The Blood-Tissue Barrier of Human Brain Tumors: Correlation of Scintigraphic and Ultrastructural Findings: Concise Communication

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Through the first 2 hr, uptake of [Tc-99m]pertechnetate and of Co-57 bleomycin were assessed in 29 brain tumors and were correlated with the ultrastructure of the tumor's capillary endothelium. No difference in uptake was found between the two tracers. Permeability of brain tumors to these agents was found to be governed by the same ultrastructural features that determine permeability in experimental brain tumors: the type of junction between contiguous endothelial cells in the capillaries. Meningiomas, which showed very high uptake of the radiotracers, demonstrated open or punctate junctions with short fusion of apposed membranes. They also showed a large number of pinocytotic vesicles and fenestrae. Capillaries of tumors without uptake had a small number of short tight junctions (<0.25 μ) between adjacent endothelial cells and a relatively large number of long junctions (>0.5 μ). In intracerebral tumors that showed relatively high uptake, the reverse was true: most of the junctions were short and only a few long junctions were found. That uptake of [Tc-99m]pertechnetate and of Co-57 bleomycin depends on tumor capillary ultrastructure (which determines the permeability) suggests the possibility of the use of radiopharmaceuticals as in vivo indicators of tumor permeability. Brain scintigraphy may help to assess brain-tumor availability to non-lipid-soluble chemotherapeutic drugs.

J Nucl Med 25: 461–465, 1984

Recent research in the use of radiopharmaceuticals for the evaluation of diseases of the central nervous system has been focused on agents that cross the blood-brain barrier (BBB) and may indicate blood flow or metabolism. Recently little attention has been paid to abnormalities of the BBB and the utility of its evaluation by scintigraphy. This probably results from the preference for transmission computerized tomography (TCT) rather than scintigraphy for the diagnosis of brain tumors.

Scintigraphy, however, provides more information than the mere establishment of the presence or absence of a tumor. Tumors differ in the rate and quantity of uptake of radiopharmaceuticals used for imaging. It has been suggested that this differential uptake can be used to indicate the nature of the tumor and that it is tumor vessel permeability that governs uptake (*1–6*). In experimental brain-tumor models, permeability is determined by the ultrastructure of a brain tumor's capillaries, and it was suggested, but not proved, that the same factors govern permeability of human brain tumors (*7–14*).

The present study was undertaken to compare the uptake of Tc-99m as pertechnetate and that of Co-57 bleomycin (Co-bleo), a labeled chemotherapeutic drug, as indicated by scintigraphy, and to correlate them with the ultrastructure of the capillaries of the human brain tumors investigated.

MATERIALS AND METHODS

Twenty-nine patients (Table 1) underwent physical and radiological examination, including head TCT. Diagnosis was established in all by light microscopy of surgical specimens.

Scintigraphic studies. Scintigraphy with [Tc-99m]

Received Aug. 22, 1983; revision accepted Nov. 30, 1983.

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TABLE 1. SCINTIGRAPHIC AND ELECTRON-MICROSCOPE FINDINGS IN 29 BRAIN TUMORS

Group	Histology	No.	Uptake in scintigraphy: Radionuclide angiography*	Uptake in scintigraphy: Early static	Uptake in scintigraphy: 2-hr study	Type of tight junction
3	Meningioma	10	+++	+++	+++	Open junctions or punctate fusion sites
2	Glioblastoma	5	+ or ++	+ or ++	++	Mainly short†
	Metastasis	2				
	Craniopharyngioma	1				
1	Glioblastoma	3	0	0	+ or ++	Short, medium and long†
	Metastasis	3				
	Oligodendroglioma	2				
	Astrocytoma Gr·II	1				
0	Astrocytoma Gr·II	1	0	0	0	Mainly long†
	Glioblastoma	1				
	Normal brain	2	0	0	0	Long pentalaminar tight junctions

* Not done with Co-bleo.

† Short tight junction <0.25 μ. Medium tight junction 0.25–0.50 μ. Long tight junction >0.50 μ.

pertechnetate included radionuclide angiography, early static study, and static study at 2 hr.

Radionuclide angiography was performed after bolus injection of 20 mCi of pertechnetate in the projection in which the tumor was closest to the surface of the brain as shown by TCT. A gamma camera was used to collect 2-sec frames for 30 sec. An early four-view study (anterior, posterior, laterals), each containing 400,000 counts, was collected immediately after the dynamic study. The same technique was used for the 2-hr study.

Co-57 bleomycin (Co-bleo) was prepared from Co-57 chloride and bleomycin by a method previously described (*15*). One mCi of Co-bleo was injected i.v. at least 72 hr after the pertechnetate study. Scintigraphy was performed 30 sec and 2 hr after the injection. Counts were collected for 10 min in each view.

Scintigrams were reviewed by two experienced nuclear medicine physicians, independently for pertechnetate and Co-bleo, without prior knowledge of histology or electron-microscopic findings.

Electron-microscope studies. Samples of the brain tumors were obtained during operation. Two samples of normal brain were also obtained at operation for deeply situated tumors that necessitated removal of normal brain. Specimens were immediately immersed in cold 2.5% glutaraldehyde in phosphate buffer (0.1 *M*; pH 7.2) for 5 min. The tissue was cut into small blocks and left in fresh fixative for 2 hr at 4°C. It was then washed by the buffer and postfixed in 1% osmium tetroxide for an hour. Dehydration by ethanol followed and the tissue was then embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

Studies were carried out by an electron microscope operating at 80 keV. The type of junction between contiguous capillary endothelial cells was determined; the length of the fusion between the adjacent endothelial cells in the tight junction was measured, and the presence of pinocytotic vesicles and fenestrae determined.

RESULTS

Scintigraphic findings. No difference was found between the uptakes of pertechnetate and Co-bleo in the 2-hr period of the study. The criteria used for evaluation of tracer uptake with three-phase brain scintigraphy have been discussed in detail in the literature and will be mentioned only briefly here. The uptake of pertechnetate was assessed for each of the stages of the study, and that of Co-bleo for early and late static images. Highest uptake is designated +++, no uptake 0, low intermediate uptake +, and higher intermediate uptake ++. Evaluation of uptake was qualitative only (*5*).

Four groups of tumors could be distinguished using these criteria.

Group 3 tumors +++ uptake in radionuclide angiography, early static, and late static studies. Ten meningiomas met these criteria.

Group 2 was characterized by + or ++ uptake in radionuclide angiography and early static study, and ++ in the 2-hr study. Five glioblastomas, two metastases, and a craniopharyngioma belonged to this group.

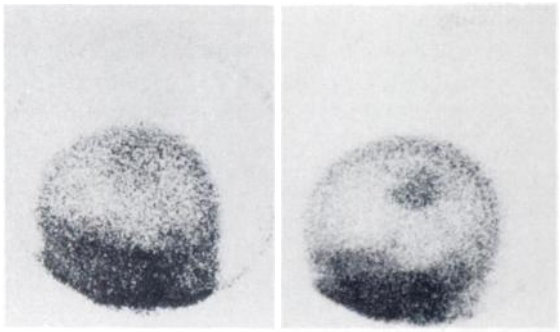

FIG. 1. Co-57 bleomycin scintigram at 2 hr. Glioblastoma in Group 1 (left) showing (+) uptake in parietal lobe. Glioblastoma in Group 2 (right) showing (++) uptake in parietal lobe.

Group 1: No uptake was detected in radionuclide angiography and in the early static study. The 2-hr study showed + or ++ uptake. Three glioblastomas, three metastases, two oligodendrogliomas, and one astrocytoma Grade II were included in this group.

Group 0: No abnormal uptake was found in these tumors. It included one low-grade astrocytoma and one glioblastoma.

Electron-microscope findings. There was a significant difference between the meningiomas (Group 3) and the rest of the tumors (Groups 0, 1, 2). The vessels of meningiomas were of the fenestrated type and showed an abundance of pinocytotic vesicles. The junctions between adjacent endothelial cells showed short apposition and the tight junctions were punctate, showing mostly one to three short fusion sites. Some junctions showed an open gap between cells.

The other tumors showed pentalaminar tight junctions with short and long fusion sites differing in number. Fusions longer than 0.5 μ were considered "long" and shorter than 0.25 μ "short". In Group 0 there were more long fusions than short. This ratio was even more evident in the capillaries of the normal brain, where only a few short fusions were detected.

The reverse was true for Group 2: most of the fusions were short, with only few long. In Group 1 an equal distribution of fusion sites was found.

Paucity of pinocytotic vesicles and fenestrae was observed in the capillaries of intracerebral tumors. The scintigraphic and electron-microscopic findings are summarized in Table 1, and illustrative tumors are shown in Figs. 1 and 2.

DISCUSSION

In organs other than the brain there are several routes of transport from the blood into the extracellular space of the tissue (*16*). Gaps between adjacent endothelial cells permit the transfer of substances with up to 20,000–40,000 molecular weight. Fenestrae provide another route for passage of macromolecules. These are regions of fusion 20 to 100 nm long between the outer and inner membrane of the endothelial cell. Transport can also occur by pinocytosis. Pinocytotic vesicles are invaginations of the endothelial cell membrane that break off and carry a small volume of plasma into the cytoplasm, then discharge it through the outer membrane of the endothelium into the extracellular space of the tissue.

The blood-brain barrier (BBB) is located in the capillary endothelium, which is structurally different from endothelium elsewhere in the body (*17–22*). The capillaries of the normal brain are composed of continuous endothelial cells without fenestrations and with only a few pinocytotic vesicles. They have extensive, long tight junctions located between the adjacent plasma membranes of neighboring cells. On electron microscopy these tight junctions are seen as pentalaminar areas of fusion of the outer leaflets of two adjacent plasma membranes. This close proximity of endothelial cells provides the capillaries of the brain with the characteristics of a plasma membrane. Molecules that are not protein bound and have a high degree of lipid solubility and low ionization at body pH enter freely into the extracellular space and rapidly achieve equilibrium. Lipophobic and protein-bound substances do not cross this membrane (*23,24*).

Radiopharmaceuticals such as pertechnetate and Co-bleo do not cross from the blood into the normal brain. They do pass into brain tumors, however, and this

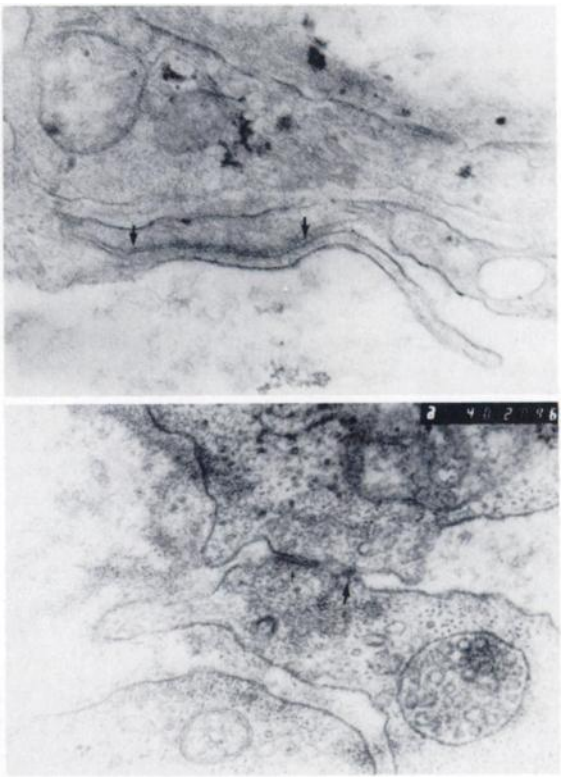

FIG. 2. Long tight junction (upper), 0.80 μ (arrows), ×82,000). Two short tight junctions (lower), 0.09 μ (wide arrow) and 0.19 μ (narrow arrow). (×82,000).

is the basis for the visualization of brain tumors by scintigraphy. This phenomenon, however, is more complex than an "all or none" phenomenon. There were various degrees of uptake of pertechnetate and Co-bleo in the tumors we investigated. Uptake was very high in the tumors of Group 3, and there was no uptake at all in Group 0. There is practically no barrier in the meningiomas, where high uptake was immediate, but a blood-tumor barrier (BTB) with increasing effectiveness has to be assumed in the tumors of Groups 2 and 1. In Group 0 it was as effective as the normal BBB.

No difference in uptake was found between pertechnetate and Co-bleo, which indicates that in the tumors investigated in the present study, the BTB has the same effectiveness, up to 2 hr, for both radionuclides.

Correlation of the permeability of the BTB as assessed by scintigraphy with the ultrastructural properties of tumor capillaries (Table 1) indicates that permeability of the BTB is affected by the same structures that affect the permeability of blood vessels in tumor models in experimental animals (*7,8,11-14*). On electron microscopy, meningiomas, which showed early and marked uptake of radiotracers, had only punctate tight junctions of apposed endothelial cells with short appositional areas, and in some cases appositional areas were entirely open. There was an abundance of pinocytotic vesicles and fenestrae in these tumors. In intracerebral tumors, pentalaminar tight junctions were observed, with few pinocytotic vesicles and no fenestrae. Uptake of radionuclides in these tumors is governed by the length of fusion of membranes of apposing endothelial cells. In Group 0 the majority of fusions are longer than 0.50 μ and there are relatively few fusions shorter than 0.25 μ. The reverse is true for Group 2. The present study demonstrates that the "leakiness" of vessels in brain tumors depends on the nature of the junctions between endothelial cells, and determines the uptake of pertechnetate used for imaging and of Co-bleo, a labeled chemotherapeutic drug.

The widespread use of new diagnostic imaging techniques such as TCT has not changed the prognosis of patients with malignant brain tumors. The effectiveness of surgery and radiotherapy are considered by some authors to have reached their peak, but chemotherapy has as yet undetermined potential for effective treatment of brain tumors. Nevertheless, chemotherapy of brain tumors has not had significant clinical success (*25-27*). For many years, brain-tumor chemotherapy was based on nitrosourea agents that penetrate the membrane of the endothelial cells of brain capillaries and thus cross the BBB. Most evaluations of chemotherapy of brain tumors used chloroethylcyclohexylnitrosourea (CCNU) or bis-chlorethylnitrosourea (BCNU), the idea being that only such lypophilic substances would be able to penetrate into the tumor tissue.

Studies of the ultrastructure of brain tumors in experimental animals showed that the vessels of the tumors were characteristically leaky, being permeable to agents such as horseradish peroxidase and lanthanum. It was then suggested that in "chemotherapy of brain tumors: the blood-brain barrier is not a factor" (*12*). Recent studies, however, suggest "the barrier to be more complex than an all-or-none phenomenon" (*28*). This view is further supported by the in vivo permeability evaluation in our study, indicating that brain tumors show great variability in their uptake of radiolabeled material. The study suggests that when chemotherapy is considered, attention should be directed to the BTB of the tumor. Treatment with water-soluble chemotherapeutic agents may be attempted in highly permeable tumors, whereas the less permeable tumors might be treated with lipid-soluble drugs.

The mode of introduction of the drug is important (*29,30*). It is suggested that tumors showing early and high uptake may benefit from flush treatment, possibly through an intracarotid injection, whereas in tumors with slow uptake, slow infusion of the drug may be preferable.

We must stress that penetration of the drug into the extracellular space of the tumor does not guarantee effectiveness in controlling the growth of the tumor cells. Yet when a drug is excluded from the tumor by the BTB, it has no chance of being effective.

CONCLUSION

Uptake of pertechnetate and Co-bleo depends on the ultrastructure of tumor capillaries. There exists blood-tumor barrier that limits the uptake of pharmaceuticals by brain tumor. The effectiveness of this barrier in human brain tumors is determined by the length of the tight junction and the existence of pinocytotic vesicles and fenestrae.

ACKNOWLEDGMENT

This study was supported by the "Bar Lamsdorf" grant of the Israel Cancer Association. Dr. D. Cook kindly reviewed the manuscript.

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