Sporidesmin concentrations in the bile of sheep resistant or susceptible to sporidesmin dosing

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ABSTRACT

Twelve ram lambs bred for resistance or susceptibility to sporidesmin dosing were operated on under general anaesthetic and T-way catheters inserted into the common bile duct to facilitate collection of bile. After a recovery period of 14 days the animals were given an oral dose of sporidesmin at a rate of 0.08 mg/kg/d over 3 consecutive days. A trace amount of ^{35}S-labelled sporidesmin was also given on the first and third day of dosing to allow quantitation of sporidesmin appearing in the bile fluid. Bile samples were collected at 0.5 to 1 hourly intervals for 24 hours after the first and third dosing. Results showed bile concentrations of sporidesmin increasing to a maximum in 1 to 3 hours and then declining over the following 20 hours. There was considerable individual variation among animals in the pattern of uptake and in the maximal concentration reached after dosing. In some animals there was a marked increase in biliary sporidesmin from the first to the third oral dosing while other animals showed little observed increase at these times. A positive correlation ($P < 0.05$) was found between liver-damage score at slaughter and maximum sporidesmin concentration in bile after the third but not the first oral dosing. These findings raise the possibility that an initial or 'potentiating' dose of sporidesmin may be required before 'susceptibility' or 'resistance' to facial eczema is expressed.

Keywords Sporidesmin; sheep; bile; facial eczema; pharmacokinetics

INTRODUCTION

The relatively high heritability value of 0.43 for animals showing 'tolerance' to facial eczema has enabled Campbell *et al.* (1981) to make rapid progress in establishing resistant and susceptible flocks at Ruakura. However the methods used to select 'resistant' animals on the farm based by either progeny testing (Campbell *et al.*, 1981) or performance testing (Towers, 1982) do have the major disadvantage that stock must first be exposed to pasture containing high levels of the toxic substance. Previous studies have indicated that the most important lesion produced in sporidesmin dosed sheep was severe necrosis and inflammation of bile ducts and the gall bladder (Mortimer, 1963). In view of these observations and the fact that unchanged sporidesmin has been detected in bile of dosed animals (Mortimer and Stanbridge, 1968) it was decided to determine sporidesmin concentrations in bile of sporidesmin dosed animals which had been bred for resistance or susceptibility to facial eczema (Campbell *et al.*, 1981).

MATERIALS AND METHODS

Animals

Twelve 5-month-old Romney rams, F_2 progeny, bred from facial eczema resistant and susceptible rams raised at Ruakura (Campbell *et al.*, 1981) were prepared for surgery. Under a general anaesthetic a modified Kehr drain T-tube was inserted into the common bile duct and the long arm exteriorised. The cystic duct was tied off and the gall bladder removed. The animals were then left for 2 weeks to recover from the operation. Bile was collected from the restrained ram via a fine PVC tube fitted into the Kehr T-tube. The rams were sampled by removing a cap at the end of the PVC catheter and allowing bile to drain into a collection bottle. The samples were transferred to a deep freeze at -15 °C within 15 min of collection.

Experimental Procedure

Sporidesmin was administered to 12 animals by ruminal intubation at a daily dose rate of 0.08 mg/kg live weight over 3 consecutive days. ^{35}S-labelled sporidesmin (0.2 μC/kg) was added to the dosing solution on day 1 and 3 for estimation of the sporidesmin levels in bile. The bile samples were collected at 0.5 hr intervals for 3 hr then at hourly intervals for the next 21 hr. The animals were killed at 6 weeks after dosing and the liver-damage score assessed on a 0 to 5 scale as described previously by Smith *et al.* (1977).

Chemical Methods and Analysis

Sulphur-35 labelled sporidesmin was prepared by

inoculating cultures of *Pithomyces chartarum* with ^{35}S-labelled sulphate. The cultures were then extracted and the labelled sporidesmin purified by column chromatography as described previously (Fairclough *et al.*, 1978). Sporidesmin concentrations in the bile of dosed animals were analysed as follows. A large excess of unlabelled sporidesmin (20μg) was added to 2 ml bile as an internal standard. The bile was then extracted, the extract dried and then fractioned by high pressure liquid chromatography using a C-18 reverse phase column and methanol:water (65:35) for the eluting solvent. The fraction containing sporidesmin was collected and counted in a β scintillation counter. These counts, when corrected for the specific activity of the solution used for dosing and losses occurring during the extraction procedure, were used to determine the concentration of sporidesmin in bile.

RESULTS

The method used for estimation of biliary sporidesmin concentrations based on the measurement of radiolabelled sporidesmin in bile was sensitive to a level around 2 ng/ml for a 2 ml sample. This limit of sensitivity was sufficient to obtain an accurate measure of sporidesmin concentrations in bile of animals dosed with sporidesmin at a rate of 0.08 mg/kg/d over 3 days. The pattern of change in biliary sporidesmin concentrations was similar for all animals in that the concentrations increased from undetectable concentrations before dosing (< 2 ng/ml) to a maximum at 1.5 to 3 hr after dosing. Thereafter the concentrations showed a decline reaching low levels after 24 hr. A typical pattern of uptake and clearance of sporidesmin in bile of 1 ram is illustrated in Fig. 1. The maximum sporidesmin concentration in bile showed considerable individual variation among animals with the concentrations ranging from 10 to 55 mg/ml after the first oral dosing with sporidesmin. This maximum concentration of sporidesmin in bile showed an increase in some animals from the first to the third dosing while other animals showed little change (Fig. 2). The relationship between maximum sporidesmin concentration and liver-damage score was statistically significant ($P < 0.05$) after the third but not the first dosing with the toxin. One ram was eliminated from the experiment because of its early atypical response and death. Those sheep bred for resistance to facial eczema showed lower liver-damage scores (mean 1.71; n = 7) and peak bile sporidesmin concentrations (mean 37 ± 14 (SD) ng/ml) than the susceptible rams which were 4.75 (n = 4) and 59 ± 16 ng/ml respectively.

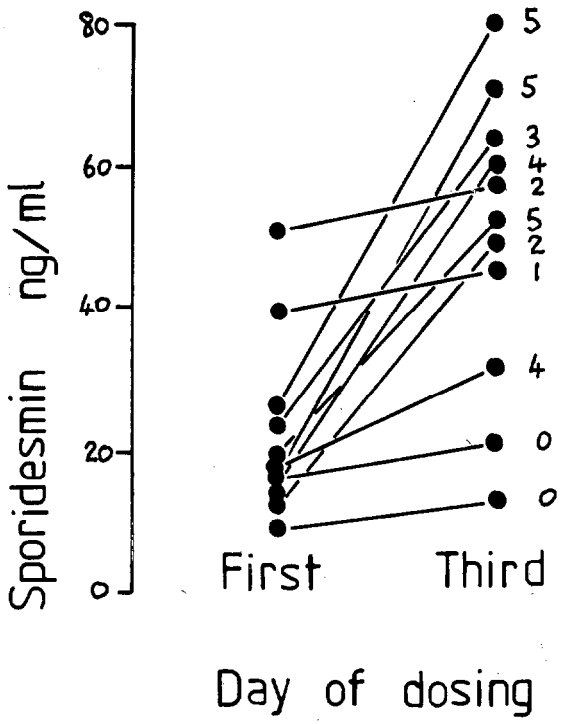

FIG. 2 Maximum concentrations of sporidesmin in bile at the first and third oral dosing of the toxin. Numbers on graph are the liver-damage scores at slaughter.

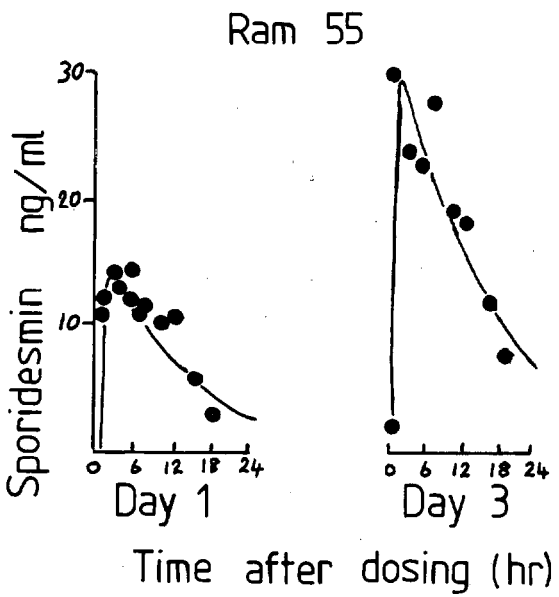

FIG. 1 Biliary concentrations of sporidesmin in ram 55 following oral dosing of the toxin on day 1 and 3. The liver-damage score of this animal was 4.

DISCUSSION

Previous studies of Lever (1968) and Mortimer and Stanbridge (1968) showed that sporidesmin was

secreted in bile when the sheep were dosed orally with 0.5 to 1 mg sporidesmin/kg live weight. Although these doses were considerably higher than the quantity used for dosing in the present experiment (0.08 mg/kg/d over 3 days), the pattern of uptake and clearance observed by Lever (1968) and Mortimer and Stanbridge (1968) in bile was essentially the same as that found in this study in that maximum concentrations in bile occurred after 1 to 2 hr and then declined over 24 to 72 hr. The maximum concentration of 2 to 20 μg/ml bile reported by these 2 investigators was, however, considerably higher than the biliary sporidesmin concentration of 10 to 80 ng/ml found in this study even allowing for the difference in the dose rate. These observations suggest that with an increase in dose rate there is a proportionately greater increase in the concentration of sporidesmin in bile.

The most interesting finding in the study was the observation of an apparent effect of the first dose of sporidesmin on the concentration of toxin appearing in bile at the third dosing. In general 'sensitive' rams (liver-damage scores 4 to 5) showed a marked increase in maximum sporidesmin concentration in bile between the first and third dosing whereas 'resistant' rams (liver-damage scores 0 to 1) showed no marked increase at these times. These observations raise the possibility that an initial or 'potentiating' dose of sporidesmin may be required before 'resistance' or 'susceptibility' is expressed. Furthermore, since it is known that the hepatocyte cell is primarily responsible for the metabolism and transport of toxins from the bloodstream to the bile ducts of the liver then it seems possible that a sporidesmin induced biochemical change within this organ is responsible for the observed variation in response among animals to a sporidesmin challenge.

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