Cancer Chemother Pharmacol (1992) 30: 251–260

Cancer Chemotherapy and Pharmacology



*Review*

# Do anticancer agents reach the tumor target in the human brain?*

**M. G. Donelli[1, 2], M. Zucchetti[1], and M. D'Incalci[1]**

[1] Istituto di Ricerche Farmacologiche Mario Negri, via Eritrea 62, Milano, Italy
[2] Dipartimento di Biologia e Genetica per le Scienze Mediche, Via Viotti 3/5, Milano, Italy

Received 20 April 1991/Accepted 20 March 1992

**Summary.** The development of effective chemotherapy for tumors of the central nervous system (CNS) is complicated in that the blood-brain barrier (BBB) hampers the penetration of most drugs into the brain and cerebrospinal fluid (CSF). This review summarizes the main reports on the distribution to CNS tumors and peritumoral normal brain of antitumor agents such as epipodophyllotoxins, *cis*-diamminedichloroplatinum(II), some nitrosoureas, bleomycin, vinblastine, and other clinically used antitumor agents as well as that of some experimental compounds with specific physicochemical properties. Drug levels were measured at surgical resection or in autopsy samples taken from patients who presented with different primary brain tumors or with brain metastases from extracerebral tumors. The observations made in each study were summarized in some detail, and the main points were then evaluated comparatively so as to highlight common aspects in the pharmacokinetic patterns of antitumor agents in human CNS tumors. Independently of their physicochemical properties, most antitumor agents appear to accumulate to a greater extent and to persist longer in intracerebral tumors than in the normal peritumoral brain. From in vitro cytotoxicity assays, it appears that epipodophyllotoxins, platinum compounds, bleomycin, and nitrosoureas reach potentially active therapeutic concentrations at the tumor target. However, all drugs have difficulty in reaching brain tissue adjacent to the tumor, as the intact BBB hampers their penetration. Plasma and CSF drug concentrations usually give little useful indication of the absolute quantity of drugs in brain tumors. To obtain a clear understanding of the CNS distribution of antitumor agents, one must determine whether the compound being measured is actually responsible for the observed activity and must consider the role of metabolites in the effect of the parent drug.

* This work was supported by the Italian Association for Cancer Research, by the Tettamenti Foundation, and by M.U.R.S.T. (60%) and was conducted under the auspices of the PAMM group of the EORTC

*Abbreviations:* AZQ, aziridinylbenzoquinone; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; BCNU, carmustine; BLEO, bleomycin; CDDP, cisplatin; DAU, 3-deazauridine; DBD, dibromodulcitol; DTIC, dimethyl triazene imidazole carboxamide; MGBG, methylglyoxal bis(guanylhydrazone); MITXR, mitoxantrone; PALA, *N*-phosphonacetyl-L-aspartate; PMM, pentamethylmelamine; TCNU, tauromustine; VLB, vinblastine; VM-26, teniposide; VP-16, etoposide

*Offprint requests to:* M. Grazia Donelli, Istituto di Ricerche Farmacologiche Mario Negri, via Eritrea 62, 20 157 MILANO, Italy

## Introduction

The clinical pharmacokinetics of anticancer agents in serum or plasma has been widely described, and relationships between pharmacokinetic profiles and therapeutic and/or toxic responses have been envisaged for several antitumor compounds [3, 8, 22, 23, 36]. Measurement of plasma drug concentrations has proved to be useful for the performance of safer and faster phase I clinical trials of new antineoplastic agents [14, 62]. However, the selective distribution of antineoplastic agents at the true target, the tumor site in patients, has been dealt with only rarely because of the virtual impossibility of collecting serial samples of patients' tissues for pharmacokinetics studies. Therefore, no evidence has yet been presented that the presence of a given drug at the tumor site is predictive of response in humans.

The development of effective chemotherapy for central nervous system (CNS) tumors is largely hindered by the blood-brain barrier (BBB), which impedes drug penetration into the brain. High concentrations in cerebral tumors can nevertheless be reached due to the frequent extensive disruption of the BBB [24, 30, 43], but tumor cells infiltrating nearby normal tissue are theoretically inaccessible to drugs that do not cross the BBB. Greig [29] extensively reviewed the factors governing drug delivery in the brain, including the time-dependent concentration profile of the free agent in plasma and the permeability of the BBB, which is a function of the drug's molecular weight, lipophilicity, degree of ionization, and protein and tissue binding as well as of local cerebral blood flow.

**Table 1.** Tumor and brain levels of the podophyllotoxins VP-16 and VM-26 in patients with brain tumors

| Reference | Drug and treatment | Patients | Analytic method | Time[a] | Tumor level (μg/g) | Brain level (μg/g) | Comments |
|---|---|---|---|---|---|---|---|
| Stewart et al. [71] | VP-16 100 mg/m² i. v. (1-h infusion) | 6 glioblastomas, 4 brain met. | HPLC after chloroform extraction; electrochemical detection | 2–7.5 h at surgery | <0.1–2.1 (pr.t.), 5.9 (necr. met.), 3.4 (viable met.) | 1.4 (distance, none), 0.1 (distance, 2 cm) | Higher uptake in brain met.; drug level higher in necr. than in viable tumor area, but always higher than levels in peritumor tissues (decrease with increasing distance from tumor); highest drug level in extra-CNS tumors |
| Stewart et al. [72] | VM-26 50–100 mg/m² i. v. (1- or 24-h infusion) | 7 gliomas (grades IV and II), 3 meningiomas, 7 brain met. | HPLC after chloroform extraction; electrochemical detection | 0.7–8 h at surgery | <0.2–11.9 (pr.t.), <0.1–2 (met.) | 0.15–1.37 | Prolonged infusion did not increase tumor penetration, but higher levels were found in tumor after oral glycerol treatment; higher drug uptake in tumor than in peritumor tissue |
| Zucchetti et al. [83] | VP-16 or VM-26 100–150 mg/m² i. v. (1-h infusion) | Glioblastomas (8 for VP-16 and 5 for VM-26), astrocytomas (4 for VP-16 and 6 for VM-26), 1 brain met. (VP-16) | HPLC after chloroform extraction; electrochemical detection | 1.5–13 h at surgery | <0.05–3.3 (VP-16), <0.05–1.68 (VM-26) | <0.05–1.19 (VP-16), <0.05–0.93 (VM-26) | For both drugs, even at later times, levels higher in tumor than in peritumor tissue; higher brain uptake of VP-16 (3.3 μg/g) in 1 patient who had received radiotherapy and BCNU |

[a] Time of excision from the end of treatment
pr.t., Primary tumor; met., metastases; necr., necrotic

Attempts have been made to overcome limited drug penetration or to manipulate the BBB, particularly in the control of meningeal leukemia. To increase drug delivery, investigators have used direct intrathecal injection [1, 4, 42, 48, 50], lipid-soluble carriers or drug-entrapping liposomes [64], high-dose systemic therapy [1, 2, 51], hyperosmolar mannitol [44, 54, 80], and previous radiotherapy [52]. These approaches have aroused heated debate and controversy [77] in view of the neurotoxicity of antineoplastic agents in the surrounding normal brain tissue [11, 34, 40]. The effect of such treatments on normal brain functions, particularly the mental status, and the quality of life [20, 49], has largely been neglected, even in patients achieving prolonged survival, but is becoming an important requirement for the design of treatment protocols for brain tumors [12]. On account of the problems that are peculiar to the cerebral area, investigations have tended to focus on the quantitative assessment of drug delivery to CNS tumors, whether used clinically in the treatment of brain tumors or undergoing investigation in the light of specific physicochemical properties.

This review summarizes representative data reported in the literature on the distribution to brain tumors of antineoplastic drugs, highlighting specific results that may have direct therapeutic implications. Drug levels were measured at surgical resection or in autopsy tumor samples taken from patients who presented with different primary brain tumors or with brain metastases of extracerebral tumors. Published studies were identified through a computer search for the period 1970–1990 using the Medline data base of the National Library of Medicine (Bethesda, Md.) and by inspecting the bibliographies of original and review articles on the pharmacology of antitumor agents in the human CNS.

## Epipodophyllotoxins

Table 1 summarizes two studies by Stewart et al. [71, 72] and one investigation by Zucchetti et al. [83] on the distribution in tumor and peritumoral brain tissue of VP-16 and VM-26, two podophyllotoxin compounds widely used in chemotherapy of human brain tumors [46, 47, 53]. Using highly specific HPLC analytical assays [21], these drugs were measured in surgical resection samples obtained from patients with glioblastomas, astrocytomas, meningiomas, or brain metastases of extra-CNS tumors who had received doses of 50–150 mg/m² as 1- or 24-h i. v. infusions.

Although only low concentrations of VP-16 or VM-26 have been attained in CSF [18, 74], at the doses used in these studies the compounds were readily detectable in human intracerebral tumors. Within 4 h of the beginning of the infusion, the highest drug levels reported were 3.4 μg/g for VP-16 (5.9 μg/g in a necrotic tumor sample) and 1.7 μg/g for VM-26, and in one patient with meningioma a level of 11.9 μg/g was detected. However, by 8–13 h after the beginning of the infusion, the concentrations had dropped to 1.1 μg/g for VP-16 and 0.6 μg/g for VM-26. Whereas levels of VP-16 seemed to be comparable in glioblastomas and in brain metastases, Stewart et al. [72]

**Table 2.** Tumor and brain levels of CDDP in patients with brain tumors

| Reference | Treatment | Patients | Analytic method | Time[a] | Tumor level (μg/g) | Brain level (μg/g) | Comments |
|---|---|---|---|---|---|---|---|
| Stewart et al. [68] | 20–25 mg/m² i.v. or 60–100 mg/m² intracarotid | 5 glioblastomas, 7 brain met. | X-ray fluorescence spectrometry | 0–2.4 h<br>2–6 days at surgery or autopsy | 0.43–1.29 (pr.t. and met.), 0.83–2.97 (pr.t. and met.) | 0.25–0.65, 0.7–1.11 | Drug level higher in tumor than in peritumor tissue, decrease with increasing distance from tumor (50%–70% decrease at 2–5 cm); detected at 6 months after therapy in one autopsied patient; barely detectable in CSF |
| Stewart et al. [73] | 75–265 mg/m² i.v. | 5 gliomas, 6 brain met. | Atomic absorption spectrometry | 2–24 h at autopsy | <0.25–0.6, <0.25–0.9 (met.) | 0.25–0.9 (distance, 0–2 cm) | Platinum level similar in tumor and peritumor tissue (decrease with increasing distance); drug uptake similar in gliomas, brain metastases, and extra-CNS tumors but not in hepatic tumors |
| Bonnem et al. [7] | 60 mg/m² i.v. (×2 days every 4 weeks) | Case report of 1 glioblastoma | Atomic absorption spectrometry | 7 weeks after the last course at surgery | 1.6, 0.09 (cyst) | Not reported | CDDP was apparently tightly bound, as it was measurable at 7 weeks after the last dose |

[a] Time of excision from the end of treatment
pr.t., Primary tumor; met., metastases; necr., necrotic

observed that VM-26 appeared to attain higher concentrations in cerebral metastases of extra-CNS tumors than in primary brain tumors. The highest levels of these compounds, however, were found in the necrotic area of brain tumors and in meningiomas.

Despite wide interpatient variability, these drugs do not seem to concentrate actively in brain tumors, as the concentrations detected in primary or secondary intracerebral tumors were generally lower than those found in extracerebral tumors and or in concurrent plasma samples. Prolonged infusion did not increase tumor penetration, but oral treatment with osmotic agents such as glycerol [72] or previous radiotherapy [83] seemed to result in somewhat higher brain uptake. Both VP-16 and VM-26 reached much lower levels in the peritumoral brain than in the tumor itself (never higher than 1.4 μg/g) for either agent), and the concentrations decreased with increasing distance from the tumor.

It is tempting to speculate that the levels of VP-16 and VM-26 attained in brain tumors or cerebral metastases of patients after i.v. infusion are high enough to produce a potential therapeutic effect, particularly in the case of VM-26, which is 5–10 times more potent than VP-16 in vitro [31, 56]. In most cases, these drugs reached the concentration required in vitro to halve the cloning efficiency of experimental neuroblastoma cells (2 μg/ml for 24 h) [31] or of human leukemic lymphoblasts (exposed for 18 h to 0.2–0.25 μg/ml for VP-16 and to 0.014–0.023 μg/ml for VM-26) [19]; these cells mostly occur as metastatic outgrowths in the cerebral area. Drug concentrations remain high for as long as 10 h after treatment, the elimination half-life in tumor and brain apparently being longer than that in plasma [83].

## *cis*-Diamminedichloroplatinum(II)

Another compound widely used in chemotherapy of brain tumors is *cis*-diamminedichloroplatinum(II) (CDDP), whose distribution into the tumor and adjacent brain of patients presenting with gliomas or brain metastases of extra-CNS tumors has been described by Stewart et al. [68, 73] and in a case report by Bonnem et al. [7] (Table 2). Brain and tumor samples were collected at surgery or at autopsy after i.v. or intracarotid treatment with either very low doses (20–25 mg/m²) or high doses of up to 265 mg/m². CDDP was measured by X-ray fluorescence or atomic absorption spectrometry from a few minutes after treatment until 6 days posttherapy (in one case, until 7 weeks after treatment). These analytical methods do not distinguish the parent compound from the drug-degradation products or metabolites.

In the three studies summarized in Table 2, platinum was barely measurable in CSF or in normal brain tissue either distant from the intracerebral tumor or in the absence of intracerebral tumors [68], but it was clearly detectable in primary and secondary brain tumors and in the adjacent brain at levels of around 1 μg/g, which more than doubled subsequently in some tumor-tissue samples (2.9 μg/g at 48 h). It is noteworthy that 2.2 μg/g compound remained detectable at 6 days after treatment and that platinum was measurable after several weeks or even months in samples obtained from two autopsied patients, indicating that the compound binds tightly to the brain-tissue proteins.

No consistent difference in drug uptake was observed between gliomas and brain metastases of extra-CNS tumors or between brain tumor and plasma or other tissue compartments, suggesting that the compound gains easy access to the brain once the BBB has been disrupted. The observation of higher drug levels in tumor tissue as compared with peritumoral brain tissue needs confirmation, as

**Table 3.** Tumor and brain levels of other clinically used anticancer agents in patients with brain tumors

| Reference | Drug and treatment | Patients | Analytic method | Time[a] | Tumor level | Brain level | Comments |
|---|---|---|---|---|---|---|---|
| Diksic et al. [16] | BCNU 10–15 mCi i. v. [$^{11}$C]-BCNU in 2 ml (140 mCi/mg) | 6 gliomas | Positron emission tomography | Tomography examination at between 0 and 35 min | 266 min ($t_{1/2\beta}$), (277, 17 min $t_{1/2\beta}$ in plasma) | 89 min ($t_{1/2\beta}$) | Drug clearance of total radioactivity from tumor slower than that from normal controlateral brain but similar to plasma clearance |
| Whittle et al. [78] | TCNU 130 mg/m$^2$ by nasogastric tube intraoperatively | 7 glioblastomas, 3 astrocytomas | HPLC after chloroform/ dichloroformethane | 10–12 min at surgery | <0.06–0.5 μg/g (viable t.), <0.01 μg/g (necr. t.) | <0.01–0.6 μg/g | Great variability in tissue and brain levels, but linear increase after administration for up to 80 min and close temporal and quantitative relation with plasma levels; no drug detected in necrotic tissue |
| Front et al. [26] | BLEO 2 mCi/2 mg i. v. [$^{57}$Co]-BLEO | 13 with glioma, meningioma, or brain met. | Single-photon emission tomography (SPECT) | SPECT examination at between 30 and 480 min | 5.1–20.5 (TCC in pr.t.), 10.3–16 (TCC in met.), 0.05–0.06 μg/g (pr.t.), 0.06 μg/g (met.) | Not reported | No correlation found between tumor and concurrent higher blood levels |
| Front et al. [25] | BLEO 2 mCi/2 mg i. v. [$^{57}$Co]-BLEO | 3 brain met. of lung carcinoma | SPECT | SPECT examination at between 10 min and 8 h | 10.3–16 (TCC), 0.04–0.07 μg/g | Not reported | Drug levels in brain metastases lower than those in primary lung tumor |
| Stewart et al. [70] | VLB 7.5 mg/m$^2$ i. v. (24-h infusion) [$^3$H] (G)-VLB | 2 brain met. | Liquid scintillation spectrometry after HPLC separation | 4 h, 4 weeks at autopsy | <1 ng/g, 68 ng/g, 22 ng/g (distance, none) 5 ng/g (distance, >4 cm) | <1 ng/g, 6 ng/g | Drug level higher in tumor tissue than in adjacent edematous brain; decrease with increasing distance from tumor; low CSF levels of VLB (0.1–0.6 ng/ml) did not reflect the level in the i. c. tumor |
| Green et al. [28] | MITXR 5–6 mg/m$^2$ i. v. | 1 glioblastoma, 4 astrocytomas, 5 brain met. | HPLC after chloroform extraction | 0.2–5.6 h, 25.8 h (1 patient at surgery) | 4 ng/g (viable t.), 31–102 ng/g (necr. t.), 25–29 ng/g (viable met.), 15–322 ng/g (necr. met.) | <0.01 ng/g in 1 patient at autopsy | In a patient autopsied at 192 days after treatment, no drug detectable in peritumor brain tissue, although 6 ng/g were detected in tumor; higher uptake in brain met., for both pr.t. and met., higher levels were found in necr. than in viable areas, higher than those in plasma |

[a] Time of excision from the end of treatment

pr.t., Primary tumor; met., metastases; necr., necrotic; t., tumor; TCC, tumor cumulative concentration (μg/cc × min)

this finding was reported in only one of the studies conducted by Stewart et al. However, the occurrence of a progressive decline in the drug concentration in the adjacent brain tissue with increasing distance from the tumor (70% decrease at 5 cm) was confirmed in both studies.

In view of their results, these authors suggest that CDDP may attain potentially therapeutic concentrations in human intracerebral tumors and adjacent edematous brain, as the levels found lie (at least) in the range of doses that result in cytotoxicity in glioblastoma cells in culture. CDDP doses that reduce the cloning efficiency of human glioblastoma cell lines on 72 h exposure range between 0.35 and 1.4 μg/ml according to Yung et al. [81] and are put at 0.8 μg/ml by Iwata et al. [33]. These values are perfectly superimposable on the concentrations achieved in the brain tumors of patients, assuming that the measurement of platinum is a good indicator of the exposure to the active species responsible for the efficacy of CDDP.

## Other clinically used anticancer agents

Data on the concentrations of other anticancer agents that have been attained in human brain tumors are sparse (Table 3). To our knowledge, the nitrosoureas, which are among the most effective drugs in the therapy of malignant tumors derived from the CNS [63], have been dealt with in only two papers, as their high chemical reactivity and the resultant difficulty in setting up specific analytical procedures has discouraged their investigation.

Diksic et al. [16] used the radiolabeled compound and positron emission tomography for detection to investigate

the delivery of carmustine (BCNU) to the tumor and normal controlateral brain in patients with gliomas. Whittle et al. [78] used a newly developed high-performance liquid chromatographic (HPLC) method for the specific measurement of a more recently developed nitrosourea compound, tauromustine (TCNU), in the tumor and peritumoral brain tissue of patients presenting with glioma or astrocytoma. Although the former technique did not quantitate the actual levels of BCNU in the brain tumors, the compound and/or its decomposition products were found to persist in the tumor ($t_{1/2\beta}$, 266 min) considerably longer than in normal brain tissue ($t_{1/2\beta}$, 89 min). The analogue TCNU reached a concentration of 0.5 μg/g in the viable part of the tumor, which was very similar to the 0.6 μg/g recorded in the brain adjacent to the tumor. Despite their wide variability, tumor and brain levels of both compounds correlated with concurrent plasma levels in the same patients, the $t_{1/2\beta}$ value for BCNU in plasma (277 min) being very similar to that in brain tumor. For TCNU, the median peak plasma level was 0.45 μg/ml at 45 min as compared with a median peak tumor level of 0.25 μg/g at 55 min. These observations suggest that plasma levels of these compounds (1–10 μg/ml for BCNU according to Levin et al. [38] and 0.1–3 μg/ml for TCNU [78]) may give some indication of the concentrations reached in the brain tumors.

The large amounts of compound and metabolites found in the brain compartment, tumor, and normal tissue together with their long persistence at the tumor site suggest that nitroso compounds may achieve chemotherapeutic concentrations in brain tumors. The results of two studies [33, 81] indicate that the doses of these compounds that are cytotoxic against human glioma cells in culture are in good agreement with these physiologically achievable concentrations. The concentrations that inhibited the colonies of human glioblastoma cells by 50% ($IC_{50}$), which represent the most reliable dose-dependent index of cell lethality [57], ranged between 4.5 and 7 μg/ml over an exposure period of 72 h [59], although less than 20% of brain tumor cells in monolayer culture responded to the same drug concentration during a 2-h incubation period.

Front et al. [25, 26] have addressed the distribution of bleomycin (BLEO) as measured by single-photon emission tomography in patients with primary or secondary brain tumors who had been treated with 2 mg [$^{57}$Co]-BLEO. The drug and its metabolites accumulated to the same extent (0.04–0.07 μg/g) in gliomas and meningiomas as in a series of metastases of extracerebral tumors. However, in primary tumors from which metastases derived, in other tissue compartments, and in plasma the compound reached concentrations higher than those detected in the brain tumor, and no correlation was found between tumor concentrations and concurrent blood levels. The doses of BLEO effective in inhibiting the growth of 50% of the cell population in a colony-forming assay of human glioma cells in vitro were reported to be around 0.9 mu/ml following 72 h exposure [33]; these findings are in good agreement with the concentrations found in vivo in the brain tumors of patients.

Stewart et al. [70] measured radiolabeled vinblastine (VLB) after i.v. injection in two patients with brain metastatic tumors. Concentrations in CSF were very low, but there was a gradual accumulation in the tumor (up to 68 ng/g). Radioactivity in the metastatic tumor had exceeded the concurrent plasma radioactivity by 2 h after drug administration and was higher than that in the adjacent edematous brain, in which the drug concentrations dropped with increasing distance from the tumor. On the basis of the current knowledge, we cannot even guess at the significance of the concentrations of VLB and/or its metabolites in the brain tumors in relation to its antineoplastic activity.

The human CNS pharmacology of mitoxantrone (MITXR), a drug that shows good activity against a series of human tumors, has been investigated by Green et al. [28] using an HPLC technique in ten patients with primary or secondary brain tumors. Drug levels were high (0.004–0.3 μg/g) in the tumor, particularly in necrotic areas, and brain metastases showed higher uptake than did the primary tumor. In spite of the accumulation and long persistence of MITXR in the tumor tissue (the drug remained detectable at autopsy on day 192 after treatment), its clinical activity was limited in adults with grade III–IV astrocytomas [65]. In agreement with the clinical observation, poor cytotoxicity against human neuroblastoma cells in vitro was reported by Von Hoff et al. [75]. No relationship between plasma pharmacokinetics and brain tumor levels was apparent in this study.

## Experimental drugs

The papers summarized in Table 4 describe the clinical CNS pharmacology of some experimental antitumor agents undergoing phase I or II trials, whose pharmacokinetic pattern in humans has not been clearly defined or which have shown high neurological toxicity in phase I clinical trials that may be ascribable to their pharmacology.

Using an HPLC technique, Stewart et al. [69] found that pentamethylmelamine (PMM) appeared to penetrate readily into human intracerebral tumors, particularly brain metastases, reaching concentrations considerably higher than those found in plasma, whereas it was barely detectable in the adjacent brain or in CSF. Demethylated metabolites attained higher concentrations in brain tumors and in CSF than did PMM itself. Overall, monomethylmelamine (MMM) was the metabolite found in highest concentrations in intracerebral tumor samples and in CSF, although it was undetectable in plasma; since it was the only metabolite found in peritumoral normal brain, it may account for the drug's neurological toxicity.

The distribution of *N*-phosphonacetyl-L-aspartate (PALA), another drug that is highly neurotoxic, into the CNS (CSF and intracerebral tumor) of patients with glioblastoma or brain metastases was investigated in an enzymatic assay by Stewart et al. [67]. The compound readily penetrated into cerebral tumors, reaching concentrations of up to 29 μg/g, which are similar to the levels attained in s.c. tumors. Lower levels were found in the normal edematous brain, which decreased with increasing distance from the tumor, and a low (12%–25%) CSF/plasma AUC ratio was observed within a few hours of treatment.

**Table 4.** Tumor and brain levels of experimental drugs in patients with brain tumors

| Reference | Drug and treatment | Patients | Analytic method | Time[a] | Tumor level | Brain level | Comments |
|---|---|---|---|---|---|---|---|
| Stewart et al. [69] | PMM 80–240 mg/m² i. v. (30-min infusion) | 1 glioblastoma, 4 astrocytomas, 4 brain met. | HPLC after extraction in perchloric acid | 0–6 h at surgery | <0.01–0.12 μg/g, 0.16–4.47 μg/g (met.) | <0.01 μg/g | In peritumor brain, PMM detectable only in 1 patient (<0.33 μg/g); the major metabolite MMM was detected in tumor, particularly in brain metastases and in peritumor brain, at higher levels than PMM; MMM was also detectable in CSF |
| Stewart et al. [67] | PALA 250–1000 mg/m² i. v. (30- to 60-min infusion) | 1 glioblastoma, 7 brain met. | Enzymatic determination of aspartate carbamoyl transferase | 0.9–3.8 h, 18 h at surgery | 1.9–29 μg/g (pr.t. or met.), 0.56–0.76 μg/g (pr.t. or met.) | 0.1–3.9 μg/g | Drug level in tumor tissue thigher than in edematous brain (decrease with increasing distance from the tumor); CSF levels (0.36–3.3 ng/ml) exceeded plasma levels only by 24 h after treatment |
| Savaraj et al. [61] | AZQ 2–4 mg/m² i. v. | 3 glioblastomas, 1 astrocytoma, 1 brain met. of melanoma | Liquid scintillation spectrometry after reverse-phase chromatography | 1–1.8 h at surgery | 9.7–106 ng/g (total AZQ), 4.4–36 ng/g (unchanged AZQ) | 30.94 ng/g (total AZQ) | AZQ, being lipid-soluble, readily penetrates brain tumors and is found in similar amounts in peritumor brain tissue adjacent to or distant from the tumor; in 1 patient at 10 days after treatment (12 mg/m²), 31 ng/g and 22 ng/g were found at autopsy in the tumor and brain, respectively |
| Csetényi et al. [15] | DBD 400 mg/m² × 1 p.o (6 patients) and 150–180 mg/m² × 3 (4 patients) | 10 glioblastomas (grade IV) | Liquid scintillation spectrometry of [³H]-DBD | 3.5–24 h at surgery | 3.9–13 μg/g | 5.3–12 μg/g (in white matter) | DBD easily penetrates the BBB and reaches similar levels in tumor tissue and brain |
| Rosenblum et al. [58] | MGBG (3-h infusion) | 2 glioblastomas, 4 brain met. | HPLC after extraction in perchloric acid | 19–130 min (from the start of infusion) at surgery | 6.2–27.5 μg/g, 0.32–11.4 μg/g (met.) | Not reported | MGBG readily penetrates brain tumor tissue and is found at consistently higher concentrations in the viable area as compared with the necrotic area; highest penetration in CSF reached only 22% of concurrent plasma levels |
| Stewart et al. [66] | DAU 2.5 g/m² i. v. (15- to 30-min infusion before surgery) | 1 astrocytoma, 3 brain met. | HPLC after extraction in perchloric acid | 0–2.5 h at surgery | 259 μg/g (pr.t.), 21.1–109.7 μg/g (met.) | 0–522 μg/g | DAU concentrates in brain intracerebral tumor and CSF to potentially therapeutic extents |

[a] Time of excision from the end of treatment
pr.t., Primary tumor; met., metastases; necr., necrotic; t., tumor

Aziridinylbenzoquinone (AZQ) is a lipophilic drug that is active against a series of experimental, intracerebrally growing tumors. In a phase I study of its CNS pharmacology [61], the radiolabeled compound readily penetrated into the tumor tissue and CSF of patients presenting with different cerebral neoplasms. Similar amounts were found in the peritumoral brain tissue (total AZQ, about 100 ng/g tissue), with only a slight decrease being observed with distance from the tumor. At 10 days after treatment, levels of about 30 ng/g were detected in the tumor and brain of one autopsied patient, indicating that the drug and/or its metabolites tended to persist in both types of tissue. This clear tropism to the CNS compartment makes this compound a potential candidate for brain tumor therapy, but its clinical efficacy appears to be limited [10, 82].

The CNS pharmacokinetics of tritium-labeled dibromodulcitol (DBD), an alkylating agent that is active against experimental ependymoblastomas [27] and is taken up to a large extent into the CNS of animals [32], was investigated by Csetényi et al. [15] in patients with glioblastoma. The radioactivity reached similar levels in tumor tissue and in white matter, but the concentrations in CSF were much lower; however, at 14 h after treatment, by which time the plasma concentrations had declined markedly, no drop in drug levels was noted in the tumor, peritumoral brain tissue or CSF.

A study by Rosenblum et al. [58] on the penetration of methylglyoxal bis(guanylhydrazone) (MGBG) into CSF and brain tumor tissue in humans as determined using an HPLC procedure indicated that the compound accumulated rapidly at potentially therapeutic levels in brain tumor tissue, reaching concentrations much higher (up to 27 μg/g) than those found in concurrent plasma samples. Viable tumor tissue contained higher concentrations of

MGBG than did necrotic areas of the same tumor. The poor penetration of the compound into CSF following its i.v. infusion (only 22% of concurrent plasma levels) may possibly be the pharmacological basis for its lack of activity against meningeal tumors.

The concentration of the uridine analogue 3-deazauridine (DAU) in CSF, surgically resected intracerebral tumors and autopsied brain specimens has been assayed by HPLC by Stewart et al. [66]. The DAU concentration delivered to intracerebral tumors and adjacent brain tissue was adequate for activity and did not differ significantly from the drug levels attained in control tissue. CSF concentrations in two patients were 22% and 59% of concurrent plasma levels, respectively.

Temozolomide has recently been reported to be very active in patients with glioblastoma [45]. This compound spontaneously decomposes to the monomethylderivative of dimethyl triazene imidazole carboxamide (DTIC), which is probably responsible for the cytotoxicity and antitumor activity of the parent drug. The preliminary results of a pharmacokinetics study (in progress in our laboratory) in patients with glioma, who were injected with an intracarotid dose of 100 mg/m$^2$, suggest a very short half-life and, therefore, high production of the active metabolite. Whereas the plasma concentration of temozolomide was 0.7 μg/ml at 3 h after injection, levels of 0.49 μg/g were found in the brain tumor, indicating a sustained distribution of the drug in brain tissue.

## Conclusions

This review summarizes the information currently available on the distribution of antineoplastic agents in brain neoplasms and in the brain tissue adjacent to the tumor. As the data are very sparse, more studies are needed to clarify the differences between various types of tumor and to investigate correlations between the drug levels reached in tumoral or normal brain tissue and those attained in CSF or plasma.

Although any extrapolation of in vitro cytotoxicity data to in vivo sensitivity must be made with great caution and the usefulness of in vitro assays for the prediction of clinical responsiveness continues to be questioned [5, 35, 60], it appears that for drugs that are widely used in brain tumor therapy, such as epipodophyllotoxin derivatives, platinum compounds, BLEO, and nitrosoureas, the minimal prerequisite for activity (i.e., adequate delivery of cytotoxic drug concentrations to the target) has been satisfied. At the doses given in clinical practice, the drug concentrations achieved in human brain tumors show a wide range of interindividual variability but, despite the differences in physicochemical properties, appear to be close to or even higher than those that are effective in vitro against human glioma cells or leukemic lymphoblasts (the main representatives of CNS metastases from extracerebral tumors). Moreover, some antitumor agents appear to persist in brain tumors, which accounts for their activity (BCNU, VP-16, DBD), and their levels decrease more slowly in cerebral neoplasms than in plasma. The difficulty of obtaining serial samples from the same patient naturally explains the limited information provided by the literature on the pharmacokinetic profile of antitumor agents.

In spite of the potentially therapeutic drug concentrations achieved in cerebral tumors, most human tumors are surgically removed, and relapses often subsequently arise from residual tumor cells that have escaped surgery or infiltrated the surrounding, apparently normal brain tissue [37, 79]. Although the results of the present studies are limited, they do suggest that drug concentrations in the peritumoral area or in normal brain tissue are mostly lower than those in the tumor, where the BBB is totally or partially inefficient; the concentrations in the brain tissue usually drop with increasing distance from the tumor. Drug concentrations in the brain adjacent to the tumor could be too low to eradicate infiltrating tumor cells and, together with other factors such as mechanisms of resistance, could be implicated in therapeutic failure.

In investigations of the distribution of antitumor agents within different areas of the same tumor, drug levels have been measured in necrotic and vegetating tumor tissue. Except for PALA [67], drugs seem to accumulate preferentially in necrotic areas, where the absence of blood vessels may hamper their release, as opposed to the tumor periphery, from which considerable amounts of drug rapidly diffuse into the drug-free surrounding brain [29, 64, 76]. The amount of drug that reaches the tumor may depend on the extent of BBB disruption, which always varies considerably.

Depending on the degree of BBB alteration induced by the tumor, it is often difficult to predict the concentration of an antitumor agent in CSF and in brain tumors on the basis of its plasma pharmacokinetics [39]. However, despite the limited data that have been obtained in a small number of patients and the insufficient time points that have been used, the results of the present studies suggest that for water-soluble agents, drug levels are generally higher in plasma than in normal brain tissue or brain tumors except at later times points and that the concentrations in brain tumors do not appear to correlate with concurrent levels in the patient's systemic circulation. Only for lipophilic compounds such as nitrosoureas, in spite of the wide variability, is there a close temporal and quantitative relationship between brain and plasma levels, with clearance being similar from both compartments.

Many antineoplastic agents that are clearly detectable in brain tumor tissue reach only low levels, if any, in CSF. The lipophilic agents DAU, PALA, and AZQ are exceptions, since they attain worthwhile concentrations in CSF. As has been found for other classes of compounds acting on the CNS [6], CSF drug levels reflect only the concentrations at the edge of the brain parenchyma and are not necessarily the same as those in the brain tissue itself; therefore, it may be inappropriate to extrapolate CSF data to the brain tissue or to brain tumors. As steady-state levels are rarely achieved and the timing of tissue sampling varies considerably, the nature of the relationships between the drug concentrations in CSF or plasma and the drug-concentration profile in brain tissue remains complex [13].

In regard to the question as to whether the compound being measured is the one that is responsible for activity at the cerebral site, very little attention has been paid to the

presence and role of metabolites of antitumor agents in the CNS. One example is PMM and its *N*-demethylated metabolites, the latter of which reach only low concentrations in blood [69] and in CSF [17] but accumulate at considerably higher levels than does PMM in cerebral tumors, which to some extent accounts for their contribution to the neurotoxicity of the parent compound. Moreover, a recent report [55] shows that idarubicinol, an active metabolite of idarubicin, is always found in the CSF of leukemic children, whereas the parent drug is mostly undetectable; again, this indicates that the metabolite may play a role in the activity and toxicity of this compound in the brain.

Current analytical techniques are still inadequate to measure most antitumor agents or their metabolites with sufficient specificity and sensitivity. In some cases, drug assays cannot identify the active species among the different molecular species, as is the case for radioactive techniques, flameless atomic absorption spectrometry, and single-photon emission tomography. It remains to be seen whether the variability observed may have been attributable to differences in the amount of drug in the cytoplasm or in the amount bound to DNA or other macromolecules, as they may not be the same as those at the receptor site, and these differences in the subcellular distribution of drugs may vary considerably in relation to the antitumor efficacy.

In conclusion, the many difficulties involved in obtaining reliable and significant information on the distribution of anticancer agents in normal and neoplastic brain tissue (measurements of the inappropriate drug moiety or at the wrong cellular/tissue sites, inappropriate sampling times, inadequate assessments of drug response, or evaluations of insufficient numbers of patients) should not discourage further research. Most currently available drugs do not possess the physicochemical properties required for significant penetration through the BBB, and this point should be given high priority in the design of new compounds or analogues of known antineoplastic agents that exhibit better distribution in the brain and lower, if any, neurotoxicity. In view of increasing efforts to limit the neurotoxic effects of antitumor agents, studies on the distribution of these drugs in the brain and on the relationships between brain drug or metabolite levels and the resultant toxicity may be helpful in attempts to reduce toxicity through the use of appropriate dosage schedules or concomitant protective agents.

Practical and ethical considerations make it difficult to obtain extensive information on drug distribution by measurements in surgical biopsies. It will therefore be essential to develop noninvasive methods for the measurement of drug concentrations in normal and neoplastic tissues of patients who are candidates for treatment. In the near future, noninvasive techniques based on technologies such as nuclear magnetic resonance [41] or positron emission tomography [9] should enable the physician to follow the time course of drug distribution in human tissues. This will provide a fuller background picture for investigations of the concentrations of antineoplastic agents in normal brain tissues and in tumors.

*Acknowledgement.* The authors would like to thank Ms. V. Pistotti of the Gustavus A. Pfeiffer Memorial Library for her valuable assistance in the collection of bibliographic material through a Medline search.

## References

1. Balis FM, Poplack DG (1989) Central nervous system pharmacology of antileukemic drugs. Am J Pediatr Hematol Oncol 11: 74
2. Balis FM, Savitch JL, Bleyer WA, Reaman GH, Poplack DG (1985) Remission induction of meningeal leukemia with high-dose intravenous methotrexate. J Clin Oncol 3: 485
3. Blasberg RG, Groothuis DR (1986) Chemotherapy of brain tumors: physiological and pharmacokinetic considerations. Semin Oncol 13: 70
4. Bleyer XWA, Poplack DG (1979) Intraventricular versus intralumbar methotrexate for central-nervous-system leukemia: prolonged remission with the Ommaya reservoir. Med Pediatr Oncol 6: 207
5. Bogdahn U (1983) Chemosensitivity of malignant human brain tumors. Preliminary results. J Neurooncol 1: 149
6. Bonati M, Kanto J, Tognoni G (1982) Clinical pharmacokinetics of cerebrospinal fluid. Clin Pharmacokinet 7: 312
7. Bonnem EM, Litterst CL, Smith FP (1982) Platinum concentrations in human glioblastoma multiforme following the use of cisplatin. Cancer Treat Rep 66: 1661
8. Borsi JD, Moe PJ (1987) Systemic clearance of methotrexate in the prognosis of acute lymphoblastic leukemia in children. Cancer 60: 3020
9. Brooks DJ, Beaney RP, Thomas DGT (1986) The role of positron emission tomography in the study of cerebral tumors. Semin Oncol 13: 83
10. Chamberlain MC, Prados MD, Silver P, Levin VA (1988) A phase I/II study of 24 hour intravenous AZQ in recurrent primary brain tumors. J Neurooncol 6: 319
11. Chiras J, Dormont D, Fauchon F, Debussche C, Bories J (1988) Intra-arterial chemotherapy of malignant gliomas. J Neuroradiol 15: 31
12. Choucair AK (1990) Proposals for evaluation of toxic effects associated with treatment of gliomas: a call for action. J Natl Cancer Inst 82: 531
13. Collins JM, Dedrick RL (1983) Distributed model for drug delivery to CSF and brain tissue. Am J Physiol 245: R303
14. Collins JM, Grieshaber CK, Chabner BA (1990) Pharmacologically guided phase I clinical trials upon preclinical drug development. J Natl Cancer Inst 82: 1321
15. Csetényi J, Afra D, Kerpel-Fronius S, Horvath IP, Institoris L, Eckhardt S (1983) The distribution of [$^3$H]-dibromodulcitol in the central nervous system of patients with brain tumour. Eur J Cancer Clin Oncol 19: 1389
16. Diksic M, Sako K, Feindel W, Kato A, Yamaoto YL, Farrokhzad S, Thompson C (1984) Pharmacokinetics of positron-labeled 1,3-bis(2-cholorethyl)nitrosourea in human brain tumors using positron emission tomography. Cancer Res 44: 3120
17. D'Incalci M, Sessa C, Beggiolin G, Mangioni C (1981) Cerebrospinal fluid levels of hexamethylmelamine and *N*-demethylated metabolites. Cancer Treat Rep 65: 350
18. D'Incalci M, Farina P, Sessa C, Mangioni C, Conter V, Masera G, Rocchetti M, Brambilla Pisoni M, Piazza E, Beer M, Cavalli F (1982) Pharmacokinetics of VP16-213 given by different administration methods. Cancer Chemother Pharmacol 7: 141
19. Dow LW, Sinkule JA, Look AT, Horvath A, Evans WE (1983) Comparative cytotoxic and cytokinetic effects of the epipodophyllotoxins 4′-demethylepipodophylotoxin-9-(4,6-*O*-2-ethylidene-β-D-glucopyranoside) and 4′-demethylepipodophyllotoxin-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) and their metabolites on human leukemic lymphoblasts. Cancer Res 43: 5699
20. Duffner PK, Cohen ME, Parker MS (1988) Prospective intellectual testing in children with brain tumors. Ann Neurol 23: 575

21. Evans WE, Sinkule JA, Crom WR, Dow L, Look AT, Rivera G (1982) Pharmacokinetics of teniposide (VM26) and etoposide (VP16-213) in children with cancer. Cancer Chemother Pharmacol 7: 147
22. Evans WE, Crom WR, Stewart CF, Bowman WP, Chen C-H, Abromowitch M, Simone JV (1984) Methotrexate systemic clearance influences probability of relapse in children with standard-risk acute lymphocytic leukaemia. Lancet I: 359
23. Evans WE, Crom WR, Abromowitch M, Dodge R, Look AT, Bowman WP, George SL, Pui C-H (1986) Clinical pharmacodynamics of high-dose methotrexate in acute lymphocytic leukemia. Identification of a relation between concentration and effect. N Engl J Med 314: 471
24. Front D, Israel O, Kohn S, Nir I (1984) The blood-tissue barrier of human brain tumors: correlation of scintigraphic and ultrastructural findings: concise communication. J Nucl Med 25: 461
25. Front D, Even-Sapir E, Iosilevsky G, Israel O, Frenkel A, Kolodny GM, Feinsud M (1987) Monitoring of [$^{57}$Co]-bleomycin delivery to brain metastases and their tumors of origin. J Neurosurg 67: 506
26. Front D, Israel O, Iosilevsky G, Even-Sapir E, Frenkel A, Kolodny GM, Feinsod M (1988) SPECT quantitation of cobalt-57 bleomycin delivery to human brain tumors. J Nucl Med 29: 187
27. Geran RI, Congleton GF, Dudeck LE, Abbott BJ, Gargus JL (1974) A mouse ependymoblastoma as an experimental model for screening potential antineoplastic drugs. Cancer Chemother Rep 4: 53
28. Green RM, Stewart DJ, Hogenholtz H, Richard MT, Thibault M, Montpetit V (1988) Human central nervous system and plasma pharmacology of mitoxantrone. J Neurooncol 6: 75
29. Greig NH (1987) Optimizing drug delivery to brain tumors. Cancer Treat Rev 14: 1
30. Greig NH, Sweeny D (1987) Brain tumors and the blood brain barrier. In: Neuwelt E (ed) The clinical impact of the blood-brain barrier and its manipulation. Plenum, New York, p 93
31. Hill BT, Whelan RDH, Rupniak HT, Dennis LY, Rosholt MA (1981) A comparative assessment of the in vitro effects of drugs on cells by means of colony assays or flow microfluorimetry. Cancer Chemother Pharmacol 7: 21
32. Instittoris L, Dzurillay E (1973) Comparative studies on the in vivo distribution pattern of dibromodulcitol and diepoxydulcitol. Z Krebsforsch 79: 49
33. Iwata K, Yung WKA, Fields WS (1988) In vitro effects of ACNU, BCNU, CDDP, and bleomycin on human glioma cells. Neurol Med Chir (Tokyo) 28: 965
34. Kaplan RS, Wiernik PH (1982) Neurotoxicity of antineoplastic drugs. Semin Oncol 9: 103
35. Kimmel DW, Shapiro JR, Shapiro WR (1987) In vitro drug sensitivity testing in human gliomas. J Neurosurg 66: 161
36. Lennard L, Keen D, Lylleyman JS (1986) Oral 6-mercaptopurine in childhood leukemia: parent drug pharmacokinetics and active metabolite concentrations. Clin Pharmacol Ther 40: 287
37. Levin VA (1975) A pharmacologic basis for brain tumor chemotherapy. Semin Oncol 2: 57
38. Levin VA, Hoffman W, Weinkam RJ (1978) Pharmacokinetics of BCNU in man: a preliminary study of 20 patients. Cancer Treat Rep 62: 1305
39. Lippens RJ, Winograd B (1988) Methotrexate concentration levels in the cerebrospinal fluid during high-dose methotrexate infusions: an unreliable prediction. Pediatr Hematol Oncol 5: 115
40. Mahaley MS, Whaley RA, Blue M, Bertsch L (1986) Central neurotoxicity following intracarotid BCNU chemotherapy for malignant gliomas. J Neurooncol 3: 297
41. McSheehy PMJ, Griffiths JR (1989) $^{19}$F MRS studies of fluoropyrimidine chemotherapy. A review. Nucl Magn Reson Biomed 2: 133
42. Miller KT, Wilkinson DS (1989) Pharmacokinetics of methotrexate in the cerebrospinal fluid after intracerebroventricular administration in patients with meningeal carcinomatosis and altered cerebrospinal fluid flow dynamics. Ther Drug Monit 11: 231
43. Neuwelt EA (1985) Therapeutic potential for blood-brain barrier modification in malignant brain tumor. Prog Exp Tumor Res 28: 51
44. Neuwelt E, Hill S (1986) Chemotherapy administered in conjuction with osmotic blood-brain barrier modification in patients with brain metastases. J Neurooncol 4: 195
45. Newlands ES, Blackledge GRP, Slack JA, Rustin GJS, Stuart NSA, Quarterman CP, Hoffman R, Stevens MFG, Brampton MH, Gibson AC (1992) Phase I trial of temozolomide. Abstract 50, Meeting of the EORTC Pharmacology and Molecular Mechanism Group, Bologna, January 9–11
46. O'Dwyer PJ, Alonso MT, Leyland-Jones B, Marsoni S (1984) Teniposide. A review of 12 years of experience. Cancer Treat Rep 68: 1455
47. O'Dwyer PJ, Leyland-Jones B, Alonso MT, Marsoni S, Wittes RE (1985) Drug therapy. Etoposide (VP-16-213): current status of an active anticancer drug. N Engl J Med 312: 692
48. Ommaya AK (1984) Implantable devices for chronic access and drug delivery to the central nervous system. Cancer Drug Deliv 1: 16
49. Packer RJ, Sutton LN, Atkins TE, Radcliffe J, Bunin GR, D'Angio G, Siegel KR, Schut L (1989) A prospective study of cognitive function in children receiving whole-brain radiotherapy and chemotherapy: 2-year results. J Neurosurg 70: 707
50. Poplack DG, Riccardi R (1987) Pharmacologic approaches to the treatment of central nervous system malignancy. In: Poplack DG, Massimo L, Cornaglia-Ferraris P (eds) The role of pharmacology in pediatric oncology. Martinus Nijhoff, Boston, p 137
51. Poplack DG, Reaman GH, Bleyer WA, Miser J, Feusner J, Wesley R, Hammond D (1984) Central nervous system (CNS) preventive therapy with high-dose methotrexate (HDMTX) in acute lymphoblastic leukemia (ALL): a preliminary report. Proc Am Soc Clin Oncol 3: 204
52. Qin DX, Zheng R, Tang J, Li JX, Hu YH (1990) Influence of radiation on the blood-brain barrier and optimum time of chemotherapy. Int J Radiat Oncol Biol Phys 19: 1507
53. Radice PA, Bunn PA Jr, Ihde DC (1979) Therapeutic trials with VP-16-213 and VM-26: active agents in small cell lung cancer, non-Hodgkin's lymphomas, and other malignancies. Cancer Treat Rep 63: 1231
54. Rapoport SI, Fredericks WR, Ohno K, Pettigrew KD (1980) Quantitative aspects of reversible osmotic opening of the blood-brain barrier. Am J Physiol 238: R 421
55. Reid JM, Pendergrass TW, Krailo MD, Hammond GD, Ames MM (1990) Plasma pharmacokinetics and cerebrospinal fluid concentrations of idarubicin and idarubicinol in pediatric leukemia patients: a Childrens Cancer Study Group report. Cancer Res 50: 6525
56. Roed H, Vindelov LL, Christensen IJ, Spang-Thomsen M, Hansen HH (1987) The effect of the two epipodophyllotoxin derivatives etoposide (VP-16) and teniposide (VM-26) on cell lines established from patients with small cell carcinoma of the lung. Cancer Chemother Pharmacol 19: 16
57. Roper PR, Drewinko B (1976) Comparison of in vitro methods to determine drug-induced cell lethality. Cancer Res 36: 2182
58. Rosenblum MG, Stewart DJ, Yap BS, Leavens M, Benjamin RS, Loo TL (1981) Penetration of methylglyoxal bis(guanylhydrazone) into intracerebral tumors in humans. Cancer Res 41: 459
59. Rosenblum ML, Wheeler KT, Wilson CB, Barker M, Knebel KD (1975) In vitro evaluation of in vivo brain tumor chemotherapy with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 35: 1387
60. Rosenblum ML, Dougherty DA, Deen DF, Hoshino T, Wilson CB (1980) Analysis of clonogenic human brain tumour cells: preliminary results of tumour sensitivity testing with BCNU. Br J Cancer 41 [Suppl IV]: 181
61. Savaraj N, Lu K, Feun LG, Leavens ME, Stewart D, Burgess MA, Benjamin RS, Loo TL (1983) Intracerebral penetration and tissue distribution of 2,5-diaziridinyl-3,6-bis(carboethoxyamino)1,4-benzoquinone (AZQ, NSC-182986). J Neurooncol 1: 15
62. Sessa C, Zucchetti M, Davoli E, Califano R, Cavalli F, Frustaci S, Gumbrell L, Sulkes A, Winograd B, D'Incalci M (1991) Phase I and clinical pharmacological evaluation of aphidicolin glycinate. J Natl Cancer Inst 81: 1160
63. Shapiro WR (1986) Therapy of adult malignant brain tumors: what have the clinical trials taught us? Semin Oncol 13: 38

64. Siegers HP (1990) Chemotherapy for brain metastases: recent developments and clinical considerations. Cancer Treat Rev 17: 63
65. Stewart DJ (1986) Phase II study of novantrone (mitoxantrone hydrochloride) in adults with grade III–IV astrocytomas. In: Walker MD, Thomas DGT (eds) Biology of brain tumor. Martinus Nijhoff, Boston, p 411
66. Stewart DJ, Benvenuto JA, Leavens M, Hall SW, Benjamin RS, Plunkett W, McCredie KB, Burgess MA, Loo TL (1979) Penetration of 3-deazauridine into human brain, intracerebral tumor, and cerebrospinal fluid. Cancer Res 39: 4119
67. Stewart DJ, Leavens M, Friedman J, Benjamin RS, Moore EC, Bodey GP, Valdivieso M, Burgess MA, Wiseman C, Loo TL (1980) Penetration of *N*-(phosphonacetyl)-L-aspartate into human central nervous system and intracerebral tumor. Cancer Res 40: 3163
68. Stewart DJ, Leavens M, Maor M, Feun L, Luna M, Bonura J, Caprioli R, Loo TL, Benjamin RS (1982) Human central nervous system distribution of *cis*-diamminedichloroplatinum and its use as a radiosensitizer in malignant brain tumors. Cancer Res 42: 2474
69. Stewart DJ, Benvenuto JA, Leavens M, Smith RG, Cabanillas F, Benjamin RS, Loo TL (1983) Human central nervous system pharmacology of pentamethylmelamine and its metabolites. J Neurooncol 1: 357
70. Stewart DJ, Lu K, Benjamin RS, Leavens ME, Luna M, Yap H-Y, Loo TL (1983) Concentrations of vinblastine in human intracerebral tumor and other tissues. J Neurooncol 1: 139
71. Stewart DJ, Richard MT, Hugenholtz H, Dennery JM, Belanger R, Gerin-Lajoie J, Montpetit V, Nundy D, Prior J, Hopkins HS (1984) Penetration of VP-16 (etoposide) into human intracerebral and extracerebral tumors. J Neurooncol 2: 133
72. Stewart DJ, Richard MT, Hugenholtz H, Dennery J, Nundy D, Prior J, Montpetit V, Hopkins HS (1984) Penetration of teniposide (VM-26) into human intracerebral tumors. J Neurooncol 2: 315
73. Stewart DJ, Mikhael NZ, Nair RC, Kacew S, Montpetit V, Nanji A, Maroun JA, Howard K (1988) Platinum concentrations in human autopsy tumor samples. Am J Clin Oncol 11: 152
74. Tirelli U, D'Incalci M, Canetta R, Tumolo S, Franchin G, Veronesi A, Galligioni E, Trovò MG, Rossi C, Grigoletto E (1984) Etoposide (VP-16-213) in malignant brain tumors: a phase II study. J Clin Oncol 2: 432
75. Von Hoff DD, Coltman CA Jr, Forseth B (1981) Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41: 1853
76. Walker MD, Weiss H (1975) Chemotherapy in the treatment of malignant brain tumors. Adv Neurol 13: 149
77. Watts C (1987) Blood-brain barrier and drug delivery in central nervous system tumors. Ann Neurol 21: 614
78. Whittle IR, MacPherson JS, Miller JD, Smyth JF (1990) The disposition of TCNU (tauromustine) in human malignant glioma: pharmacokinetic studies and clinical implications. J Neurosurg 72: 721
79. Workman P (1985) The pharmacology of brain tumor chemotherapy. In: Bleehen NM (ed) Tumors of the brain. Springer, Berlin Heidelberg New York, p 183
80. Yamada K, Takahama H, Nakai O, Takanashi T, Hosoya T (1989) Intra-arterial chemotherapy of malignant glioma after osmotic blood-brain barrier disruption. Gan To Nakagu Ryoho 16: 2692
81. Yung W-KA, Shapiro JR, Shapiro WR (1982) Heterogeneous chemosensitivities of subpopulations of human glioma cells in culture. Cancer Res 42: 992
82. Yung W-KA, Harris MI, Bruner JM, Feun LG (1989) Intravenous BCNU and AZQ in patients with recurrent malignant gliomas. J Neurooncol 7: 237
83. Zucchetti M, Rossi C, Knerich R, Donelli MG, Butti G, Silvani V, Gaetani P, D'Incalci M (1991) Concentrations of VP16 and VM26 in human brain tumors. Ann Oncol 2: 63