Cancer Chemother Pharmacol (1982) 8:251–252

Cancer Chemotherapy and Pharmacology

Short Communication

Razoxane Penetration Into the Cerebrospinal Fluid of Rats

Nigel Greig[1], Fu Xiao-Chang[2], and Kurt Hellmann[1]

[1] Cancer Chemotherapy Dept., Imperial Cancer Research Fund, London, W2, Great Britain
[2] Institute of Drug Control, Chengdu, Sichuan, China

Summary. *Razoxane (100 mg/kg) was administered to rats as a single IP dose. Plasma and CSF samples were obtained at intervals varying from 15 min to 24 h after dosing, and the razoxane was assayed by a new and simple high performance liquid chromatography (HPLC) technique.*

Razoxane was absorbed quickly from the peritoneal cavity; it reached a peak concentration within 0.5 h and decayed with a half-life of 1.6 h. Only 10% of the plasma razoxane available entered the CSF, reaching a peak concentration by 2 h, which was maintained for a further 4 h, and finally decaying to reach 10% of its peak value by 8 h.

Introduction

Razoxane [(±)1,2-bis-(3,5-dioxopiperazin-1-yl) propane], is one of the few antileukaemic agents to show activity against the intracerebral L1210 leukaemia in the mouse (B. Abbott, unpublished work). Although the effect is modest, it may be of value when leukaemic cells are sequestered in the brain. Razoxane has shown activity against acute lymphoblastic and myeloblastic leukaemia in children [1, 3, 4], and although it can induce complete remission, this is almost always followed by relapse within 6 months. It may be that insufficient razoxane reaches the reservoirs of leukaemic cells, especially those within the brain. It is not known to what degree razoxane penetrates the blood-brain barrier. Measurements of razoxane concentration in the cerebrospinal fluid (CSF) have been hampered by lack of a suitable analytical technique, but the recent development of a cold assay on HPLC (Fu X-C et al., unpublished work) has made it possible to analyse the pharmacokinetic behavior of this drug in the CSF.

Reprint requests should be addressed to K. Hellmann

Materials and Methods

Adult Sprague-Dawley rats weighing approximately 200 g received single IP injections of razoxane (100 mg/kg) in 0.5% CMC saline) and were anaethetized with sodium pentobarbital (40 mg/kg). At 0.25, 0.5, 1, 2, 4, 6, 8, 16, and 24 h after razoxane administration, blood and CSF were taken and the animals killed.

Blood was withdrawn by cardiac puncture, placed in a heparinized vial, and centrifuged at 7,000 *g* for 5 min at 4° C. The plasma was removed and placed in solid CO_2. CSF was obtained by percutaneous sampling from the cisterna magna. The animal was clamped into a stereotaxic instrument and a lateral midline incision was made, exposing the muscles of the head and neck. The lateral cervical muscles (clavotrapezeus, sternomastoideus, and cleidomastoideus) and the deeper anterior vertebral muscles (Longus colli, Longus capitis, and Rectus capitis) were cut transversely and retracted to expose the occipital-atlanto membrane. This membrane was pierced with a 25 gauge needle and clear CSF (approximately 100 µl) was withdrawn from the cisterna magna into a Hamilton syringe and placed in solid CO_2 prior to analysis. The complete procedure took approximately 2 min.

Razoxane Analysis. Plasma samples were filtered prior to HPLC analysis in Chemlab Ultrafiltration cells (Model C10) through UM05 Diaflow membranes (mol. wt. 500) at 25–30 psi nitrogen pressure. Plasma razoxane (mol. wt. 268) passed freely across the membrane. CSF contains minimal protein and required no ultrafiltration.

HPLC separation was carried out on a Waters Assoc. liquid chromatograph (Waters Assoc., Milford, Mass., USA), consisting of two Model 6000A solvent pumps, a Model 660 programmer, a Model U6K injector, and two LKB Bromma 2138 UV detectors. Signals from the detectors were recorded by a LKB Bromma 2210 two-channel recorder. All separations were accomplished on a Varian MicroPak MCH-10 C18 reverse-phase column (4 mm × 30 cm) with an ODS guard column.

Samples were eluted in 20% methanol in 0.01 *M* phosphate buffer, pH 7.1 (helium-degassed) at 1,400 psi pressure and a flow rate of 1.0 ml/min. The absorbances of the eluate at 206 nm and 278 nm were recorded simultaneously (chart speed 10 mm/min).

Standard curves were constructed for CSF and control plasmas from each Diaflow membrane (with a minimum of five points). Quantitation was achieved by measuring peak heights.

0344-5704/82/0008/0251/$ 01.00

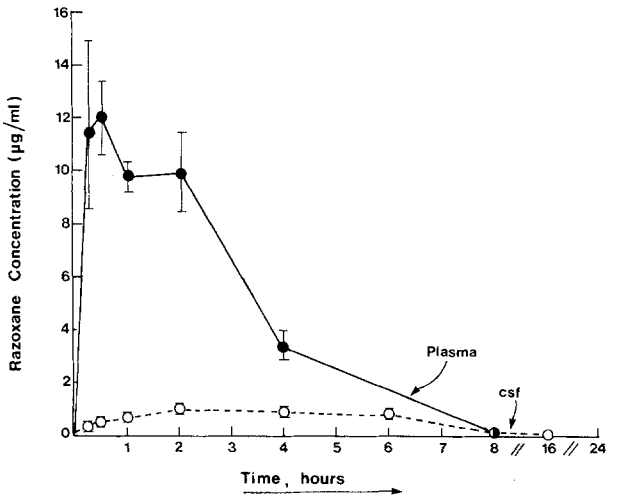

Fig. 1. CSF and plasma levels of razoxane (mean + SEM) in rats following IP administration of 100 mg razoxane/kg body weight in 0.05% CMC

Results

The razoxane retention time was 6.5 min measured at 206 nm absorbance, the detection limit being 0.1 µg/ml.

No razoxane was detectable in either blood or CSF at time zero (before razoxane administration). Figure 1 shows the razoxane plasma and CSF concentration in rats between 0.25 and 24 h following IP administration. Three animals were killed at each of time points 0.25, 0.5 and 1 h, four at 2 h, and two at 4, 6, 8, 16, and 24 h.

Razoxane was absorbed quickly from the peritoneal cavity, reaching high plasma concentrations within 15 min and a peak concentration of 12.0 µg/ml by 30 min. Razoxane levels then declined mono-exponentially according to first-order kinetics. The half-life was approximately equal to 1.6 h, with a decay constant of 0.43/h; complete elimination occurred by 8 h. CSF razoxane levels rose more slowly and reached a peak of 0.975 µg/ml by 2 h with a $t^1/_2$ influx of 1.1 h, by which time approximately 40% of the plasma concentration had been eliminated. The CSF/plasma ratio at the time of the peak CSF value was 0.1, and the peak CSF/plasma ratio (at 2 h CSF and 30 min plasma) was 0.08. The CSF razoxane concentration fell slowly. During the first 6 h razoxane concentrations fell imperceptibly from 0.975 to 0.855 µg/ml, and then fell more quickly between 6 and 8 h. No razoxane was detectable at 16 h.

Discussion

After IP administration razoxane was clearly found to enter the CSF. Not surprisingly, the concentrations reached were much lower than the corresponding plasma levels, because razoxane, a relatively non-polar substance with poor aqueous solubility, is minimally lipophilic with an octanol/water partition coefficient of log P −1.85 [2]. A drug with such a low partition coefficient, even with a small molecular weight (268), would be expected to show low brain capillary permeability [5].

Only free, unbound, razoxane was measured in the plasma after ultrafiltration. It is probable that most plasma razoxane is not protein-bound (though acylation is a possibility), but only 10% of the free plasma razoxane available entered the CSF. Nonetheless, CSF razoxane concentrations, although low, were maintained for 6 h after administration, even though plasma levels started to decline after 30 min. CSF penetration of ICRF-187, the *d*-isomer of the racemic razoxane, has also been shown to be limited to 10% of the (bound and unbound) plasma concentration (in the rhesus monkey), even after maintenance of high plasma levels with 2-h IV infusions of ICRF-187 [6].

This study suggests that razoxane and its analogs are unlikely to be effective in the treatment of malignant disease protected by an intact blood-brain barrier.

References

1. Bakowski MT, Prentice HG, Lister T (1979) Limited activity of ICRF-159 in advanced acute leukemia. Cancer Treat Rep 63: 127
2. Creighton A, Jeffery W, Long J (1978) Bisdioxopiperazines. Proc. 6th Int. Symp. Med. Chem. Cotswolds Press, Oxford, p 281
3. Hellmann K, Newton KA, Whitmore DN, Hanham IW, Bond JV (1969) Preliminary clinical assessment of ICRF159 in acute leukaemia and lymphosarcoma. Nature 222: 384
4. Krepler P, Pawlowsky J (1975) Clinical trials with bis-dioxopiperazine propane (ICRF 159; NSC 129,943) in acute leukemias. Oesterreichische Zeitschrift für Onkologie 2: 112
5. Levin VA (1980) Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. J Med Chem 23: 682
6. Von Hoff DD (1980) Pharmacokinetics of ICRF-187 in the cerebrospinal fluid of subhuman primates. Cancer Treat Rep 64: 734

Received January 25/Accepted February 3, 1982