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

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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 trichinellosis 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 (creatinine 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 trichinellosis and 10 serum samples from individuals known not to have trichinellosis 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 digested as described above.

Results. All 92 infected individuals tested positive for both IgM and IgG antibodies, and all of them presented with myalgia and fever. Other symptoms were facial edema (n = 77; 84%), arthralgia (n = 40; 43%), gastrointestinal disorders (n = 39; 42%), headache (n = 28; 30%), hemorrhages in the conjunctivae (n = 5; 5%), rash (n = 2; 2%), and neurological disorders (n = 2; 2%).

Four weeks after the ingestion of infected horse meat, the patients’ WBC counts ranged from 6200 cells/mL to 25,760 cells/mL. Eosinophilia, ranging from 4% to 48.8%, was detected in 85 individuals (92%). High creatine phosphokinase levels (range, 198–3480 IU) were detected in 51 individuals (55%), and high lactate dehydrogenase levels (range, 460–2562 IU) were detected in 44 (48%). High IgE total values (range, 11–680 ng/mL) were detected in 51 individuals (55%), in 85 individuals (92%).

Table 1 lists the specific drug regimens given to the 4 individuals who underwent a biopsy, which were chosen based on current recommendations for therapy [9]; the specific dosage and the duration of treatment varied according to clinical characteristics. During the course of treatment, most signs and symptoms in all 92 infected individuals promptly resolved; however, 2 weeks after the end of treatment, myalgia was still present in 41 (45%), arthralgia in 3 (3.2%), and 2 patients (2%) had fever, facial edema, headache, and neurological disorders.

Muscle biopsy was performed twice for 1 individual and once for the other 3 (table 1). Motile larvae were found in all biopsy specimens after artificial digestion. The number of larvae ranged from 13.4 to 36.1 per gram of muscle tissue. After electrophoresis, the PCR amplification of larva DNA showed a product of 173 bp, which is specific for T. spiralis. The mice injected with larvae from the 4 muscle biopsy specimens showed Trichinella larvae in their muscles 5 weeks after infection.

Light microscopic observations of semithin sections of muscle tissue revealed the presence of encapsulated larvae. The nurse-cell parasite complex appeared to be surrounded by a thin capsule of connective tissue, and inflammatory cells were observed to have infiltrated the outer zone of the collagen capsule (figure 1A). 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 very 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>Treatment regimen</th>
<th>Days from infection to:</th>
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<tbody>
<tr>
<td></td>
<td>Mebendazole</td>
<td>Corticosteroid</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>
</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>
</tr>
<tr>
<td>3</td>
<td>250 mg t.i.d. for 10 d</td>
<td>50 mg Pred for 7 d</td>
</tr>
<tr>
<td>4</td>
<td>500 mg t.i.d. for 10 d</td>
<td>50 mg Pred for 7 d</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

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

plexes, 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.

**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
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, which corresponds to day 16 or 17 after infection [11]. Further studies indicate that once encapsulation has begun, the collagen capsule does not develop in nurses cells in humans, and that in swine, the number of intestinal worms greatly decreases after 1–2 weeks. Therefore, it appears that the nurse cell acts as a barrier against treatment could inhibit glucose metabolism and kill larvae. This appears to be true even in humans, as in swine, the number of intestinal worms greatly decreases after 1–2 weeks. In fact, the larvae in the muscle cells are at the same stage (i.e., L1) as those that are ingested and that are present 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 present in our study.

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. 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 antihelminthic 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

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**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>
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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;c&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 d–30 y</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup> According to [3-6].
<sup>b</sup> Only suspected, on the basis of clinical results after treatment and experimental results in laboratory animals.
<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.
<sup>d</sup> Results of the present study.
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