Enhancement of Ketoconazole Penetration across the Blood-Brain Barrier of Mice by Dimethyl Sulfoxide

PETER C. IWEN and NORMAN G. MILLER*

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68105

Received 9 September 1985/Accepted 10 July 1986

Mice were treated with ketoconazole with and without dimethyl sulfoxide. Concentrations of ketoconazole at 3 and 5 h after treatment were significantly higher in serum (P < 0.05) and brain tissue homogenate (P < 0.01) of mice treated with dimethyl sulfoxide than in those of mice not treated with dimethyl sulfoxide. Differences observed in the concentrations of KTZ in serum and BTH between DMSO-treated and untreated mice were analyzed by using the Wilcoxon signed-rank test (6). A P value of >0.05 was not considered significant.

To determine whether DMSO interfered with the antifungal activity of KTZ in the bioassay, various concentrations of KTZ and DMSO were combined and added to the serum and BTH. Identical concentrations of only KTZ in the serum and BTH were assayed at the same time. No significant differences were seen in the diameters of zones of inhibition with comparable concentrations of KTZ with and without DMSO.

The results showed that the concentrations of KTZ in the BTH and serum of DMSO-treated mice were consistently higher when compared with those of mice not treated with DMSO at each time interval (Table 1). The highest concentration of KTZ in both the BTH (11.8 μg/g) and serum (46.6 μg/ml) was achieved at 3 h after treatment of mice receiving DMSO. This is a statistically significant increase over the concentration of KTZ at 3 h in BTH (P < 0.01) and serum (P < 0.05) in mice not treated with DMSO. Although the concentration of KTZ decreased at 5 h whether DMSO was given or not, mice given DMSO still had a significantly higher concentration of KTZ in both BTH (P < 0.01) and serum (P < 0.01) than did mice not receiving DMSO.

A 9.8% mortality rate as a result of toxicity was observed in DMSO-treated mice, with no difference seen between KTZ-treated and untreated mice. No mice died that were treated only with KTZ. Immediately after intravenous inoculation with DMSO, a temporary paralysis of the hind legs was observed. Hematuria began on about day 2 of treatment but disappeared after DMSO treatment was discontinued. The cause of death was usually seizures that developed immediately following intravenous inoculation.

Of particular interest was the high concentration of KTZ maintained in the serum (12.9 μg/ml) and BTH (6.7 μg/g) as long as 5 h after treatment with KTZ and DMSO. The concentration of KTZ in BTH exceeds the MIC required for in vitro inhibition of fungi such as Cryptococcus neoformans which commonly invade the brain. It might appear that the higher concentrations of KTZ in BTH in DMSO-treated mice reflects the higher concentration of KTZ in the serum. However, the insignificant amount of blood (0.1 μg/g of tissue) present in BTH suggests that increased permeability of the BBB may be important in increasing the concentration of KTZ.

Attempts to penetrate the BBB with a variety of compounds by using DMSO have met with varied levels of success (2, 9, 15, 17). Broadwell et al. (4) showed that

Ketoconazole (KTZ), a relatively nontoxic and easily administered antifungal drug, has been shown to be effective for the treatment of a variety of systemic fungal infections (11, 18); however, it does not readily penetrate the blood-brain barrier (BBB) (1, 8). It has been shown experimentally that nontoxic concentrations of dimethyl sulfoxide (DMSO) have opened the BBB to a variety of compounds (3, 4). The purpose of this study was to determine whether DMSO enhances the penetration of KTZ across the BBB so that effective therapeutic concentrations of this antifungal drug can be maintained in the brain.

This study was presented at the 86th Annual Meeting of the American Society for Microbiology, 23 to 28 March 1986, Washington, D.C.

Eight-week-old CFW mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were treated by gavage at 8:30 a.m. and 4:00 p.m. for 3 days with 120 mg of KTZ (Janssen Pharmaceutica, New Brunswick, N.J.) per kg suspended in 0.3% Noble agar (Difco Laboratories, Detroit, Mich.). Mice treated with DMSO were given a 15% concentration of DMSO (Sigma Chemical Co., St. Louis, Mo.) intraperitoneally (0.5 ml) 30 min before treatment with KTZ and intravenously (0.25 ml) immediately after treatment with KTZ (4).

For each experiment, 30 mice were treated with KTZ. Three groups of five mice received DMSO, and three groups of five mice received 0.85% NaCl only. As a control, 15 mice were treated with DMSO and 0.3% Noble agar without KTZ. This experiment was repeated three times. At 1, 3, and 5 h after treatment, mice were bled from the orbital sinus and killed by cervical dislocation. The brains were removed and washed four times in saline to remove surface blood. The sera and brains from each group of mice were pooled and stored at −70°C until assayed.

Before the assay for KTZ, the sera and brain tissue were thawed. The brain tissue was homogenized with 0.85% NaCl by the method of Craven et al. (7). Concentrations of KTZ in serum and brain tissue homogenate (BTH) were determined by a bioassay method of Jorgensen et al. (13) using a strain of Candida parapsilosis isolated from a patient at the University of Nebraska Medical Center. A standard curve was prepared for each bioassay by using 1.25 to 20 μg of KTZ per g of brain tissue and up to 40 μg of KTZ per ml of serum. The concentrations of KTZ in serum and BTH were determined from the standard curves. The amount of blood within the brain was determined by the method of Lowry and Hastings (14).

* Corresponding author.
compounds such as horseradish peroxidase with a mass of less than 70,000 daltons readily penetrated the BBB; however, Greig et al. (10) could not duplicate those results. Ionization, lipid solubility, protein binding, size, and steric complexity along with the weight of compounds may have an effect on penetration of the BBB (16).

Because uninfected animals were used in this study, the antifungal effect of KTZ after enhancement with DMSO in an infected host is not known. It is possible that the pharmacokinetics of KTZ is altered by DMSO, causing it to be inactive against fungi in the host, although this was not seen in vitro. Also, the toxicity of the 15% concentration of DMSO must be addressed, since this would not be acceptable for human use. Because 10% concentrations of DMSO reduced toxicity considerably in experimental animals (5), it will be interesting to determine whether a 10% DMSO concentration in combination with KTZ will result in a significant survival rate in a murine model of cryptococcosis (12).

**LITERATURE CITED**


