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Effective Ribavirin Concentration in Hamster Brains for Antiviral Chemotherapy for Subacute Sclerosing Panencephalitis

TAKANORI ISHII,[1] MITSUAKI HOSOYA,[1]* SHUICHI MORI,[2] SHIRO SHIGETA,[2] AND HITOSHI SUZUKI[1]

Departments of Pediatrics[1] and Microbiology,[2] Fukushima Medical College, Fukushima 960-12, Japan

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The ribavirin concentration in hamster brains was measured by a high-performance liquid chromatography (HPLC) system and a bioassay system. When ribavirin was administered intracranially at a dosage of 10 mg/kg of body weight per day for 10 days, a dosage which results in 100% survival of hamsters infected with subacute sclerosing panencephalitis (SSPE) virus and which inhibits the replication of SSPE virus in hamster brains, the ribavirin concentration in the brains estimated by HPLC and bioassay was kept higher than 50 μg/g for 10 days. The effective concentration in vivo corresponds to the concentration at which ribavirin completely inhibits the replication of SSPE virus in vitro. The maximal tolerable ribavirin concentration for hamsters was calculated to be 150 μg/g. Although ribavirin shows toxicity to the animals at a relatively low concentration (250 to 400 μg/g), intrathecal or intraventricular administration of ribavirin should be explored for potential use in the treatment of patients with SSPE, while the ribavirin concentration in cerebrospinal fluid or brain tissue should be monitored.

Subacute sclerosing panencephalitis (SSPE) is a progressive and fatal central nervous system disorder that results from a persistent measles (SSPE) virus infection. SSPE virus strains are genetically altered from measles virus, particularly in the viral genes coding for the envelope proteins. The alteration appears to be important in the pathogenesis of the persistent central nervous system infection that yields the syndrome of SSPE. Several compounds, including inosiplex (4, 10) and interferon (11, 13), have been claimed to prolong the lives of patients with SSPE, but definitive evidence of their efficacies is lacking. We examined a wide variety of antiviral compounds for their inhibitory effects on measles and SSPE virus strains in vitro and found that several nucleoside analogs, including ribavirin, inhibited the replication of various SSPE virus strains (3, 8). SSPE virus can replicate in hamster brains (6) and causes an encephalitis which results in hyperirritability, myoclonus, convulsion, and then death (6, 14). Similar signs are observed in patients with SSPE (9). We also examined ribavirin for its anti-SSPE virus activity in the hamster SSPE model (6). Ribavirin did not improve the survival of infected hamsters when it was administered intraperitoneally at the maximal nonlethal dosage of 50 mg/kg of body weight per day for 10 days, but it did improve their survival when it was administered intracranially, and this improvement occurred in a dose-dependent manner. The administration of ribavirin at a dosage of 10 mg/kg/day for 10 days completely prevented mortality and inhibited the replication of SSPE virus in the brains of infected hamsters. In the present study, we evaluated the effective and tolerable concentrations of ribavirin in hamster brains by using a high-performance liquid chromatography (HPLC) system and a bioassay system.

Ribavirin [1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide] was provided by Yamasa-Shoyu Co., Chiba, Japan. Golden Syrian hamsters (age, 3 weeks; females) were used for these experiments. Under ether anesthesia, 50 μl of ribavirin solution at dosages of 5, 10, and 20 mg/kg/day was injected for 10 days intracranially to a depth of 2 mm by using a 27-gauge needle and was placed within the subarachnoid space. At 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were sacrificed. The brains were aseptically removed, washed twice with phosphate-buffered saline (PBS), homogenized, and suspended in PBS. The suspension was centrifuged at 1,600 × *g* for 10 min. The supernatant was collected, ethanol was added to remove proteins, and the mixture was heated at 90°C to evaporate the ethanol. The protein-free samples were used to evaluate the effective ribavirin concentration in brain tissue by HPLC and bioassay. To determine the toxic concentration of ribavirin in hamster brains, a dose of 10 mg of ribavirin per kg was injected every 3 or 6 h (total daily dose of 80 or 40 mg/kg, respectively). When a hamster died, its brain was removed to measure the drug concentration. The brains were treated in the same way in which they were treated when we evaluated the effective concentration. The protein-free samples were suspended in 0.025 M phosphate buffer with 2% acetonitrile (pH 2.5) and were centrifuged at 8,300 × *g* for 5 min. Ten microliters of the supernatant was loaded onto a reverse-phase column (TSKgel ODS-120T column; Tosoh, Tokyo, Japan) and was eluted with the same buffer. The flow rate was 1.0 ml/min. The A_{226} was measured with a UV detector. Ribavirin revealed its absorbance peak to be at 280 s after loading. A smaller absorbance peak (13.2 ± 0.69 mm) was also observed in PBS-treated control hamster brain samples 280 s after loading. The optical density of ribavirin in the sample was defined as the value remaining after subtracting the average peak value of the PBS-treated control samples from the peak value of the ribavirin-treated sample. The ribavirin concentration of a sample was estimated from the standard curve of the optical density-ribavirin concentration. The correlation coefficient of optical density to ribavirin concentration was 0.999 in the range of 0 to 500 μg/ml. The lower limit of detection for the assay was 10 μg/g. To confirm whether the samples contained ribavirin in an active form, we also measured the anti-influenza virus activities of the samples. MDCK cells were grown in a 96-well microtiter tray. Each well was inoculated with 100 μl of an influenza virus

* Corresponding author. Mailing address: Department of Pediatrics, Fukushima Medical College, Hikarigaoka 1, Fukushima 960-12, Japan. Phone: (81) 245-48-2111. Fax: (81) 245-48-6578.

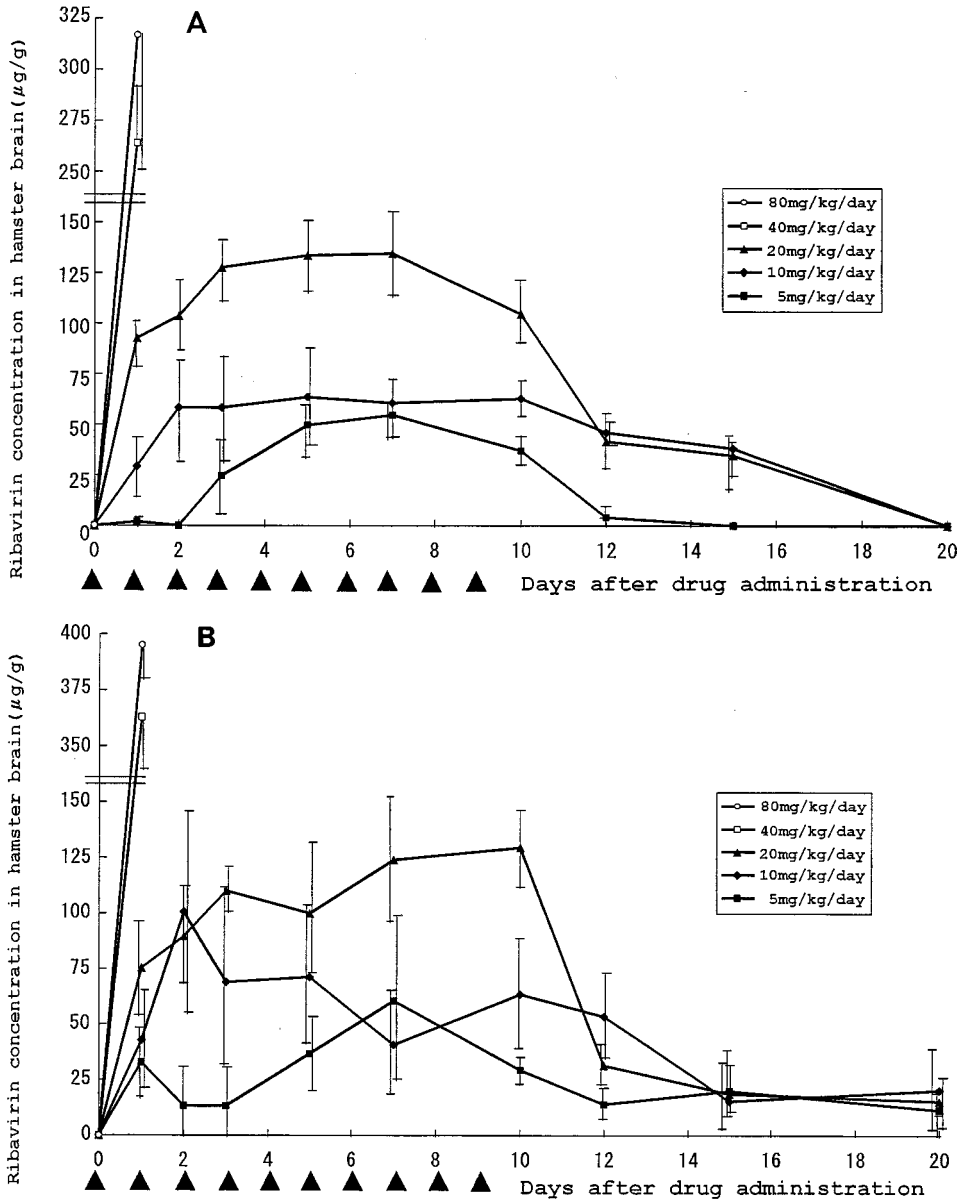

FIG. 1. Evaluation of ribavirin concentration in hamster brains. Hamsters were given 50 μl of ribavirin solution by intracranial injection at dosages of 5, 10, and 20 mg/kg/day for 10 days. At 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were sacrificed, and their brains were removed aseptically to measure the effective ribavirin concentration by HPLC (A) and bioassay (B). Ribavirin solution at a dose of 10 mg/kg was injected every 3 or 6 h (total daily dose, 80 or 40 mg/kg, respectively). Each hamster's brain was removed aseptically when the hamster died to measure the toxic ribavirin concentration by HPLC (A) and bioassay (B). The arrowheads on the *x* axes indicate the times of intracranial drug administration.

type B (strain Singapore) suspension containing 100 times the 50% tissue culture infectious dose and either 100 μl of the protein-free sample suspended in a maintenance medium or serial dilutions of ribavirin. After 5 days of incubation at 35°C, the viable cells were measured by modifications of a tetrazolium-based method (7). The antiviral activities of the samples were evaluated by their ability to protect the cells from virus-induced destruction and were converted into the corresponding ribavirin concentrations. The correlation coefficient of cell viability to ribavirin concentration was 0.932 in the range of 2.0 to 20 μg/ml. The lower limit of detection for the assay was 18 μg/g.

When ribavirin was administered intracranially at a dosage of 5 mg/kg/day for 10 days, a dosage which improves the survival of infected hamsters by 80%, ribavirin in the brain tissue was detectable by HPLC from days 3 to 10 after initial administration. The peak concentration was about 50 μg/g (Fig. 1A). When ribavirin was administered at 10 mg/kg/day for 10 days, a dosage which results in 100% survival of infected hamsters and inhibits completely the replication of SSPE virus in brains, the concentration of ribavirin was kept higher than 50 μg/g for 10 days (Fig. 1A). By the administration of ribavirin at a dosage of 20 mg/kg/day, which is the maximal tolerable dose for hamsters, the ribavirin concentration was maintained at 100 to 150 μg/g for 8 days (Fig. 1A). Hamsters administered ribavirin at a dose of 10 mg/kg every 3 or 6 h died after three or four doses. The toxic concentration measured by HPLC was 250 to 350 μg/g. Similar results were obtained by a bioassay of the

ribavirin concentration (Fig. 1B). When ribavirin was administered at a dosage of 10 mg/kg/day, the concentration of ribavirin was maintained at 40 to 100 μg/g for 10 days. The toxic concentration was found to be 300 to 400 μg/g.

We reported previously that ribavirin inhibits the replication of various strains of measles and SSPE viruses in cell culture (8). Virus replication was reduced to 50% of that of the control at a ribavirin concentration of 8 μg/ml and was completely inhibited at 50 μg/ml. Ribavirin was claimed to be effective for the treatment of naturally occurring uncomplicated measles virus infection (1). However, oral ribavirin therapy for patients with SSPE was reported to be ineffective. The maximal concentrations achieved in the cerebrospinal fluid of those patients (0.8 to 2.5 μg/ml) were below the concentrations that inhibit SSPE virus strains in vitro (12), although ribavirin seemed to cross the blood-brain barrier (2). Recently, we found that intraperitoneal administration of ribavirin slightly extended the survival period of infected hamsters but that it did not improve the survival rate even if the maximal nonlethal dose was administered (6). The ribavirin concentration in the brain when it was administered intraperitoneally was below the level detectable by HPLC (<10 μg/g). In contrast, ribavirin administered intracranially decreased the signs of disease and improved the survival rate in a dose-dependent manner. In particular, hamsters administered ribavirin at a dosage of 10 mg/kg/day for 10 days had 100% survival and no infectious virus was detectable (6). These results strongly suggest that the concentration of ribavirin in brain tissue correlates well with its curative effect for hamsters infected with SSPE virus. The ribavirin concentration in the brain of hamsters administered the drug at a dosage of 10 mg/kg/day was kept higher than 50 μg/g, that is, a concentration at which ribavirin completely inhibits the replication of SSPE virus in vitro. Therefore, ribavirin should be further pursued for its potential use in the therapy of patients with SSPE by intrathecal or intraventricular, or possibly aerosol (5), administration, but the ribavirin concentration in cerebrospinal fluid or brain tissue should be strictly monitored, since ribavirin shows toxicity to hamsters when it is used at a concentration five to eight times higher than the effective antiviral concentration.

The experiments described here were reviewed and approved by the Animal Research Committee of Fukushima Medical College in accordance with The Guideline on Animal Experiments in Fukushima Medical College, Japanese Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6).

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