The effects of thiabendazole, mebendazole and cambendazole in normal and immunosuppressed dogs infected with a human strain of Strongyloides stercoralis

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Abstract
The effects of three benzimidazole anthelmintics in dogs infected with a human strain of *Strongyloides stercoralis* were investigated. Cambendazole, but not thiabendazole or mebendazole, abrogated the subsequent development of a patent infection when administered at the same time as infection to immune competent dogs. None of the drugs eradicated infection when given after the onset of patency in immunosuppressed animals, although worm burdens were greatly reduced in dogs treated with cambendazole. The implications of these findings for the treatment of patients with strongyloidiasis, particularly those with disseminated infections, are discussed.

Introduction
*Strongyloides stercoralis*, one of the major human intestinal nematodes, is resistant to most anthelmintics. The benzimidazole compound, thiabendazole, has been the prime agent for the therapy of strongyloidiasis since its introduction in 1963 (Franz, 1963). Nevertheless, this drug has significant toxicity (Grove, 1982b) and is not always effective in either normal or immunosuppressed patients with strongyloidiasis (Scowden et al., 1978; Igra-Siegman et al., 1981; Panyathanya et al., 1983). Consequently, two related compounds, mebendazole (Chongsuphajaisiddhi et al., 1976; Musgrave et al., 1979; Louzado, 1981; Marava, 1983) and cambendazole (Louzado, 1981; Martirani & Rodrigues, 1976; Amato Neto et al., 1978; Huggins, 1979; Bicalho et al., 1983), have been used with variable success in recent years.

Experimental studies in mice infected with *S. ratti* and *S. stercoralis* provided a means for investigating the effects of these compounds in *vivo* (Grove, 1982a). Thiabendazole had no effect on migrating *S. ratti* larvae and did not expel adult worms from the gut but did reduce their fecundity. In contrast, mebendazole and cambendazole eliminated *S. ratti* adult worms from the intestine but had little effect on migrating larvae. Furthermore, whereas thiabendazole and mebendazole had no significant effect on *S. stercoralis* third-stage larvae in the muscles of mice, cambendazole eradicated this parasite from the tissues. The proposition that cambendazole may have significant advantages over the other two benzimidazole compounds in the treatment of strongyloidiasis was supported by observations on the effects of these drugs in *vivo* (Grove & Northern, 1986). Only cambendazole impaired the viability of first- and second-stage *S. ratti* larvae and only this drug abrogated the subsequent development of infection when *S. ratti* and *S. stercoralis* infective larvae were pre-incubated with drug.

Since only limited information can be derived from *in vitro* studies, observations on *S. ratti* infections in mice, or on *S. stercoralis* in mice (in which infective larvae migrate mostly to the muscles and do not develop any further) (Grove et al., 1986), further studies were undertaken in dogs infected with *S. stercoralis*. In these animals, patent infections develop; furthermore, infections persist if the dogs are immunosuppressed (Grove et al., 1983). 2 experiments were performed with each drug. Firstly, the effect of administration of drug concurrently with infection with third-stage larvae on the subsequent development of a patent infection was observed. Secondly, patent infections were produced in immunosuppressed dogs, anthelmintics were then administered, and the animals were followed long-term for evidence of infection, assessed by excretion of larvae in the faeces. Eventually, the dogs were killed and the presence or absence of worms in the bowel was determined.

Materials and Methods
*Parasites.* *S. stercoralis* worms were recovered originally from a human subject who had been infected while a prisoner-of-war in southeast Asia over 40 years ago (Grove, 1980). This parasite has since been passaged in mongrel dogs; the methods of preparation of larvae, infection of animals and counting of larvae in the faeces have been described by Grove & Northern (1982).

*Dogs.* Male mongrel dogs, obtained from the local dog pound, were washed, treated with the anthelmintics buminamide hydrochloride and pyrantel pamoate, and then immunized with canine measles, parvovirus, distemper and hepatitis vaccines. Their faeces were examined to ensure that they were free of helminths. Dogs were provided with food and water *ad libitum*, and housed in individual cubicles which were washed thoroughly each day, so that there was negligible chance of either cross-infection or external reinfection. The technique for examination of the bowel for *Strongyloides* has been described by Grove et al. (1983).

*Drugs.* Thiabendazole and cambendazole were supplied by Merck, Sharp and Dohme (South Granville, New South Wales), and mebendazole was obtained from Janssen Pharmaceutica (Lane Cove, New South Wales). Prednisolone tablets (25 mg) were manufactured by Fawns and McAllan (Croydon, Victoria). Thiabendazole, mebendazole and prednisolone tablets and cambendazole paste were administered orally directly by hand.
Results

Treatment at the time of infection

A series of experiments was undertaken in which dogs were infected with 5000 infective larvae percutaneously and treated at the same time with thiabendazole 25 mg/kg twice daily for 3 days, mebendazole 100 mg twice daily for 3 days, or cambendazole 25 mg/kg daily for 3 days. The numbers of larvae in the faeces 3 weeks later, and whether or not S. stercoralis infective larvae developed in faecal cultures, are shown in Table 1. Only cambendazole abrogated the development of a patent infection; when the numbers of infected dogs in the control and treated groups were compared by Fisher’s exact test, there was a significant difference ($P = 0.008$).

Treatment of established infections in immunosuppressed dogs

In a preliminary experiment, a dog in which a patent infection had been maintained for 6 months by immunosuppression was treated with thiabendazole 25 mg/kg twice daily for 3 days and kept on 50 mg prednisolone daily. One week later, larvae could not be identified in its faeces. 8 weeks after thiabendazole treatment, larvae were again seen in the faeces.

A series of experiments was therefore undertaken in which dogs were infected with 10,000 infective larvae percutaneously and were given, beginning on the day of infection, 50 mg of prednisolone daily, which was increased to 100 mg daily 8 weeks later. All dogs developed patent infections and were then treated with anthelmintics on 2 or 3 occasions, beginning 6 weeks after infection (Table 2). Thiabendazole and, to a lesser extent, mebendazole transiently cleared larvae from the stools but, several months later, larvae were easily demonstrable in the stools and plentiful adult worms and rhabditiform larvae were seen in the bowel at autopsy. Parasites could not be identified in the stools of dogs treated with cambendazole, and only very sparse numbers of adult worms and rhabditiform larvae were found in the bowel at autopsy.

Discussion

S. stercoralis contrasts starkly with almost all other worm infections as it can replicate within the human host. Consequently, unless all the worms are erad-

Table 1—Effects of administration of anthelmintics at the time of percutaneous infection with Strongyloides stercoralis on the development of a patent infection 3 weeks later

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of dogs</th>
<th>Larvae per g faeces mean±SD</th>
<th>Number of positive faecal cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiabendazole</td>
<td>3</td>
<td>26±9</td>
<td>3</td>
</tr>
<tr>
<td>None (controls)</td>
<td>3</td>
<td>55±28</td>
<td>3</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>3</td>
<td>120±150</td>
<td>3</td>
</tr>
<tr>
<td>None (controls)</td>
<td>3</td>
<td>230±320</td>
<td>3</td>
</tr>
<tr>
<td>Cambendazole*</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None (controls*)</td>
<td>6</td>
<td>165±300</td>
<td>5</td>
</tr>
</tbody>
</table>

*2 experiments were combined as one control dog failed to develop a patent infection.

Table 2—Effects of anthelmintics administered to immunosuppressed dogs with established infections of Strongyloides stercoralis. Excretion of larvae in faeces and the presence of rhabditiform larvae and adult worms in the small intestine at autopsy are shown at various times after treatment

<table>
<thead>
<tr>
<th>Weeks of infection</th>
<th>Drug treatment</th>
<th>No. of dogs</th>
<th>Larvae per g faeces</th>
<th>No. of positive faecal cultures</th>
<th>Worms in bowel at autopsy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Thiabendazole</td>
<td>3</td>
<td>160-410</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>3</td>
<td>&lt;=58</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>24</td>
<td>3</td>
<td>&lt;=230</td>
<td>2</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Mebendazole</td>
<td>3</td>
<td>170-380</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>3</td>
<td>&lt;=56</td>
<td>3</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Cambendazole</td>
<td>3</td>
<td>170-430</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>2**</td>
<td>0-3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>28</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*± = Very sparse; ++ = plentiful.

**One dog developed an intercurrent illness and was removed from the study.
cated, the few parasites remaining may multiply and build up numbers again to pre-treatment levels.

**Strongyloidiasis** is difficult to diagnose (Grove, 1980), so, although clearance of worms from the stools after administration of anthelmintics could indicate eradication of the parasite, it may equally well reflect merely a reduction in adult worm burden or inhibition of fecundity of adult worms.

A major problem in interpreting clinical assessment of the efficacy of thiabendazole is the short duration of follow-up. In 2 studies in which patients living in a non-endemic area were observed for 6 months after treatment, a significant proportion of individuals was shown to be persistently infected (Domart et al., 1967; Grove, 1982b). The present investigation has confirmed the suggestion from these papers, and from the in vitro and mouse studies mentioned earlier, that thiabendazole is likely to be ineffective in the treatment of strongyloidiasis. When thiabendazole was administered during the migratory phase of infection, i.e. on the day of infection and for the following 2 days, it did not impair subsequent development of patent infections. Furthermore, there was no significant difference in worm burdens, assessed by larval excretion in the faeces, between treated and control dogs. Therefore, thiabendazole has no effect on migrating larvae. It is probable that patients with strongyloidiasis would harbour worms in all stages of development. Thus, even if thiabendazole were active against adult worms, tissue larvae would subsequently produce patent infections. This occurred when thiabendazole was given to immunosuppressed dogs with patent infections. There was a transient clearance of worms from the stools, but after 8 weeks all dogs were still infected. Since S. ratti larvae migrate transiently through the central nervous system of mice (Dawkins et al., 1982), and as S. stercoralis infective larvae have been recovered from human cerebrospinal fluid (Meijer et al., 1979), and because thiabendazole could conceivably not pass through the blood-brain barrier, dogs were re-treated and then given a second course of thiabendazole one week later, by which time it was hoped that any intracranial parasites would have migrated elsewhere. Again, however, larvae reappeared in the stools, and large numbers of parasites were found in the small intestine at autopsy. Clearly thiabendazole is of limited value in the treatment of strongyloidiasis, although it may keep down the worm burden. The most logical way of using this drug, especially in immunosuppressed patients, would be to give repeated courses of treatment.

In their review of mebendazole, Keystone & Murdoch (1979) remarked that the place of this agent in the treatment of strongyloidiasis was uncertain. The drug is poorly absorbed in dogs and humans and may therefore have little opportunity to affect infective larvae, even if intrinsically active (Van den Bossche, 1986). Response to treatment with mebendazole in dogs was disappointing. It had no effect on the subsequent development of patent infection when given concurrently with infective larvae. Moreover, there was a very poor response in terms of temporary elimination of rabbitiform larvae from the stools in immunosuppressed dogs with patent infection, and plentiful parasites were found in the small intestine at autopsy. Thus, mebendazole appears to have little place in the treatment of strongyloidiasis.

The initial experiment with cambendazole was encouraging and seemed to confirm previous indications of its superior effectiveness. In contrast to thiabendazole and mebendazole, cambendazole completely inhibited the subsequent development of a patent infection when given concurrently with infective larvae. However, cambendazole did not eradicate the infection in immunosuppressed dogs with established infections. Nevertheless, the worm burdens were greatly reduced when compared with those in dogs treated with thiabendazole or mebendazole. Following the second and third courses with cambendazole (given twice, one week apart, like thiabendazole in case the drug could not cross the blood-brain barrier), no larvae were detected in the stools during the ensuing 9 weeks, but very sparse numbers of parasites were seen in the bowel.

Cambendazole, like other benzimidazoles, affects helminth mitochondrial electron transport by inhibiting phosphoenolpyruvate carboxykinase and fumarate reductase (Rahman & Bryant, 1977). Furthermore, like mebendazole, cambendazole binds to tubulin, the dimeric subunit protein of microtubules, and inhibits assembly of these microtubules resulting in their loss from the intestinal cells of nematodes and the cells' subsequent degeneration (Irland et al., 1979). However, the various observed differences in the effects of cambendazole on Strongyloides suggest that this drug may act in a different manner.

The few worms found in the bowel of cambendazole-treated dogs at autopsy may have been intrinsically resistant worms that had been selected. A much less probable explanation is that these worms developed from larvae that had been residing in a pharmacologically inaccessible site at the time of treatment. The existence of resistance against benzimidazoles has been well recognized in a number of usually susceptible species of nematodes, including Haemonchus, Ostertagia and Trichostrongylus. It has been postulated that benzimidazole resistance is mediated by a reduced affinity of the nematodes' tubulin for the drug (Sangster et al., 1985). The translation of such a mechanism into clinically significant effects is yet to be observed. Presumably, if dogs or humans were followed for long enough, worm numbers might increase sufficiently to reach clinically significant levels.

Despite the fact that cambendazole failed to eradicate infection in canine strongyloidiasis, it is clearly the best of the 3 benzimidazoles tested. Unfortunately, cambendazole has caused rare idiosyncratic reactions in ruminants (Hogg, 1978; Main & Vass, 1980), and the drug has been withdrawn by the manufacturers. However, cambendazole has been used in hundreds of patients, particularly in South America, apparently without any serious adverse effect. Since many patients with overwhelming strongyloidiasis fail to respond to treatment with thiabendazole, more consideration ought to be given to making cambendazole available for the management of such patients. An alternative benzimidazole is albendazole. This drug was not available to us when we began these experiments and we are currently studying its effectiveness in experimental strongyloidiasis. The only other classes of compound which seem to offer much hope for the management of this
difficult infection are the avermectins (GROVE, 1983) and the cyclosporins (SCHAD, 1986).

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References


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