Ocular effects of scrapie agent in hamsters: preliminary observations

N. Buyukmihci, R. F. Marsh, D. M. Albert, and K. Zelinski

Scrapie is caused by one of a group of so-called slow viruses responsible for the subacute spongiform encephalopathies. In the present study, young hamsters were inoculated intracerebrally with hamster-adapted scrapie agent. At termination, all inoculated animals showed signs and central nervous system pathology compatible with scrapie infection. The eyes appeared well developed grossly, but histologically the retina and optic nerve were abnormal. There were varying degrees of thinning of the retina, with the photoreceptor layer being most severely affected. Although the ganglion cell layer was not much different from the controls, the optic nerve appeared more cellular than that of the controls.

Key words: hamster, scrapie, retina, optic nerve, degeneration.

Scrapie is a transmissible disease of sheep and goats caused by a viruslike agent and characterized by a nonfebrile, chronic course ending in complete debilitation and death. Clinical signs include behavioral changes, generalized pruritus, impairment of locomotion, and terminal anorexia.1 Scrapie has an incubation period of several months to several years and thus is classified as a slow virus disease. Four slow virus diseases have such similarity that they have been further subdivided into a group known as the subacute spongiform encephalopathies2 with scrapie as the prototype. The other diseases in this group include kuru (a disease of New Guinea natives), transmissible mink encephalopathy (TME), and Creutzfeldt-Jakob disease.
The pathology seen in natural scrapie includes vacuolation and noninflammatory degeneration of the neurons of the central nervous system (CNS), accompanied by astrocytic hypertrophy, and a spongy appearance in the neuroparenchyma. The visual system is affected in this disease but generally as a result of CNS pathologic conditions rather than ocular. There is only one report describing ocular changes in scrapie-infected animals. This report described bilateral blisterlike areas in the tapetal retina of two sheep that were clinically blind. Histologically, these lesions consisted of areas where a lipidlike material separated the photoreceptor outer segments from the pigment epithelium. The photoreceptor outer segments were said to be hypertrophied, presumably in the abnormal areas. Amorphous material and degenerating outer segment material were seen near the pigment epithelium with the electron microscope. Unfortunately, no real association between the ocular lesions and the systemic disease could be established.

The following is a report of the ocular changes seen in scrapie-affected hamsters killed in the terminal stages of the disease.

**Methods**

The animals used were outbred (LVG, LAK) golden Syrian hamsters (Lakeview Hamster Colony, Newfield, N. J.). The scrapie agent used was originally derived from the Chandler strain of mouse-adapted scrapie. After transmission of the agent to rats for several serial passages, hamsters were inoculated, and brain from these animals was used for the present experiment. Five weanling animals were inoculated (0.05 ml.) intracerebrally with a saline brain suspension containing 10^7 LD_50 of hamster-adapted scrapie agent. Five animals of the same age were injected intracerebrally with saline and were handled in a manner identical to the brain-inoculated animals. The animals were killed at 13 weeks of age when showing advanced signs of scrapie disease. Brain, spinal cord, and eyes were fixed by immersion in neutral buffered 10 percent formalin. All tissue was paraffin embedded (the eyes were sectioned vertically) and cut at 6 to 8 \( \mu \text{m} \). Hematoxylin and eosin (H & E) was used on all tissues, and in addition periodic acid–Schiff (PAS) was used for eye sections.

**Results**

All saline-injected animals remained normal throughout the observation period. Gross and histologic findings were also normal (Figs. 1 and 2).

Clinical signs in the scrapie-inoculated animals consisted of ataxia, hypersensitivity to outside stimuli, and, at the time of termination, severe debilitation and somnolence. Histologic examination of the CNS revealed astrocytic hypertrophy accompanied by spongiform and neuronal degeneration with a predilection for brain stem and cerebellum as has been reported previously.

The eyes of the scrapie-inoculated hamsters appeared grossly similar to those of the controls. Histologic abnormalities were limited to the retina and optic nerve. The pigment epithelium appeared to be normal as compared to the controls. The neural retina showed diffuse thinning, with the photoreceptor layer being the most severely involved (Fig. 3). The inner and outer segments of the photoreceptor cells were...
markedly reduced in numbers; those present were shorter than normal and displayed a disorderly array. There were a variable decrease in the number of photoreceptor nuclei and vacuolation of the outer and inner nuclear layers (Fig. 3). The outer plexiform layer was generally less than one-half the thickness of control eyes. The inner nuclear layer was thinned and vacuolated, but not to the degree seen in the outer nuclear layer. The appearance of the inner plexiform layer varied in different specimens, sometimes being similar in thickness to the controls but usually thinned. The ganglion cell and nerve fiber layers were basically similar to the controls. The optic nerve was normal in size but showed increased cellularity, possibly due to astrocytic hypertrophy (Fig. 4, A). Also, a markedly greater degree of vacuolation was present in the nerve substance than was seen in the controls, most of the vacuoles appearing larger than the few seen in the controls (Fig. 4, B). The vacuolation seen in the nuclear layers and optic nerve appeared to be intracellular, but ultrastructural confirmation was not done.

Discussion

Prior to this experiment, ocular pathologic conditions associated with scrapie have only been reported once. In most other studies on scrapie, there has been no mention of histologic examination of the eyes although visual deficits were reported. One study did show wallerian-type degeneration of the optic tracts. It is interesting, although not surprising, that in the present experiment, the optic nerve showed increased cellularity and vacuolation similar to that seen in the CNS.

To explain the retinal pathology, one might consider that this viruslike agent may be affecting development of the retina, as many viruses are known to do. For example, bluetongue virus of sheep causes extensive retinal degeneration and dysplasia when injected into sheep feti early in gestation. As the retina becomes more mature, the virus-induced changes are
Fig. 3. Scrapie-infected hamster retina. Photomicrographs showing varying degrees of pathological state. (H & E; original magnification x250.) A, Note reduction in width of inner and outer segment region and outer and inner nuclear layers (compare with Fig. 1). B, Affected layers thinner than in A. Note presence of vacuolation within outer plexiform and both nuclear layers, and nuclei among the outer and inner segments. C, Extreme thinning of photoreceptor and outer plexiform layers with vacuolation of inner nuclear layer. Arrows indicate abnormal, stubby remains of photoreceptor cell processes.

lessened, and there is complete resistance late in gestation. The causes for the age-related effects were speculated to be either a reduced susceptibility by maturation of retinal cells or an increased immunologic competence. A similar age-related degeneration of the retina occurred in rats infected with simian virus 40. A review of other developmental disturbances of the retina is available. At the age at which the hamsters in this study were injected (21 days), the retina is completely differentiated, so that it is unlikely that the pathologic conditions seen are due to an arrest of development or other related causes.

Light-induced retinopathy is a well-known phenomenon but is more readily produced in the albino retina. The animals in this study were pigmented and kept in cyclic light. In addition, none of the similarly housed control animals showed retinal disease. There is the possibility that the scrapie agent may have altered the photoreceptor membranes in such a way as to render them more susceptible to light damage. However, pigmentation alone would probably not be responsible, since no ap-
preciable difference in retinal pigment epithelial or other pigmentation was observed between controls and those showing disease.

Hereditary, toxic, or other environmental factors also appear to be unlikely avenues of direct pathogenesis in that there was such a clear-cut distinction between controls and inoculated animals. The retina is one of the most metabolically active tissues in the body. The inner segment of the rod photoreceptor cell continually synthesizes visual pigment protein in the production of outer segment disks.19,23 An agent interfering with any step in the protein synthesis process could conceivably cause degeneration of the photoreceptor cells. It is not inconceivable that the scrapie agent might have a cytopathogenic effect at this level. Alternatively, it could affect the photoreceptor perikaryon or nucleus. Not enough is known about the agent (or others in
the subacute spongiform encephalopathy group) to suggest a specific, primary pathologic process.

The importance of continued study of this group of diseases has become evident in light of recent findings. Creutzfeldt-Jakob disease has been transmitted from one person to another inadvertently by corneal transplantation.2 Experiments in animals have shown that these agents can persist in corneal epithelium, most likely in the numerous nerve endings.23 We have reviewed eyes from three patients dying of Creutzfeldt-Jakob disease. Although one eye had optic atrophy, lesions similar to those seen in the experimental animals were not found. Nevertheless, the findings in the present study warrant further investigation of what may prove to be a useful model of acquired retinal and optic nerve degeneration due to slow virus infection.

REFERENCES