinetics [5]. Because the binding to serum albumin is reversible it may serve to prolong drug action, especially in the case of teniposide. These data suggest that the more pronounced clinical toxicity of teniposide than of etoposide is based partly on prolonged drug action and could thus indicate comparable cellular exposure of the two drugs at equitoxic doses.

It has been shown that a schedule of 3 consecutive days per week or 5 consecutive days every 3 weeks of etoposide is superior to treatment once or twice weekly in the treatment of SCCL [14]. This schedule dependency corresponds to the observation that concentrations used in the 1-h incubation experiments are about 100-fold the concentrations used in the experiments with continuous drug exposure to obtain the same degree of cell kill [11]. Schedule dependency could also be suggested by the fact that the dose-response curves obtained with 1-h incubation reach a saturation value, whereas the curves obtained with continuous incubation are exponential, a pattern seen when cell cycle phase-specific drugs are tested [10, 20]. These phenomena are observed with etoposide as well as with teniposide, indicating that the optimal schedule for teniposide is probably also daily administration for several consecutive days.

Cells resistant to etoposide are normally also resistant to teniposide, although a few clinical cases with no cross resistance between etoposide and teniposide have been reported [9]. The results in the present investigation indicate that etoposide and teniposide act in an identical manner against SCCL, because ranking of the cell lines according to their sensitivity to etoposide is identical with ranking according to sensitivity to teniposide.

Although this study suggests a cellular cross resistance, it is still not clear whether there is a clinical cross resistance between etoposide and teniposide in SCCL. One study [15] compared two groups of patients with SCCL treated with teniposide. One group was clinically resistant to etoposide treatment, whereas the other had never received etoposide. Response rates in the two groups were 5% and 25% respectively. Although the confidence intervals do overlap, this difference in response rates could indicate clinical cross resistance.

In conclusion, the superiority of teniposide to etoposide in vitro against a number of SCCL cell lines has been demonstrated. Pharmacologic evidence suggests that the difference in antineoplastic potency is more pronounced than the toxicologic difference.

So although early indications of the activity of etoposide have attracted investigators interest to etoposide, with the result that clinical research with teniposide has lagged far behind [13], this investigation, together with promising results achieved in a recent phase II trial of teniposide in previously untreated patients with SCCL [3], justifies additional clinical trials to compare teniposide and etoposide in the treatment of SCCL.

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