Capillary Physiology of Human Medulloblastoma

Impact on Chemotherapy

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BACKGROUND. Advances in the treatment of medulloblastoma have largely been attributed to the introduction of chemotherapy, although Phase III trials have shown advantages for chemotherapy only in subgroups. Because the efficacy of chemotherapy depends on tumor vascularization, the vascular physiology of human medulloblastomas was evaluated.

METHODS. Seven patients with histologically proven medulloblastomas underwent measurements of capillary permeability and vascular plasma volume using contrast-enhanced dynamic computer tomography. Regional blood flow was measured in 5 patients using xenon computed tomography (CT).

RESULTS. The capillary permeability-surface product for water-soluble compounds ranged from 1.7 ± 5.5 to 17.6 ± 12.3 μL/g/min with a mean of 10.5 ± 6.3 μL/g/min. The vascular plasma volume ranged from 0.02 ± 0.021 to 0.045 ± 0.049 mL/g with a mean of 0.03 ± 0.01 mL/g. The efflux rate ranged from 0.012 ± 0.007 to 0.065 ± 0.064 1/min with a mean of 0.039 ± 0.020 1/min. Regional tumoral blood flow showed a mean of 19.86 ± 6.8 mL/100g/min as compared with normal cerebellum with 45.4 ± 12.03 mL/100g/min ($P < .005$).

CONCLUSIONS. The current study demonstrated a low capillary permeability and blood flow in medulloblastomas that could explain the limited response rates of partially resected tumors even after aggressive high-dose chemotherapy, as recently reported. ***Cancer* 2006;107:2223–7.** © *2006 American Cancer Society.*

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Medulloblastomas or infratentorial primitive neuroectodermal tumors (PNETs) are the most common malignant brain tumors in childhood.[1,2] Although major advances have been made in the surgical and radiation treatment of these tumors, improved median survival has mainly been ascribed to chemotherapy,[3–6] which has been used over the last 10 years employing predominantly hydrophilic compounds such as cyclophosphamide, vincristine, cisplatin, carboplatin, and etoposide. Applying evidence-based medicine criteria, it is not entirely clear what role chemotherapy plays in the treatment of medulloblastoma, and most recent articles have shown that despite remarkable advances in the recurrence-free interval, a much higher percentage of patients develop recurrent disease, even after multimodality treatment, than previously perceived.[7,8] Also, the response rate of partially resected tumors in young children treated with primary chemotherapy are limited, with only 46% demonstrating a complete response.[9] In view of this clinical scenario, it is important to evaluate the vascular physiology of medulloblastomas as 1 of the crucial determinants of drug delivery both for hydrophilic and lipophilic compounds, because the degree by which these compounds enter the tumor depends on the capillary permeability of the tumor

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vessels and the area of exchange (hydrophilic drugs) or the tumoral blood flow (lipophilic drugs). We therefore prospectively studied untreated medulloblastomas as to their bidirectional capillary permeability, vascular plasma volume, and regional cerebral blood flow. To the best of our knowledge, this is the first quantitative study of permeability and blood flow in human medulloblastoma.

MATERIALS AND METHODS

Seven patients with histologically proven medulloblastomas were enrolled in the study. Patients had de novo tumors and were studied before surgical debulking and were taken off steroids for at least 4 days. The study was approved by the local ethics committee and informed consent was provided by all patients or parents. The median age of the patients was 9.4 ± 5.7 years. Five patients were male and 2 were female. All patients underwent measurements of vascular plasma volume (V_p) and bidirectional capillary permeability (i.e., the blood-to-tissue-transfer constant [K_1]) and the tissue-to-blood-efflux rate (k_2) using contrast-enhanced dynamic computer tomography (CT) (Siemens Somatom HiQ, Erlangen, Germany). Using iopamidol (MW 777, octanol/water partition coefficient 0.0038, 2 mL/kg bodyweight; iodine concentration, 300 mg/mL) as the contrast agent, the above parameters were determined at each image location (voxel) using a 2-compartment model as described by Warnke et al.[10] All scans were taken with a gantry tilt to exclude the optic nerves and chiasm within the measurement slice. Absorbed doses per slice and scan were approximately 15 to 25, milligrays (mGy), resulting in a local dose of approximately 0.375 to 0.625 grays (Gy).

Regions were selected from morphologic images and superimposed on the parameter images. Regions of interest (ROIs) for the entire tumor were chosen by visual outlining of the contrast-enhancing mass. The ROIs were then checked against the histologic findings from those areas to ensure that vital tumor was present in these regions. ROI analysis was performed separately by 2 investigators (P.C.W. and K.K.) with an interobserver variability of $< 3\%$.

Regional cerebral blood flow (rCBF) was measured using stable-xenon CT with a 6-minute wash-in protocol and a modified Kety-Schmidt equation in 5 patients.[11] In 2 patients the rCBF could not be measured because the patients moved during the examination, which was detected on the error function maps of the xenon images, resulting in erroneous values. A concentration of 30% xenon, 30% O_2, and 40% air was used in all patients. Again, a Siemens Somatom HiQ was used for all blood flow studies. Scanning parameters were 80 kiloelectron volts (keV) and 500 milli-ampere-second (mA-s) with a slice thickness of 10 mm. End-tidal O_2, CO_2, respiratory rate, and volume were continuously monitored. The end-tidal Xe-concentration was recorded using an Fe55 analyzer. Using commercial software (Siemens), parameter maps for blood flow were generated from the CT images and tumor and contralateral mirror regions of normal cerebellum were analyzed for rCBF. All examinations were performed under normocapnic conditions as documented by blood gas examinations to eliminate blood flow activation or suppression by the xenon inhalation. An area of identical size in the right cerebellar hemisphere comprising cerebellar cortex and white matter was used as the normal reference.

Despite the additional radiation exposure and the existence of alternative magnetic resonance imaging (MRI)-based approaches for measurement of capillary permeability[12–14] and blood flow,[15–17] the above methods were chosen as they are validated against the respective "gold standard" (i.e., quantitative autoradiography).

RESULTS

Capillary permeability values for the entire tumor were ranged widely from 1.7 ± 5.5 μL/g/min to a maximum of 17.6 ± 12.3 μL/g/min. Table 1 shows the distribution of values across the 7 patients. The mean K_1 value was 10.5 ± 6.3 μL/g/min and the mean vascular plasma volume was 0.03 mL/g ± 0.01 mL/g. The tissue-to-blood-efflux rate, k_2, varied from 0.012 to 0.065 1/min, with a mean of 0.045 ± 0.070 1/min. Figure 1A shows the native CT of an individual tumor and Figure 1B the corresponding color-coded representation of permeability values. As can be seen, the permeability values are heterogeneously distributed intratumorally and, as demonstrable from the histogram distribution (Fig. 1C), the majority of pixels demonstrate a rather low permeability. $rCBF_{tumor}$ ranged from 9.9 to 28.1 mL/100 g/min, with a mean value of 19.86 ± 6.8 mL/100 g/min. In comparison to normal cerebellum in the same patients, which demonstrated a mean $rCBF_{cerebellum}$ of 45.4 ± 12.03 mL/g/min, the tumor was significantly less perfused ($P < .005$).

No correlation was found between tumor size and any of the physiologic variables measured (rank correlation test, $P > .1$).

DISCUSSION

Capillary permeability and vascular plasma volume can be measured reliably in human brain tumors using CT and iodinated markers in posterior fossa tumors. A potential drawback of CT, especially in very young children, is the radiation exposure, which,

TABLE 1
Distribution of Capillary Permeability Values for the Current Study Patients*

Patient no.	K_1 [µl/g/min]	V_P [ml/g]	k_2 [1/min]	$rCBF_{tumor}$ [ml/100 g/min]	$rCBF_{cerebellum}$ [ml/100 g/min]	V_{tumor} [ml]
1	5	0.028	0.032	28.1	41.3	17.1
2	17.6	0.023	0.053	22.5	62.8	45.2
3	11.9	0.02	0.042	22	46.6	12.8
4	5.8	0.023	0.016	16.8	29.4	18.1
5	14.9	0.025	0.056	9.9	47	9.1
6	16.6	0.045	0.065			27.4
7	1.7	0.044	0.012			7.4
Mean	10.5 ± 6.3	0.03 ± 0.1	0.044 ± 0.017	19.9 ± 6.8	45 ± 12	

K_1 indicates the blood-to-tissue-transfer constant; V_p, vascular plasma volume; k_2, tissue-to-blood-efflux rate; *rCPF*, regional cerebral blood flow; V_{tumor}, tumor volume.
* Patients in the current study harbored medulloblastoma (values of physiologic parameters represent the average over the tumor region as displayed in a slice through the maximum dimension).

nonetheless, is very small and biologically rather negligible if the optic nerve and chiasm are avoided. In this particular setting, radiation exposure was rendered insignificant by the fact that all patients were to receive fractionated radiotherapy as part of their standard treatment, to which the CT measurements added only a maximum of 1%.

Our study of the quantification of vascular physiology in human medulloblastomas demonstrates a surprisingly low capillary permeability compared with another intracerebral tumor entity proven to be chemosensitive (i.e., primary central nervous system [CNS] lymphoma). Using the identical methodology and patients who were not receiving steroids, we found the mean capillary permeability of 29.47 ± 10.6 µL/g/min in patients with primary lymphomas of the CNS to be significantly higher than the mean value of 10.5 ± 6.3 µL/g/min in patients with medulloblastomas (Student t test, $P < .005$).[10] Although some areas of the tumors studied may have quite high capillary permeability, this pertains only to a small number of pixels studied, as illustrated in the histogram of permeability. Hence, these small "islands" of high permeability have no overall pharmacokinetic impact. Blood flow in lymphomas was 43.16 ± 10.5 mL/100 g/min, which again was significantly (Student t test, $P < .01$) higher than in medulloblastomas and was similar to that of normal gray matter.

Previous studies in experimental medulloblastomas have shown increased permeability of alpha-isoaminobutyric acid and reduced blood flow using iodoantipyrine and quantitative autoradiography.[18] However, this model was hampered by a mixture of medulloblastoma and rhabdomyosarcoma cells and most likely is not an accurate reflection of medulloblastoma physiology. For example, solid tumors showed a massively increased permeability of 80 µL/g/min, whereas perivascular tumors had a K_1 of 4 µL/g/min. This explains why this experimental tumor model was quite sensitive to chemotherapy with water-soluble compounds.[19,20] Furthermore, these experimental tumors were implanted into the supratentorial compartment, which again rendered them not adequately comparable to the human situation in the posterior fossa. Using iopamidol as a marker, we found human medulloblastomas to be significantly less permeable than experimental tumors and to have a reduced blood flow compared with the 36.8 ± 5.3 mL/100 g/min in these tumors. Because the permeability of the blood-brain barrier is a function of molecular weight and water/octanol partition coefficient over a wide range of parameter values (molecular weight < 1000, log octanol/water < 0.05), decreased permeability for iopamidol translates directly into decreased permeability for water-soluble drugs used for the treatment of brain tumors that fall into these categories of molecular weight and octanol/water partition coefficients.[21] This encompasses the majority of drugs used in the treatment of medulloblastoma. For example, vincristine (with a molecular weight of 824), etoposide (with a molecular weight of 588), methotrexate (with a molecular weight of 454), cyclophosphamide (with a molecular weight of 261), and carboplatin (with a molecular weight of 371), which are currently used in chemotherapy protocols, behave similarly to our marker and are transported via passive diffusion across the blood-brain/blood-tumor barrier.

It is important to bear in mind that our measurements reflect the preoperative situation and, therefore, unless significant tumor remnants remain might not be entirely applicable to the postoperative situation. But when examining all tumor stages M0-M3, only approximately 50% of tumors can be resected completely, resulting in remaining tumor in a considerable proportion of children.[9] In addition, the effects

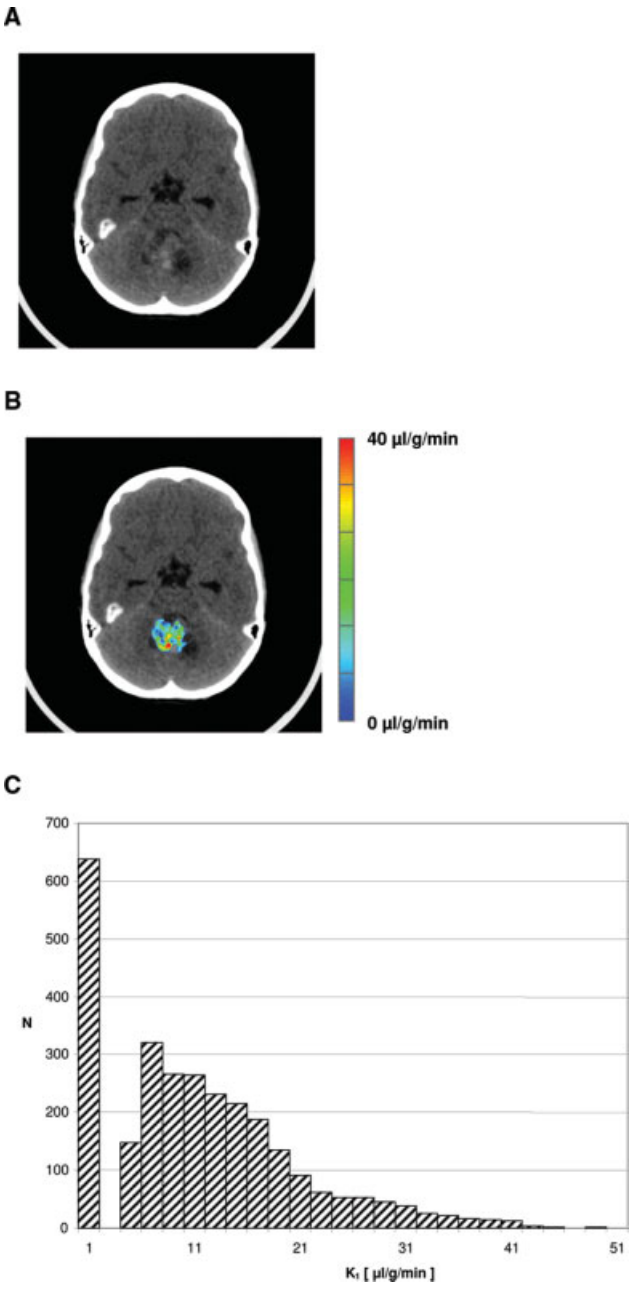

FIGURE 1. Native computed tomography (CT) of a medulloblastoma and (B) color-coded representation of the blood-to-tissue-transfer constant (K_1 values). (C) The wide distribution of K_1 values demonstrates the physiologic heterogeneity of medulloblastomas. The peak at $K_1 = 0-2$ µL/g/min most likely results from areas of intact blood-brain barrier ($K_1 = 0$ µL/g/min).

of radiotherapy, although most likely negligible with regard to permeability, are not accounted for. Nevertheless, the postoperative situation is most likely to be even less favorable to the pharmacokinetics of water-soluble drugs because infiltrative tumor remnants have an intact blood-brain barrier, limiting drug delivery even further—radiotherapy will lead to reduced blood flow and hyalinized vessels less favorable for molecular exchange. On the positive side, the mass effect exerted by the tumor will have disappeared by that time.

Therefore, the pharmacokinetic situation for the treatment of medulloblastomas by hydrophilic compounds is anything but ideal. For lipophilic compounds, delivery to the tumor is almost entirely dependent on blood flow, which is fairly reduced in medulloblastomas compared with normal surrounding cerebellum, again providing an adverse pharmacokinetic situation. Whereas clinical efficacy of chemotherapy in medulloblastomas has been shown in children treated with chemotherapy alone, the response rate is at best in the range of 60% and the pharmacokinetic basis for in vivo chemosensitivity of medulloblastoma is not well established. Our data actually indicate adverse physiologic conditions in human medulloblastoma to be responsible for the lack of chemotherapeutic efficacy in the unresponsive cases.

It will be most enlightening to perform a prospective study correlating individual physiologic parameters in medulloblastomas (preferably before and after surgery and radiotherapy) with a therapeutic response to chemotherapy. In this context it is feasible to measure drug levels (e.g., methotrexate) and derive an area under the curve that can be used as an input for measurements of the net extraction fraction and estimate tumoral drug levels and their dependency on individual tumor physiology.

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