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Human autopsy tissue distribution of the epipodophyllotoxins etoposide and teniposide

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Abstract. Autopsy tissues were collected from ten patients who had received etoposide, 150–3480 mg, from 1 to 412 days antemortem and from five patients who had received teniposide, 234–1577 mg, from 3 to 52 days antemortem. Tissues were assayed for etoposide and teniposide using high-pressure liquid chromatography with electrochemical detection. Etoposide was detectable in tissues of three of four patients dying <5 days after their last etoposide treatments to cumulative doses of 150–432 (median, 280) mg but was detectable in tissues of only one of six patients dying 7–412 (median, 37) days after their last etoposide treatment to a cumulative dose of 607–3600 (median, 1553) mg. The highest tissue concentrations were in the small bowel, prostate, thyroid, bladder, spleen, and testicle. Intermediate concentrations were found in the lymph node, skeletal muscle, adrenal gland, stomach, tumor, liver, lung, pancreas, and kidney, and the lowest concentrations were found in the heart, brain, diaphragm, vagina, and esophagus. Teniposide was detectable in one patient dying 3 days after a cumulative teniposide dose of 576 mg (spleen, prostate, heart > large bowel, liver, pancreas > thyroid, adrenal, stomach, small bowel, bladder, testicle, and skeletal muscle) but was not detectable in any tissue from four patients dying 5–52 (median, 8) days after their last treatment to a cumulative teniposide dose of 234–1577 (median, 520) mg. The very short tissue half-life contrasts with our previous observations for human autopsy tissue concentrations of mitoxantrone, doxorubicin, menogaril metabolites, diaziquone, and amsacrine. The short tissue half-life may help explain the schedule dependency of epipodophyllotoxin efficacy and may also help explain the lack of visceral toxicity of these compounds.

Introduction

The epipodophyllotoxins etoposide and teniposide are useful antineoplastic agents [2] that are thought to kill cells primarily through inhibition of topoisomerase II [9]. The efficacy of both drugs is schedule-dependent [10]. The major toxic effect of both drugs is myelosuppression [10]. Toxicity of these drugs to organs such as the brain, lungs, heart, kidneys, and liver is very uncommon. We have previously studied the autopsy tissue distribution of several antineoplastic agents and have found that each of the compounds cisplatin [5, 13, 21, 25], vinblastine [15], mitoxantrone [22], amsacrine [17], doxorubicin and its metabolite doxorubicinol [27], diaziquone [11], and the menogaril metabolite 7-deoxynogarol [28] are retained in human tissues for prolonged periods after the last drug administration. These previous autopsy studies suggest that chronic organ toxicity associated with these drugs in many cases may be related to their concentrations in the affected tissue [5, 17, 21, 22, 27, 28]. In light of the relative lack of toxicity of the epipodophyllotoxins, we were interested in also determining their tissue concentrations.

Materials and methods

Sample collection and storage

Autopsy tissue samples were collected from ten patients who had received cumulative lifetime etoposide doses of 150–3600 mg, with the last treatment having been given from 1 to 412 days antemortem. Tissue samples were also collected from five patients who had received cumulative lifetime teniposide doses of 234–1577 mg, with the last treatment having been given from 3 to 52 days antemortem. Tissues were stored at –20°C until assayed. Complete sets of autopsy tissues were not available from each patient since all of the patients had received more than one chemotherapy drug, and for some organs from each patient, all tissues had previously been utilized for measurement of other drugs.

Chemicals. Etoposide and teniposide for use as standards in our assays were kindly provided by Bristol-Myers Sqibb (Wallingford, Conn.). Standard solutions of etoposide (1 mg/ml) and teniposide (0.25 mg/ml) were prepared in methanol and stored at 4°C. Potassium phosphate

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Table 1. Concentration of etoposide in human autopsy tissues

Patient	1	2	3	4	5	6	7	8	9	10
Etoposide[a]	400	160	432	150	607	3480	1485	1620	665	3600
Time (days)[b]	0.8	1	5	5	7	21	26	48	84	412
Tissue	Tissue etoposide (ng/g)[c]									
Small bowel		1339		0		0	0			0
Prostate	2229	1371			0			392		
Thyroid		1266		0	0					
Liver			154	0	0	0	0	556		0
Bladder	2652	1217	0	0		0		477	0	0
Spleen	1955	2022	90	0	0	0	0	0	0	0
Testicle	2209	674			0			132		
Lymph node		900			0	0				
Muscle	1965	815	38	0	0	0		214		0
Adrenal	752			0	0	0	0	597		0
Stomach	993	795	0	0		0	0			0
Lung	360	1093	126		0	0	0	179		0
Pancreas	163		0	0	0	0		572	0	0
Kidney				0	0		0	197		
Heart			0	0	0	0		199	0	0
Brain					0	0		71		0
Diaphragm			45							
Vagina			0							
Esophagus			0				0			
Large bowel			0	0		0				0
Ovary				0						
Cervix					0					
Aorta			0				0			
Tumor[d]				0	0		0	473		

[a] Cumulative life-time dose of etoposide (mg)
[b] Time in days from last etoposide treatment to death
[c] Where no value appears, no tissue was available for assay. The lower limit of accurate detection was 100 ng/g. Values less than this must be interpreted with caution
[d] Non-small-cell lung cancer

monobasic and dibasic were obtained from J.T. Baker Chemical Company (Phillipsburg, N.J.). Methanol and acetonitrile were purchased from BDH Inc. (Toronto, Ontario).

Extraction of drug from tissue samples Autopsy tissues (1–2.5 g) were minced with a pair of scissors, then homogenized in 3 vol. (w/v) of ice-cold 0.065 *M* potassium phosphate buffer (pH 7.0) with a Brinkmann Polytron (setting 7–8). Aliquots (1 ml) in triplicate were transfered to 15-ml Corex centrifuge tubes (number 8441) containing teniposide (1.5 μg) or etoposide (1 μg) as internal standards. Protein was precipitated by adding 0.1 ml methanol plus 3 ml acetonitrile and allowing samples to stand on ice for 10 min. The tubes were vortexed (1 min) and centrifuged at 11,000 rpm (10 min) in a Jouan MR 14.11 refrigerated centrifuge using an RPM TR/MN 11500 rotor.

The supernatants were diluted with distilled water (25 ml) in Labconic 50-ml copolymer centrifuge tubes, then passed through preconditioned Bond Elut C18 columns (100 mg) that were fitted with 8-ml reservoirs and placed in a Vac Elut Apparatus (Analytichem International) under low vacuum. An additional 3 × 8 ml distilled water was then added, and nitrogen was blown through to remove as much residual water as possible. Etoposide and teniposide were eluted with 1 ml potassium phosphate buffer (0.065 M, pH 7.0) containing 40% acetonitrile.

High-pressure liquid chromatographic analysis. Eluate (100 μl) from the Bond Elut C18 columns was analyzed for etoposide and teniposide by high pressure liquid chromatography (HPLC) using an electrochemical detector [7]. The HPLC system consisted of a Shimadzu LC-6A pump, an SLC-6A system controller, an SIL-6A autosampler, a CR-5A chromatography integrator recorder, and a BAS (Bioanalytical Systems Inc.) LC-4B amperometric detector with a glassy carbon working electrode and an Ag/AgCl reference electrode. The compounds were separated on a Waters μ Bondapak phenyl column (300 × 3.9 μm) coupled with a Guard-Pak Resolve CN precolumn insert guard column at a flow rate of 1 ml/min.

The mobile phase consisted of potassium phosphate buffer (0.065 *M*, pH 7.0) and methanol (40:60, v/v). It was degassed under vacuum (10 min) before use. Etoposide and teniposide were detected at an oxidation potential (0.75 V, 10-nA range), and were identified by their retention times of 5.6 and 8.5 min, respectively. The compounds were quantitated by comparison of the peak height of the sample with standard curves constructed by adding drug to control human autopsy tissues. All data points weighted with the reciprocal of concentration were used in regression. Recoveries of etoposide and teniposide internal standards and standards from homogenized control human autopsy tissues were 85%–100%. The lower limit of detection was 100 ng/g for etoposide and 150 ng/g for teniposide. No drug metabolite standard was available; hence, no attempt was made to assay metabolites and none was identified.

Results

Tissue concentrations of etoposide are presented in Table 1. Figure 1 presents a summary of the etoposide concentrations for the four patients in whom etoposide was detectable. As noted above, not all organs were available for assay in all patients, since in some cases, all available tissue had previously been utilized for assay of other drugs. Etoposide was detected in tissues of three of four patients who had received their last dose of etoposide 5 days before

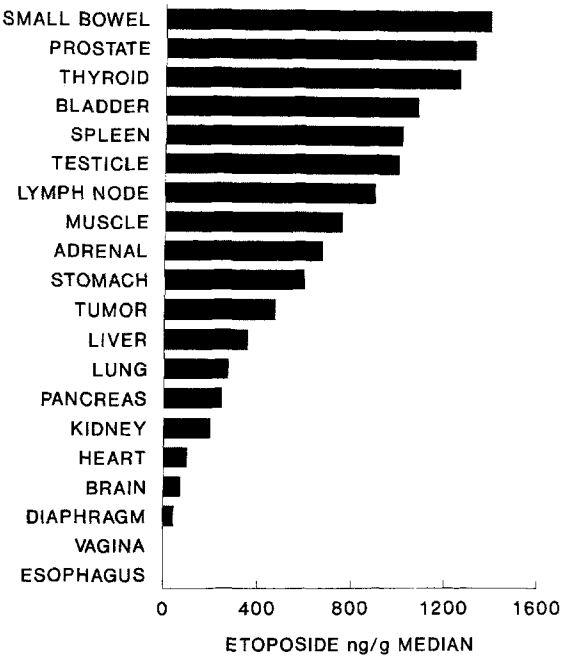

Fig. 1. Median tissue concentrations of etoposide for the 4 patients with etoposide detectable in their tissues. Data are not available for some organs from many of the patients since tissue samples had previously been used to assay other drugs received by the patient

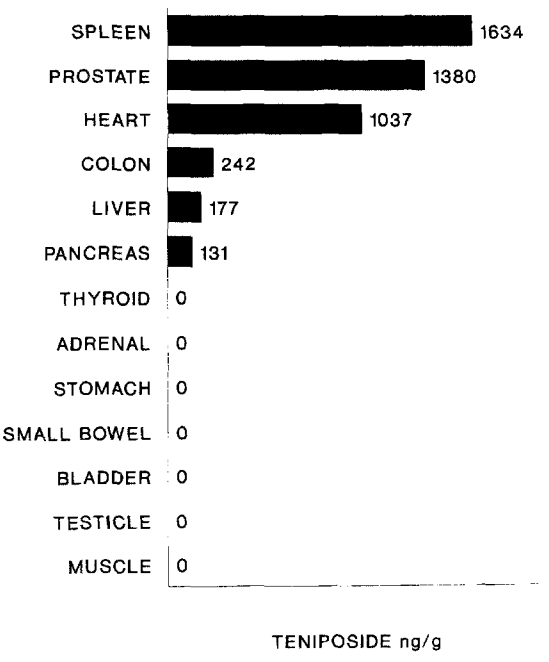

Fig. 2. Concentrations of teniposide in tissues of the only patient with detectable teniposide concentrations

death. These three patients in whom etoposide was detectable had received cumulative lifetime etoposide doses of 160, 400, and 432 mg, respectively, whereas etoposide was not detectable in the tissues of a patient who died 5 days after receiving a single dose of 150 mg. Etoposide was generally not detectable in the tissues of patients dying >5 days after receiving their last dose of etoposide. The only exception was a patient who had received a total cumulative etoposide dose of 1,620 mg, with the last dose having been given 48 days prior to death. Five additional patients who had received cumulative lifetime etoposide doses of 607–3600 (median, 1075) mg, with their last etoposide dose having been given between 7 and 412 (median, 26) days antemortem, had no detectable etoposide in their tissues.

There was no definite pattern to the distribution of drug for those patients in whom etoposide was detectable, but some generalizations can perhaps be made. For the two patients in whom the liver was assayed, liver etoposide concentrations were relatively high as compared with etoposide concentrations in other organs. Prostate, spleen, and bladder concentrations also tended to be relatively high. In three men, the etoposide concentration in the testicle was relatively high in the patient for whom the shortest interval from the last treatment to death was recorded (19 h), but it was relatively low in the other two men with detectable tissue etoposide concentrations.

Only one of the four patients with detectable etoposide had tumor and brain tissue assayed (since all tumor and brain tissue collected from the other patients had previously been used to assay other drugs). His tumor (non-small-cell lung cancer) etoposide concentration was comparable with the concentration of etoposide in his other tissues, whereas his brain etoposide concentrations were lower than the concentrations in most other tissues.

Teniposide was detectable in the tissues of only one of the five patients studied. Tissue teniposide concentrations for the patient dying 3 days after his last teniposide treatment to a total cumulative dose of 576 mg are presented in Fig. 2. As observed for etoposide, the spleen and prostate had relatively high concentrations of teniposide in the patient in whom teniposide was detectable. Unlike our findings for etoposide, heart concentrations of teniposide were also moderately high. Four other subjects (teniposide patients 2, 3, 4, and 5) dying 5, 5, 11, and 52 days after having completed teniposide treatment to cumulative lifetime doses of 234, 240, 520, and 1573 mg, respectively, had no teniposide detectable in their tissues. Organs assayed for teniposide in these patients were the tumor, brain, kidney, and stomach in patient 4 only; the small bowel, skeletal muscle, uterus, and ovary in patient 2 only; the thymus and prostate in patient 5 only; the thyroid in patients 2–5; the spleen in patients 2, 4, and 5; the bladder in patients 2, 3, and 5; the lung and colon in patients 3–5; the heart and liver in patients 3 and 4; the adrenal in patients 2 and 3; and the pancreas and lymph node in patients 4 and 5. As was the case for etoposide, some patients had only limited numbers of tissues available since tissue samples had also previously been assayed for other compounds.

Discussion

The findings of this study indicate that unlike most other compounds we have studied [5, 11, 13, 15, 17, 21, 22, 25,

27, 28], the epipodophyllotoxins etoposide and teniposide are retained in human tissues for only a very short period after the last drug administration. Very limited data that have previously been published in animals [1] and humans [4] also suggest that etoposide has a short tissue half-life, although it is possible that the relative insensitivity of the assay methodology could have partially explained the limited detection of these drugs in tissues. A more recent animal study in mice suggested a somewhat longer terminal tissue half-life [3], but this study was done using tritiated etoposide, and steps were not taken to ensure that the radiolabel represented intact etoposide. On the other hand, our assay methodology did not detect epipodophyllotoxin metabolites, and it remains possible that there could have been substantial amounts of such metabolites in our samples.

This apparent short retention time of the epipodophyllotoxins in our human tissue samples could possibly help explain why these drugs rarely result in chronic organ toxicity [10]. For many other drugs that we have studied, patterns of prolonged tissue retention appeared to bear a relationship to the types of long term toxicity caused by those agents [5, 17, 21, 22, 27, 28]. For example, cisplatin's nephrotoxicity may be related to kidney cortex platinum concentrations [21], cisplatin's peripheral neuropathy may be related to dorsal root-ganglion platinum concentrations [5]; amsacrine's neurological and cardiac toxicity may be related to its concentrations in the brain and heart, respectively [17]; menogaril's pulmonary toxicity could possibly be related to high lung concentration of its metabolite, 7-deoxynogarol [28]; mitoxantrone's cardiotoxicity might be related to high heart muscle concentrations of the drug [22]; and doxorubicin's cardiotoxicity might be related to heart muscle concentrations of both doxorubicin and its metabolite, doxorubicinol [27].

One might also speculate that this low retention time in tissues could possibly play a role in the marked schedule dependency of the antineoplastic activity of etoposide and teniposide [10], i.e., since the drugs are not retained in tissues for prolonged periods, repeated exposure is needed for an optimal therapeutic effect. New analogues with longer tissue retention times could potentially have greater antineoplastic activity than the current generation of epipodophyllotoxins, but they could also have substantially different patterns of toxicity.

We have previously published data on the human central nervous system pharmacology of etoposide [18] and teniposide [19] following administration of low doses of these drugs during surgical resection of brain tumors. We found moderately high concentrations of these drugs in human brain tumors, although the drugs do not generally reach high concentrations in the normal central nervous system. In this autopsy study, we also found only poor penetration of etoposide in the normal human central nervous system for the single patient in whom brain tissue was available for assay. In our studies of the human central nervous system pharmacology of several antineoplastic agents, we have found no consistent correlation between human brain tumors and the normal central nervous system with respect to drug concentrations attained [8, 12, 14, 16, 20, 23, 24, 26]. Although we found somewhat lower concentrations of etoposide in brain tumors than in extracerebral tumors in humans [18], the difference was relatively minor.

It has previously been suggested that the testicle is a pharmacological sanctuary and that the low concentrations of antineoplastic agents achieved in the testicle result in the protection of intratesticular tumor cells from chemotherapy [6]. Our patient for whom the shortest interval between the last treatment and death was recorded actually had quite high concentrations of etoposide in the testicle. However, with increasing time after the last treatment, the relative concentration of etoposide in the testicle as compared with other tissues decreased. Because of the small number of patients involved, this observation may be attributable to chance alone. However, it is possible that a chemotherapeutic agent may be actively extruded from the testicle at a faster rate than is the case for other tissues or, alternatively, it may be less tightly bound to the testicle than to other tissues. We have also found no evidence of a blood-testicular barrier for cisplatin [13], amsacrine [17], or mitoxantrone [22].

The concentration of etoposide in tumor tissue (for the single patient with etoposide detectable in tissues who had tumor available for assay) was within the range of etoposide concentrations found in the other tissues assayed. No drug was detected in any of the tumor samples or normal tissues in either the three other patients who had received etoposide or the single patient who had received teniposide for whom tumor samples were available for assay. Overall, this suggests that the handling of these drugs by tumor tissue is comparable with their handling by normal tissues. Although the limited amount of data available requires that caution be exercised in the interpretation of our results, this is a pattern that we have seen for almost every other drug that we have studied [11, 13, 15, 17, 22, 25, 27, 28], and is also in keeping with previous limited data published for etoposide [4].

In summary, as for other drugs we have studied, human autopsy-tissue distribution studies of epipodophyllotoxins provide a possible pharmacological explanation for their pattern of toxicity and efficacy.

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