Anti-Cancer Drugs 1996, 7, pp. 213–219

Modification of the blood–brain barrier permeability by vinorelbine: effect of intracarotid infusion compared with intravenous infusion

C Mouchard-Delmas, B Gourdier,[1] R Vistelle and M Wiczewski

Laboratoire de Pharmacologie, UFR de Pharmacie, 51 rue Cognacq Jay, 51096 Reims Cedex, France. Tel: (+33) 26053599; Fax: (+33) 26053552. [1]Laboratoire de Pharmacie Clinique, Pharmacie Centrale, Hôpital Robert Debré, CHR Reims, 51092 Reims Cedex, France.

Brain levels of the antineoplastic compound, vinorelbine, and its effects on the permeability of the blood–brain barrier (BBB) were studied. Preliminary experiments were carried out to define the dose of 10 mg/kg and the delay of 3 h after infusion required to induce BBB disruption. Vinorelbine was infused by i.c. or i.v. infusion to anesthetized male Sprague-Dawley rats. BBB disruption was evaluated qualitatively by the presence in the infused hemisphere of i.v. administered Evans blue dye (2%) and vinorelbine intratissular levels were measured by HPLC. After an i.c. infusion, there is an important variability in the degree of extravasation of Evans blue albumin complex, which is correlated with vinorelbine levels ($p < 0.01$). The percent of dose in brain tissue is less than 1%. After an i.v. infusion, the parenchyma is globally affected as shown by the uniform faint bluish staining of the two hemispheres. Vinorelbine levels are homogenous and similar to levels of brains graded +1 after an i.c. infusion. These results seem to indicate that an i.c. infusion induces localized BBB disruptions while the effect of an i.v. infusion is global and that the gain in tissue level after an i.c. infusion is low compared with i.v. infusion.

Key words: Blood–brain barrier, chemotherapy, drug delivery, intracarotid infusion, vinorelbine.

Introduction

Many observations suggest that the incidence of metastatic brain tumors increases when primary peripheral tumors are treated successfully.[1] The fact that most brain metastases originate as blood-borne emboli led us to investigate the effects of antineoplastic drugs on the blood–brain barrier (BBB) permeability. Nevertheless, this may be also the result of the brain being a sanctuary site for tumor growth because the BBB limits the access of drugs to the brain.[2] Therefore, the importance of having drugs that can reach sufficient high levels for the treatment of brain tumors become increasingly important. However, a limiting step in delivering more chemotherapy to brain tumors is the neurotoxicity of the agents used.[3]

Vinorelbine, the vinca-alkaloid with the minor neurotoxicity,[4] is used today in the treatment of non-small cell lung cancer[5] and breast cancer.[6] As yet, only one set of data has been published about vinorelbine levels in brains of monkeys.[7] Since vinorelbine increases the permeability of a confluent culture of human endothelial cells to bovine serum albumin,[8] the question arose whether vinorelbine could have an effect on the permeability of the BBB. As this drug has an important effect on two central nervous system tumor cell lines and seems to be devoid of central adverse effects at doses used presently, a possible 'self-induced' increased BBB permeability could be advantageous with respect to treatment of brain tumors.

In this study, we examined the effect of vinorelbine on BBB permeability after an intracarotid (i.c.) and an intravenous (i.v.) injection of a high dose of the drug. Altered BBB permeability was documented qualitatively by the appearance in the brain parenchyma of the systemically administered dye, Evans blue. The intratissular drug level was determined to evaluate the incidence of the BBB disruption on the brain tissue distribution of vinorelbine.

Materials and methods

Drugs

Vinorelbine was supplied by Pierre Fabre Médicament (Paris, France). Teniposide (internal standard) was kindly provided by Sandox Laboratories (Rueil-Malmaison, France). Evans blue was obtained from Sigma (Saint Quentin Fallavier, France).

Correspondence to C Mouchard-Delmas

Animals

Fifty four male Sprague-Dawley rats (Elevage Dépré, Saint Doulchard, France) weighing between 280 and 350 g were used. Animals were housed in a temperature-controlled room at 22 ± 2°C with normal diurnal–nocturnal illumination and had free access to pellet food (Alimentation UAR, Epinay sur Orge, France) and tap water. They were acclimatized for at least 1 week under these conditions before the start of the experiment.

Animal Experiments

Preliminary experiments were carried out in 18 rats to define the dose of vinorelbine and the delay after i.c. infusion of the drug required to induce a BBB disruption. Three groups were used: a control group ($n=6$) receiving normal saline and two test groups ($n=6$ for each group) receiving vinorelbine at doses of 5 and 10 mg/kg, respectively. Rats were anesthetized with ethyle carbamate (1.25 g/kg; i.p.). A catheter was inserted in a retrograde manner into the left external carotid artery to the birfurcation of the common carotid artery as described previously.[9,10] Vinorelbine (1.25 or 2.5 mg/ml in saline for 5 and 10 mg/kg, respectively) was i.c. injected at a constant rate for 2 min. Immediately after the vinorelbine infusion, 2% Evans blue in saline, in a volume of 0.5 ml, was injected into the penis vein as a bolus over about 30 s. Three rats of each group were sacrificed at 2 and 3 h after the end of the vinorelbine injection by perfusion with saline through a left aortic catheter with blood drainage through a right atrial incision. After removal of the brains, staining of the cerebral hemispheres by Evans blue was evaluated by direct visual inspection.

The effect of vinorelbine on the BBB permeability was studied by an i.c. and i.v. injection of the drug. The animals were divided into three groups: two groups of 13 rats each received, in the same experimental conditions as reported in the preliminary study, an i.c. injection of normal saline (control group) or vinorelbine at the dose of 10 mg/kg (i.c. group). One group of 10 rats received an i.v. injection of vinorelbine at the dose of 10 mg/kg over 2 min (i.v. group). Rats were sacrificed, as previously described, at 3 h after the administration of the drug. Brains were graded for the presence of Evans blue staining and were stored at −20°C until analysis. Grading of Evans blue and determination of intratissular drug level was done blindly.

Evans blue scoring

At sacrifice, the brains were inspected visually for the presence of Evans blue dye. The dorsal and ventral faces of the brain were examined. In addition, coronal section was made and inspected visually. The staining of each hemisphere was graded as follows: Grade 0, no staining; Grade 1+, just noticeable staining; Grade 2+, moderate staining; Grade 3+, extensive blue staining with the presence of Evans blue staining in the contralateral hemisphere.

Analytical method

The intratissular vinorelbine level was measured by HPLC with coulometric detection.[11] Briefly, each hemisphere, added with teniposide as an internal standard (100 μl; 625 ng/ml), was rapidly homogenized in 1 ml of methanol. After centrifugation for 10 min at 1500 *g* (4°C), an aliquot of the supernatant (100 μl) was injected directly in the HPLC system equipped with a coulometric detector. The chromatograms were recorded and peak areas integrated on an integrator. Chromatographic separations were performed on an octadecylsilane column (LiChrospher 100 RP-18, 250 × 4 mm I.D., 5 μm; Merck Darmstadt, Germany). The mobile phase was a mixture of sodium dihydrogenphosphate buffer (20 g/l pH 3)–acetonitrile–methanol (50:30:20; v/v/v). Heptane sulfonic acid was added to the aqueous phase (0.8 g/l). The flow rate was 1 ml/min. Electrochemical detection was performed with a potential of +0.70 V. Calibration curves were constructed from spiked rat brains in the range of 78–1250 ng/ml.

Calculations and statistical analysis

Intratissular vinorelbine levels were determined in each hemisphere of the brains infused with vinorelbine by i.c. injection and in the total brains of animals receiving the drug by i.v. injection. They were expressed as micrograms of vinorelbine per gram of fresh tissue. The correlation coefficient (r) between Evans blue staining and drug level was calculated and the statistical significance of the correlation was tested using the r test. Comparison of means used the Mann–Withney test. The variability of results was compared by analysis of variance. A probability of 0.05 or less was considered significant.

Table 1. Frequency and grade of left hemisphere staining with Evans blue dye during preliminary assays.

Vinorelbine (mg/kg)	No. of animals: 2 h after injection	No. of animals: 3 h after injection	No. of rats at each grade of Evans blue staining[a]: 0	+1	+2	+3
0	3		3	0	0	0
0		3	3	0	0	0
5	3		3	0	0	0
5		3	3	0	0	0
10	3		1	0	1	1
10		3	0	1	0	2

[a] Staining grades: 0, none; +1, just noticeable; +2, moderate; +3, extensive.

Results

Preliminary experiments

The effect of i.c. infusion of vinorelbine on the BBB as measured by the presence of Evans blue dye in the hemispheres is shown in Table 1.

Intracarotid injection of 0.9% saline at the rate used in the study had no effect on the integrity of the rat's BBB. After 2 and 3 h following the end of the administration of the drug, the brains removed from animals receiving i.c. saline injections demonstrated no Evans blue staining on their surfaces or cut sections. Similarly, i.c. injection of vinorelbine at a dose of 5 mg/kg failed to disrupt the BBB. In contrast, the opening of the barrier occurs at the dose of 10 mg/kg. However, 2 h after the i.c. infusion, BBB disruption only appears in two of three animals. In contrast, 3 h after the drug administration, BBB disruption take place in all of the animals and is more marked as shown by the shift to a higher grade of staining.

BBB disruption

The results obtained 3 h after the end of the i.c. injection of vinorelbine (10 mg/kg) are shown in Table 2. None of the rats in the control group infused with saline had any Evans blue staining. In contrast, all of the animals infused with vinorelbine had disrupted BBBs. Levels of eight brains were graded as having a just noticeable staining. Three brains exhibited a moderate staining. No disruption to Evans blue dye was noted in the right hemispheres of these 11 animals. Two brains demonstrated extensive blue staining of the ipsilateral hemisphere and a contralateral staining within the distribution of the anterior cerebral artery. Although the most prominent areas of staining varied, the white matter seen by coronal section was systematically affected.

Drug levels

A representative chromatogram resulting from the analysis of brain tissue is depicted in Figure 1. The retention times of teniposide (internal standard) and vinorelbine were about 8.6 and 12.3 min, respectively. Vinorelbine levels in brain tissue are reported in Table 2 for the i.c. infusion. The brain levels of vinorelbine in the ipsilateral and contralateral hemispheres and in the total brain ranged between 0.45 and 22.82, 0.15 and 1.72, and 0.30 and 12.69 $\mu g/g$, respectively. They were correlated with Evans blue staining: $r = 0.8870$ ($p < 0.01$) for the total brain (Figure 2), $r = 0.8737$ ($p < 0.01$) for the ipsilateral hemisphere and $r = 0.9103$ ($p < 0.01$) for the contralateral hemisphere. Moreover, the vinorelbine levels in the ipsilateral hemisphere were correlated with those in the contralateral hemisphere ($r = 0.8090$; $p < 0.01$).

In the i.v. group, all of the brains exhibited an uniform faint bluish staining of the two hemispheres. Vinorelbine levels in brain tissue are reported in Table 3 for the i.v. infusion. The mean levels of the drug in the total brain, after i.v. or in brains graded +1 after i.c. infusion, were not statistically different (i.v. group: 0.59 ± 0.09 $\mu g/g$, mean ± SD; brains graded +1 of I.C. group: 0.85 ± 0.50 $\mu g/g$). In contrast, taking into account all of the brains in each group, the mean level of vinorelbine in the total brain in the i.v. group was significantly lower ($p < 0.01$) than that (4.22 ± 3.79 $\mu g/g$) determined in the i.c. group. Three hours after the administration of the drug, the amount of vinorelbine in the total brain tissue expressed as percent of the dose administered was $2.66 \times 10^{-2} \pm 0.39 \times 10^{-2}\%$ and $3.7 \times 10^{-2}\% \pm 2.0 \times 10^{-2}\%$ for the I.V. and the I.C. group graded +1, respectively.

Table 2. Grade of Evans blue staining and drug levels in rat brain after an i.c. infusion of vinorelbine 10 mg/kg

Evans blue staining[a]	Vinorelbine levels (μg/g of tissue)			
	Ipsilateral hemisphere	Contralateral hemisphere	Total Brain	Percent of dose (× 100)
+1	0.45	0.15	0.30	1.37
+1	0.52	0.22	0.37	1.70
+1	0.77	0.24	0.51	2.43
+1	1.21	0.21	0.74	3.37
+1	1.24	0.19	0.73	3.45
+1	1.67	0.18	0.96	4.15
+1	2.46	0.74	1.60	6.41
+1	2.69	0.48	1.55	6.72
+2	2.32	0.80	1.49	6.87
+2	3.11	1.05	2.07	9.42
+2	11.50	0.43	6.15	25.09
+3	22.82	1.72	12.69	57.87
+3	13.96	1.64	8.14	35.81

[a] Staining grades: 0, none; +1, just noticeable; +2, moderate; +3, extensive.

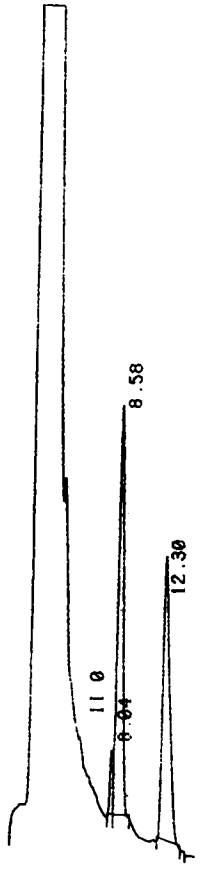

Figure 1. Representative chromatogram of the analysis of brain tissue. Peaks: 8.58 min, internal standard; 12.30 min, vinorelbine.

Discussion

The i.c. infusion of an antineoplastic compound in combination with the Evans blue dying technique is commonly used to evaluate the ability of the drug to disrupt the BBB.[9,10,12–15] using this approach, the results of the present study indicate that vinorelbine administered in sufficient concentration induces a disruption of the BBB in the rat. The results of the preliminary study suggest that a threshold concentration is required for the barrier disruption and that vinorelbine produces graded opening of the BBB during the 3 h following drug administration.

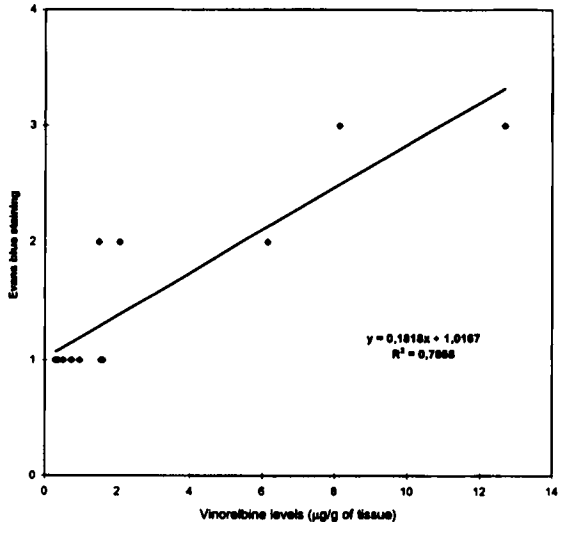

Figure 2. Correlation between Evans blue staining and vinorelbine levels in total brain.

Table 3. Total brain levels of vinorelbine in rats ($n=10$) after an i.v. infusion of a dose of 10 mg/kg

Vinorelbine levels (μg/g of tissue	Percent of dose (× 100)
0.52	2.51
0.60	3.07
0.74	3.41
0.635	2.53
0.595	2.88
0.67	2.68
0.46	1.99
0.65	2.67
0.46	2.36
0.59	2.46

An i.c. infusion of vinorelbine at a dose of 10 mg/kg opens the BBB to the Evans blue–albumin complex in all of the animals within the 3 h following the drug administration. Although vinorelbine can drastically affect the BBB permeability in some of the animals, in fact this effect seems to be moderate since most of the rats exhibited a just noticeable staining. The variability of the degree of the extravasation of the Evans blue–albumin complex may reflect individual differences in susceptibility to vinorelbine's effect and/or to the numerous arteries like pterigopalatine that normally siphon substantial blood from the internal carotid.[16] Evans blue staining of the contralateral hemisphere reported for the brains graded +3 is common to techniques of BBB disruption based on i.c. infusion and would be due to bilateral drug delivery through the anterior communicating artery or because rats frequently have a common anterior artery.[17]

The mechanism of vinorelbine induced enhanced permeability is not yet known. The effect of vinorelbine on BBB permeability cannot be attributed to a hyperosmolar insult. Vinorelbine solution employed is formulated in 0.9% saline and its osmolality ranged from 0.235 to 0.262 Osm. This value is far below that (1.4 Osm) reported by Rapoport[18] as necessary for hyperosmotic disruption of the BBB with mannitol or arabinose. In the same way, the changes in BBB permeability are not due to hypertensive effect. The barrier opens at i.c. pressures above 180 mm Hg, conditions which are obtained, for instance, by rapid infusion of 1 ml blood during 3 s.[13] A maximum volume of 1.4 ml being infused over 2 min cannot induce such a hypertension. In contrast, this volume should completely displace the intravascular volume of the cerebroarterial system on the ipsilateral side of the brain and allows complete exposure of the cerebral vasculature to the infused vinorelbine solution. Finally, the lack of extravasation of Evans blue in brain tissue of control animals would indicate that the experimental conditions of anesthesia did not cause any breakdown of the barrier. Consequently, BBB disruption does not depend on the experimental conditions but is due to an intrinsic effect of the drug which has not been established yet.

Although many studies on the pharmacokinetics of vinorelbine are available in the literature,[19–21] data on the level of the drug in the brain tissue are very scarce. As expected, the intratissular levels of vinorelbine determined in the ipsilateral hemisphere are statistically higher than those in the contralateral hemisphere and are related to the Evans blue staining. In spite of the high dose administered, the percentage of the dose in brain tissue is less than 1% whatever the staining of the brain. However, it has been shown that some brain tumors are very sensitive to vinorelbine[22] and that the brain tissue displays a capability to retain vinorelbine for a long period of time.[7] In these conditions, it may be hypothesized that the repetition of infusions should induce an accumulation of vinorelbine, allowing it to reach an effective level in the brain.

However, it is noteworthy that there is an important variability of the brain drug levels for the same staining grade. The ratio between the higher and the lower value is about 5 for the brains graded +1 and +2 and about 2 for the brains graded +3. This important variability of the drug level could be due to the fact the localized increase in BBB permeability caused by vinorelbine occurs most consistently and marked 3 h after the administration of the drug. In these conditions, the brain drug levels are under the influence of the rats of elimination from this tissue, which may be subject to important interindividual variation. These findings pose the question of the real benefit of an intraarterial injection for drugs such as vinorelbine which require a delay of several hours to obtain maximum BBB opening. Indeed, the penetration of the drug into the brain will be minimized by the fact that, when the increase of the BBB permeability is optimal, the blood concentration of the drug is not maximal and has decreased to a variable level according to the patient. The therapeutic gain of such an approach remains to be evaluated for vinorelbine by taking into account the non-selective opening of the barrier, and thus, the possible penetration into the CNS of blood-borne metastatic emboli and of endogenous compounds which can produce undesirable side effects into the brain.

The effect of an i.v. infusion of the same dose of vinorelbine on the BBB permeability was unexpected since a disruption of the BBB appears in all of the brains of tested animals as shown by the uniform faint bluish staining of the two hemispheres. A BBB disruption was also reported by MacDonnel after i.v. injection of 5-fluorouracil in cats[23] but these findings are controversed since they could not be reproduced by Neuwelt in a rat model.[24] Although the degree of staining suggests a less marked BBB opening than that observed for the brains graded +1 of the i.c. group, the total brain levels of the drug were similar. These results seem to indicate that an i.c. infusion of a high dose of vinorelbine induces localized BBB disruptions while the effect of an i.v. infusion globally affects the parenchyma. Moreover, the analysis of variance shows a variability of data significantly lower ($p < 0.001$) after an i.v. infusion. These different effects could influence the intratissular distribution of drug in brain. In addition, the gain in brain levels after an i.c. infusion was low in relation to the gravity of the side effects due to the method of administration.

Conclusion

These results indicate that an i.c. infusion induces localized BBB disruptions while the effect of an i.v. infusion is global. Moreover, the gain in tissue level after an i.c. infusion is low compared with i.v. infusion. Since the BBB is already altered in tumor vessels,[25] vinorelbine distribution could be modified with higher levels in tumor tissue. Thus, it is necessary to perform these experiments in a rat brain tumor model to evaluate the potential effect of vinorelbine in brain neoplasm.

References

1. Paterson AHG, Agarwal M, Lees A, Hanson J, Szafran O. Brain metastases in breast cancer patients receiving adjuvant chemotherapy. *Cancer* 1982; **49**: 651–4.
2. Siegers HP. Chemotherapy for brain metastases: recent developments and clinical considerations. *Cancer Treat Rev* 1990; **17**: 63–76.
3. Moynihan TJ, Grossman SA. The role of chemotherapy in the treatment of primary tumors of the central nervous system. *Cancer Invest* 1994; **12**: 88–97.
4. Binet S, Fellous A, Lataste H, Krikorian A, Couzinier JP, Meininger V. *In situ* analysis of the action of Navelbine® on various types of microtubules using immunofluorescence. *Semin Oncol* 1989; **16**: 5–8.
5. Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin JC. Efficacity of Navelbine® (NVB) in non small cell lung cancer. *Semin Oncol* 1989; **16**: 26–9.
6. Gasparini G, Caffo O, Barni S, *et al.* Vinorelbine is an active antiproliferative agent in pretreated advanced breast cancer patients: a phase II study. *J Clin Oncol* 1994; **12**: 2094–101.
7. Krikorian A, Rahmani R, Bromet M, Bore P, Cano JP. Pharmacokinetics and metabolism of Navelbine®. *Semin Oncol* 1989; **16**: 21–5.
8. Devie I, Mouchard-Delmas C, Minardi L, Duffer J. Effects of the anticancer agent vinorelbine on human endothelial cells permeability and tissue factor production. *Throm Haemostas* 1995; **73**: 1281 (Abstr).
9. Spigelman MK, Zappulla RA, Malis Ll, *et al.* Intracarotid dehydrocholate infusion: a new method for prolonged reversible blood–brain barrier disruption. *Neurosurgery* 1984; **12**: 606–12.
10. Spigelman MK, Zappulla RA, Johnson J, Goldsmith SJ, Malis Ll, Holland JF. Etoposide-induced blood–brain barrier disruption: effect of drug compared with that of solvents. *J Neurosurg* 1984; **61**: 674–8.
11. Mouchard-Delmas C, Gourdier B, Vistelle R. Determination of vinorelbine in rabbit plasma by high-performance liquid chromatography with coulometric detection. *J Chromatogr* 1995; **663**: 390–4.
12. Spigelman MK, Zappulla RA, Straucehn JA, *et al.* Etoposide induced blood–brain barrier disruption in rats: duration of opening and histological sequelae. *Cancer Res* 1986; **46**: 1453–7.
13. Hardebo JE. A time study in rat on the opening and reclosure of the blood–brain barrier after hypertensive or hypertonic insult. *Exp Neurol* 1980; **70**: 155–66.
14. Ogasawara H, Uozumi T, Kiya K, *et al.* Analysis and distribution of etoposide in rat brain tumor model: intracarotid versus intracarotid with angiotension II-induced hypertension. *Cancer Invest* 1993; **11**: 299–305.
15. Strausbaugh LJ. Intracarotid infusions of protamine sulfate disrupt the blood-brain barrier of rabbits. *Brain Res* 1987; **409**: 221–6.
16. Greene EC. *Anatomy of the rat.* New York: Hafner 1955.
17. Zemen W, Innes JRM. *Craigie's neuroanatomy of the rat.* New York: Academic Press 1963.
18. Rapoport SI, Fredericks WR, Ohno K, Pettigrew KD. Quantitative aspects of reversible osmotic opening of the blood–brain barrier. *Am J Physiol* 1980; **238**: R421–31.
19. Rahnmani R, Martin M, Barbet J, Cano JP. Radioimmunoassay and preliminary Pharmacokinetic studies in rats of 5'-norhydrovinblastine (Navelbine®). *Cancer Res* 1984; **44**: 5609–13.
20. Jehl F, Quoix E, Leveque D, Pauli G, Breillout F, Krikorian A. Pharmacokinetic and preliminary metabolic fate of navelbine in humans as determined by high performance chromatography. *Cancer Res* 1991; **51**: 2073–6.
21. Van Telligen O, Kuijpers AV, Beignen JH, Nooijen WJ, Bult A. Plasma pharmacokinetic, tissue disposition, excretion and metabolism of vinorelbine in mice as determined by high performance liquid chromatography. *Invest New Drugs* 1993; **11**: 141–50.
22. Cros S, Wright M, Morimoto M, Lataste H, Couzinier JP, Krikorian A. Experimental antitumor activity of Navelbine®. *Semin Oncol* 1989; **16**: 15–20.
23. MacDonnel LA, Potter PE, Leslie RA. Localized changes

in blood–brain barrier permeability following the administration of antineoplastic drugs. *Cancer Res* 1978; **38**: 2930–4.

24. Neuwelt EA, Barnett DA, Barranger J, MacCormick C, Pagel M, Frenkel E. Inability of dimethyl sulfoxide and 5-fluorouracil to open the blood–brain barrier. *Neurosurgery* 1983; **12**: 29–34.

25. Petersdorf SH, Livingston RB. High dose chemotherapy for the treatment of malignant brain tumors. *J Neurooncol* 1994; **20**: 155–63.

(Received 17 October 1995; accepted 26 October 1995)