Evaluation of Lithium as an Inhibitory Agent of Herpes Simplex Virus in Cell Cultures and During Reactivation of Latent Infection in Rabbits

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Lithium carbonate inhibited plaque formation of herpes simplex virus types 1 and 2 in rabbit kidney and Vero cells (50% effective dose, 435.5 to 490 μg/ml). Plasma lithium levels of 67 to 134 μg/ml were achieved by oral therapy in rabbits. However, neither ocular virus shedding nor virus-positive trigeminal ganglia were reduced after intentional reactivation of latent herpes simplex virus infection.

The remission of recurrent herpes simplex virus (HSV) infection has been reported in humans during systemic use of lithium (4, 5). Also, Skinner et al. have reported that lithium inhibits the replication of HSV type 1 (HSV-1), HSV-2, pseudorabies, and vaccinia at concentrations which permit host cell growth (9). The purpose of this communication is to report on the activity of lithium carbonate against HSV-1 and HSV-2 in cell cultures as determined by plaque reduction tests and its capacity to block intentional reactivation of latent HSV-1 infections in rabbits.

Confluent monolayers of secondary rabbit kidney (RK) and Vero cells were inoculated with 100 PFU of virus and allowed to adsorb for 1 h at room temperature. The virus inoculum was removed, and the cell monolayers were overlaid with 2% methylcellulose medium containing a threefold serial dilution of the lithium (11). After incubation for 72 h at 37°C in a humidified 5% CO2 atmosphere, the medium was discarded. Cells were fixed with methanol-acetic acid (3:1) and stained with 0.5% crystal violet. The number of plaques formed in the presence and absence of the drug was determined and plotted as a percentage of the control. The theoretical concentration of the drug which would give a 50% plaque reduction (i.e., 50% effective dose) was determined in this manner for two strains each of HSV-1 and HSV-2 (McKrae, RE6, HG52, and 333, respectively). The 50% effective doses varied depending on the virus strain and host cell. The 50% effective dose of lithium carbonate for HSV-1 (McKrae) was 435.5 and 187.6 μg/ml in RK and Vero cells, respectively, and against HSV-1 (RE6), 361.8 and 187.6 μg/ml, respectively. The 50% effective dose of lithium carbonate for HSV-2 (HG52) was 187.6 and 234.5 μg/ml, respectively, and against HSV-2 (333), 469.0 and 187.6 μg/ml, respectively. This antiviral activity is similar to that reported for lithium chloride in baby hamster kidney (BHK) cells (9). Lithium had a toxic effect on both RK and Vero cells at 2.68 mg/ml, although lithium carbonate was more toxic for RK and Vero cells than lithium chloride was for BHK cells. Toxicity determinations were based on inhibition of cell replication and the observation of abnormal morphology by microscopic examination.

New Zealand White male rabbits (2 to 3 kg) were inoculated with 108 PFU of HSV-1 (McKrae) per eye without scarification. Infection was confirmed by virus isolation from the tear films on day 3 postinoculation as previously described (10) or by rinsing the cornea and lower cul de sac with 0.2 ml of physiological saline containing penicillin (200 U/ml), streptomycin (200 μg/ml), and gentamicin (100 μg/ml). These ocular specimens were inoculated onto RK cell culture monolayers and incubated at 37°C in a 5% CO2 atmosphere for 7 days.

From days 28 to 63, nine rabbits with confirmed ocular infections received lithium carbonate in drinking water at a concentration of 1 to 5 mg/ml (J. T. Baker Chemical Co., Phillipsburg, N.J.). The concentration was adjusted to achieve a lithium level in plasma of approximately 67 μg/ml. Lithium concentrations in plasma, blood, cerebrospinal fluid, and brain tissue were measured by atomic absorption spectrophotometry (3). Plasma samples were collected and lithium determinations were made weekly throughout the study. The mean lithium concentration in plasma ranged from approximately 67 to 134 μg/ml during the period of therapy, whereas the lithium concentration in cerebrospinal fluid was 4.02 to 16.75 μg/ml.

During oral lithium therapy but before intentional stimulation to induce virus shedding, only one spontaneous occurrence of ocular HSV shedding was detected. During the same time period no spontaneous occurrences were observed in the three rabbits (no. 12 to 14) not on lithium therapy. On day 58 after inoculation, an attempt was made to intentionally reactivate the latent HSV infection by the method of Shimomura et al. (8). Briefly, iotophoresis of 6-hydroxydopamine was performed bilaterally on rabbit corneas, and this was followed by topical treatment with 2% epinephrine. Four of six eyes from stimulated latently infected control rabbits (no. 12 to 14), i.e., not treated with lithium, shed HSV in the tear films (Table 1). Oral lithium therapy did not appear to significantly reduce (P = 1.0) the frequency of induced reactivation in experimental animals (no. 1 to 11) in that 12 of 18 eyes were HSV-1 positive after iotophoresis. After day 68 postinoculation, all rabbits were sacrificed, and the trigeminal ganglia were removed as previously described (11). Seven of eight lithium-treated rabbits had at least one HSV-1-positive trigeminal ganglion. Rabbit no. 6 died before the trigeminal ganglia were studied. Two of three control rabbits not on lithium therapy had at
least one HSV-1-positive trigeminal ganglion. Again, no statistically significant differences ($P = 0.5$) could be demonstrated between experimental and control animals.

Our interest in lithium as an antiviral agent was enhanced when we confirmed in vitro findings that lithium inhibits both HSV-1 and HSV-2 (9). Since lithium is known to accumulate in the cerebrospinal fluid and brain (2) and remission of recurrent HSV-1 infections during systemic lithium treatment has been reported for humans (4, 5), it was felt that lithium had potential to be useful as an inhibitory agent of HSV. However, the frequency at which HSV-1 was shed in the tears by stimulated latently infected rabbits was apparently not affected by a lithium concentration in plasma of 67 to 134 $\mu$g/ml, which is approximately the desired therapeutic level in humans (7). We had hoped that a beneficial therapeutic response might be demonstrated by showing a decrease in the number of episodes of induced reactivations; however, this was not the case. The clinical usefulness of lithium as an agent to prevent HSV-1 shedding seems doubtful when to these negative results one adds the numerous side effects and large individual variations in the transport of this agent (1, 6).

**LITERATURE CITED**