Failure of Mebendazole in the Treatment of Humans with Trichinella spiralis Infection at the Stage of Encapsulating Larvae

E. Pozio,1 D. Sacchini,2 L. Sacchi,2 A. Tamburrini,1 and F. Alberici2

1Laboratory of Parasitology, Istituto Superiore di Sanità, Rome; 2Unità Operativa Malattie Infettive, Azienda Unità Sanitaria Locale, Piacenza; and 3Department of Animal Biology, University of Pavia, Pavia, Italy

Trichinella spiralis larvae infective for laboratory mice were collected from muscle biopsies performed at different times (from 1 day to 16 months) following the end of treatment, indicating the failure of mebendazole to kill Trichinella parasites when they are encapsulating in muscles.

Trichinosis is a parasitic zoonosis that is present throughout the world. When a host ingests raw meat infected with Trichinella larvae, within 5 or 6 days the larvae develop into adults in the gut of the host. The females then produce new larvae, some of which, after passing through the lymphatic vessels and then through the blood vessels, reach muscle cells. In these cells, after 2–3 weeks, the larvae become infective and the host cell develops into a nurse cell, which, in some cases, allows infective larvae to survive for years.

In humans, Trichinella infection still occurs in both industrialized and nonindustrialized countries; it is mainly associated with the consumption of pork, horse meat, and game [1, 2]. In countries where human trichinosis is endemic, physicians are more likely to recognize the infection early; in countries where infections are only sporadic, the diagnosis and, consequently, the treatment are often delayed. For example, in the northern hemisphere, most Trichinella infections occur during the winter season, when pigs are slaughtered on family farms, and during hunting season. Since the first signs and symptoms of Trichinella infection are similar to those of viral and bacterial infections, which are very common during this time of year, diagnosis is often made late.

The drugs most commonly used for treating human trichinosis are benzimidazole derivatives (i.e., albendazole, flubendazole, mebendazole, and thiabendazole, although thiabendazole is no longer on the market because of its side effects). However, studies in which mice were experimentally infected with Trichinella spiralis have shown that these drugs are apparently unable to kill encapsulating larvae (i.e., larvae in an advanced stage of development) [3–5]. The objective of the present study was to evaluate, among individuals infected with T. spiralis, the effectiveness of mebendazole when administered late in the course of an infection (i.e., when muscle larvae are at an advanced stage of development).

Patients and methods. In late February 1998, an outbreak of human trichinosis occurred in the town of Piacenza (in northern Italy) [6] in 92 individuals who had purchased and eaten raw horse meat in the last week of January (i.e., 3–4 weeks earlier). The meat had been purchased at a single butcher’s shop. Twenty-four individuals had severe symptoms and were admitted to the local hospital.

For all 92 individuals, blood samples were taken in order to measure the WBC count, eosinophilia, and muscular enzyme levels (creatine phosphokinase and lactate dehydrogenase). An ELISA with an excretory/secretory antigen was used to detect specific IgM and IgG antibodies to Trichinella, as described elsewhere [7]. Ten serum samples from individuals with trichinosis and 10 serum samples from individuals known not to have trichinosis infected were used as controls.

After diagnosis, all 92 individuals were treated with both mebendazole and an anti-inflammatory and antiallergic drug (methylprednisolone or prednisone). Immediately after the end of the treatment and/or 10–16 months after infection, 4 of the hospitalized individuals underwent biopsy of the deltoid muscle. Each specimen was cut into several pieces for performance of histologic analysis, transmission electron microscopy (TEM) examination, identification of larvae at the species level, and evaluation of the level of infectivity.

For the histologic analysis, the muscle biopsy specimen was fixed in 10% formalin. For TEM examination, the specimen was fixed in 5% glutaraldehyde in 0.1-M cacodylate buffer (pH, 7.2) for 4 h at 4°C; the sample was then washed 3 times in cacodylate buffer and postfixed with 1% OsO4 in the same buffer for 1.5 h at 4°C, dehydrated in ethanol, transferred to propylene oxide, and embedded in Epon 812 (Merck); sections
of 1 μm were stained with toluidine blue in 1% borax. Ultrathin sections stained with uranyl acetate and lead citrate were examined with use of a Zeiss EM 900 electron microscope (Zeiss).

In order to collect larvae, the muscle biopsy specimen was artificially digested by placing it in 1.0% pepsin (1:10,000 National Standard Formulary) and 0.5% hydrochloric acid, at a ratio of 25 g of tissue per liter, and then mixing on a magnetic stirring plate for 30 min at 40°C. After digestion, larvae were allowed to settle and the digestion fluid was decanted. Larvae were washed several times with warm tap water and counted in the final sediment by microscopy (magnification, ×50). Four larvae were used for species identification, which was accomplished with use of multiplex PCR analysis [8]. The remaining larvae from each biopsy specimen were orally inoculated by gavage into 4-week-old female CD1 mice to evaluate their infectivity. Five weeks after inoculation, the mice were killed by cervical dislocation; each carcass was skinned, eviscerated, and the muscle biopsy specimen was orally inoculated by larval capsules around the nurse-cell larva complex had thickened remarkably (figure 2A). Neither light microscopy nor electronic microscopy revealed partially or totally calcified larvae or larvae with signs of degeneration or morphological changes. TEM showed that the structure of the nurse cell was typical: an enlarged endoplasmic reticulum, a large strand of mitochondria, and hypertrophic nuclei with prominent nucleoli (figure 1B). The encapsulated larva did not show any morphological alterations and appeared to be surrounded by a thin capsule of connective tissue (figure 1C). Biopsy specimens examined 16 months after infection (about 15 months after the end of treatment) revealed that the collagen capsule around the nurse-cell larva complex had thickened remarkably (figure 2A). Electron micrographs of the nurse cell showed the presence of smooth and rough endoplasmic reticulum and a large amount of vesicles, ribosomes, Golgi com-

Table 1. Treatment regimen (anthelmintic and anti-inflammatory drugs) for individuals infected with Trichinella spiralis who underwent a muscle biopsy.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Mebendazole</th>
<th>Corticosteroid</th>
<th>Days from infection to:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Treatment regimen</td>
<td>Start of treatment</td>
<td>Biopsy of muscle</td>
</tr>
<tr>
<td>1</td>
<td>500 mg t.i.d. for 10 d</td>
<td>40 mg Mpred t.i.d. for 12 d</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>200 mg t.i.d. for 5 d, 500 mg t.i.d. for 7 d</td>
<td>50 mg Pred for 5 d, 80 mg Mpred for 3 d, 60 mg Mpred for 2 d, 50 mg Pred for 2 d, 25 mg Pred for 2 d</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>250 mg t.i.d. for 10 d</td>
<td>50 mg Pred for 7 d</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>500 mg t.i.d. for 10 d</td>
<td>50 mg Pred for 7 d</td>
<td>34</td>
</tr>
</tbody>
</table>

NOTE. Mpred, Methylprednisolone; Pred, prednisone.
before they develop in muscle cells [3–5]. By contrast, adult worms in the intestine and larvae in muscle cells appear to be less responsive or not responsive at all to benzimidazole treatment (table 2). Our results are consistent with these findings. We found that mebendazole had no effect on T. spiralis larvae in human muscles (table 2). In fact, even 16 months after infection, neither light microscopy nor TEM showed any morphological change in the Trichinella larvae present in the muscles of individuals who began treatment 20–34 days after the

FIGURE 1. Encapsulated larva of Trichinella spiralis in the deltoid muscle. A, Nurse-cell larva complex 42 days after the ingestion of infected meat; visible are inflammatory cells (arrows), larva (arrowhead), collagen capsule (asterisk), and nurse cell (nc; bar, 0.125 mm). B, Ultrastructural detail of a nurse-cell larva complex 42 days after the ingestion of infected meat; the nurse cell shows a hypertrophic nucleus (nu) with a prominent nucleolus (arrow), larva (l), and nurse cell (nc; bar, 5 μm). C, detail of the interface between the collagen capsule (cc) and the nurse cell (nc) 42 days after the ingestion of infected meat (bar, 5 μm).

FIGURE 2. Encapsulated larva of Trichinella spiralis in the deltoid muscle. A, The collagen capsule (cc) is remarkably thickened 481 days after the ingestion of infected meat (bar, 5 μm). B, Nurse cell with nucleolus (n) in the transcription phase, 481 days after the ingestion of infected meat (bar, 5 μm). C, Nurse cell with smooth reticulum (sr) and rough reticulum (arrows) and a large number of vesicles (v) and Golgi complexes (arrowheads), 481 days after the ingestion of infected meat (bar, 2.2 μm).

Discussions. In animals experimentally infected with T. spiralis, treatment with benzimidazole derivates at different times after infection has been shown to be effective against L₁–L₄ larvae at their intestinal stage and against newborn larvae complexes, and hypertrophic nuclei in the transcriptional phase (figures 2B and 2C). The encapsulated larva did not show any morphological alteration, which confirmed the results of infectivity in vivo.
results show that, once encapsulation has begun in humans, which corresponds to day 16 or 17 after infection [11]. Our detected 11 days after the larva invades the muscle cell [10], the same stage (i.e., L1) as those that are ingested and that are in our study. In fact, the larvae in the muscle cells are at the mebendazole, at least for 12 days and at the dosage administered. Thus it appears that the nurse cell acts as a barrier against treatment could inhibit glucose metabolism and kill larvae. The mode of action of this drug consists of its binding to -tubulin, which inhibits the polymerization of tubulin and the microtubule-dependent uptake of glucose. This apparently did not occur in our study, despite the fact that treatment lasted 10–12 days (the standard duration) [9]. However, we cannot exclude the possibility that a longer duration of treatment could inhibit glucose metabolism and kill larvae. Thus it appears that the nurse cell acts as a barrier against mebendazole, at least for 12 days and at the dosage administered in our study. In fact, the larvae in the muscle cells are at the same stage (i.e., L1) as those that are ingested and that are susceptible to mebendazole when they are still in the small intestine (table 2).

Table 2. Activity of mebendazole as related to developmental stage of Trichinella species in laboratory mice and humans.

<table>
<thead>
<tr>
<th>Developmental stage, location</th>
<th>Days after infection</th>
<th>Mebendazole activity in:</th>
</tr>
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<tbody>
<tr>
<td>Larva (L1–L4), in the small intestine</td>
<td>0–4</td>
<td>Yes</td>
</tr>
<tr>
<td>Adult, in the small intestine</td>
<td>5–28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Newborn larva, in lymph and blood vessels</td>
<td>6–21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
</tr>
<tr>
<td>Larva (L1), in muscle cells</td>
<td>7–30</td>
<td>No</td>
</tr>
</tbody>
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<sup>a</sup> According to [3-5].<br><sup>b</sup> Only suspected, on the basis of clinical results after treatment and experimental results in laboratory animals.<br><sup>c</sup> In humans, the duration of adult survival and the consequent time of new larva production were estimated on the basis of the results of experimental infections in pigs. The number of newborn larvae produced by females greatly decreases after 1–2 weeks.<br><sup>d</sup> Results of the present study.

ingestion of infected meat. Furthermore, the increased RNA synthesis in the nurse-cell nuclei was consistent with the finding of an increased number of polyribosomes and Golgi complexes and the presence of an active endoplasmic reticulum, all of which are crucial to the parasite’s survival (figure 1). Larvae recovered from these infected individuals were infective for laboratory mice.

Experimental studies in mice have shown that in nurse cells infected with T. spiralis larvae, the collagen capsule can first be detected 11 days after the larva invades the muscle cell [10], which corresponds to day 16 or 17 after infection [11]. Our results show that, once encapsulation has begun in humans, T. spiralis larvae are resistant to mebendazole treatment.

Although, in this study, muscle biopsy specimens were not collected from a control group (i.e., infected individuals who were not treated), the lack of dead or even partially destroyed larvae, as revealed by means of both light microscopy and TEM, indicates that all of the larvae that were present in the muscle cells when treatment began had survived; moreover, these larvae were infective for laboratory mice.

The mode of action of this drug consists of its binding to free β-tubulin, which inhibits the polymerization of tubulin and the microtubule-dependent uptake of glucose. This apparently did not occur in our study, despite the fact that treatment lasted 10–12 days (the standard duration) [9]. However, we cannot exclude the possibility that a longer duration of treatment could inhibit glucose metabolism and kill larvae. Thus it appears that the nurse cell acts as a barrier against mebendazole, at least for 12 days and at the dosage administered in our study. In fact, the larvae in the muscle cells are at the same stage (i.e., L1) as those that are ingested and that are susceptible to mebendazole when they are still in the small intestine (table 2).

In light of these findings, the resolution of symptoms is apparently a result of corticosteroid therapy and not anthelmintic therapy, which suggests that the clinical picture observed in infected persons is almost exclusively due to inflammatory and allergic reactions, as observed in other parasitic infections [12]. This observation has also been confirmed by the detection of 1460 larvae per gram of Trichinella britovi in a specimen of quadriceps muscle from an asymptomatic person who acquired trichinellosis during corticosteroid therapy following renal transplantation [13].

Mebendazole remains the drug of choice against L1–L4 larvae in the intestinal phase (table 2). However, this phase lasts for only ~4 days after the ingestion of infected meat, and diagnosis and treatment this early are unlikely. Nonetheless, mebendazole treatment is effective in reducing the risk of increased larval deposition (table 2), which is especially high for persons receiving corticosteroid therapy, although the half-life of mebendazole is only 2–9 h, and most of it is excreted in the feces. Although adult worms, including gravid females, have been detected up to 17 weeks after infection in the small intestine of persons who died of trichinellosis, it is reasonable to assume that in humans, as in swine, the number of intestinal worms greatly decreases ~3–4 weeks after ingestion of infective larvae [14].

In a randomized trial that compared treatment albendazole with thiabendazole plus flubendazole as treatment for persons infected with T. spiralis, albendazole was found to be more effective, although 1 larva was recovered from a muscle biopsy specimen from a person treated with albendazole 16 months after infection [15]. In a mouse model, a significant decrease (of 94.7%) in the worm burden in mouse muscles was obtained only with albendazole treatment (100 mg/kg) [5]; however, the acceptable dosage for human beings is ~10 times lower [15]. In another experimental study in mice, treatment with mebendazole or flubendazole was found to be more effective than treatment with albendazole against larval and adult stages in the intestine [16].

Albendazole, flubendazole, and mebendazole differ mainly in their steric hindrance of the presence of a propylthio in the
first drug, and a benzoyl in the other 2 drugs, at position 5 of the benzimidazole carbamate. This suggests that not only mebendazole but probably also albendazole and flubendazole are incapable of killing encapsulating larvae in humans.

Physicians must be made aware of the fact that mebendazole is only effective against newborn larvae, and that larvae in the muscle cells can survive for up to 30 years [17], until the host induces the calcification process that, starting from the nurse cell, provokes the progressive calcification of the larvae.

References