Rabies: ocular pathology

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SUMMARY Ocular pathology in the first European case of human bat-borne rabies is described. The patient was a 30-year-old bat scientist who seven weeks after bat bite developed neurological symptoms and died 23 days later. Rabies virus antigens were detected in brain smears. After extensive virological studies the virus turned out to be a rabies-related virus, closely resembling the Duvenhage virus isolated from bats in South Africa in 1980. By light microscopy focal chronic inflammatory infiltration of the ciliary body and of the choroid was found. PAS-positive exudate was seen in the subretinal and in the outer plexiform layers of the retina, and retinal veins showed endothelial damage and perivascular inflammation. Many of the retinal ganglion cells were destroyed. The presence of rabies-related viral antigen in the retinal ganglion cells was shown by positive cytoplasmic immunofluorescence, though electron microscopy failed to identify definite viral structures in the retina. By immunohistochemistry gial fibrillary acidic protein was observed in the Müller's cells, which are normally negative for this antigen but express it as a reactive change when the retina is damaged. Synaptophysin, a constituent of presynaptic vesicles of normal retinal neurons, was not detected in the retina.

Rabies, a neurotropic rhabdovirus infection of worldwide occurrence, is usually transmitted to humans by animals, most often by dog or cat bite. In addition to transmission via animal bites the virus can penetrate through intact mucous membranes or abraded skin from the saliva of a rabid animal. There are also cases of airborne transmission. However, the only reported cases of human-to-human transmission have occurred via corneal transplants and rabies virus has been experimentally shown to spread to cornea by axonal transport. In view of this it is surprising that, to the best of our knowledge, there are no reports of ocular pathology in human rabies.

Though human rabies caused by bat bite has long been recognised on the American continent it has only recently become evident that even insectivorous European bats are carriers of rabies-related viruses constituting a potential hazard to humans.

The purpose of the present paper is to describe the ocular pathology in the first European case of human bat-borne rabies.

Case report

The patient was a 30-year-old man who had previously been in excellent health. He was a zoologist who had been studying bats in different parts of the world but had never been vaccinated against rabies. Four and a half years before his illness he had been bitten by a bat in Malaysia and one year before in Switzerland. Fifty one days before the onset of his neurological symptoms he had been caring for a bat (Myotis daubentonii) at his home. The bat had behaved oddly and had bitten him several times. After apparent improvement of the bat the patient had let it go and it was never examined virologically.

Seven weeks later the patient felt numbness and pain in his right arm and neck and developed an ascending paralysis of the Guillain-Barré type. He was admitted to the Helsinki University Central Hospital, where he developed hyperexcitability, hyperventilation, and spasms. Rabies was suspected and the patient was treated intensively on this hypothesis, though virological investigations during life could not confirm the diagnosis. The patient died 23 days after the onset of the first neurological symptoms.
Post-mortem neuropathological and virological studies showed severe encephalitis and peripheral neuritis. Rabies virus antigens were detected in brain smears. After inoculation of brain material into newborn and 3-week-old mice the virus was further characterised by electron microscopy and a set of monoclonal antibodies (Roine et al. and Haltia et al., in preparation). It turned out to be closely related to the Duvenhage virus isolated from bats in South Africa in 1980. It was antigenically similar but not identical with the rabies related viruses recently isolated from bats in Germany, Poland, and Denmark.14

Materials and methods

The eyes were fixed two hours after death in 4% phosphate buffered formaldehyde solution. After fixation they were cut in the horizontal plane and embedded in paraffin. Small selected specimens were postfixed in 2% phosphate-buffered glutaraldehyde and routinely processed for transmission electron microscopy. Paraffin sections were stained with haematoxylin-eosin, van Gieson, and periodic acid-Schiff (PAS) methods. The direct immunofluorescence method was used to detect the presence of rabies antigen in the ocular tissues, by means of a commercial antirabies immune globulin labelled with fluorescein isothiocyanate, isomer I (BBL Microbiology Systems, Cockeysville, MD, USA).

The immunohistochemical stainings to demonstrate neuron-associated and glia-associated antigens in formalin-fixed and paraffin-embedded tissues were carried out with a commercial version (Vectastain ABC Kits for Rabbit and Mouse IgG, Vector Laboratories, Burlingame, CA, USA) of the avidin-biotinylated peroxidase method17 as previously described in detail.18-20 The primary antibodies used were antineuron specific enolase (Code A589, Lot 084, dilution 1:500) and anti-S-100-protein antisera (Code Z311, Lot 113, dilution 1:100) from Dakopatts a/s (Glostrup, Denmark), monoclonal antisynaptophysin antibodies (Code 902322, Lot 10644526-01, dilution 1:5) from Boehringer-Mannheim Biochemica (Mannheim, FRG), as well as monoclonal antiglial fibrillary acidic protein21 and anti-200 kD neurofilament triplet protein antibodies (NFI21, both diluted 1:50) generously provided by Dr Ismo Virtanen (Department of Pathology, University of Helsinki). Prior to immunohistochemical staining the sections were pretreated with 0-4% pepsin (E Merck, Darmstadt, FRG) in 0-01 N hydrochloric acid for 15 min at 37°C to reduce background and to enhance the intensity of specific staining. Five formalin-fixed and paraffin-embedded human eyes obtained from necropsies at the Department of Forensic Medicine,
University of Helsinki, and previously referred to the Ophthalmic Pathology Laboratory, were used as control post-mortem material. Except for autolytic changes observed in photoreceptor cells and cystic degeneration of the ora serrata region in some specimens, these eyes were entirely normal under the light microscope.

Results

Morphological Findings
Macroscopic findings were within normal limits. By light microscopy a chronic inflammatory reaction consisting of lymphocytes and plasma cells was seen in the ciliary body and as foci in the choroid (Figs. 1, 2). The pigment epithelium of the retina showed some loss of pigment especially in the posterior fundus (Fig. 2). The overall structure of the sensory retina, including the visual cells, was rather well preserved. PAS-positive exudate was seen as foci in the subretinal layers as well as in the outer plexiform layer (Fig. 3). In the retinal nerve fibre layer perivascular inflammatory foci consisting of lymphocytes and plasma cells were seen. The endothelium of the retinal veins was destroyed and the lumen almost totally occluded in places (Figs. 4, 5). In semithin sections many of the ganglion cells were seen to be destroyed and the nuclei of many bipolar cells were altered (Fig. 6). As evidence of absence of post-mortem autolysis good preservation of the retinal visual cells can be seen. Electron microscopic studies failed to reveal structures positively identifiable as viral elements.

Immunohistochemical Findings
Sections stained by the rabies antibody showed small

Fig. 3 PAS-positive exudation in the outer plexiform layer and subretinal space (arrows). PAS, x475.

Fig. 4 Good preservation of the visual cells as well as only a slight inflammatory involvement of the choroid are seen. In the nerve fibre layer perivascular inflammatory infiltration is present. PAS, x108.
Vasculitis and perivasculitis have resulted in an almost complete obstruction of the retinal vessel lumen. Exudate in the outer plexiform layer. PAS, ×477.

green intensely fluorescing granules in the cytoplasm of many of the remaining retinal ganglion cells (Fig. 7) (as well as in the patient's brain and in a rabies-infected mouse brain) but not in non-infected control tissues.

In human necropsy eyes of normal appearance on light microscopy the monoclonal antibody to glial fibrillary acidic protein (GFAP) reacted with astrocytes in the nerve fibre and ganglion cell layers (Fig. 8A), as well as with a few Müller's cells close to the ora serrata. In the central retina all Müller's fibres were negative for GFAP. However, in the eye from the rabies patient, Müller's cells expressed GFAP throughout the retina (Fig. 8B). The antiserum to S-100 protein, another marker for retinal glia, labelled retinal astrocytes and a few Müller's cells in all specimens studied.

The polyclonal antiserum to neuron-specific enolase reacted strongly with photoreceptor cells, but labelled the inner nuclear, inner plexiform, and nerve fibre layers only weakly. A positive reaction was also seen in the remaining ganglion cells. The 200 kD neurofilament triplet protein was detected in neuronal processes of the nerve fibre layer and optic nerve, as well as in occasional nerve fibres in the inner plexiform layer. While these neuronal markers were identically expressed in all retinas examined, both plexiform layers reacted positively for synaptophysin in the control eyes (Fig. 8C), but no reactivity was detected in the eye from the rabies patient (Fig. 8D).

Discussion

Several viruses are known to cause intraocular
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Fig. 7 A remaining ganglion cell shows a strongly fluorescent cytoplasmic inclusion body after staining with rabies antibody. Direct immunofluorescence method, ×660.

lesions. Herpes simplex virus (type 1) is probably the most common cause of viral intraocular inflammation. Uveitis associated with keratitis is characterised by a non-granulomatous inflammatory infiltrate composed of lymphocytes and plasma cells. Necrotising retinitis in adults occurs only with immunodeficiency. Varicella zoster may induce intraocular changes when the nasociliary branch of the trigeminal nerve is involved. When choroiditis is present, it is focal and of the granulomatous type, usually without necrosis. In terms of the nature of the uveal inflammatory lesion this type of uveitis is very different from that found in the present case. In cytomegalic inclusion disease a focal progressive

Fig. 8 Differences in antigenic properties of human retina from the rabies patient and from a control necropsy eye (immunoperoxidase staining, ×210). A. In the necropsy eye only astrocytes express glial fibrillary acidic protein (GFAP). B. In the rabies eye an additional positive reaction is observed in slender Müller’s fibres reaching towards the outer limiting membrane. C. In the necropsy retina both plexiform layers express synaptophysin. D. The entire rabies infected retina is negative for synaptophysin.
necrotising inflammation is strikingly confined to the retina. It has been repeatedly shown that the vitreous and the choroid are usually spared from any inflammatory changes even in the areas of severe retinal necrosis.\textsuperscript{25,26} Retinal necrosis is also the main ocular feature of measles retinopathy.\textsuperscript{27} These findings bear no reflections to the present case, with focal non-granulomatous cyclitis and choroiditis as well as involvement mainly of the inner retina.

To our knowledge no descriptions of ocular pathology in human rabies are available. After inoculation against rabies, neuroretinitis has been described\textsuperscript{28} as well as bilateral optic neuritis and retinal haemorrhages.\textsuperscript{29} Rabbits inoculated with rabies virus by Dejean showed extracellular muscle palsy, disappearance of the corneal sensitivity, corneal clouding, venous congestion of the retina and of the optic nerve as well as clouding of the vitreous. At histopathological study Negri-like bodies could be identified in the retinal ganglion cells.\textsuperscript{30} Argüello and Melita\textsuperscript{31} described two cases, a boy bitten on the right side of his face with facial paralysis of the same side and mydriasis, and an adult showing conjunctival congestion and retinal haemorrhages. In their 100 rabbits inoculated with rabies virus choroidal haemorrhages were found. In comparison with the rabbit study of Dejean\textsuperscript{32} our findings agree only with regard to the involvement of the retinal ganglion cells. No changes in the vitreous or in the cornea could be identified. Our immunofluorescence studies showed the presence of rabies virus antigen in the cytoplasm of many of the remaining retinal ganglion cells in the form of small intensely fluorescing granules. They apparently correspond to the Negri-like bodies found by Dejean\textsuperscript{33} in rabies infected rabbits and indicate that the retinal lesions are directly induced by the virus.

Two further immunohistochemical deviations from the usual pattern seen in human necropsy eyes appearing normal on light microscopy were evident. Glial fibrillary acidic protein (GFAP) was observed in Müller’s cells, which are normally negative for this antigen.\textsuperscript{34,35} In the control necropsy eyes only a few GFAP-positive Müller’s cells were seen at the ora serrata, confirming previous reports.\textsuperscript{36,37} It appears that Müller’s cells in the rabies eye have accumulated GFAP in response to the retinitis and retinal vasculitis observed. Previous observations have shown that GFAP is a relatively sensitive indicator of damage to retina that results from mechanical trauma,\textsuperscript{38} photoreceptor degeneration,\textsuperscript{39,40} intraocular tumours,\textsuperscript{41} glaucoma, retinal detachment, or inflammatory reaction involving the vitreous cavity.\textsuperscript{42}

On the other hand synaptophysin, a constituent of presynaptic vesicle membranes that is normally present in synapses of both plexiform layers of the mammalian retina,\textsuperscript{43} was absent in the rabies eye. This was unexpected, since synaptophysin was detected in all control necropsy eyes, obtained after comparable delays after death, albeit less strongly than we have seen in surgical specimens. There is a possibility that the amount of synaptophysin in retinal neurons was diminished before death, though this remains conjectural at present. There was no obvious change in the expression of other neuronal markers examined. The inner retinal layers, however, reacted more weakly with antiserum to neuron-specific enolase than is seen in surgical specimens.\textsuperscript{18} This may reflect virus-induced degeneration and loss of the retinal ganglion cells and bipolar cells in addition to post-mortem change.

References


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