Plasma and cerebrospinal fluid pharmacokinetics of intravenous temozolomide in non-human primates

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Summary

Temozolomide is a prodrug that undergoes spontaneous chemical degradation at physiologic pH to form the highly reactive alkylating agent, methyl-triazenyl imidazole carboxamide (MTIC). In clinical trials, temozolomide has activity in gliomas and is approved for recurrent anaplastic astrocytoma. We, therefore, studied the penetration of temozolomide into the cerebrospinal fluid (CSF) as a surrogate for blood–brain barrier penetration in a non-human primate model. Three Rhesus monkeys with indwelling Ommaya reservoirs received 7.5 mg/kg (150 mg/m²) of temozolomide as a 1 h intravenous infusion. Frequent blood and CSF samples were obtained over 24 h, plasma was immediately separated by centrifugation at 4°C, and plasma and CSF samples were acidified with HCl. Temozolomide concentration in plasma and CSF was measured by reverse-phase high-pressure liquid chromatography. Plasma temozolomide concentration peaked 0.5 h after the end of the infusion and was 104 ± 3 µM. The mean peak CSF temozolomide concentration was 26 ± 4 µM at 2.5 h. The mean areas under the temozolomide concentration–time curves in plasma and CSF were 392 ± 18 and 126 ± 18 µM h, respectively, and the CSF : plasma ratio was 0.33 ± 0.06. Clearance of temozolomide was 0.116 ± 0.004 l/kg/h, and the volume of distribution at steady state was 0.254 ± 0.033 l/kg. In this non-human primate model, temozolomide penetrated readily across the blood–brain barrier. These findings are consistent with the activity of temozolomide in brain tumors.

Introduction

Temozolomide, which is a non-classical alkylating agent that has schedule-dependent antitumor activity in brain tumors, is approved for recurrent anaplastic astrocytoma [1–3]. Temozolomide is structurally related to dacarbazine, and both temozolomide and dacarbazine are prodrugs. Dacarbazine requires enzymatic N-demethylation in the liver to generate the reactive methylating species, 5-(3-methyl-triazen-1-yl) imidazole-4-carboxamide (MTIC); whereas temozolomide undergoes spontaneous chemical degradation at physiological pH to MTIC (Figure 1) [4]. MTIC forms methyl adducts at the N²-position of guanine, the N³-position of adenine and the O⁶-position of guanine. O⁶-methylguanine appears to be the critical cytotoxic lesion [5].

In clinical trials temozolomide was administered orally at doses of 150–200 mg/m²/day, daily for 5 days [6]. The absorption of oral temozolomide is complete [7]. Peak plasma concentrations ranged from 3 to 15 µg/ml (15–77 µM), and the terminal half-life was 1.6–1.8 h [8–10]. Although an intravenous formulation of temozolomide was not developed, an intravenous preparation in 3% dimethyl sulfoxide (DMSO) was administered to patients at doses of 50–200 mg/m² in the initial phase I trial [7]. The clearance (CL) and volume of distribution of temozolomide were 12 l/h and 28 l, respectively. Plasma concentrations of the active metabolite, MTIC, are considerably lower than concentrations of the parent drug. Exposure (as measured by the area under the concentration–time curve) to MTIC is only 1–2% of temozolomide exposure [11].

Because of the activity of temozolomide in brain tumors in clinical trials, we studied the central nervous system pharmacology of temozolomide in a non-human primate model to determine its ability to penetrate across the blood–brain barrier, using cerebrospinal fluid (CSF) penetration as a surrogate.
Materials and methods

Drugs
Temozolomide was supplied by the Pharmaceutical Resources Branch, National Cancer Institute, Bethesda, MD. The purity of the parent compound was 100%. A stock solution of temozolomide (30 mg/ml) was prepared in 100% DMSO, and then filtered through a 0.22 µm filter. Two milliliter aliquots were placed in sterile Eppendorf tubes and stored at −70°C. Immediately prior to intravenous infusion, the stock drug solution was thawed, diluted in normal saline to a final concentration of 1 mg/ml and filter sterilized again.

Monkeys
Three adult male Rhesus monkeys (Macaca mulatta) ranging in weight from 8.1 to 10.3 kg were used in these experiments. The animals were fed Purina Monkey Chow twice daily and were group housed in accordance with the Guide for the Care and Use of Laboratory Animals. Blood samples were drawn from a central venous catheter placed in either the femoral or the saphenous vein contralateral to the site of drug infusion. CSF samples were drawn from a subcutaneous Ommaya reservoir attached to an indwelling Pudenz catheter with its tip in the fourth ventricle [12].

Experiments
Each animal received 7.5 mg/kg temozolomide (adult human equivalent dose of 150 mg/m²) infused intravenously over 1 h. Blood samples were collected in heparinized tubes prior to temozolomide infusion, then at 30 min, at the end of infusion, and 5, 15, 30 min and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after the end of the infusion. Plasma was immediately separated by centrifugation at 4°C and acidified with 1 N HCl to give a final concentration of 0.1 N HCl. CSF samples were drawn prior to the infusion, then at the end of infusion, and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after the end of the infusion. The reservoir was pumped four times before and after each sample collection to ensure adequate mixing with ventricular CSF. The CSF samples were acidified as described above. All samples were frozen at −70°C until assayed. Temozolomide is stable under these conditions [13]. Blood counts, electrolyte and hepatic panels were obtained prior to and weekly for 4 weeks following drug administration to monitor for toxicity.

Sample analysis
Temozolomide concentrations in plasma and CSF were measured with a reverse-phase high-pressure liquid chromatography (HPLC) assay slightly modified from published methods. Plasma samples were thawed at room temperature. One milliliter of the acidified plasma was placed in a 15 ml glass tube and 8 ml of ethyl acetate was added. The sample was vigorously vortexed for 10–15 s and centrifuged at 2000 rpm at 4°C for 15 min. The organic phase was aspirated, placed in a 15 ml glass tube, and evaporated to dryness under nitrogen at 40°C for 45 min (TurboVap LX,