Pharmacokinetics of Cytosine Arabinoside, Methotrexate, Nimustine and Valproic Acid in Cerebrospinal Fluid during Cerebrospinal Fluid Perfusion Chemotherapy

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This report investigates the pharmacokinetics of cytosine arabinoside (Ara-C), methotrexate (MTX), nimustine (ACNU) and valproic acid (VPA) in cerebrospinal fluid (CSF) during CSF perfusion chemotherapy. A 28-year-old Japanese woman with disseminated glioblastoma was, on admission, on a stable oral regimen of prolonged-release VPA tablets (DepakeneTM-R), 400 mg twice a day, for seizure control. Twelve courses of CSF perfusion chemotherapy with Ara-C, MTX, and ACNU were administered. Plasma samples and CSF samples via Ommaya reservoirs were obtained during the eleventh course of treatment. The Ara-C and ACNU concentrations were measured by HPLC. The MTX and VPA concentrations were measured by fluorescence polarization immunoassay.

During CSF perfusion chemotherapy, the highest CSF concentrations of Ara-C, MTX, and ACNU were observed at the end of the perfusion and decreased in a nonexponential pattern. The half-lives of Ara-C, MTX, and ACNU were 2.65, 3.52, and 0.71 h, respectively. No anticancer drugs were detectable in plasma during CSF perfusion chemotherapy. Before CSF perfusion chemotherapy, the free VPA concentration in plasma was 14.4% of the total VPA concentration. The mean total and free VPA concentrations in plasma were 78.0±0.8 and 10.9±0.3 μg/ml, respectively. The free VPA concentrations in plasma and in CSF were of similar values. At the end of perfusion, the lowest free VPA concentration in CSF was 30.3% of that at the initiation of perfusion. The free VPA concentrations in CSF at 3, 7, 23, and 47 h after the end of perfusion were 79.8, 94.5, 100.9, and 100.9%, respectively of that at the initiation of perfusion. During CSF perfusion chemotherapy, the ratio of free VPA concentrations to the total VPA in CSF was 86.3±6.9%. The VPA concentrations in CSF rapidly decreased during the CSF perfusion but recovered to pre-treatment levels within 7 h.

Key words cerebrospinal fluid perfusion chemotherapy; pharmacokinetics; valproic acid; cytosine arabinoside; methotrexate; nimustine

Clinical responses to valproic acid (VPA) appear to be poorly correlated with plasma drug concentration. While seizure control is generally achieved at plasma VPA concentrations exceeding 50 μg/ml (or 350 μmol/l), the minimum effective concentration varies considerably between patients. VPA is known to disperse quite rapidly into the central nervous system (CNS), specifically, the brain and cerebrospinal fluid (CSF). Shen et al. reported that in patients receiving chronic VPA therapy, VPA transmission between blood and brain was relatively low and more variable compared with the transmission of other commonly used anticonvulsants, such as carbamazepine, phenytoin, and phenobarbital, in spite of rapid VPA transmission into the CNS.23 Clarification of the transport mechanism in blood-CNS partitioning of VPA is essential for investigation of the causes of low and variable distribution of VPA into the CNS.

Recently, a pilot study was conducted (using cytosine arabinoside (Ara-C) and methotrexate (MTX)) to evaluate the efficacy and side effects of CSF perfusion chemotherapy in patients with meningial carcinomatosis.19 It has been reported that CSF perfusion chemotherapy is effective in improving cerebral, cranial nerve and spinal root signs and symptoms. In addition, we have reported on the pharmacokinetics of Ara-C, MTX, and nimustine (ACNU) in lumbar CSF during CSF perfusion chemotherapy in patients with disseminated brain tumors.25,6 Most patients undergoing perfusion chemotherapy receive anticonvulsants for seizure control, but during CSF perfusion chemotherapy the patient's CSF is replaced with artificial CSF for long periods (72 h4 or from 1 to 3 h30). If the patients were attacked by a convulsive seizure during CSF perfusion chemotherapy, they would be exposed to a dangerous situation. Even so, there have been few reports on the pharmacokinetics of the anticonvulsants, concomitantly administered for seizure control, during CSF perfusion chemotherapy. In the present study, we investigated the pharmacokinetics of Ara-C, MTX, ACNU, and VPA in CSF during CSF perfusion chemotherapy in a patient with a disseminated brain tumor.

MATERIALS AND METHODS

Chemicals Ara-C and ACNU were kindly supplied by Nippon Shinyaku (Kyoto, Japan) and Sankyo (Tokyo, Japan), respectively. Methanol and acetonitrile were of HPLC grade. All other chemicals were of analytical grade. Prolonged-release VPA tablets (DepakeneTM-R tablets, containing 200 mg of VPA), Ara-C (CyloicideTM injection), MTX (MethotrexateTM parenteral), and ACNU (NidranTM injection) were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), Nihon Shinyaku Co., Ltd. (Kyoto, Japan), Wyeth Lederle Japan, Ltd. (Tokyo, Japan), and Sankyo Co., Ltd. (Tokyo, Japan), respectively.

Patient A 28-year-old Japanese woman (164 cm, 45 kg) with disseminated glioblastoma, treated previously by partial surgical resection and radiation therapy, underwent twelve courses of CSF perfusion chemotherapy with intervals of at
least one week between each treatment. Prior to chemotherapy, she was receiving a stable oral regimen of prolonged-release VPA tablets (containing 200 mg of VPA), taking 400 mg twice a day.

**CSF Perfusion Protocol** The CSF perfusion solution was perfused from an indwelling tube (Ommaya reservoir) placed in the anterior horn of the lateral ventricle and drained from the tumor removal chamber of the temporal lobe. Informed written consent for the treatment was obtained from the patient. In the eleventh course of treatment, the CSF perfusion chemotherapy solution, consisting of 30 mg of Ara-C, 10 mg of MTX, and 10 mg of ACNU which was dissolved in 100 ml of artificial CSF (99.5 ml of lactated Ringer’s, 0.4 ml of 7% sodium bicarbonate, and 0.1 ml of 50% glucose) was perfused for 1 h immediately after the dissolution in artificial CSF. The patient clinically showed a partial response according to magnetic resonance imaging performed 4 months after treatment, and there were no complications related to treatment.

**Sampling** Blood samples (5.0 ml) were collected in heparin-containing tubes at baseline and at 30, 60, and 120 min after the initiation of perfusion. The samples were centrifuged and stored at -20°C until analysis, which was performed within 1 week. CSF samples were obtained, using an Ommaya reservoir, from the tumor removal chamber of the temporal lobe at 10 min intervals from 0 to 120 min, then at 150, 180, 240, and 480 min, and at 24 and 48 h after the initiation of perfusion. The CSF samples (2.0 ml) were collected in tubes, frozen immediately and stored at -20°C until analysis. The ultrafiltrates from plasma and CSF for free VPA analysis were obtained by centrifugation at 1500 g for 30 min using an Amicon Centriffree™ MPS-1 micropartition system (cutoff, 10000 Da, MA, U.S.A.).

**Measurements of Anticancer Drugs and VPA Concentrations** The concentrations of Ara-C and ACNU in plasma and CSF were measured by HPLC assay according to methods previously reported.5 The concentrations of MTX in CSF were measured by a fluorescence polarization immunoassay (FPIA, Tdx Abbott, North Chicago, IL) according to the method previously reported.5 The concentrations of total VPA and free VPA in plasma and CSF were measured by FPIA. The limit of detection was 0.7 μg/ml for total VPA and 0.1 μg/ml for free VPA. The standard curves were between 0.7 and 150 μg/ml for total VPA, and between 0.1 and 25.0 μg/ml for free VPA. The within and between-day coefficients of variation were less than 5%.

**Pharmacokinetic Analysis** Values for the pharmacokinetics of the anticancer drugs in CSF were estimated by a modified microcomputer program (MULTIT)7 with a microcomputer, NEC Model PC-9801, as follows,5

\[
C_{CSF} = 0 \quad (0 \leq t < t_l)
\]

\[
C_{CSF} = A(1 - \exp(-k(t - t_l))) \quad (t_l \leq t < t_p)
\]

\[
C_{CSF} = A(1 - \exp(-k(t - t_l))) + \exp(-k(t - t_p)) \quad (t_p \leq t)
\]

where \(C_{CSF}\) is the CSF concentration of the anticancer drug, \(A\) is the monoeponential equation constant, \(t_l\) is the lag time, \(t_p\) is the perfusion time, and \(k\) is the elimination rate constant.

Values for the pharmacokinetics of free VPA in CSF were estimated as follows,

\[
C_{CSF} = B \quad (0 \leq t < t_l)
\]

\[
C_{CSF} = B - C(1 - \exp(-k(t - t_l))) \quad (t_l \leq t < t_p)
\]

\[
C_{CSF} = B - C(1 - \exp(-k(t_l - t_p))) \exp(-k(t - t_p)) \quad (t_p \leq t)
\]

where \(C_{CSF}\) is the CSF concentration of free VPA, \(B\) is the concentration of free VPA at steady state, \(C\) is the monoeponential equation constant, \(t_l\) is the lag time, \(t_p\) is the perfusion time, and \(k\) is the recovery rate constant.

**RESULTS**

The CSF concentrations of anticancer drugs and VPA in the patient are shown in Figs. 1 and 2.

No anticancer drugs remaining in the CSF were detectable at the initiation of each perfusion because the interval between each treatment was at least 1 week. During CSF perfusion chemotherapy, the highest CSF concentrations of Ara-C, MTX, and ACNU were observed at the end of the perfusion and decreased in a monoeponential pattern. The half-lives of Ara-C, MTX, and ACNU were 2.65, 3.52, and 0.71 h, respectively. No anticancer drugs were detectable in plasma during CSF perfusion chemotherapy.

Before CSF perfusion chemotherapy, the total and free
VPA concentrations in plasma were 79.0 and 11.4 μg/ml, respectively (free VPA was 14.4% of the total VPA concentration). The mean total VPA concentrations in plasma at baseline and at 30, 60, and 120 min after the initiation of perfusion, were 79.0, 77.5, 78.1, and 77.2 (78.0±0.8 (mean±S.D.)) μg/ml, respectively. The mean free VPA concentrations in plasma at the same points in time were 11.4, 11.0, 10.7, and 10.6 (10.9±0.3 (mean±S.D.)) μg/ml, respectively. Before CSF perfusion chemotherapy, the total and free VPA concentrations in CSF were 15.2 and 10.9 mg/ml, respectively (free VPA was 71.7% of the total VPA concentration). The free VPA concentrations in plasma and in CSF were of similar values. At the end of perfusion, the lowest free VPA concentration observed in CSF was 3.3 μg/ml, which was 30.3% of that at the initiation of perfusion. The VPA concentrations in CSF at 3, 7, 23, and 47 h after the end of perfusion were 10.1, 12.0, 14.5, and 14.4 μg/ml, respectively. The VPA concentrations in CSF at 3, 7, 23, and 47 h after the end of perfusion were 8.7, 10.3, 11.0, and 11.0 μg/ml, respectively, which were 79.8, 94.5, 100.9, and 100.9%, respectively, that at the initiation of perfusion. During CSF perfusion chemotherapy, the ratio of free VPA concentrations to the total VPA in CSF was 86.3±6.9% (mean±S.D., n=19). The recovery rate constant (k) and the recovery half-life of free VPA in CSF were estimated at 0.48 h⁻¹ and 1.44 h, respectively.

DISCUSSION

While there are numerous reports of VPA concentrations in CSF from lumbar puncture studies, data on the distribution of VPA in human brain tissue is lacking. Vajda et al. reported biopsy data from a group of neurosurgical patients undergoing tumor resection. The observed brain concentrations of VPA were remarkably low and variable between patients, ranging from 6.8 to 27.8% of total plasma level. Shen et al. reported brain-to-serum concentration ratios varying from 5.2 to 22.4%, and the mean brain-to-serum ratio was 11.1±5.1%. In our patient, the results agree with the earlier observations of low VPA in CSF, and that of the free VPA concentrations in CSF being correlative with the free VPA concentrations in plasma. However the total VPA concentrations in CSF were always higher than the free VPA concentrations in CSF. The amount of protein contained in CSF is generally small (about 15 to 45 mg/dl), but that of our patient had increased (to about 1000 to 2000 mg/dl before the CSF perfusion chemotherapy) because of the dissemination of glioblastoma in the spinal canal. The difference in concentrations between total VPA and free VPA in CSF was from some of the VPA binding with the increased CSF protein. The process mediating the translocation of VPA between brain and blood appears not to follow the generally assumed concept of diffusional exchange of free drugs across the cerebral capillary endothelium. There is some evidence to suggest that movement of VPA into and out of CNS may involve specialized transport mechanisms. It is well known that ketone bodies and other endogenous fatty acids are transported across the blood-brain barrier by a so-called monocarboxylic acid carrier system. It is conceivable that the passage of a branched medium-chain fatty acid such as VPA across the brain capillary endothelium may also be facilitated by the monocarboxylic acid transporter. Cornford et al. reported on CNS elimination of VPA between blood and brain. Furthermore, it was noted that since brain-to-blood transport of VPA was greater than that of water, VPA efflux was presumed to occur via an active transport system. Although our patient's blood-brain barrier was locally destroyed by prior surgical resection and radiation therapy, the free VPA concentrations in CSF were of similar values to those in plasma before the eleventh course of CSF perfusion chemotherapy. Therefore, we speculated that the movement of VPA into and out of CSF could depend on such a carrier system, even though our patient's blood-brain barrier had been locally destroyed.

The treatment of disseminated malignant brain tumors generally entails intrathecal chemotherapy with MTX alone or in combination with Ara-C, together with local radiotherapy. Recently, CSF perfusion chemotherapy has been conducted to evaluate efficacy and side effects, and it has been reported that this treatment was effective in improving cerebral, cranial nerve, and spinal root signs and symptoms. In our patient, with CSF perfusion chemotherapy using a volume of 100 ml of perfusion solution and a 1 h duration time, the concentration profiles of anticancer drugs in CSF were in agreement with previous reports. Because a maximum or steady-state concentration of the anticancer drugs was achieved in CSF at the end of perfusion, we thought that the maximum amount of the drug had reached the CSF space. However, the concentration of ACNU in CSF had degraded after 1 h of perfusion, due to the extremely short half-life of ACNU. Therefore, when the perfusion solution of anticancer drugs includes ACNU, the duration of perfusion should be shortened to about 1 h. Although the CSF was replaced with artificial CSF during CSF perfusion chemotherapy, there have been few reports on the pharmacokinetics, during CSF perfusion chemotherapy, of anticonvulsants concomitantly administered for seizure control. In our patient, the lowest free VPA concentration in CSF was at the end of perfusion, being 30.3% of that at the initiation of perfusion. The free VPA concentrations in CSF rapidly decreased during CSF perfusion and recovered to the initial, pre-treatment levels within 7 h. Further, complications such as seizures were not experienced during treatment. It appeared that the rapid decrease and recovery of VPA concentrations in CSF during CSF perfusion chemotherapy did not affect the clinical response to VPA. We then speculated that the intra- cephalic concentration of VPA did not change according to the rapid change of VPA concentration in CSF, because the amount of VPA in the brain was larger than that in CSF, and the diffusion rate of VPA in the brain was slower than that in CSF.

The present case is the first to measure concentrations of VPA in CSF during CSF perfusion chemotherapy. Although these findings require confirmation in a larger patient population, our present results may be a useful guide for CSF perfusion chemotherapy in patients concomitantly administered anticonvulsant drugs such as VPA.

REFERENCES


