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Concentrations of vinblastine in human intracerebral tumor and other tissues

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Summary

Uptake of vinblastine into human cerebrospinal fluid, intracerebral tumor and autopsy tissues was quantitated radiochemically after separating vinblastine from its metabolites by high pressure liquid chromatography. Only low concentrations of vinblastine were found in cerebrospinal fluid from a single patient. A second patient who received a tracer dose of radiolabelled vinblastine prior to surgical resection of an intracerebral tumor had slightly less radioactivity in tumor than in temporalis muscle, but more in tumor than in edematous brain surrounding the tumor. The radioactivity in tumor increased gradually and exceeded concurrent plasma radioactivity by 2 hr after drug administration. A third patient died 4 hr into a planned 24-hr infusion of radiolabeled vinblastine. Highest vinblastine concentrations were found in organs with high blood flow such as kidney and heart. Intermediate concentrations were found in liver and lung, and low concentrations were found in prostate, gastrointestinal tract, spleen, muscle, bladder, and hepatic and lymph node metastases. A fourth patient died one month after receiving radiolabeled vinblastine. Highest concentrations were in liver and next highest concentrations were in intracerebral tumor. Moderately high concentrations were found in pancreas, thyroid, lung, spleen, ovary, kidney, and kidney metastases. Lowest concentrations were found in omental metastases, heart, breast, and brain. Vinblastine concentration decreased with increasing distance into brain from the brain metastases. Thus, vinblastine was not selectively localized in tumors. The concentrations in tumor did not reflect the concentration in the organ in which the tumor was located. There was no indication that uptake into intracerebral tumor was impaired. Cerebrospinal fluid and brain concentrations of vinblastine did not give any indication of the concentration attainable in intracerebral tumor.

Introduction

While it is relatively easy to determine the tissue distribution of drugs in animals, it is considerably more difficult to do so in humans. Tissue distribution studies can be helpful, since they may provide a pharmacologic explanation for toxicity or activity. Moreover, knowledge of a drug's tissue distribution may provide a pharmacologic rationale in planning further therapeutic studies with the drug. One of the most important potential applications of information on tissue concentrations of drugs is in the field of in vitro sensitivity testing, since such data may provide guidelines for choosing concentrations of drug to be tested.

Vinblastine is an antineoplastic agent with a wide range of activity, including carcinomas of the breast, kidney and testicle, as well as leukemia and lymphoma (1). Its major toxicities are myelosuppression, alopecia, neuropathy, and local tissue

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destruction upon extravasation (1). In humans, vinblastine pharmacokinetics appear to be consistent with a three-compartment open model system, with a $(t_{1/2})\alpha$ of 3.9 min, $(t_{1/2})\beta$ of 53 min and $(t_{1/2})\gamma$ of 1173 min (2). The major metabolite is deacetylvinblastine (2).

In this paper we describe the distribution of vinblastine in human tissue and cerebrospinal fluid.

Materials and methods

Patient population

Four patients were given radiolabeled [^{3}H(G)] vinblastine sulfate for pharmacology studies after granting informed consent. Patient 1 was a 39-year-old female with adenocarcinoma of the breast who was in complete remission from meningeal carcinomatosis and still had an Ommaya reservoir in place. She received vinblastine 7.5 mg/m^2 by continuous intravenous infusion over five days including 250 μCi (1.5 mg/m^2) of radiolabeled drug on the first day of the infusion. Serial plasma samples were obtained before, during, and after treatment, and cerebrospinal fluid was obtained from the Ommaya reservoir before drug infusion, 4.5 hr after initiation of the infusion, and 1 and 72 hr after the end of the infusion.

Patient 2 underwent surgical resection of a brain metastasis from malignant melanoma. He was given a tracer dose of tritiated vinblastine, 27 μg (200 μCi) intravenously during the surgery. Intracerebral tumor, edematous brain adjacent to tumor, temporalis muscle and plasma were assayed for vinblastine.

Patient 3 was started on a 24-hr continuous intravenous infusion of tritiated vinblastine for small cell undifferentiated carcinoma of the lung but died 4 hr into the infusion. He had received a total of approximately 35 μCi. Antemortem he had markedly abnormal hepatic function but normal renal function. At autopsy, tissues were collected for pharmacology studies.

Patient 4 had an epitheloid sarcoma. She had mildly abnormal hepatic function but normal renal function and received a 24-hr infusion of vinblastine (1.7 mg/m^2) that included 200 μCi of radiolabeled drug. The patient died four weeks later and tissue samples for vinblastine assay were collected at autopsy.

Drug

Generally tritiated vinblastine sulphate (8.2 Ci/mmole) was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. It was purified by a published procedure (3) until its radiochemical purity exceeded 95% as determined by high pressure liquid chromatography. Glass-distilled chromatographic solvents were purchased from Burdick Jackson Laboratories, Muskegon, MI. Other chemicals and reagents were from regular commercial sources.

Extraction of vinblastine from tissues

To extract vinblastine from tissues, a weighed specimen was blotted dry, minced with scissors, and mixed with 10 ml of normal saline. The mixture was placed in a Pyrex flask, externally cooled with ice, and homogenized with a Virtris model 23 high-speed homogenizer (Virtris Co., Inc., Gardiner, NY) equipped with stainless steel blades at 15 000–20 000 rpm for 5–10 min. The homogenate was centrifuged at 12 000 $\times$ g for 10 min and the supernatant was removed. The recovery was 75–80%. The supernatant was deproteinated with 10% sulfosalicylic acid and neutralized with KOH. All biological samples were filtered through a 0.45-μm Millipore filter.

Drug assay

Radioactive vinblastine was separated from its metabolites using a Waters Associates model 204 liquid chromatography system equipped with the following accessories from Varian Instruments: a Varichron variable wavelength detector, a CDS 111 integrator, and a model 9176 recorder. The column used was a Waters μBondapak C_{18} reverse phase column (30 cm $\times$ 4 mm internal diameter). The solvent system consistend of a linear gradient from solvent A (20% acetonitrile in 0.001M phosphate buffer pH 7.5) to solvent B (80% acetonitrile in 0.001M phosphate buffer, pH 7.5). The program time was 10 min and the flow rate was 2.5 ml/min. A UV detector set at 254 nm was used to monitor the effluent from the column. A large quantity of unlabeled vinblastine was added to all samples so that UV detection could be used to identify the time at which unchanged vinblastine was eluting from the column. When the vinblastine peak was detect-

ed, eluate was collected into a scintillation vial as a single fraction. Collection was terminated when the entire vinblastine peak had eluted. Radioactivity was determined in the collected fraction using a Packard Model 2650 Tri-Carb Liquid scintillation spectrometer equipped with an automatic self-calibration quenching device. Disintegrations per minute (dpm) were computed. Plasma and urine (0.2 mg) were counted in 11 ml of 'PCS,' a commercial phase-combining solution available from Amersham Corporation, Arlington Heights, IL. The recovery of radioactivity from tissues using this procedure was greater than 85%. To determine *total* radioactivity in tissues, tissues were combusted in a Packard Sample oxidizer model 306B and were counted in 11–15 ml of Monophase, a product of Packard, Downsgrove, IL.

Results

Concentrations of vinblastine in the cerbrospinal fluid of patient 1 are presented in Table 1. Although vinblastine concentration in the cerebrospinal fluid was only 16% of that in plasma by 1 hr after the end of the infusion, vinblastine appeared to be cleared far more slowly from cerebrospinal fluid than from plasma such that by 72 hr after the infusion the cerebrospinal fluid concentration of vinblastine was 50% of the plasma concentration.

In patient 2 (Table 2) it was possible to determine only the total radioactivity in the tissue sample obtained during the removal of the intracerebral tumor since samples were too small to permit extraction. Radioactivity in intracerebral tumor increased with time, and by 2 hr after drug administration more radioactivity was present in tumor than in plasma. At that time, radioactivity was greater in intracerebral tumor than in surrounding brain but less than in temporalis muscle.

Autopsy tissue distribution of vinblastine for patients 3 and 4 is illustrated in Figure 1. For patient 3, the highest concentrations were found in kidney, and heart; intermediate concentrations were found in lung and liver, and low concentrations were found in prostate, gastrointestinal tract, spleen, muscle, hepatic and lymph node metastases, and bladder. For patient 4, by far the highest vinblastine concentration was in liver followed by intracerebral tumor. Moderately high levels were also found in pancreas, kidney, renal metastasis, thyroid, lung, spleen, and ovary. There were lower concentrations in heart, omental metastases, breast, brain adjacent to tumor and cerebellum. Generally, concentration of vinblastine in brain decreased with increasing distance from the intracerebral tumor (Table 3).

In patient 4, unchanged vinblastine accounted for 70% to 89% of total radioactivity.

Table 1. Vinblastine concentrations in cerebrospinal fluid and plasma from a patient who received radiolabeled vinblastine 1.5 mg/m² by 24-hr continuous intravenous infusion.

Time from start of 24-hr infusion (Hr)	Vinblastine ng/ml		Cerebrospinal fluid vinblastine as % of plasma vinblastine
	Cerebrospinal fluid	Plasma	
5	0.1	2.3	4
25	0.8	5.0	16
96	0.6	1.2	50

Table 2. Radioactivity in surgically resected intracerebral tumor, edematous brain, temporalis muscle, and plasma from a patient who received radiolabeled vinblastine intravenously prior to surgery.

Time (Hr)	Radioactivity DPM/g			
	Tumor	Brain	Muscle	Plasma
1	1430			1982
1.5	1800			2025
2	3114	1245	4616	1382

Table 3. Concentration of vinblastine in brain of patient 4 as a function of distance into brain from necrotic tumor.

Distance from tumor (cm)	Vinblastine (ng/g)
Tumor	68
0– 0.5	22
0.5–1	9
1– 2	8
2– 4	6
> 4	5
Cerebellum	11

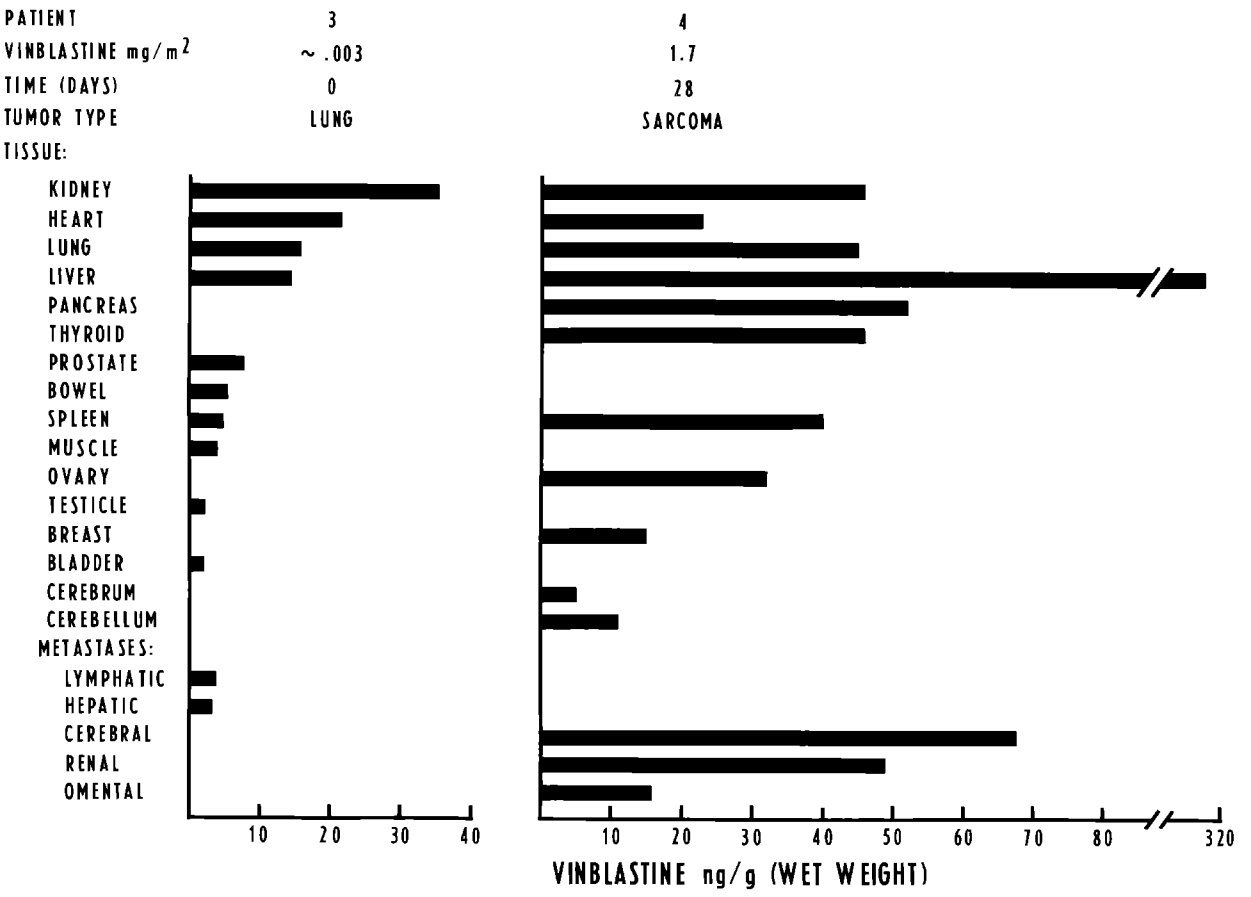

Fig. 1. Tissues were obtained at autopsy from two patients who had received radiolabeled vinblastine I.V. antemortem. Patient 3 died 4 hr into a planned 24-hr continuous I. V. infusion of vinblastine and patient 4 died four weeks after a 24 hr infusion of vinblastine. Concentration of vinblastine in various organs was measured using radiochemical techniques following separation of vinblastine from its metabolites by HPLC. Absence of a value beside an organ indicates that tissue from that organ was not assayed. All evaluated tissue had detectable concentrations of vinblastine.

Discussion

To our knowledge, the localization of vinblastine and other vinca alkaloids in human tissues has not been reported previously. Our results must be interpreted cautiously because of the small numbers of patients studied. Because of the limited amount of radiolabelled vinblastine available to us, additional patients could not be studied. Based on the known blood flow to various organs (4), it appears that the tissue concentration of vinblastine in patient 3 who died early during a 24-hr vinblastine infusion may have been flow-limited. In patient 4, who died one month following drug infusion the tissue concentrations no longer corresponded to flow rates, suggesting that redistribution of the drug may have occurred, particularly to the liver. It is felt that the liver is predominantly responsible for the metabolism of vinblastine to its major metabolite, deacetylvinblastine (2). It is not clear whether or not the markedly abnormal hepatic function in patient 3 affected the concentration of vinblastine found in his liver. It should not have affected the distribution to other organs although it could have altered the amount of metabolite present. In patient 4, 70–90% of radioactivity represented unchanged vinblastine.

Our ability to detect vinblastine in the tissues of a patient who had received the drug one month earlier suggests that at least in some patients vinblastine may be tightly bound to tissues. This is compatible with the previous observations of a large apparent

volume of distribution and a long terminal half-life for vinblastine (2) and is also compatible with the previous observation that only 30% of a dose of vinblastine is excreted in the first 6 days after drug administration (2).

In patient 3, vinblastine concentration was similar in viable tissue from metastases in two different sites, but was less than in the organ of origin (lung). The hepatic metastasis also had a lower concentration than did surrounding liver. In patient 4, more vinblastine was present in highly necrotic intracerebral tumor than in more viable renal metastasis, suggesting that membrane phenomena may have been responsible for concentration differences. Lower blood flow would be expected to the necrotic intracerebral lesion than to the renal metastasis. The concentration of vinblastine in the renal metastasis was slightly higher than the concentration in kidney and far higher than the concentration in omental metastases. Hence, vinblastine does not appear to be preferentially concentrated in or excluded from human tumors. The variability of drug concentration from one metastatic site to another in the same patient could help explain why mixed responses are seen in some patients. Nevertheless all tumor samples showed concentrations of vinblastine that have previously been shown to be potentially cytotoxic in vitro (2).

The fact that tissue specimens were collected at autopsy performed several hours after death, should not significantly alter the tissue distribution of radioactivity, since all blood-flow mediated redistribution would cease at the time of death. However, diffusion could continue as could post-mortem metabolism within the tissues and the events leading up to death could possibly have potentiated drug redistribution.

Despite the lipophilicity of vinblastine (5), little penetrated into brain distant from intracerebral tumor in patient 4. This may have been caused by vinblastine's relatively high molecular weight. As with many agents that do not readily cross the blood–brain barrier (6), the concentration of vinblastine in brain in patient 4 decreased with increasing distance from tumor and was less in adjacent brain than in intracerebral tumor in patient 2. It has been postulated that the low concentration of drugs attained in brain adjacent to intracerebral tumors may help explain the relative resistance of intracerebral tumors to chemotherapy (7), but this postulate remains unproven. The role of pharmacologic factors in the resistance of intracerebral tumors remains unclear. As in vitro sensitivity testing techniques improve it will be necessary to assess whether intracerebral and extracerebral metastases respond similarly in vitro. In any event, our limited data in patients 2 and 4 do not suggest that penetration of vinblastine into intracerebral tumor is limited by its relative inability to cross the intact blood-brain barrier. Similar observations have previously been noted for other antineoplastic agents (8, 9, 10, 11). Also similar to the experience with other drugs (8, 9) was the observation that cerebrospinal fluid drug concentration did not accurately predict drug concentration attainable in intracerebral tumors. The related drug, vincristine, also attains only low concentration in the cerebrospinal fluid (12), yet this drug is effective against intracerebral tumors (13). To our knowledge the ability of vincristine to penetrate into brain tumors has not been studied.

In summary, the human tissue distribution of vinblastine appeared to be flow-limited early during drug administration but not later. Drug persisted in tissue for a long time after administration and at least 70% of the radioactivity present represented unchanged vinblastine. Despite attaining only low concentration in cerebrospinal fluid, vinblastine penetreated readily into intracerebral tumor but decreased in concentration with increasing distance into brain from the tumor. Cerebrospinal fluid concentration of a drug should not be used as a basis for deciding whether or not to use a drug therapeutically against intracerebral tumors. Vinblastine concentration varied from one metastatic site to another in a single individual. In addition, drug concentration in tumor differed not only from that in the tissue of origin, but also from that in the organ in which the metastasis was located.

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