Pharmacokinetics of Etoposide and Carboplatin in Cerebrospinal Fluid and Plasma during Hyperosmotic Disruption of the Blood Brain Barrier and Intraarterial Combination Chemotherapy

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The present paper investigates the pharmacokinetics of etoposide (VP-16) and carboplatin (CBDCA) in plasma and the cerebrospinal fluid (CSF), in the space left by tumor removal, of patients with glioma. Eight Japanese patients with glioma received a course of hyperosmotic disruption of the blood-brain barrier (HODBBB) and intraarterial combination chemotherapy with 60 mg/m² of VP-16 and 300 mg/m² of CBDCA. All patients were initially administered mannitol, followed by infusion of the anticancer drugs into the right internal carotid artery. Blood samples and samples of CSF in the space left by tumor removal were obtained. VP-16 and CBDCA concentration were measured by HPLC, and the pharmacokinetic parameters of these drugs estimated in CSF and plasma.

The plasma concentrations of VP-16 and CBDCA peaked at the end of infusion, then decreased in a bi-exponential decay pattern during the remainder of the treatment period. Both VP-16 and CBDCA were detectable in CSF beginning 0.5 h after the initiation of each infusion, and were then slowly eliminated from the space left by tumor removal. The mean maximum CSF concentration of VP-16 and CBDCA was 0.17 and 15.25% of that in plasma, respectively. The mean area under the time-CSF concentration curve from 0 to 24 h after VP-16 and CBDCA infusion was 1.91 and 113.6% of plasma, respectively. In two of the eight patients, the clinical response to treatment was a partial response and other patients showed no change. HODBBB and intraarterial combination chemotherapy with VP-16 and CBDCA may be useful in patients with brain tumors for maintenance chemotherapy.

Key words: etoposide; carboplatin; pharmacokinetics; cerebrospinal fluid; hyperosmotic disruption; intraarterial combination chemotherapy

The blood-brain barrier (BBB) can impair the delivery of chemotherapeutic agents, even in the presence of a high systemic blood concentration of drugs. In an attempt to increase the delivery of drugs to tumors and the surrounding brain tissue, hyperosmotic disruption of the BBB (HODBBB) has been conducted for patients with brain tumors, with some success.1-2 In addition, combinations of etoposide (VP-16) and cisplatin (CDDP), which have synergistic effects, have been administered intraarterially to raise the tumor exposure level over that obtained by the intravenous route.3-4 Carboplatin (CBDCA) is reported to cross the BBB more readily than CDDP, and thus seems to offer an advantage over CDDP in treating primary or secondary tumors of the brain.3-7 Currently, we routinely select VP-16 and CBDCA as anticancer drugs for use in HODBBB and intraarterial combination chemotherapy in patients with malignant glioma. As in a case report, we have previously reported that in two patients with glioma, the maximum cerebrospinal fluid (CSF) concentrations of VP-16 and CBDCA in the space left by tumor removal following HODBBB and intraarterial drug administration were higher than those following intravenous administration.8 However, the pharmacokinetics of these drugs in the space left by tumor removal during this treatment have not been investigated in detail. Thus, to clarify the pharmacokinetics and to evaluate their permeability to CSF in the space left by tumor removal, we measured the concentrations of VP-16 and CBDCA in plasma and the CSF using an Ommaya’s reservoir in eight patients with glioma, following HODBBB and intraarterial combination chemotherapy.

MATERIALS AND METHODS

Chemicals Standard grade VP-16 and CBDCA were kindly supplied by Nippon Kayaku Co. Ltd. (Tokyo, Japan) and Bristol-Myers Squibb K.K. (Tokyo, Japan), respectively. Methanol and acetonitrile were of HPLC grade. All other chemicals were of analytical grade.

Subjects Eight brain tumor patients were examined, aged 46 to 69 years (mean: 54.9 ± 9.3 years) and weighing 35 to 66 kg (mean: 49.9 ± 6.1 kg). Patients had been previously treated by surgical removal of almost the entire tumor and insertion of an Ommaya’s reservoir into the space left by tumor removal. After the surgery, radiation therapy was examined. Informed written consent was obtained to HODBBB and intraarterial combination chemotherapy from all patients.

Chemotherapy Following radiation therapy (40—60 Gray), the patients were administered a course of HODBBB with mannitol, and intraarterial combination chemotherapy with VP-16 (Lastetrade; Nippon Kayaku Co., Ltd., Tokyo, Japan), CBDCA (Paraplatintrade; Bristol-Myers Squibb K.K., Tokyo, Japan), and nimustine (Nidrantrade; Sankyo Co., Ltd., Tokyo, Japan). Each anticancer drug was reconstituted in 100 mL of 0.9% NaCl. Patients were initially administered 50 mL of 20% mannitol for 10 min, and then sequentially followed by infusions of 60 mg/m² of VP-16 for 30 min, 300 mg/m² of CBDCA for 30 min, 30 mg/m² of nimustine for 30 min, and 125 mg of methylprednisolone (Solud Medroltrade; Pharmacia & Upjohn Co., Ltd., Tokyo, Japan) for 10 min into the right internal carotid artery. No complications were observed during treatment.

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Sampling  In all patients, an Ommaya’s reservoir was inserted into the space left by tumor removal to sample the CSF. Samples of plasma and CSF were obtained before treatment and again at 0.5, 1, 1.5, 2, 4, 8 and 24 h after the initiation of perfusion of VP-16. The blood samples were drawn into heparin-containing tubes, centrifuged, and plasma stored at -40°C. The CSF samples were immediately frozen and stored at -40°C until assay, which was performed within 1 week.

Measurement of VP-16 and CBDDA Concentrations HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisted of a 600 E pump, 717 plus autosampler, 486 UV detector, ECD-100 electrochemical detector (Eicom Co., Kyoto, Japan) and 805 data station. The stationary phase for VP-16 was a μBondasphere C18 column (150×3.9 mm i.d., Waters Assoc., Milford, MA, U.S.A.) and that for CBDDA was a LiChrosorb diol column (250×4.1 mm i.d.; Merck, Darmstadt, Germany).

Plasma and CSF concentrations of VP-16 were measured by HPLC assay according to the method of Holthuis et al. Briefly, 0.1 ml of plasma was mixed with 0.1 ml of acetonitrile, shaken for 2 min and centrifuged at 1500 g for 5 min. After centrifugation, 20 μl of the supernatant was analyzed by HPLC. 0.5 ml of CSF was mixed with 3.0 ml of dichloroethane (DCE), shaken for 2 min and centrifuged at 1500 g for 5 min. After centrifugation, 2.4 ml of the DCE phase was transferred into a test tube and the DCE layer then evaporated. The residue was dissolved in 100 μl of mobile phase solution, and 20 μl analyzed by HPLC. The mobile phase for etoposide was a mixture of 65% methanol 35% phosphate buffer (pH 7.0) (45:55 v/v). The eluate was monitored at 282 nm for plasma concentration of VP-16 and at an applied oxidative potential of 700 mV with an electrochemical detector for CSF concentration of VP-16. The flow rate was 1.0 ml/min and the column temperature was 40°C. The limit of detection in plasma was 100 ng/ml and the coefficient of variation was 3.3% at 5 μg/ml (n=5). The limit of detection in CSF was 5 ng/ml and the coefficient of variation was 5.0% at 20 ng/ml (n=5).

Plasma and CSF concentrations of CBDDA were measured by the HPLC assay described by Gaver et al. 0.5 ml of plasma or 0.3 ml of CSF ultrafiltrate were obtained by centrifugation at 1500g for 30 min using an Amicon Centrifree™ MPS-1 microparticulation system (cutoff, 10000 Da, MA, U.S.A.). After centrifugation, 20 μl of the ultrafiltrate was analyzed by HPLC. The mobile phase for CBDDA was a mixture of acetonitrile in distilled water (92:8 v/v) and the eluate was monitored at 229 nm. The flow rate was 1.0 ml/min and the column temperature was 40°C. The calibration curve of CBDDA was linear up to 100 μg/ml. The limit of detection was 100 ng/ml. The within- and between-day coefficients of variation were less than 5%.

Pharmacokinetic Analysis  The pharmacokinetic parameters of VP-16 and CBDDA in plasma were estimated using a modified microcomputer program (MULTI(11)) with a model NEC PC-9801 microcomputer, as follows:

\[
C_p = A \cdot (\exp(-\alpha \cdot (t-t_1)) - \exp(-\alpha \cdot t)) + B \cdot (\exp(-\beta \cdot (t-t_1)) - \exp(-\beta \cdot t)) \quad (t \geq t_1)
\]

where \(C_p\) is the plasma concentration, \(t\) is the time after infusion, \(t_1\) is the infusion time, \(A\) and \(B\) are the biexponential equation constants, \(\alpha\) and \(\beta\) are the elimination rate constants of the first and second decay phase in the plasma. We established \(t_{1/2\alpha}\) (half-life in plasma of first-phase decay) as 0.693/\(\alpha\) and \(t_{1/2\beta}\) (half-life in plasma of second-phase decay) as 0.693/\(\beta\). The area under the time–concentration curve from 0 to 24 h after infusion in plasma (\(AUC_{0-24}\)) and CSF (\(AUC_{0-24}\)) for VP-16 and CBDDA were calculated using the trapezoidal rule.

RESULTS

The plasma concentrations of VP-16 and CBDDA peaked at the end of infusion, then decreased in a bi-exponential decay pattern during the remainder of the treatment period. Both VP-16 and CBDDA were detectable in CSF beginning 0.5 h after the initiation of each infusion. VP-16 then slowly increased for 24 h after infusion and CBDDA then slowly eliminated from the space left by tumor removal (Fig. 1). The mean maximum CSF concentration of VP-16 and CBDDA was 0.17 (at 24 h) and 15.25% (at 2 h) of plasma, respectively. However, 4 h after the end of infusion, the level of CBDDA in CSF exceeded that in plasma. The mean \(AUC_{0-24}\) of VP-16 and CBDDA in CSF was 1.91 and 113.6% of those in plasma, respectively. The mean half-life of VP-16 and CBDDA in plasma were 1.08 and 0.21 h for \(t_{1/2\alpha}\), respectively, and 16.41 and 4.68 h for \(t_{1/2\beta}\), respectively (Table 1).
The half-life of CBDCA in CSF ($t_{1/2,\text{CSF}}$) was 7.93 h but that of VP-16 could not be estimated because the concentration of VP-16 in CSF gradually increased until 24 h.

In two of eight patients, the patient's clinical response to HODBBB and intraarterial combination chemotherapy was partial response, according to magnetic resonance imaging and the other patients showed no change.

DISCUSSION

CDDP has marked anti-tumor effects and has been administered intraarterially to patients with malignant glioma. The response rate reportedly varies from 30% to 70%. The penetration of CBDCA, another platinum agent, into the CSF of children with brain tumors following intravenous administration of a high dose (600 mg/m²) of this drug has been reported, as has the complete remission of brain metastases of ovarian cancer following high-dose CBDCA administration. We reason that CBDCA penetrates into the CSF, and possesses similar anti-tumor effects to CDDP against malignant glioma. VP-16 has been shown to penetrate the BBB of rats. In addition, VP-16 administered intravenously demonstrated anti-tumor effects in patients with brain tumors in a clinical trial, with the intraarterial route showing no superiority over intravenous administration.

In our patients, CBDCA and VP-16 were detectable in CSF during HODBBB and intraarterial chemotherapy. However, CBDCA readily penetrated the CSF, whereas VP-16 penetrated the CSF with difficulty. Four hours after the end of infusion of CBDCA, the level of CBDCA in CSF exceeded that in plasma. The level of CBDCA in plasma decreased quickly after the end of infusion, while that in CSF increased after the initiation of infusion, and was maintained at a high level for at least 4 h. The level of VP-16 in CSF was always low as compared with the plasma level. However, it was assumed that these levels of VP-16 in CSF were higher than those after intravenous administration. We previously reported that in two glioma patients, the maximum CSF concentrations of VP-16 and CBDCA following HODBBB and intraarterial administration were twice as high as those obtained by intravenous administration when these drugs were given in equal doses to the same patient. The penetration of VP-16 into the CSF after intravenous administration to patients presenting with malignant brain tumors is minimal, with a very low concentration of $0-0.13$ μg/ml being observed in CSF following the standard dose of $<300$ mg/m². Furthermore, the VP-16 concentration in CSF remains low, ranging from 0.08 to 1.4 μg/ml, even when a higher dose, such as 300—2500 mg/m², is administered intravenously. Intrinsic and acquired multidrug resistance in many human cancers may be due to expression of the multidrug transporter P-glycoprotein. The endothelial cells of the central nervous system (CNS) express P-glycoprotein, which is thought to contribute to the BBB in the CNS. In addition, VP-16 is known to be a substrate for the P-glycoprotein transporter. Therefore, it may be predicted that VP-16 would not penetrate the CNS, as a reflection of P-glycoprotein expression in the CNS, the substrate specificity of VP-16 for P-glycoprotein, and the probable expression of P-glycoprotein in the patient's brain tumor. CBDCA appeared to readily penetrate the CSF, and was maintained at a higher level in CSF than CDDP. Thus, CBDCA may have an advantage over CDDP in treating malignant glioma because of the presence of a large quantity in CSF.

We reasoned that HODBBB with mannitol, and intraarterial administration of combination chemotherapy with VP-16 and CBDCA, may represent a useful approach to treating glioma because hyperosmotic infusions will increase drug delivery across the BBB, thereby exposing the tumor and surrounding brain tissue that is susceptible to invasion. However, this therapy may also increase the exposure of normal brain to these drugs. Thus, the dose of anticancer drugs injected may need to be reduced to avoid injury to normal brain when using HODBBB. Furthermore, studies of the synergistic effect of combined anticancer drugs during HODBBB and intraarterial administration of combination chemotherapy are required. We used combination chemotherapy with 60 mg/m² of VP-16 and 300 mg/m² of CBDCA in eight patients and we acquired positive responses in two patients. These dosages of anticancer drugs for the HODBBB and intraarterial combination chemotherapy may be useful in many patients with brain tumors for maintenance chemotherapy. The findings of the present study may help establish the optimal dose and schedule for administering a combination of VP-16 and CBDCA by HODBBB and the intraarterial route in the adjuvant treatment of patients with malignant glioma.

REFERENCES

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