VIRULENCE OF TWO STRAINS OF COWDRIA RUMINANTIUM IN MICE AND THEIR USE TO PREDICT DRUG ACTIVITY AGAINST HEARTWATER

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SUMMARY

A study was made of the infectivity of two mouse-adapted strains of Cowdria ruminantium in mice. The Kwanyanga strain was most virulent in Balb/C mice which died nine days after infection with homogenate of liver from infected mice. CD-1 mice were least susceptible of six strains tested. The du Plessis strain of C. ruminantium was equally virulent in all six mouse strains. The du Plessis strain in CD-1 mice was used as the basis of a drug screen to detect activity against heartwater (C. ruminantium infection) and was highly predictive when active compounds were tested in sheep infected with the Ball 3 strain of C. ruminantium.

INTRODUCTION

Research on heartwater (Cowdria ruminantium infection) has been severely restricted by the absence of a convenient laboratory model of the infection since C. ruminantium is so specific to ruminants as its host. Attempts to adapt it to grow in cell culture have all failed. It has been recognised for many years that the organism remained viable in mice and ferrets (Mason and Alexander, 1940) for several weeks but the infection is usually asymptomatic and not lethal. Then in 1971 du Plessis and Kumm reported that they had isolated the organism from an infected goat in laboratory mice; the infection was readily passaged between mice by the injection of blood or a variety of homogenised tissues, liver by choice. The infection killed mice in 10 to 14 days. Sheep and goats infected with this organism developed typical heartwater but calves did not. There was therefore some doubt as to whether it was C. ruminantium. In 1980 MacKenzie isolated another strain of Cowdria in mice this time from a sheep naturally infected in the Eastern Cape region of South Africa. It behaved remarkably similarly to du Plessis strain and was infective to calves as well as sheep (MacKenzie and van Rooyen, 1981). This strain designated the “Kwanyanga strain” (Kwa) satisfied Koch’s postulates. It is now accepted that both these strains are C. ruminantium. MacKenzie isolated a second lethal strain of Cowdria from sheep in the Nonile area of Natal in mice but was unable to make a second lethal isolation at Kwanyanga. This report investigates the nature of the Kwanyanga and du Plessis strains of C. ruminantium in mice and describes their use in a screen for compounds effective for the treatment of heartwater. The efficacy of active compounds was confirmed by experiments in sheep artificially infected with the Ball 3 strain of C. ruminantium.

MATERIALS AND METHODS

Initial studies at Kwanyanga, South Africa were conducted in male albino mice randomly bred and of unspecified origin. Later work at Beckenham, UK used initially male CD-1 mice randomly bred and in-bred male mice of seven strains—CBA/CA, C3H/HE, C57 BL/6, DBA2, Balb B, C and K. All weighed 15
to 20 g; they were boxed in groups usually of 10 mice, fed a pelleted diet and water
*ad lib.* Dorper and merino lambs aged three to 12 months of both sexes were used.

The Kwanyanga and du Plessis strains of *C. ruminantium* were used in mice; the
Kwa and Ball 3 blood-passaged laboratory strains were used in sheep. Mice were
infected intravenously (i/v) unless otherwise specified. Infected blood from
moribund mice was withdrawn into a solution of heparin to give a final
concentration of 10 iu/ml. Infected mouse liver was prepared by removing the
entire liver from a moribund mouse, homogenising it in 10 ml of physiological
saline in a glass tissue grinder and allowing the debris to settle by standing on the
bench at room temperature for about 5 min. The standard inoculum was 0·1 ml of
blood or 0·1 ml of liver homogenate supernatant. When necessary infective
material from other tissues was prepared as for liver. Sheep were infected by the i/v
injection of 5 ml blood from a donor sheep clinically sick with heartwater.
Experimental compounds, all of them dithiosemicarbazones, were micronised and
prepared for injection as aqueous suspensions appropriately diluted. They were
administered subcutaneously (s/c) to mice, i/v or *per os* (p/o) to sheep.

*C. ruminantium* is extremely difficult to demonstrate in infected mice although
infrequent organisms are observed in stained smears of liver, spleen and peritoneal
fluid (du Plessis and Kumm, 1971) but not in brain capillaries, a predilection site in
ruminants. The only quantitative parameter of infection in mice is therefore time to
death. In sheep the course of infection is monitored by daily rectal temperature
readings, general clinical observation and time to death or recovery. A temperature
of 40·5 ° or higher was regarded as significant pyrexia. The infection was confirmed
at autopsy by typical symptoms of heartwater, hydropericardium, hydrothorax and
ascites, and demonstration of colonies of parasites in capillary endothelial cells in
stained brain squashes. None of these symptoms was seen in any strain of mice. The
cause of death in mice is not known.

**RESULTS**

*Experiment 1.* To observe the course of development of infective organisms in
South African white mice 30 were infected with liver homogenate from a moribund
mouse infected with the Kwa strain. Daily at random two mice were sacrificed and
supernatant prepared from the pooled livers. Groups of 10 mice were injected i/v
with 0·1 ml of the supernatant on each day. No donor mice survived beyond day 10.
From day 6 onwards the liver was consistently lethally infective and a major

### TABLE I

**Susceptibility of strains of mice to infection with Kwa strain**

<table>
<thead>
<tr>
<th>Mouse strain (groups of 10)</th>
<th>Infected i/v</th>
<th>Infected i/v</th>
<th>Infected i/p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality</td>
<td>Days to death</td>
<td>Mortality</td>
</tr>
<tr>
<td>Balb/C</td>
<td>10</td>
<td>9·0 ± 0·00</td>
<td>10</td>
</tr>
<tr>
<td>CBA/CA</td>
<td>10</td>
<td>12·1 ± 1·10</td>
<td>10</td>
</tr>
<tr>
<td>C3H/HE</td>
<td>10</td>
<td>11·8 ± 1·23</td>
<td>10</td>
</tr>
<tr>
<td>C57 BL/6</td>
<td>10</td>
<td>11·5 ± 0·71</td>
<td>10</td>
</tr>
<tr>
<td>DBA/2</td>
<td>10</td>
<td>11·2 ± 0·92</td>
<td>10</td>
</tr>
<tr>
<td>CD-1</td>
<td>8</td>
<td>12·6 ± 1·60</td>
<td>7</td>
</tr>
</tbody>
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