Cold Stress-Induced Recurrences of Herpetic Keratitis in the Squirrel Monkey

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Purpose. Models of recurrent herpetic keratitis that depend on tissue damage or immunosuppression have been described. The authors report a model that depends only on minimal temperature stress to produce clinical recurrences in a small primate.

Methods. Squirrel monkeys (Saimiri sciureus) infected with the ocular route with the Rodanus strain of herpesvirus type 1 (HSV-1) were exposed to temperatures approximately 5°C lower than the usual ambient temperature for periods as short as 12 hours.

Results. The corneas showed more or larger lesions typical of recurrent herpetic keratitis than are usually seen in these animals under normal conditions. Statistical analysis showed that there were significantly higher frequencies of epithelial keratitis at 18°C and 20°C (P < 0.0001).

Conclusions. A minimal temperature change produced significant recurrences in this small animal within a short time. Tissues were not damaged to produce the recurrences. This approach may provide an efficient primate model for rapid testing of drugs to prevent the clinical recurrence of ocular herpes keratitis. Invest Ophthalmol Vis Sci. 1995;36:1181–1183.

Present models of recurrent herpetic keratitis generally fall into three categories. In one, animals are infected and spontaneous clinical recurrences are examined. The frequency of these recurrences is often suboptimal, requiring a long period of observation and extended periods of drug treatment to obtain statistically analyzable results. Thus, drug testing with this type of model is expensive and difficult.

A second category involves trauma to the tissue that produces recurrent disease. Whether the injury involves direct surgical trauma, ioxtophoresis, ultraviolet light, or other mechanisms, however, the pathway of trauma-induced recurrence is unclear, and the possibility that injury alters the mechanism of spontaneous recurrence is real. The third category, immunosuppression—with antimetabolites, corticosteroids, or both—can produce recurrences of herpes, but again the similarity to human disease is unclear.

One model, in which temperature and exercise stress produce repeated virus shedding in the mouse, has been reported. In the very small mouse, however, it is virtually impossible to observe and quantitate ocular disease. We report here the inducement of recurrences of herpes keratitis in previously infected monkeys with relatively mild temperature stress.

MATERIALS AND METHODS. Animals used in these studies were cared for in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The corneas of 20 young adult squirrel monkeys (Saimiri sciureus) were infected with the Rodanus strain of HSV-1, as previously described. Before infection, all corneas were normal.

Nonanesthetized monkeys were examined by slit lamp biomicroscopy after the corneas were stained with fluorescein (Fluor-I-Strip; Ayerst Laboratories, New York, NY). Examination on the third and fourth days after inoculation showed that all corneas had typical dendritic keratitis. From days 15 to 39, 10 of the 20 monkeys were treated with systemic 5′-ethynylthymidine, and the remaining 10 were treated with balanced salt solution placebo in a coded, placebo-controlled study to determine the effect of the viral thymidine kinase inhibitor on the ocular recurrences of herpetic keratitis.

On day 35, the room temperature was noted to be 18°C at 6 AM, and the size and number of recurrent ocular lesions was noted to be increased. Observations of corneal recurrences was continued after the treatment study was completed through day 410. During the observation period, some of the monkeys died from the effects of the systemic viremia that followed the initial ocular infection. On day 161, room temperature was again 18°C at 6 AM, and there were eight corneas with dendritic keratitis or punctate lesions in the 16 surviving monkeys. These first two episodes of lower than ideal room temperature were determined to be caused by a malfunction of the air handling system during nights when outside temperatures were in the freezing range. Personnel of the Division of Animal Care had the defect repaired, and the room temperature did not drop again in cold weather.

Observation of clinical recurrences continued. Room temperature was lowered to 20°C (with permission of the Director of the Division of Animal Care) from the normal range of 25°C to 27°C by 5 PM of day 192, and it was kept at 20°C until day 198. Room temperature was again lowered, this time to 18°C, on...

the evening of day 409, and the surviving 14 monkeys were examined the next morning. During the periods of reduced temperature, there was no increase in the death rate from viremia or other causes.

**Statistical Methods.** Frequencies of HSV-1 epithelial keratitis, number of days after infection, and ambient temperatures were analyzed using Statistical Analysis System programs and procedures. A chi-square analysis of frequencies of recurrent herpetic keratitis compared to the ambient temperature was conducted on the 29 observation days of normal temperatures and the three instances of below-normal temperatures. Chi-square analysis was performed on a $2 \times 3$ level table of frequencies of outcomes of the slit lamp examination on each eye (negative or positive) and ambient temperature ($23^\circ$C or one of two reduced temperatures, $18^\circ$C or $20^\circ$C).

**RESULTS.** On the morning of day 35, four corneas showed herpetic recurrences, and two of these lesions were much larger than those usually seen in this animal with this strain of virus (Fig. 1). Two of these corneas were in the group treated with ethynyl thymidine, and the other two were in the placebo-treated group. As seen in Figure 1, spontaneous clinical recurrences (tiny dendrites, punctate lesions, disciform keratitis, keratic precipitates) increased each time the temperature was lowered, including the second overnight reduced temperature caused by a malfunction in the air handling system (day 161) and the two intentional periods of temperature reduction (days 192 to 198 and day 409).

**Statistical Results.** Chi-square analysis of the $2 \times 3$ level frequency table of outcome of the slit lamp examination (negative or positive) and the ambient temperature ($23^\circ$C, $20^\circ$C, or $18^\circ$C) showed that there were significantly higher frequencies of HSV-1 epithelial keratitis at the lower temperatures ($P < .0001$) (Table 1).

**DISCUSSION.** The ability to induce recurrences of herpetic keratitis in primates with relatively small reductions in ambient temperature provides for the first time a primate model of recurrent herpetic disease that is temperature-stress induced. The Rodanis strain of HSV-1 produces spontaneous clinical recurrences in the squirrel monkey, an animal that is more similar to humans than are rabbits or mice. Squirrel monkeys are small (500 g to 800 g) and can be handled for infection and undergo examination without anesthesia. The corneal recurrences seen in these animals are typical herpetic lesions consisting of dendrites, geographic or punctate lesions, keratic precipitates, and disciform edema. Using this primate model, we were able to show that a thymidine kinase inhibitor could reduce the incidence of clinical recurrent herpetic keratitis. In preliminary experiments, we were unable to reproduce this model—that is, to induce increased recurrences of epithelial keratitis—in New Zealand white rabbits by lowering room temperature. Although we know of no published documentation, observations in connection with previous studies suggest that human herpetic disease, like some other viral infections in temperate climates, seems to occur...
Expression of bFGF and TGF-β2 in Experimental Myopia in Chicks

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Purpose. To determine factors that control ocular enlargement in experimental form-deprivation myopia and to clarify the mechanism of form-deprivation myopia.

Methods. After the left eyes of 20 chicks were monocularly occluded for 2 weeks, protein, basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-β2 contents in samples of constant area (circular button, diameter = 8.5 mm) in the retina-retinal pigment epithelium (RPE)-choroid and the sclera in the posterior region of control and myopic eyes were determined by enzyme-linked immunosorbent assay.

Results. The bFGF content (per circular button) and bFGF concentration (per mg protein) were significantly lower in the sclera in the posterior region of the myopic eyes than in control eyes. The bFGF content and concentration were similar in the retina-RPE-choroid in myopic and control eyes. The TGF-β2 content and concentration were significantly higher in myopic eyes in both the retina-RPE-choroid and the sclera (P < 0.05).

Conclusions. These results are consistent with the possibility that bFGF and TGF-β2 regulate ocular enlarge-