AMAOUROSIS IN SHEEP RESULTING FROM TREATMENT WITH RAFOXANIDE

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ABSTRACT


Amaurosis occurred in sheep on various farms in the Republic of South Africa after treatment with rafoxanide. Histopathological examination revealed a status spongiosus of varying severity in the central nervous system in all the cases, having a predilection for certain areas such as the periventricular area of the lateral ventricles, optic tracts, lateral geniculata and optic fasciculi. The retina was the only ocular tissue affected and lesions observed in the retina included necrosis of nerve cells in the ganglion layer. In chronic cases of amaurosis this layer showed a complete absence of nerve cells. The possible pathogenesis of the lesions and their differentiation from those found in certain plant toxicoses are discussed.

INTRODUCTION

In 1969, Mrozik, Jones, Friedman, Schwartskopf, Schärz, Patchett, Hoff, Yakstis, Riek, Ostlind, Plishek, Butler, Cuckler & Campbell announced the discovery of a new fasciolicidal compound, rafoxanide [3,5-diocthyl-2-chloro-4-(p-chlorophenoxy)-salicylanilide], one of the halogenated salicylanilides.

Since its appearance and general use, rafoxanide has proved to be an effective fasciolicide in both sheep and cattle (Snijders, Horak & Louw, 1971; Horak, Snijders & Louw, 1972; Boray, Wolff & Trepp, 1973). Boray (1971) emphasized that its anthelmintic efficacy was dependent on the age and susceptibility of the host.

Toxicity trials proved rafoxanide to be a safe drug (Sutherland & Batty, 1971; Snijders et al., 1971; Mrozik et al., 1969; Guilhon, Jolivet & Barnabé, 1971; Boray, 1971). According to Mrozik et al. (1969), sheep not infected with liver fluke survived treatment at a dosage of 200 mg/kg, while death occurred in sheep with natural liver fluke infection when treated at the same dosage. Some of the non-infected sheep treated with a dosage of 200 mg/kg and some of the infected sheep that received dosages of 100 mg/kg or more showed signs of blindness. These authors made no mention of lesions in the eyes or central nervous system (CNS) that could explain the visual impairment.

Brown, Rubin, Hite & Zwickiey (1972) reported that Washko & Norcross (1968), using a single dose of 100 mg/kg of rafoxanide in sheep and multiple doses of 250 mg/kg in the rat, found vacuoles in the white matter of the brain and opacity of the lens. Blindness and vacuolation of the CNS were briefly mentioned in sheep with another halogenated salicylanilide, cloroxylen, in Australia (O’Brien, 1970). The fact that no detailed report on the lesions caused by rafoxanide in sheep were to be found in the literature and the absence of any references to retinal changes motivated the present study.

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RESULTS

Gross pathology
No gross lesions were observed in any of the sheep apart from bulging of the optic fasciculi around the circumference of the optic foramen in 3 of the animals.

Microscopic pathology

Brain
The most prominent lesion in the brains examined was a bilateral status spongiosus unaccompanied by any inflammatory reaction in the white matter affecting different areas of the brain (Fig. 1). Vacuoles in the areas of status spongiosus varied considerably in size, from 7-75 μm, and some were even larger. Luxol fast blue-Holmes' stained preparations showed the vacuoles to be transected by thin strands of material but none of the other special staining techniques employed showed up any other material within the cavities.

Three of the brain specimens showed dilated perivascular spaces, while the neuropil around the bloodvessels was Lusitane and in the septum pellucidum. Neuronal loss in the various parts of the CNS in all the specimens examined were unaffected, but glia cells with enlarged eosinophilic cytoplasm in the areas showing status spongiosus were present in 3 of the animals.

The extent of the spongy lesions varied considerably in different animals. They were particularly prominent in 7 of the brain specimens examined but only mild in 6 others. Spongy lesions showed a predilection for the periventricular areas (Fig. 1) which included the fornix and septum pellucidum. Other areas in which lesions occurred less commonly were the optic radiations, lateral and medial geniculate, and optic tracts. In pronounced cases the entire white substance of the brain was affected, although the extent of the lesion varied in the different areas.

Optic fasciculi
Marked lytic necrosis with mobilization of gitter cells and severe myelin loss were present in 1 animal. Spongy lesions of varying severity, without gitter cell reaction, were seen in the optic fasciculi of 5 of the animals (Fig. 3). They were more prominent at the periphery of the optic fasciculi and were probably due to swelling and pressure against the optic foramen. Areas showing lytic necrosis had a moth-eaten appearance (Fig. 4). One animal only had no lesions in the optic fasciculi.

Spinal cord
Spongy lesions similar to those in the brain were present in the spinal cord of 8 of the 12 cases from which this structure was available for examination (Fig. 2). The dorsal tracts were the areas most often affected except in 1 animal where the entire peripheral zone of white matter showed a narrow band of status spongiosus. Although representative samples of the whole spinal cord of only 3 animals were examined, it appeared that lesions were not present at all levels of the spinal cord and that the lumbar region was the area most consistently affected.

Spinal nerves
Spongy lesions corresponding to the lesions observed in the brain were present in 1 animal only (Fig. 2).

Eyes
The retinas of the eyes of 6 of the 7 animals available for examination, were the only intra-ocular tissues affected. No lesions at all were present in one animal. Lesions observed in the eyes of the 3 sheep autopsied 2½ years after treatment showed a complete absence of nerve cells in the ganglionic cell layer of the retina (Fig. 7). In one of these chronic cases the fact that the inner and outer nuclear layers appeared less dense was apparently due to a diminished cellular content. In 2 animals autopsied approximately 2-6 weeks after treatment, vacuoles of different size containing an eosinophilic material were seen in the cytoplasm of the neurones in the ganglionic cell layer. Neurophagocytosis was prominent and in focal areas of the retina there seemed to be an increase of glial cells (Fig. 6). Oedema, seen as the accumulation of homogeneous eosinophilic material between the neurones in the retina, severe haemorrhages and congestion were present in an animal examined 2 days after dosing (Fig. 8). Vacuoles, as previously described, were present in the cytoplasm of odd neurones in the ganglionic cell layer. A prominent feature in the retina of this case was the presence of numerous necrotic neurones in the ganglionic cell layer (Fig. 9). The entire cytoplasm of these dead cells consisted of an eosinophilic amorphous material. Nuclei of the necrotic neurones commonly exhibited pyknosis and karyorrhexis, while in some the fading of the nucleus gave evidence of chromatolysis. Only a vague outline of the nucleus or no evidence of it at all was present in these instances. A few of the necrotic neurones were infiltrated or surrounded by neutrophils. Some of the glia cells in the ganglionic layer showed enlarged cytoplasm. Small purplish granules of different size, probably representing nuclear debris, were irregularly scattered between the dead neurones.

Lens
There were no lesions present in the 2 lens specimens available for examination.

DISCUSSION

Status spongiosus, microscopically characterized by vacuoles in the white and/or grey matter which gives the tissue its spongy or sieve-like appearance, may be found in a variety of conditions and therefore does not represent a specific CNS lesion (Jellinger & Seitelberger, 1970). Spongy changes without primary loss of neural elements may be due to swollen astrocytes (Klatzo, 1967), swollen astrocytic processes (Klatzo, 1967), vacuolation of oligodendroglia (Luse & Harris, 1960), intramyelin vacuoles (Suzuki & Kikikawa, 1969), intra-axonal vacuoles (Hirano, Levine & Zimmerman, 1967), distended extracellular spaces (Bogaert & Bertrand, 1949, cited by Jellinger & Seitelberger, 1970) or the result of several of these (Adachi, Wallace, Schneck & Volk, 1966). Suzuki & Kikikawa (1969) stated that intramyelinic vacuoles induced by cuprizone could be explained by a metabolic disturbance of the oligodendroglia which showed vacuoles in their cytoplasm. At the light microscope level, status spongiosus is similar in appearance in many of these instances (Suzuki & Kikikawa, 1969). The exact location of the vacuoles cannot be determined accurately at this level, but it appeared to be within the myelin sheaths in the present material studied.
FIG. 1 Periventricular area (corpus callosum) with severe status spongiosus. HE × 75
FIG. 2 Spinal cord and spinal nerves showing mild status spongiosus. HE × 75
FIG. 3 Optic fasciculus with central area showing lytic necrosis. HE × 75
FIG. 4 Optic fasciculi showing severe lytic necrosis. HE × 75
FIG. 5 Normal retina. HE × 200
FIG. 6 Retina of sheep autopsied approximately 4 weeks after treatment with rafloxanide. Note increase of glia cells in the ganglionic cell layer. HE × 200
FIG. 7 Retina of sheep autopsied 2½ years after treatment with rafloxanide. Note absence of neurons in the ganglionic cell layer. HE × 200
FIG. 8 Severe hemorrhages and congestion in the retina of a sheep autopsied 2 days after administration of rafloxanide. HE × 200
FIG. 9 Retina of the same animal shown in Fig. 8. Note swelling of neurons and gliosis in the ganglionic cell layer. HE × 500
studies showed that activity of adenosine triphosphatase (ATPase) within mitochondria of astrocytic processes was decreased in areas showing spongy lesions. Jellinger & Seitelberger (1970) reported that a membrane-bound ATPase, activated by Na+ and K+, plays an important role in the active transport of ions and water across cell membranes. In 1965 Torack (cited by Jellinger & Seitelberger, 1970) observed that membrane-bound ATPase, when inhibited, evoked a form of brain oedema. Adachi & Volk (1968) mentioned the possibility that enzyme defects, especially ATPase in astrocytes and basement membranes of cerebral blood vessels, might play an important role in the pathogenesis of status spongiosus in infancy.

In 1973, Sanderson (cited by Yorke & Turton, 1974) proved that many fasciolicidial or anti-cestode anthelminitics act by uncoupling electron transport-linked phosphorylation and in doing so, inhibit the uptake of oxygen at high levels. Earlier, Miert & Groenewald in 1969 (cited by Yorke & Turton, 1974) used mammalian mitochondria in experiments and came to the same conclusion. As rafaxonide was the most potent inhibitor of ATPase production in experiments carried out by Yorke & Turton (1974), it is possible that the status spongiosus observed in the CNS of the sheep examined may be due to ATPase inhibition by this drug.

The high incidence of spongy lesions in the nerve tracts involved in the conduction of light impulses in the cases reported here was of considerable interest. Those included in these pathways were the optic fasciculi, optic chiasma, optic tracts, lateral geniculate and optic radiation, all of which showed pronounced Papilloedema, associated with increased cerebro-spinal fluid pressure, spongy changes of the periventricular white matter, corpus callosum, fornix, periphery of the optic chiasma and perivascular areas throughout the CNS in dogs after experimental treatment with rafaxonide was reported by Brown et al. (1972).

The blindness observed in the sheep could be due to the lesions in the retina or optic nerves, or to the spongy lesions in the CNS. Unfortunately no definite correlation between the severity of the eye and brain lesions could be made as the eyes of the animals exposed to rafaxonide. Brown et al. (1972) described slight bilateral equatorial lenticular degeneration (cataract) in 11 of the 12 dogs given rafaxonide and superficial retinal oedema and haemorrhages in others. According to these authors, Washko & Norcross in 1969 observed lens opacities in sheep and cattle. Pathology.

Liver fluke-infested sheep are more susceptible to rafaxonide than normal animals (Mrozik et al., 1969). Non-infested sheep tolerated rafaxonide at a dosage rate of 200 mg/kg. Death occurred in some animals naturally-infested with liver fluke at the same dosage rate. One sheep inoculated with 1000 metacercariae died after treatment with rafaxonide at 100 mg/kg. In contrast Boray (1971) reported a maximum tolerated dose of 45 mg/kg (recommended dosage 7.5 mg/kg). Horak, Snyders & Louw, 1972). Campbell, Ostlind & Yakstis (1970) proved that the formulation of rafaxonide also influenced its toxicity. In the present cases under review, however, it became clear that faulty handling of the drug, over-estimation of the animal's body mass, the use of faulty drenching equipment and failure to read the directions on the label were the main causes of poisoning.

In Southern Africa amoarousis is a feature of various plant poisonings. According to Steyn (1949), Brassica spp. and Ornithogalum spp. can give rise to blindness in sheep but the lesions caused by these plants which may be responsible for amoarousis have not yet been documented. Helichrysum argyrosphaerum (Basson, Kellerman, Albl, Von Maltitz, Miller & Welman, 1976), when ingested in large quantities by sheep, causes status spongiosus of the brain, spinal cord, optic fasciculi and nerves, followed by paralysis. The lesions in this condition resemble those of rafaxonide in many respects and should be kept in mind as an important differential diagnosis since various species of Helichrysum are widely distributed in South Africa. The lesions present in the eye in rafaxonide toxicity, however, differ from the lesions described in H. argyrosphaerum poisoning. Necrosis commencing in the layer of rods and cones and extending to the inner nuclear layer was observed in the latter condition, but no lesions were present in the ganglionic cell layer. In comparison, the ganglionic layer of the retina was most severely affected with rafaxonide intoxication and no changes were present in the layer of rods and cones. Cataracts were observed 2–3 months after the initial outbreaks of Helichrysum poisoning under natural conditions but this lesion could not be reproduced experimentally (Basson et al., 1976). No cataracts were found in sheep poisoned with rafaxonide under field conditions.

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References


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