Justification for 20 rabbits in Column E

35K0024

Registration No: 35-R-0029

IX. **Write a brief description of the procedures to be utilized on the animals in your research project. Indicate any physical or physiological impairment or loss of function occurring to the animal from your procedure, i.e., diabetes, blindness, loss of motor abilities. Use additional paper if necessary and attach the methods and vertebrate animal portions from your grant proposal.**

In these studies, the corneas of rabbits will be infected with the McKrae strain of herpes simplex virus type 1. Herpetic keratitis will be allowed to develop for 7 days prior to initiating therapy. During this time the rabbits will experience some pain and discomfort. The level of discomfort the animals experience is hard to quantify but they do not rub their eyes excessively, suggesting the discomfort is not severe.

Therapy with an antiviral agent and an anti-inflammatory agent will be initiated on day 7 post-infection and continued until days 14 to 20. The natural course of acute disease is symptomatic during the period of 3 to 20 days post infection. The disease is self-limiting and generally becomes asymptomatic in rabbits about 20 days post-infection.

It is necessary to allow the disease to develop untreated in order to mimic the human situation. Patients do not come for therapy until they become symptomatic. By this time, the immune/inflammatory response has been triggered and latent infection of the sensory nerves that serve the infected dermatome have been established.

The use of topical or systemic analgesics is not a viable option in these studies because HSV is a neurotrophic virus. Any treatment that includes drugs that alter nerve function could affect the outcome of experiments designed to evaluate antiviral/anti-inflammatory therapy. Similarly, studies of viral latency could be dramatically influenced by neuroactive drugs. Initiation of antiviral therapy on the day of infection or within a day or two of infection would prevent the disease from developing, thus negating the model.

Justification for 5 rabbits in Column E

35R0029

Registration No: 35-R-0029

APPLICATION FOR APPROVAL OF THE USE OF ANIMALS IN RESEARCH AND TEACHING
Principal Investigator: [redacted]
Date: 4/3/97

Page 2, supplement

NOT A FOIA DELETION

III. b. If survival surgery is performed, outline postoperative care including use of analgesics and list the names of personnel who will provide the care.

I really don't know if the procedure of infection is considered surgery. Animals will be anesthetized as described below. After injection of virus in the subalveolar mucosa and/or intrastromally, rabbits will be observed hourly until ambulatory.

III. e. Will there be any procedure that would cause the animal pain or discomfort greater than that caused by inducing anesthesia or drawing blood? (If yes, describe and justify.)

Rabbits are anesthetized with ketamine and xylazine and then given a primary herpetic infection by injection of 10^5 pfu of herpes simplex virus at the midline of the alveolar mucosa. A subclinical gingivitis, neutralizing antibody titer, and latent ganglial infection result from this primary disease. These animals demonstrate little pain or discomfort and eat normally. About 30 days post primary infection, the corneas of rabbits will be infected. The corneas of rabbits will be infected with herpes simplex virus type 1 by intrastromal injection of 20 microliters containing 10^3 pfu of virus. The intrastromal injection of virus may cause transient pain, but the animals will be anesthetized with ketamine and xylazine. The local anesthetic proparacaine will be used to reduce corneal sensation. Upon regaining consciousness the rabbits display no discomfort. Over the next 15 days the rabbits develop and recover from stromal keratitis. During this time they likely experience some reduced vision due to viral infection. HSV-1 is a neurotrophic virus which establishes latent infections in neurons. During latency, however, no pain or sense of latent infection is experienced by humans, and it is unlikely that animals experience pain. Because our studies focus on latency, no anesthetics can be given because they can affect the state of latency. The rabbits experience impaired vision with only about 2% developing monocular blindness. Bilateral blindness is very rare and, if it develops, the animal will be sacrificed. Administration of antivirals could defeat the purpose of the studies.

V. a. Describe the applicable substance (i.e., infectious agent, isotope).

Herpes simplex virus type 1 is considered a Class II pathogen by NIH guidelines; therefore, containment is the principal issue. All procedures will be done in the assigned biocontainment room, and tissues removed from the room for further analysis will be contained for transport to our lab.