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Etoposide (VP16-213) and Teniposide (VM26) Comparative in vitro Activities in Human Tumors

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Summary. *In order to determine if any inherent sensitivity differences may exist between VP16-213 and VM26 individual human tumors were grown in vitro and drug sensitivities were determined using the soft agar clonogenic assay method. Only nine of the 34 tumors tested so far showed a differing sensitivity to VP16-213 and VM26 as measured by a 25% or greater colony number reduction. However in none of these tumors did this added reduction result in a 70% decrease over control plate colony numbers. As yet we have been unable to demonstrate any clinically meaningful inherent in vitro sensitivity difference between VP16-213 and VM26 in any tumor type tested.*

Introduction

The podophyllotoxin derivatives VP16-213 and VM26 differ chemically only by the substitution of a methyl group on the glucopyranoside by a thenylidine group. The preclinical antitumor sensitivity profiles are similar in the transplanted murine systems and there is no evidence of any cellular or subcellular effect difference in studies so far reported [5]. During their 10 years of clinical development each compound has been tested in predominantly different tumor types for largely historical reasons.

The composite clinical response rates for each compound may suggest a superiority of our compound over another in certain tumor types. However these rates are compiled from historical studies using different doses and schedules, some of which would now be considered suboptimal. Furthermore there is considerable inter-study variation of patient and tumor characteristics within each composite tumor group. Whether one drug has a different activity as compared to the other in a specific tumor type has not yet been determined in a clinical study sufficiently adequate to answer this question. In order to help direct the further clinical development of VP16-213 and VM26 we decided to use the human in vitro soft agar clonogenic assay to determine if one compound may be more active in a specific tumor type. This assay has been shown to be highly predictive for in vivo patient drug response [1, 7]. This paper outlines the interim results of an ongoing study.

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Materials and Methods

The soft agar clonogenic assay has been previously described in detail elsewhere [3, 4, 6]. Briefly, tissue samples for culture were obtained from malignant effusions or from surgically resected solid tumor tissue. Effusions were collected in preservative-free heparinized containers. Singlecell suspensions from solid tissue were obtained by a mechanical disaggregating procedure that was modified from the previously described methods and is as follows. The specimen was cut into cubes of 2 mm or less with scalpels, suspended in nutrient medium, and further disaggregated by being forced hydrostatically through a series of "syringe screens" ranging from 20-mesh to 400-mesh in size. A syringe screen consisted of a 50 ml syringe barrel with the needle end cut off and replaced by a stainless steel screen.

Tumor cell suspensions were incubated in serum-free nutrient medium containing 2.0 μg/ml of VP16-213 or VM26 at 37° C for 1 h. Drug concentrations of 2.0 μg/ml were chosen based on 10% peak-serum concentrations obtainable following in vivo drug administration [2].

After drug incubation, the cells were washed twice in medium. Either 1×10^6 or 2×10^6 cells, depending on the viability previously determined by the trypan blue exclusion method, were mixed in 1.0 ml of enriched medium containing 0.3% Low Melting Point Agarose™ (Bethesda Research Laboratories Rockville, Maryland, USA) and placed on a 2 ml 0.5% agar containing nutrient feeder layer in 35 mm plastic petri dishes. The 35 mm assay dishes were evaluated for day 0 debri or cell aggregates and then placed in 150 mm humidified petri dishes and incubated in 5% CO_2 at 37° C. They were first monitored for preparation quality and were left undisturbed until counting 15 and 21 days later.

Colonies were defined as tight aggregations of greater than 30 cells. Assay dishes and controls were run in triplicates, a procedure

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which we have generally found gives individual dish variability of less than 15%. A positive drug effect was defined as a 70% or more decrease in the number of colonies of the drug-treated plates compared to control plates. This percentage decrease had been previously found to predict for a positive patient effect [8].

Results

Over 200 separate tumor specimens from either solid tumor excision, malignant effusion aspiration, or malignant bone marrow sampling have been received for assay by our laboratory. Seventy-four percent of the samples received grew colonies. In 53% of the total samples, there were multiple plates of sufficient numbers of colonies for satisfactory drug testing. Virtually all the major histological tumor types tested have been successfully cultured. Comparative VP16-213 and VM26 testings on 34 separate human tumors are completed to date.

Table 1

Tumor type	Source	Number control colonies	% Decrease of control	
			VP16-213	VM26
Acute lymphoblastic leukemia	Marrow	51	38[a]	28E
Lymphoma				
DHL	Solid	68	57	70
DHL	Solid	115	53[a]	27
DHL	Solid	15	36	40
DHL	Solid	12	49[a]	6
DML	Solid	76	72	82
Lung				
Small cell	Solid	35	31E	56E
Adenocarcinoma	Pleural effusion	84	41	69[a]
Large cell	Solid	36	16	12
Non-small cell	Solid	94	45	60
Breast	Solid	27	33[a]	9
Breast	Solid	38	0	5
Breast	Solid	493	12E	20
Breast	Solid	34	4	3E
Gastric	Solid	74	31E	37E
Pancreatic	Ascites	19	58	37
Colorectal	Solid	214	27	24
Colorectal	Solid	55	4	37[a]
Colorectal	Solid	179	16	21
Colorectal	Solid	49	12	6
Colorectal	Solid	15	37	62[a]
Germ cell	Solid	22	9	60[a]
Renal cell	Solid	1,680	12	28
Endometrial	Solid	307	21E	18E
Ovarian	Ascites	115	8	21
Ovarian	Ascites	7,220	57	33
Ovarian	Solid	793	0	5
Ovarian	Ascites	37	25	14
Rhabdomyosarcoma	Solid	37	46	54
Neuroblastoma	Pleural effusion	50	38	45
Melanoma	Solid	42	47	41
Melanoma	Solid	127	51	29
Brain	Solid	77	16	46[a]
Anaplastic unknown primary	Solid	61	9	20E

[a] 25% or greater difference
E = Enhancement of growth compared to control

Table 2. Compound ranking (25% or greater difference) (n = 34)

Ranking	Frequency	Tumor types
VP16-213 > VM26	4	ALL, breast, 2 DHL
VM26-213 > VP16	5	Brain, germ cell, adeno lower case lung, 2 colorectal

The tumor types tested, sources of tumor cells, number of control colonies, and decrease in colony numbers compared to controls for VP16-213 and VM26 are outlined in Table 1. Table 2 outlines the comparative ranking of VP16-213 and VM26 based on a 25% or more difference in drug sensitivity for each tumor type. VM26 showed a superiority to VP16-213 in five tumors and VP16-213 showed a superiority to VM26 in four tumors. There is no evidence from our experience or from other reported data that these in vitro differences would be reflected by different in vivo patient responses to the podophyllotoxin derivatives tested.

Discussion

The inability of transplanted murine tumors to predict relative activities of anticancer drugs in patients is a major problem of cancer drug development. The introduction of the soft agar clonogenic assay with its high in vitro-in vivo correlation now provides a technology with considerable potential for selecting drugs relevant to the treatment of patients. It is true that further understandings of the biology of the assay and additional technological refinements are required before its results can be considered with utmost confidence. Nevertheless, in comparison with our previously available methods, this system could well constitute a major breakthrough.

In addition to its utilization in helping to select appropriate drugs for individual cancer patients, the assay is also being used in our laboratory to aid the selection of analogs and novel compounds for development at the preclinical and phase I clinical stages. It is intended to test compounds head-to-head against at least ten individual tumors of several signal histological types. To aid the supply of a sufficient

number of tumors of each histological type, the validity of using thawed frozen specimens is also under investigation.

As is the case with VP16-213 and VM26, there are instances where analogs have undergone substantial clinical development without established evidence of any superiority for one over another. This is mainly because the compounds were developed sequentially and historical trends had established the utilization of each compound in separate tumor types. Head-to-head clinical comparisons were rarely carried out in a single tumor type. An important question often asked by clinicians is, can one of the analogs with a more convenient form of administration (oral) substitute for its solely parenterally formulated analog in all patients? The hope that the clonogenic assay may help answer these questions form the rationale for this study.

The results presented here are interim and therefore largely inconclusive. However, no clinically meaningful in vitro sensitivity difference between VP16-213 and VM26 has yet been detected in an individual tumor.

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