Acta Medica Okayama

Volume 53, *Issue* 1 1999 *Article* 2

FEBRUARY 1999

Effect of intracarotid infusion of etoposide: modification of the permeability of the blood-brain barrier and the blood-tumor barrier in rat brain tumor model

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Abstract

The effect of intracarotid infusion of etoposide on the permeability of the blood-brain barrier (BBB) and brain-tumor barrier (BTB) was investigated using a model of rats injected with C6 glioma cells. Fifty four glioma-bearing rats were divided into 3 groups and treated with 0, 3, or 15 mg/kg of etoposide infused into the internal carotid artery. BBB or BTB permeability was evaluated qualitatively by the leakage of Evans blue (6 animals in each group) or quantitatively by the diffusion of carboplatin [cis-diammine (1,1-cyclobutane-dicarboxylato) platinum(II); CBDCA] (12 animals in each group) into the normal brain or the tumor tissue. BBB and BTB disruption augmented significantly in proportion to the dose of etoposide. The degree of disruption of BTB was greater than that of BBB, but the rate of disruption of BBB in proportion to increasing the dose of etoposide was higher than that in the BTB. Histopathologically, no obvious changes were observed in the animals of either the control group or the 3 mg/kg group but degenerative changes in the neurons of the hippocampus of the infused hemisphere were seen in the 15 mg/kg group. This change is thought to be caused by apoptosis because of the positive reaction with TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method. Our results suggest that intracarotid infusion of etoposide can increase drug delivery of concurrent antitumor agents into tumor tissue, but cerebral parenchymal cell damage is expected with a higher dosage of etoposide. Therefore, the dosage of etoposide for intracarotid infusion should be lower than 15 mg/kg in order to reduce neurotoxicity of both etoposide and concurrent anticancer drugs.

KEYWORDS: etoposide, intracarotid infusion, blood-brain barrier, blood-tumor barrier, apoptosis

ACTA MED OKAYAMA 1999; 53(1): 5-11

Effect of Intracarotid Infusion of Etoposide: Modification of the Permeability of the Blood-Brain Barrier and the Blood-Tumor Barrier in Rat Brain Tumor Model

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The effect of intracarotid infusion of etoposide on the permeability of the blood-brain barrier (BBB) and brain-tumor barrier (BTB) was investigated using a model of rats injected with C6 glioma cells. Fifty four glioma-bearing rats were divided into 3 groups and treated with 0, 3, or 15 mg/kg of etoposide infused into the internal carotid artery. BBB or BTB permeability was evaluated qualitatively by the leakage of Evans blue (6 animals in each group) or quantitatively by the diffusion of carboplatin [cis-diammine (1, 1-cyclobutane-dicarboxylato) platinum(II); CBDCA] (12 animals in each group) into the normal brain or the tumor tissue. BBB and BTB disruption augmented significantly in proportion to the dose of etoposide. The degree of disruption of BTB was greater than that of BBB, but the rate of disruption of BBB in proportion to increasing the dose of etoposide was higher than that in the BTB. Histopathologically, no obvious changes were observed in the animals of either the control group or the 3 mg/kg group but degenerative changes in the neurons of the hippocampus of the infused hemisphere were seen in the 15 mg/kg group. This change is thought to be caused by apoptosis because of the positive reaction with TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method. Our results suggest that intracarotid infusion of etoposide can increase drug delivery of concurrent antitumor agents into tumor tissue, but cerebral parenchymal cell damage is expected with a higher dosage of etoposide. Therefore, the dosage of etoposide for intracarotid infusion should be lower than 15 mg/kg in order to reduce neurotoxicity of both etoposide and concurrent anticancer drugs.

Key words: etoposide, intracarotid infusion, blood-brain barrier, blood-tumor barrier, apoptosis

One of the possible reasons for the limited effects of chemotherapy on central nervous system (CNS) malignancies is insufficient drug delivery to the tumor due to the blood-brain barrier (BBB). It has been reported that the BBB is completely intact in small CNS neoplasms and at least partially intact in larger tumors (1). To enhance the effect of chemotherapy on malignant brain tumors, several methods have been tried to increase drug delivery to brain tumors, such as reversible osmotic opening of the BBB (2, 3), augmentation of cerebral blood flow by angiotensin II (4), and increasing drug accumulation by means of calcium channel blockers (5).

Spigelman *et al.* (6) showed evidence that the intracarotid infusion of an anti-neoplastic compound, etoposide (4 demethyl-epipodophyllotoxin-β-D-ethylidine glucoside), could disrupt the BBB in the normal brain without causing parenchymal damage. On the other hand, Ogasawara *et al.* (4) recently reported that intracarotid infusion of high-doses of etoposide was capable of producing irreversible neuronal damage in a rat model. There have been few reports on the effects of etoposide on blood-tumor barrier (BTB) permeability and the action of the drug on the BBB and BTB is not yet clearly understood.

The present study was designed to examine the effects of intracarotid infusion of etoposide on the permeability of the BBB and BTB in a model of rats injected with C6 glioma cells (rat C6 glioma model). BBB or BTB permeability was evaluated qualitatively by observing the leakage of Evans blue into the normal brain and tumor tissues (18

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animals) or quantitatively by measuring the diffusion of carboplatin [cis-diammine (1, 1-cyclobutane-dicarboxylato) platinum (II); CBDCA] penetrated into normal brain and tumor tissue (36 animals). Both Evans blue and carboplatin were administered systemically. CBDCA, which is frequently administered against a variety of neoplasms in combination with etoposide (7, 8), is reported to show only limited penetration into brain parenchyma (9). Lastly, we investigated histopathological changes of normal brain tissue infused by etoposide.

Materials and Methods

Cells and animals. Cells: C6 glioma cells were maintained as a monolayer culture in Eagle's minimum essential medium supplemented with 10 % fetal bovine serum in an atmosphere of 5 % CO_2 and 95 % room air at 37 °C.

Animals: Fifty four male Wister rats (250–350 g) were used for qualitative study (18 animals) and quantitative study (36 animals). All animals were kept in pathogen-free animal facilities at Okayama University and were handled in a humane fashion in accordance with the university's modified version of the National Institute of Health guidelines.

Brain tumor model. Animals were anesthetized by intraperitoneal administration of pentobarbital (50 mg/kg). A burr hole was made at 4 mm to the right from midline and 2 mm posterior to the bregma. Suspended tumor cells of 5×10^5 in 5 μl phosphate buffered saline were implanted into the right frontal lobe at a depth of 5 mm vertically from the brain surface through the burr hole.

Intracarotid administration of etoposide. Twelve to 14 days after tumor inoculation, the animals were anesthetized with pentobarbital. A polyethylene catheter was inserted retrogradely down the right external carotid artery to the bifurcation of the common carotid artery. Etoposide at dosages of 0, 3, or 15 mg/kg in 5 ml of normal saline were given at a constant rate for 20 min into the right internal carotid artery through the catheter. The right pterygopalatine artery was clipped temporarily during the intracarotid infusion.

Qualitative and histopathological studies on BBB and BTB disruption. Three group of 6 rats each were used for the control and the above two etoposide dose in the studies. Immediately after the intracarotid infusion of etoposide, 2 ml/kg of 2 % solution of Evans blue was injected into the femoral vein. Animals were sacrificed 2 h after the end of the intracarotid infusion of etoposide. The brain was removed and sliced coronally at the maximum diameter of the tumor. Staining of the tumor and cerebral hemispheres by Evans blue was evaluated by direct visual inspection without knowledge of which group the rat belonged to, and graded as follows: grade 0, no staining; grade 1+, just noticeable staining; grade 2+, moderate staining; grade 3+, dark staining (10). The sections were then fixed with 10 % formalin, embedded in paraffin, stained with hematoxylin and eosin, and observed microscopically. Selected sections were also processed for the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL) method, which detects nuclear DNA breakdown, using an *in situ* apoptosis detection kit (Takara Shuzo Co. Ltd., Japan). The TUNEL method is described in detail elsewhere (11). Briefly, sections were deparaffinized, protein was digested with proteinase K, and endogenous peroxidase was inactivated using 0.3 % hydrogen peroxide. DNA fragments were then linked to fluorescein isothiocyanate (FITC)-dUTP by TdT and incubated with an anti-FITC antibody conjugated with peroxidase. The reaction was visualized using diaminobenzidine (DAB) and the sections were counterstained with hematoxylin.

Quantitative study on BBB and BTB disruption using CBDCA. Three groups of 12 rats each were used in this experiment. After the carotid infusion of etoposide, 16 mg/kg of CBDCA was administered through the femoral vein. Thirty minutes after the end of CBDCA injection, specimens of 2 ml of blood were collected from the femoral vein catheter, and then the rats were sacrificed. Brains were removed and the right cerebral hemisphere was separated into tumor tissue and normal tissue. The same was also done for the left cerebral hemisphere. The CBDCA concentrations in the tissue and blood samples were measured by flameless atomic absorption spectrophotometry. The data are expressed as mean $\pm$ standard deviation. Statistical comparisons among the groups were performed using the Student's *t*-test or nonparametric methods (Mann-Whitney's U test). A probability of 0.05 or less was considered significant.

Results

Qualitative and histopathological studies on BBB and BTB disruption. Table 1 presents

the relationship between the dose of etoposide and BBB disruption evaluated by Evans blue staining of the tumor and the normal brain in the right ipsilateral cerebral hemisphere. While the staining of all the tumors were graded as 1+, the normal brain was not entirely stained in the control group. In the 3 mg/kg etoposide group, the tumors were stained as 1+ in one animal, 2+ in four, and 3+ in one. In the 15 mg/kg etoposide group, the staining of tumors increased and was graded as 2+ in one and 3+ in five. In proportion to the doses of etoposide, the staining of the normal brain tissue also progressively increased. The grade of staining of the normal brain was 0 in two, 1+ in three, and 2+ in one in the 3 mg/kg group, whereas it was 1+ in one, 2+ in four, and 3+ in one in the 15 mg/kg group. No staining was observed in the non-infused (left) hemisphere except for one in the 15 mg/kg group.

Microscopically, degenerative changes in the neurons such as shrinkage of cytoplasm and pyknosis were seen in the hippocampus in the infused hemisphere of two animals with 2+ staining in the 15 mg/kg group (Fig. 1). Those cells were positively stained with the TUNEL method and showed the common morphological features of apoptosis (Fig. 2).

Quantitative study on BBB and BTB disruption using CBDCA. Fig. 3 presents the relationship between the dose of etoposide and the CBDCA concentrations in tissue and serum. The serum CBDCA concentrations in the control, 3 mg/kg, and 15 mg/kg groups were statistically similar [$7.54 \pm 1.10\,\mu g/g$ (mean $\pm$ SD), $7.56 \pm 1.29\,\mu g/g$ and $7.88 \pm 1.70\,\mu g/g$, respectively]. The CBDCA concentrations in the tumors of the control, 3 mg/kg, and 15 mg/kg groups were $2.15 \pm 0.82\,\mu g/g$, $3.43 \pm 1.71\,\mu g/g$ and $4.76 \pm 1.23\,\mu g/g$, respectively. As etoposide dose increased, CBDCA concentration progressively increased in tumors. Statistically significant differences were found among them. The ratios of concentrations in tumor to serum (T/S) were $28.9 \pm 11.0\,\%$ in the control group, and significantly increased to $44.2 \pm 17.2\,\%$ in the 3 mg/kg group ($P < 0.05$) and $61.5 \pm 17.0\,\%$ in the 15 mg/kg group ($P < 0.01$). The difference of T/S ratio between the 3 mg/kg group and the 15 mg/kg group was also significant ($P < 0.05$). The CBDCA concentrations in the normal brain tissues of the ipsilateral (right) hemispheres in the control, 3 mg/kg, and 15 mg/kg groups were $0.66 \pm 0.28\,\mu g/g$, $1.58 \pm 0.90\,\mu g/g$ and $2.30 \pm 0.58\,\mu g/g$, respectively. The ratios of concentrations in normal brain tissues of the ipsilateral hemispheres to serum (I/S) were $9.1 \pm 4.3\,\%$ in the control group, and also significantly increased to $20.6 \pm 9.9\,\%$ in the 3 mg/kg group ($P < 0.05$) and $29.8 \pm 8.5\,\%$ in the 15 mg/kg group ($P < 0.01$). The difference of I/S ratio between the 3 mg/kg and 15 mg/kg group was also statistically significant ($P < 0.05$). The concentration of CBDCA was higher in the tumor tissue than in normal tissue but the rate of increase of the concentration was greater in normal tissue (from 2.3-fold to 3.3-fold compared to the control) than in tumor tissue (from 1.5-fold to 2.1-fold) as the dose of etoposide increased from 3 mg/kg to 15 mg/kg. The CBDCA concentrations in the contralateral (left) hemisphere of the control, 3 mg/kg, and 15 mg/kg group were $0.58 \pm 0.32\,\mu g/g$, $0.54 \pm 0.25\,\mu g/g$ and $0.62 \pm 0.26\,\mu g/g$, respectively. The ratios of concentrations in the contralateral hemispheres to serum (C/S) of the control, 3 mg/kg, and 15 mg/kg group were $7.9 \pm 4.8\,\%$, $7.5 \pm 2.5\,\%$ and $8.2 \pm 3.7\,\%$, showing no significant differences among the groups.

Table 1 Etoposide dosage and tissue staining with Evans blue

Site/Etoposide dosage (mg/kg)	Number of rats	Evans blue staining grade			
		0	1+	2+	3+
Tumor					
0	6	0	6	0	0
3	6	0	1	4	1
15	6	0	0	1	5
Ipsilateral hemisphere					
0	6	6	0	0	0
3	6	2	3	1	0
15	6	0	1	4	1

Evans blue staining, grade 0: No staining; grade 1+: Barely noticeable staining; 2+: Moderate staining; 3+: Dark staining.

Discussion

Etoposide is synthesized from podophyllotoxin and has been used for the treatment of malignant brain tumors by intravenous or intracarotid administration in recent years (7, 12, 13). It is difficult for this drug to pass the BBB because of its relatively high molecular weight (588.57) and high binding capacity with serum proteins (14). However, it can alter BBB permeability significantly when infused into the carotid artery. Spigelman *et al.* (6) reported that intracarotid infusion of etoposide pro-

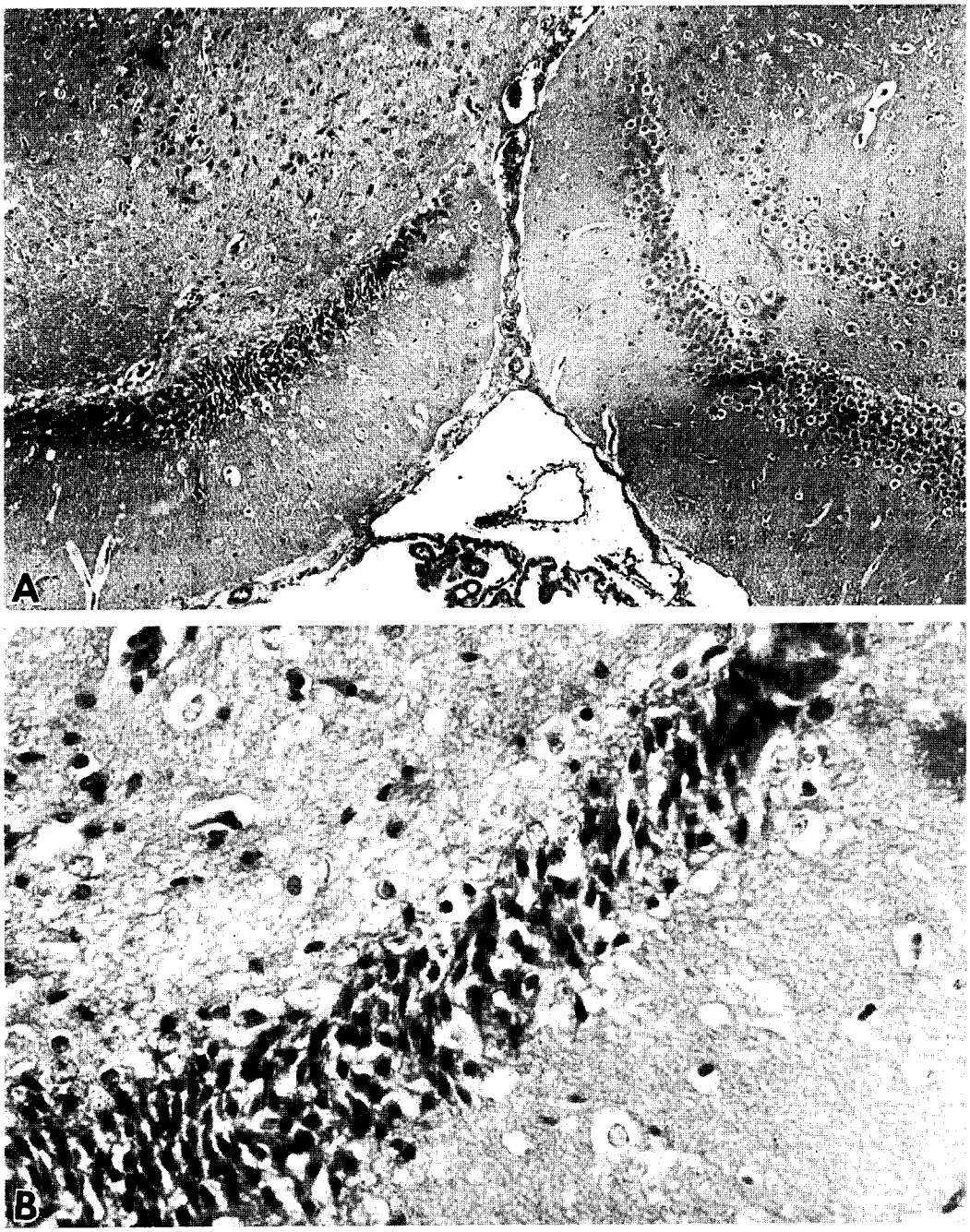

Fig. 1 Photomicrographs of brain sections obtained 2h after the intracarotid infusion of 15mg/kg of etoposide. Degenerative changes of neurons in the hippocampus are seen in the infused hemisphere, specifically cytoplasmic shrinkage and nuclear pyknosis. H & E stain; × 100 (**A**), × 400 (**B**).

duced dose-dependent and reversible disruption of the BBB in the normal rat brain.

Using Evans blue staining and CBDCA-diffusion analysis, the present study demonstrates that intracarotid infusion of etoposide induces a dose-dependent disruption of both the BTB and BBB. The leakage of systematically administered Evans blue into tumor and normal brain tissues gives a qualitative understanding of BBB and

Fig. 2 Photomicrograph of the neurons in the hippocampus after intracarotid infusion of 15 mg/kg of etoposide. Brown Tdt-mediated dUTP-biotin nick-end labeling-positive cells are visible and show nuclear condensation and fragmentation. Counterstained with hematoxylin, × 1000.

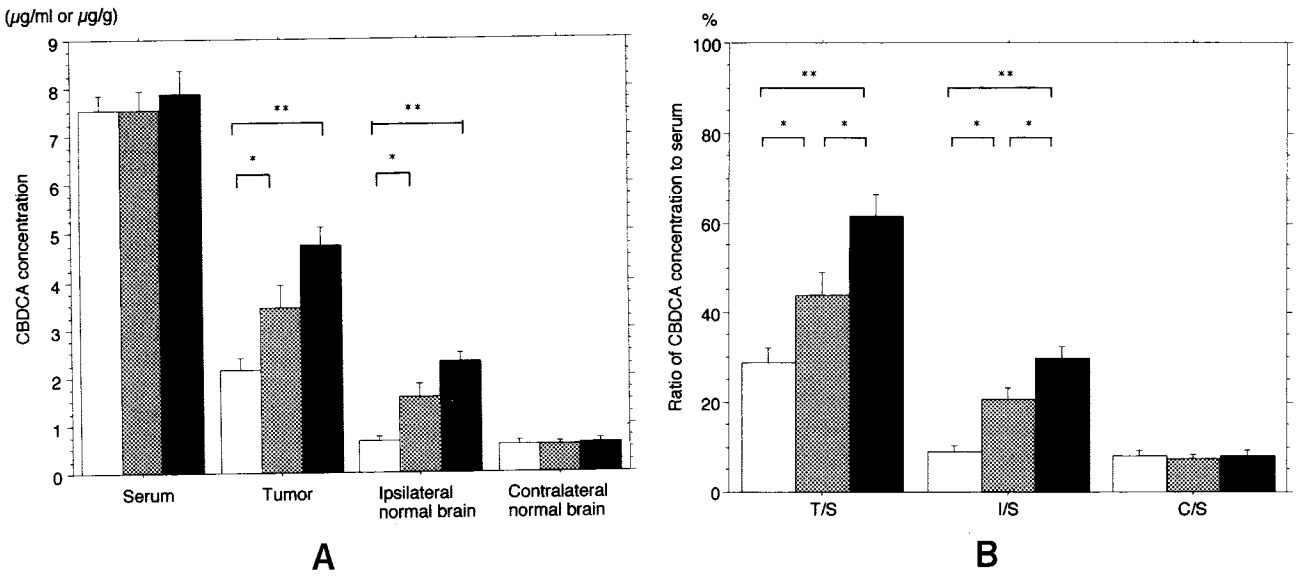

Fig. 3 **A**: Carboplatin (CBDCA) concentrations in tissue and serum. As etoposide dosage increases, CBDCA concentration progressively increases in the tumor and in the normal ipsilateral hemisphere. $*P < 0.05$; $**P < 0.01$. **B**: Ratio of carboplatin concentration in tissue to serum. The ratios of tumor to serum concentration in the 3 mg/kg and 15 mg/kg groups were approximately 1.5 and 2.1 times higher than that of the control group, respectively. Ratios of normal brain concentrations in the ipsilateral hemisphere to serum were approximately 2.3 and 3.3 times control for the 3 mg/kg and 15 mg/kg groups, respectively. T/S: Ratio of concentration in tumor to serum; I/S: Ratio of concentration in ipsilateral normal brain to serum; C/S: Ratio of concentration in the contralateral hemisphere to serum, $*P < 0.05$; $**P < 0.01$. □: control (n = 12), ▨: 3 mg/kg (n = 12), ■: 15 mg/kg (n = 12).

BTB disruption. CBDCA, which is also used to treat malignant brain tumors (8, 15) but shows limited passage through normal BBB (9), was used as a tool to estimate the disruption of the BBB and BTB because it contains platinum and can be measured quantitatively by flameless atomic absorption spectrophotometry. In this study, the staining of the tumor and the normal brain tissue with Evans blue increased progressively in proportion to increases in the dose of etoposide. The concentration of CBDCA also increased in both the tumor and the normal tissue of the right (etoposide-infused) hemisphere but not in the opposite hemisphere. Although the concentration of CBDCA in the tumor tissue was higher than in normal tissue, the rate of increase of concentration was greater in the normal brain tissue than in the tumor tissue. These results suggest that intracarotid infusion of etoposide can induce the BBB or BTB permeability and increase drug delivery to the brain tumor, but may also increase the exposure of normal brain tissue to anticancer agents and induce toxic effects in normal cells.

The mechanism of etoposide-induced BBB disruption is still unclear. Etoposide, utilized in this experiment, is formulatedas a complex solvent solution containing polysolvate 80, alcohol, citric acid and macrogol 300. Because of the evidence that alcohol alone can affect BBB permeability, the solvent solution may contribute to enhanced BBB permeability. But Spigelman *et al.* (16) reported that, while the solvent solution alone is capable of altering BBB permeability, the major effect is due to the drug itself.

In intracarotid infusion of anticancer drugs, their neurotoxicity should be taken into consideration. Savaraj *et al.* (17) reported that when 2 mg/kg of etoposide was infused into the internal carotid artery and femoral vein of normal dogs, the concentration of etoposide in the brain tissue of the intracarotid infusion group was at least four times higher than that of the intravenous infusion group. However, none of the animals in the intracarotid infusion group showed neurologic toxicity or histopathological changes in the brain. Spigelman *et al.* (6) reported that intracarotid infusion of etoposide of up to 22.5 mg/kg produced a reversible disruption of the BBB without damage to the cerebral parenchyma except for a mild perivascular lymphocytic infiltration in the infused hemisphere. However, Hollis *et al.* (18) reported that when 25 mg/kg of etoposide was infused into the internal carotid artery, a significantly higher incidence of cerebral edema and increase in the brain water content was observed in the infused hemisphere. Recently, Ogasawara *et al.* (4) reported that intracarotid infusion of high-dose etoposide (75 mg/m^2) induced BBB disruption and irreversible neuronal damage in a rat model without showing any focal neurological deficits except for some decrease of daily activity. They observed demyelination and edema in areas largely concurrent with areas of Evans blue leakage, as well as degenerative necrosis of neurons in part of the hippocampus and cerebral cortex. In the present study, neuronal damage in the hippocampus of the infused hemisphere was also seen in two animals with 2+ staining in the 15 mg/kg group and the mechanism of etoposide-induced neuronal damage is thought to be apoptotic cell death.

These results suggest that intracarotid infusion of high-dose etoposide can cause cerebral parenchymal damage. Therefore, the dose of etoposide for intracarotid infusion should be lower than 15 mg/kg in order to reduce neurotoxicity of the etoposide itself and concurrent anticancer drugs. Further investigation should be done to decide the proper dosage of etoposide for intracarotid infusion in clinical use.

References

1. Hasegawa H, Ushio Y, Hayakawa T, Yamada K and Mogami H: Changes of the blood-brain barrier in experimental metastatic brain tumors. J Neurosurg (1983) **59**, 304-310.
2. Neuwelt EA, Barnett PA and Frenkel EP: Chemotherapeutic agent permeability to normal brain and delivery to avian sarcoma virus-induced brain tumors in the rodent: Observations on problems of drug delivery. Neurosurgery (1984) **14**, 154-160.
3. Inoue T, Fukui M, Nishio S, Kitamura K and Nagara H: Hyperosmotic blood-brain barrier disruption in brains of rats with intracerebrally transplanted RG-C6 tumor. J Neurosurg (1987) **66**, 256-263.
4. Ogasawara H, Kiya K, Kurisu K, Hotta T, Mikami T, Sugiyama K, Nakahara T and Uozumi T: Effect of intracarotid infusion of etoposide with angiotensin II-induced hypertension on the blood-brain barrier and the brain tissue. J Neuro-Oncol (1992) **13**, 111-117.
5. Matsukado K, Nomura T, Ikezaki K and Fukui M: Selective increase in blood-tumor barrier permeability by calcium antagonists in transplanted rat brain tumors. Acta Neurochir (1994) **60** [Suppl], 403-405.
6. Spigelman MK, Zappulla RA, Strauchen JA, Feuer EJ, Johnson J, Goldsmith SJ, Malis LI and Holland JF: Etoposide induced blood-brain barrier disruption in rats: Duration of opening and histological sequelae. Cancer Res (1986) **46**, 1453-1457.
7. Jeremic B, Grujicic D, Jevremovic S, Stanisavljevic B, Milojevic L, Djuric L and Mijatovic L: Carboplatin and etoposide chemotherapy regimen for recurrent malignant glioma: A phase II study. J Clin Oncol (1992) **10**, 1074-1077.
8. Williams PC, Henner WD, Roman-Goldstein S, Dahlborg SA, Brummett RE, Tableman M, Dana BW and Neuwelt EA: Toxicity and efficacy of carboplatin and etoposide in conjunction with disruption of blood-brain tumor barrier in the treatment of intracranial neoplasms.

Neurosurgery (1995) **37**, 17–28.

9. Riccardi R, Riccardi A, Di Rocco C, Carelli G, Tartaglia RL, Lasorella A, Servidei T and Mastrangelo R: Cerebrospinal fluid pharmacokinetics of carboplatin in children with brain tumors. Cancer Chemother Pharmacol (1992) **30**, 21–24.
10. Rapoport SI, Fredericks WR, Ohno K and Pettigrew KD: Quantitative aspects of reversible osmotic opening of the blood-brain barrier. Am J Physiol (1980) **238**, 421–431.
11. Gavrieli Y, Sherman Y and Ben-Sasson SA: Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J Cell Biol (1992) **119**, 493–501.
12. Madajewicz S, Chowhan N, Iliya A, Roque C, Beaton R, Davis R, Fertman S, Meek A, Alvarez O, Pampati M and Tyson G: Intracarotid chemotherapy with etoposide and cisplatin for malignant brain tumors. Cancer (1991) **67**, 2844–2849.
13. Nakagawa H, Fujita T, Kubo S, Tsuruzono K, Yamada M, Tokiyoshi K, Miyawaki Y, Kanayama T, Kadota T and Hayakawa T: Selective intra-arterial chemotherapy with a combination of etoposide and cisplatin for malignant gliomas: Preliminary report. Surg Neurol (1994) **41**, 19–27.
14. Feun LG, Lee YY, Yung WK, Savaraj N and Wallace S: Intracarotid VP-16 in malignant brain tumors. J Neuro-Oncol (1987) **4**, 397–401.
15. Warnick RE, Prados MD, Mack EE, Chandler KL, Doz F, Rabbitt JE and Malec MK: A phase II study of intravenous carboplatin for the treatment of recurrent gliomas. J Neurooncol (1994) **19**, 69–74.
16. Spigelman MK, Zappulla RA, Johnson J, Goldsmith SJ, Malis LI and Holland JF: Etoposide-induced blood-brain barrier disruption. Effect of drug compared with that of solvents. J Neurosurg (1984) **61**, 674–678.
17. Savaraj N, Lu K, Feun LG, Burgess MA and Loo TL: Comparison of CNS penetration, tissue distribution, and pharmacology of VP 16-213 by intracarotid and intravenous administration in dogs. Cancer Invest (1987) **5**, 11–16.
18. Hollis PH, Zappulla RA, Spigelman MK, Feuer EJ, Holland JF and Malis LI: Effects of etposide-induced blood-brain barrier disruption on brain water, intracranial pressure, and cerebral vasomotor tone. Exp Neurol (1988) **99**, 428–439.

Received August 18, 1998; accepted September 28, 1998.