Central nervous system pharmacology of Baker's antifolate (NSC139105) in man\textsuperscript{1,3}

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Abstract

Radiolabelled Baker’s Antifolate (BAF) was administered to 6 patients undergoing surgical resection of intracerebral tumors. Levels of radioactivity in resected tumor and edematous brain adjacent to tumor were generally higher than levels in concurrent plasma samples and were generally comparable to levels in temporalis muscle. Levels in tumor cyst fluid were far lower than concurrent plasma levels and levels in surrounding tumor. Chromatography was performed on tumor from 2 patients and revealed that only a small proportion of the radioactivity represented unchanged BAF. The major metabolite present in tissues was 1 000 times less potent as an inhibitor of dihydrofolate reductase than was BAF.

Five patients had cerebrospinal fluid (CSF) sampled following administration of tracer doses of radiolabelled BAF. Radioactivity levels were far lower in CSF than in plasma. Levels of radioactivity in the CSF were also far lower than levels in tumor and brain samples from other patients and were slightly lower than tumor cyst fluid levels. Two patients had CSF collected after they received therapeutic doses of BAF. In these patients, both CSF and plasma were assayed using a dihydrofolate reductase inhibition assay. As with tracer dose studies, CSF concentrations of BAF were substantially lower than were concurrent plasma concentrations.

Thus it appears that only very low concentrations of BAF are attainable in human CSF and intracerebral tumor, although a metabolite which is a very weak inhibitor of dihydrofolate reductase attains high concentrations in tumor.

Introduction

Baker’s antifolate (BAF\textsuperscript{4}, triazinate, α-(2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazine-1 (2 H)-yl)phenoxy)-N,N-dimethyl-m-toluamide ethanesulfonate, NSC 139105) is one of a series of dihydrofolate reductase inhibitors synthesized by Baker (1). Unlike methotrexate, it is designed to enter cells by passive diffusion and accordingly does not require an active carrier mechanism for its transport. It is slightly lipid-soluble but the n-octanol-water partition coefficient is less than 1; thus, only modest penetration of the blood-brain and blood-CSF barriers would be expected. In dogs, CSF concentrations have been reported to be 15% of concurrent plasma concentrations following intravenous administration (2), although only very low concentra-

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\textsuperscript{3} The abbreviations used are:

- BAF: Baker’s Antifolate
- CNS: Central Nervous System
- CSF: Cerebrospinal Fluid

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tions have been noted in human CSF (3). Because of its acute central nervous system toxicity (3, 4) we felt that pharmacologically significant tissue concentrations might be attainable in human brain. In addition, it is known that the blood-brain barrier is disrupted to a variable extent within intracerebral tumors (5) and that lipid insoluble compounds of high molecular weight may attain high concentrations in brain tumors (6, 7). BAF is presently being investigated in the treatment of human astrocytomas (8). We performed a study designed to evaluate the penetration of BAF into human brain, intracerebral tumors, and cerebrospinal fluid (CSF).

Materials and methods

Drug administration, patient population, and sample collection

Radioactive (4,6 ¹⁴C) BAF was supplied by the Drug Development Branch of the National Cancer Institute. It had a specific activity of 6.5 millicuries per millimole and was 98% pure by radioautography. Informed consent was obtained from patients as required by institutional policy. Six patients received tracer doses of BAF (8–18 mg/m²) containing 200–400 μCi of labelled drug over 15–30 min shortly before surgical resection of intracerebral tumor. Four patients had glioblastoma multiforme, one had a meningeal sarcoma, and one had adenocarcinoma of the lung metastatic to the brain. Fluid was obtained from a cyst within the tumor of 2 patients. Removal of small amounts of edematous white matter of brain from the immediate environs of the tumor was performed in all 6 patients, permitting measurement of radioactivity in brain. Temporalis muscle samples were also analyzed. Generally, infusion of the drug was commenced on the opening of the dura. Timing of removal of samples was dictated by the ease of locating and resecting the tumor.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture from 1 patient after a tracer dose of 13 mg/m² of BAF labelled with 200 μCi of radioactive drug and by ventricular puncture after a tracer dose of 13 mg/m² in one patient undergoing surgical resection of intracerebral tumor. Three patients receiving intravenous tracer doses of BAF (10–13 mg/m²) had multiple CSF samples collected by puncture of Ommaya reservoirs implanted for the treatment of meningeal carcinomatosis. Blood samples were obtained concurrently by venipuncture.

Radiochemical assay

Tissue samples were prepared for analysis by adding 1.0 ml of the tissue solubilizer Soluene-350 (Packard, Downer Grove, IL) to each 100 mg portion of tissue. Samples were incubated at 60 ° for 75 minutes. The digests were allowed to cool to room temperature and 0.2 ml of isopropanol was added to each followed by hydrogen peroxide (0.2 ml of a 30% solution) to effect bleaching. The vials were swirled and allowed to stand at room temperature for 15 minutes and then heated to 40 ° for 15 min. Eleven ml of PCS (Amersham Corp, Arlington Heights, IL), a commercial xylene based scintillation counting solution, was added to each sample which was then counted in a Packard 2650 liquid scintillation spectrometer (Packer Instruments, Downer Grove, IL) for 2 min. Radioactivity of samples was automatically computed to disintegrations per min (dpm) by built in efficiency correlation curves. Recovery of radiochemical was 80–85%.

Dihydrofolate reductase inhibition assay of CSF samples

A single CSF sample was obtained from one patient and three CSF samples were obtained from a second patient receiving therapeutic doses of BAF (240–500 mg/m²). BAF was assayed by dihydrofolate reductase inhibition since these patients had not received radiolabelled drug.

Livers of Spray-Dawley rats were homogenized in 2 vol of ice-cold 0.25 M sucrose containing 0.001 M EDTA and 0.33 M Tris buffer, pH 8.0. The homogenate was centrifuged for 20 min. at 16 000 × g. The supernatant solution was again centrifuged for 1 h at 100 000 × g. The final supernatant was used as the source of dihydrofolate reductase. All of the above procedures were performed at 4 °.

The enzymatic assay used was similar to one described previously (9). The 0.5-ml assay mixture contained 0.04 μmoles NADP, 0.02 μmoles glucose-6-phosphate, 0.023 units glucose-6-phosphate