THE SAFETY OF INJECTABLE RAFOXANIDE IN CATTLE*


Thirty-two weaned bull calves were injected subcutaneously with rafoxanide solution to determine the lethal dosage. The recommended therapeutic dosage is 3 mg/kg. Eight of 12 calves treated at 45 to 60 mg/kg displayed signs of toxicity 24 hours to 8 days after treatment. These included recumbency, poly-pnoea, muscle tremors and clonic spasms, opisthotonus, paddling movements of the feet, and blindness with mydriasis and death. An easily detected histopathological lesion was status spongiosus of the central nervous system. This study demonstrated that injectable rafoxanide has a wide safety margin when used at the recommended therapeutic dosage.

INTRODUCTION

The usual recommended dosage of rafoxanide in a preformed suspension (Ranide, MSD) for cattle is 7.5 mg/kg by the oral route (as compared to 3 mg/kg by subcutaneous injection). At that dosage the compound is used to treat cattle infected with Fasciola gigantica, Fasciola hepatica and Haemonchus placei. It appears that the 7.5 % solution of rafoxanide (Ranide Injectable, MSD) is 2.5 times more bioavailable than the oral suspension when injected subcutaneously4. At 3 mg/kg injectable rafoxanide is effective against Bunostomum phlebotomum and Oesophagostomum radiatum, in addition to the previous 3 parasites.

In order to satisfy requirements for registration under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36/1947), the following experiment was performed to determine the lethal dose of injectable rafoxanide in cattle.

MATERIALS AND METHODS

Experimental animals

Thirty-two weaned Friesian bull calves, 3-15 months of age, were kept in concrete floored pens. They were fed a mixture of lucerne and Eragrostis curvula hay and maize silage and had free access to borehole water in steel troughs.

Treatment and observation

Each calf was identified with a numbered ear tag and individually weighed prior to treatment. The calves were treated in 4 groups with doses of rafoxanide ranging from 15-21, 24-30, 33-42 and 45-60 mg/kg. Two calves were treated at each dosage. The trial extended over 2½ months. The calves were injected subcutaneously on the neck with half the dose immediately cranial to the left shoulder. The other half of the dose was injected caudal to the shoulder. There were no untreated controls.

The first 3 groups were examined at least once every day for 10-20 d after treatment for signs of toxicity. The pupillary reflex of each calf was tested 2-4 d after treatment. The fourth group of calves was examined every 2 h from 22-28 h after treatment, and thereafter once or twice daily until 10 d after treatment.

Calf 32 was killed by exsanguination for necropsy because it displayed most of the spectrum of clinical signs.

Necropsy

All calves (8) that died were necropsied. Tissue specimens were taken for histopathology from most parenchymatous organs and fixed in 10 % formalin. From the central nervous system, specimens were taken of the optic chiasma, hippocampus, colliculus oralis (corpora quadrigemina), and cerebellum.

Tissue specimens of 5 calves were sectioned and stained with haematoxylin and eosin. Additional liver blocks were subsequently frozen, sectioned and stained with Oil Red O by a private medical pathology laboratory (Dr W.J. Pepler and partners, Pretoria).

RESULTS

Clinical signs

No signs of toxicity were seen in any of the 20 calves treated at 15-42 mg/kg, i.e. 5-14 times the recommended therapeutic dosage. One each of the 2 calves treated at 36 and 42 mg/kg became moribund because of pneumonia, and were killed for humane reasons. Eight of the 12 calves treated at 45-60 mg/kg started displaying signs of intoxication 24 h to 8 d after treatment. These signs consisted of recumbency (8/8), rapid shallow respiration (6/8), muscle tremors and clonic spasms (6/8), foaming at the mouth (4/8), and in 2 cases opisthotonus, paddling movements of the feet, nystagmus, prolapse of the nictitating membrane, and blindness with mydriasis.

The clonic muscle spasm occurred spontaneously at 30-60 second intervals, but could be elicited by external stimuli such as touch and sound, and apparently affected all skeletal muscle.

With the exception of calf 32 that was killed, the other 7 calves that showed clinical signs eventually died. The data from these animals are summarized in Table 1. Calves treated at 60 mg/kg died within 25-66 h after treatment, and calves treated at 45 mg/kg within 2-8 d.

Necropsy findings

Seven of the 8 calves that had shown clinical signs of acute toxicity had grossly visible subdural oedema at necropsy. Another prominent lesion was a mild to moderate hepatomegaly (roundness of the liver's edges, bulging of cut surfaces). In 4 calves there were subepicardial petechiae or ecchymoses, and 3 calves had a mild pulmonary oedema.

Histological examination revealed a mild to moderate status spongiosus in all the sections of nervous tissue (see Fig. 1). A marked feature in many sections was the wide Virchow-Robin spaces, indicating pericapillary

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oedema. In the hippocampus the status spongiosus was confined to the fimbria, alveus and lacunar layer.

Acute cellular swelling was seen in the hepatocytes from all the livers that were examined. In some livers the periacinar hepatocytes had large intracytoplasmic vacuoles that stained negative for lipids, and resembled hyaline droplets. In 3 of the livers the spaces of Disse were conspicuously widened, which is compatible with perisinusoidal oedema (see Fig. 2).

No other histological lesions were seen which could be attributed to the treatment.

TABLE 1: DOSAGE USED, PERIOD FROM TREATMENT TO DEATH AND THE CLINICAL SIGNS IN CALVES INJECTED SUBCUTANEOUSLY WITH RAFOXANIDE SOLUTION

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Dosage (mg/kg)</th>
<th>Period from treatment to death</th>
<th>Recumbency</th>
<th>Polyphagia</th>
<th>Clonic spasms</th>
<th>Frothing at the mouth</th>
<th>Blindness</th>
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<tr>
<td>14 calves</td>
<td>15.33</td>
<td>No signs seen</td>
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<td>18</td>
<td>36 Euthanasia (7d)</td>
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<td>+</td>
<td>+</td>
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<td>96</td>
<td>42 Euthanasia (7d)</td>
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<td>124</td>
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<tr>
<td>32</td>
<td>54 Euthanasia (48h)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>37</td>
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<td>26h</td>
<td>+</td>
<td>+</td>
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</table>

+ = Present, - = Absent

*Clinical signs of the 8/12 animals that died.

DISCUSSION

A variety of differential diagnoses exist for the range of clinical signs and gross lesions observed in this experiment. Those animals that were in lateral recumbency and paddled with their feet while displaying opisthotonus and nystagmus were very similar to "typical" cases of heartwater. The blindness and pronounced mydriasis, which are the most obvious signs of rafoxanide toxicity in sheep, are similar to descriptions of sheep suffering from progressive retinal degeneration ("bright blindness", thought to be caused by the plant *Pteridium aquilinum*), or *Helichrysum argyrosphaerum* toxicity.

The best known differential diagnosis of the status spongiosus of the central nervous system in this country is *H. argyrosphaerum* toxicity in sheep. This lesion may, however, also be caused by a variety of other toxic substances.

In view of the list of possible differential diagnoses, it is advisable that any diagnosis of rafoxanide toxicity be confirmed by chemical assay for plasma rafoxanide concentrations. Such assays were not performed on any of the animals in this experiment.

The overall impression one gets from the above descriptions of gross and microscopic lesions, is one of oedema, probably as a result of increased vascular permeability. Rafoxanide toxicity is associated with papilloedema and vacuolation of nervous tissue in dogs, sheep and cattle. This oedema can possibly be explained by the fact that rafoxanide has been shown to inhibit adenosine triphosphatase (ATPase) production, and that a membrane-bound ATPase plays an important role in the active transport of ions and water across cell membranes.

With regard to the therapeutic index, the results of this experiment are in agreement with those published for sheep, where it was concluded that the toxic dose of rafoxanide by the oral route lies in the region of 15-20 times the therapeutic dosage, which is 7.5 mg/kg.

From the data in this trial it can be concluded that injectable rafoxanide solution possesses a wide safety margin in cattle. Cases of intoxication present clinical signs and lesions that are easily recognisable, but which should be confirmed through plasma rafoxanide assay.
ACKNOWLEDGEMENTS

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REFERENCES


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