Efficacy of levamisole alone and in combination with mebendazole against Gongylonema pulchrum infection in rabbits

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ABSTRACT. Gongylonema pulchrum is an important parasite of captive primates. Twelve rabbits were infected with 30 third-stage larvae of G. pulchrum. At 4–7 months post-infection, animals were administered levamisole at a single dose of 12 mg/kg, levamisole at 8 mg/kg three times at 2-day intervals, levamisole at a single dose of 8 mg/kg after administration of mebendazole at 70 mg/kg for 3 days or 8 ml of distilled water for 3 days (control). Necropsy at 14 days after treatment revealed that single and multiple dosages of levamisole reduced nematode burdens by 68.4% and 89.5%, respectively. The combined regimen of mebendazole and levamisole exhibited high efficacy for treating G. pulchrum located widely within the upper digestive tract, with a reduction of 98.2%. These results suggest that this combined chemotherapy treatment may be effective against G. pulchrum infection, including buccal and lingual gongylonemiasis in primates.

KEYWORDS: combined treatment, Gongylonema pulchrum, levamisole, mebendazole


Gongylonema pulchrum (gullet worm) is a cosmopolitan parasite that occurs in the upper digestive tract of a variety of mammals, including domestic and wild ruminants, equids, swine, primates, squirrels, rabbits, bears, skunks, hedgehogs and humans [4]. In Japan, it is most commonly found in cattle, but it has also been reported in wild deer (Cervus nippon), wild macaques (Macaca fuscata) and captive primates (Saimiri boliviensis) [8, 13, 15–17]. The life cycle of G. pulchrum includes dung beetles and cockroaches as intermediate hosts [4].

While G. pulchrum is typically found in the esophageal mucosa of definitive hosts without any apparent pathogenicity, the nematode causes buccal and lingual gongylonemiasis associated with pathologic changes and clinical signs in primates, including humans. To date, numerous cases of Gongylonema spp. infection have been identified in captive primates at zoological parks [1, 13], and fatal cases of gongylonemiasis due to severe inflammation of the lips and tongue have been reported in Goeldi’s monkeys (Callimico goeldii) and Common marmosets (Callithrix jacchus) [3, 5]. According to Brack [3], clinical signs of disease in the infected Common marmosets consisted of intensive itching and scratching of the edematous and hyperemic periportal tissues, with inflammation of the lips aggravated by the intense scratching. Duncan et al. [5] suggested that lingual gongylonemiasis associated with oral inflammation and irritation in Goeldi’s monkeys might have predisposed the animals to Pasteurella septicaemia, which resulted in their death. In addition, in humans, infection by G. pulchrum is usually associated with local irritation of the buccal mucosa [4]. Nematode infection of the tongues of slaughtered pigs accompanied by mild and chronic inflammation of the lingual mucosa has also been reported [18].

Although anthelmintic treatment of G. pulchrum infection is not generally recommended in livestock, treatments using ivermectin, mebendazole and fenbendazole have been reported in callitrichid primates with gongylonemiasis [1, 3, 5]. In these studies on naturally infected callitrichids, a combined regimen of ivermectin and mebendazole was more effective for improving clinical signs than either monotherapy with each drug or combined chemotherapy with ivermectin and fenbendazole. In addition, clinical signs in human infections were resolved following treatment with levamisole and albendazole [2, 6]. However, because these reports lacked comprehensive data on the efficacy of anthelmintic treatment and the extent of the reduction in the number of nematodes after treatment, more detailed studies are necessary to assess the therapeutic efficacy of anthelmintics against gongylonemiasis. We previously demonstrated that levamisole is more effective against G. pulchrum infection than thiabendazole, mebendazole or ivermectin, by post-mortem examination of experimentally infected rabbits [11].

Experimental infection of rabbits with G. pulchrum is considered to be well suited for examining the therapeutic characteristics of buccal and lingual gongylonemiasis, because of the high susceptibility of rabbits to infection by this nematode and the location of the nematode burdens in the buccal mucosa and tongue of the animals [9, 10]. This paper presents additional information on the efficacy of levamisole monotherapy and a combined chemotherapy regimen of levamisole and mebendazole against G. pulchrum infection using rabbits.

The G. pulchrum used in this study was originally isolated from naturally infected dung beetles (Aphodius rectus and...
A. haroldianus) in Aomori Prefecture, Japan. The nematodes were maintained in our laboratory using cockroaches (Blattella germanica) as an intermediate host and rabbits as the definitive host. Using a stomach tube, 12 Japanese white rabbits (10-week-old males) were individually inoculated with 30 third-stage G. pulchrum larvae (L3) that were obtained from experimentally infected cockroaches. The prepatent period of G. pulchrum is 72–81 days in rabbits [10]. At 4–7 months post-infection, when the larvae were fully developed, the animals were divided into 4 groups containing 3 animals each. The following treatments were administered orally to each group: Group 1 was administered levamisole hydrochloride (powdered levamisole hydrochloride, Yuko Chemical Industries Co., Ltd., Nishinomiy, Japan) at a single dose of 12 mg/kg body weight; Group 2 was administered levamisole hydrochloride at a dose of 8 mg/kg three times at 2-day intervals; Group 3 was administered levamisole hydrochloride at a single dose of 8 mg/kg after administration of mebendazole (Mebendazole administered levamisole hydrochloride at a single dose of 12 mg/kg) in this study. On the other hand, the efficacy of levamisole administered three times at a dose of 12 mg/kg levamisole (>0.05). Compared with the blood cell counts of group 4 (control) and the reference ranges for serum biochemistry values in normal rabbits [12], the blood tests revealed no side effects in drug-treated rabbits. In our previous study, necropsy of experimentally infected rabbits at 14 days PT revealed that monotherapy with levamisole at a single dose of 8 mg/kg, or mebendazole at 70 mg/kg for 3 days, reduced G. pulchrum burdens by 63.2% and 22.8%, respectively [11]. Compared with those results, no significant dose-dependent effect was observed when rabbits were treated with a higher single dose of levamisole (12 mg/kg) in this study. On the other hand, the efficacy of levamisole administered three times at a dose of 8 mg/kg was similar to that obtained using a single dose of 8 mg/kg. It therefore appears that a multiple dosage of levamisole has an additive effect in the treatment of G. pulchrum. Furthermore, the rate of nematode reduction obtained by combining mebendazole and levamisole was higher than that obtained with monotherapy. 

A similar synergistic effect between mebendazole and levamisole in the treatment of G. pulchrum infection. Indeed, a similar synergistic effect between these drugs has been reported in the treatment of Trichuris muris in mice [7].

While the number of nematodes recovered from the rabbit esophagi in groups 1 and 2 was markedly reduced compared to the number in the control group, some nematodes were found in the buccal mucosa, tongue and pharyngeal mucosa in both of these groups as well as in the control. These results indicate that multiple dosage of levamisole (group 2) may only be moderately effective for treating buccal and lingual gongylonemiasis in primates. On the other hand, except for the esophageal mucosa in group 3 (administered
CHEMOTHERAPY OF *G. PULCHRUM* INFECTION

Table 2. Recovery of *Gongylonema pulchrum* from the tissue and contents of the digestive tract of levamisole-treated rabbits at 12 and 48 hr after treatment

<table>
<thead>
<tr>
<th>Tissue of digestive tract</th>
<th>12 hr post-treatment</th>
<th>48 hr post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal mucosa</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Tongue</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pharyngeal mucosa</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Esophageal mucosa</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Walls of stomach and intestine</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Contents of digestive tract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>21</td>
</tr>
</tbody>
</table>

Rabbits were inoculated with 100 L3 of *G. pulchrum* per animal before treatment with levamisole (8 mg/kg) at 7 months post-inoculation. a) Not examined. b) Number of dead nematodes is shown in parenthesis.

mebendazole and levamisole), no nematodes were found in the upper digestive tract, implying that mebendazole acted to reduce the number of nematodes in the buccal mucosa, tongue and pharyngeal mucosa. There is little information on the efficacy of mebendazole treatment related to the distribution of nematode parasites within hosts. However, it has been reported that albendazole, which is structurally similar to mebendazole, stimulates the outward migration of *Gnathostoma spinigerum* to the dermis in humans [14]. In the same way, in this study, mebendazole may have caused the migration of nematodes from the tongue and buccal cavity to the esophagus where they were then reduced by levamisole. Further studies are therefore necessary in order to clarify the efficacy of mebendazole in stimulating migration of *G. pulchrum*.

The nematodes recovered from each group were all alive, and no dead nematodes were found in any of the tissues examined at 14 days PT. We therefore performed an additional examination to investigate the elimination of *G. pulchrum* in rabbits after levamisole treatment. Two rabbits were infected with 100 L3 of *G. pulchrum* per animal and treated with levamisole at a single dose of 8 mg/kg at 7 months after infection. The animals were sacrificed and examined for worms at 12 and 48 hr PT as described above, and the contents of the digestive tract were examined under a dissecting microscope.

As shown in Table 2, many nematodes were found in the contents of the digestive tract, with a few nematodes recovered from the tissues of the upper digestive tract at 12 hr PT. However, by 48 hr PT, worm recovery was restricted to the tissues of the upper digestive tract. Dead nematodes were only found in the contents of the stomach, small intestine and large intestine at 12 hr PT, indicating that the nematodes in the gut mucosa migrate into the lumen of the upper digestive tract immediately after levamisole treatment before being eliminated in the feces within 48 hr PT. According to Adkesson *et al.* [1], there is no reliable ante-mortem test for evaluating drug treatment against gongylonemiasis in callitrichid primates. Examining the feces for worms within 48 hr after levamisole treatment is therefore considered useful for evaluating the efficacy of drugs against *G. pulchrum* infection in these primates.

In conclusion, the combined chemotherapy regimen of mebendazole and levamisole exhibited high efficacy for treating *G. pulchrum*, which was located widely within the upper digestive tract. These findings suggest that this combined chemotherapy treatment may be effective against *G. pulchrum* infection, including buccal and lingual gongylonemiasis in primates. Indeed, it is considered that this drug regimen is able to contribute to the prevention and control of gongylonemiasis in captive primates, provided that coprophagous beetles and cockroaches are prevented from entering the primate enclosures.

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