THE EFFICACY AND TOLERABILITY OF TRICLABENDAZOLE IN CUBAN PATIENTS WITH LATENT AND CHRONIC FASCIOLA HEPATICA INFECTION

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Abstract. Current chemotherapy for the treatment of infections caused by the liver fluke Fasciola hepatica is not satisfactory. Therefore, the efficacy and tolerability of triclabendazole (TCZ) was assessed for this indication. Eighty-two patients (51 female, 31 male, age 15–81 yr, mean 42 yr) with chronic or latent F. hepatica infection refractory to previous anti-helminthic chemotherapy were enrolled in a 60-day open, non-comparative trial. Patients received 20 mg/kg TCZ as two doses of 10 mg/kg administered after food 12 hr apart. Efficacy of treatment was assessed by stool microscopy, determination of Fasciola excretory-secretory antigen (FES) in feces, and by ultrasonography (US) which were systematically performed pre-therapy and on Days 1–7, 15, 30, and 60 post-therapy. For continuous safety assessment, patients were hospitalized during the first week after therapy and then monitored at home for the appearance of any adverse events. Clinical chemistry and hematology tests were carried out on Days 1, 3, 7, 15, and 60, and whenever an adverse effect occurred possibly related to therapy. Seventy-one (92.2%) of the 77 patients who completed the 60-day follow-up period became egg-negative. Efficacy of therapy was supported by the disappearance or decrease of FES antigen and of ultrasonography abnormalities. In the 6 remaining patients, parasitological cure was achieved by another single TCZ dose of 10 mg/kg on Day 60. A total of 74 adverse events possibly related to therapy was reported by 54 patients. The most important adverse event was colic-like abdominal pain (40 patients [49%]) consistent with the expulsion of the parasite through the bile ducts as confirmed by US on Days 2–7. Most adverse events (53) were graded as mild, 20 as moderate, and only 1 as severe (a biliary colic responding to spasmolytic therapy within two hours). Triclabendazole 20 mg/kg is an effective therapy for the treatment of F. hepatica infection in patients who have failed to respond to other antihelminthic agents. Biliary colics reflecting the expulsion of dead or damaged parasites usually occur during Day 3–7 and respond well to spasmolytic therapy.

INTRODUCTION

Fascioliasis, a common zoonosis of ruminants occurring worldwide, is caused by the liver flukes Fasciola hepatica or Fasciola gigantica. Humans may ingest the infective larvæ (metacercariae) by eating contaminated aquatic vegetables (especially watercress), by drinking contaminated water, or by washing vegetables and kitchen utensils with metacercariae-carrying water.1,2 Human disease is endemic in many developing countries in relation to particular alimentary habits and/or poor hygienic conditions where there is a lack of access to safe water and the presence of outdoor defecation.1 Epidemic outbreaks have primarily been reported in Iran, the Far East, Egypt, South America, and the Caribbean.1,4 Recent environmental changes and modifications in human behavior have increased the risk in many populations.1,2,5 In Europe, infections related to the consumption of wild watercress occur sporadically, most frequently in France, Spain, and Portugal.1,2,5 In the United Kingdom fascioliasis has been identified in imported vegetables kept moist during transport.1 The metacercariae penetrate the intestines, circulate inside the body, and migrate through the liver, settling in the biliary system where the hermaphrodite flukes mature and start oviposition. Clinically, two stages of fascioliasis are distinguished: an acute stage characterized by fever, eosinophilia, and hepatosplenomegaly which coincides with the invasion of the liver by the larvae and a chronic-latent stage where symptoms (e.g., biliary colics and obstruction, intermittent jaundice, intercurrent cholangitis, gallstones) are induced by the presence of the adult flukes in the biliary system.9

Current chemotherapy options for the treatment of fascioliasis are not satisfactory: Emetine and dicyclomine are effective, but their use is restricted by the need for parenteral administration and limited tolerability, especially cardiotoxicity requiring electrocardiographic monitoring.2 Bithionol requires a 5–30 day regimen, and has a cure rate as low as 50%.2,4,6 Furthermore, these drugs are no longer commercially available. Other antihelminthics, such as praziquantel, metronidazole, and albendazole, are virtually ineffective.2,4,6,9 Very recently, nitazoxanide, an antiparasitic drug marketed in Mexico, has been reported to be effective against fascioliasis in a very limited number of patients.10 However, before any conclusion can be drawn, confirmation by investigations regarding its efficacy is required, and its tolerability in this indication needs to be assessed. The duration of the 7-day nitazoxanide treatment is a disadvantage in remote areas with limited health care facilities. There is, therefore, a need for a simple oral drug regimen with good tolerability and high efficacy for patients with fascioliasis.

Triclabendazole (TCZ), a benzimidazole with selective action against trematodes, has been used in veterinary medicine since 1983 for treating fascioliasis in sheep and cattle. In this setting, it is effective as a single dose therapy against both mature and immature forms of adult worms.11 The helminthicidal mechanism of action is at present unclear, but
the pharmacologically active TCZ sulfoxide and sulfone metabolites may act either by uncoupling oxidative phosphorylation in mitochondria, leading to loss of respiratory control or by binding to parasite tubulin monomers, thereby disrupting the tegument of both immature and mature flukes.12–14

MATERIALS AND METHODS

Study area and patients. In the town of La Palma in the Pinar del Rio province of western Cuba an outbreak of acute fascioliasis had occurred. Heavy rainfalls led to flooding, contaminating the lettuce fields situated at the foot of a hill where cattle grazed and defecated. Eighty-two patients (51 female, 31 male, age 15–81 yr, mean 42 yr), presumably infected by eating contaminated lettuce 9–11 months before, were enrolled in a 60-day trial to investigate the safety and efficacy of TCZ. An open, non-comparative study design was chosen because although all patients had already undergone at least one unsuccessful course of antiparasitic therapy, symptoms persisted and the worm burden did not decrease as determined by Fasciola antigen concentration. A placebo arm would neither have been accepted by the patients, nor was it ethically acceptable because one patient infected during the outbreak had already died, presumably in relation to the disease. Before the commencement of the study, the protocol was approved by the Cuban Ethics Committee and the International Ethics Committee in Freiburg, Germany.

Only patients who fulfilled all major eligibility criteria: 1) excretion of ova of Fasciola hepatica in stools, 2) written informed consent, and 3) the failure of at least one antihelminthic therapy completed more than 30 days before enrollment, were enrolled. Exclusion criteria were inability to comply with the study protocol; pregnancy; lactation; body weight < 20 kg; severe anemia (Hb < 5 mol/L); severe liver disease of other etiology; and severe renal, pulmonary, or cardiac dysfunction. To exclude the interference of prior antihelminthic drugs, patients who had taken any compounds with known antihelminthic activity less than 30 days prior to study entry or during the study period were not enrolled. Patients who at trial entry were receiving regular medication for conditions not related to Fasciola hepatica infection were allowed to continue their medication, if it was not expected to interfere with hepatic or biliary function. Patients were permitted to receive antiemetics to prevent nausea and vomiting, spasmolytics to reduce the symptoms associated with parasite expulsion, and/or antibiotics to prevent or treat bacterial superinfection.

Antiparasitic treatment. A standard dose of TCZ for treating human fascioliasis has not yet been established. Dosages from 5 mg/kg to 20 mg/kg have been applied (Muller and others, unpublished data). In the present study, patients received 20 mg/kg TCZ as two doses of 10 mg/kg administered after a fatty meal 12 hr apart. The rationale of this dosage which exceeded the dosage used in previous studies15–17 was to achieve maximum efficacy in our patients with a recent infection who had already proved refractory to other antihelminthic drugs. A previous therapy trial using a single dose of 10 mg/kg TCZ yielded a cure rate of 79% in asymptomatic patients.18 Absorption of TCZ, a highly lipophilic molecule, has been shown to increase greatly when taken with a meal rich in lipids. Based on pharmacodynamic studies, the 12-hr span between the two doses appeared to be best in achieving a maximum peak of the drug 4–12 hr later.19 Plasma concentrations of TCZ and its metabolites were determined in order to control the absorption of TCZ.

Evaluation of efficacy. Evaluation of efficacy relied on the assessment of primary criteria supported by additional parameters. A patient was deemed to be cured only if all primary criteria were fulfilled: clearance of ova from stools,20–22 decrease of Fasciola excretory-secretory antigens (FES) in feces below the cut-off level,23–25 and the absence of parasites at ultrasonography. The outcome of cure was supported by a decrease of anti-Fasciola antibody concentration,23 disappearance of eosinophilia, and intercurrent biliary colic accompanied by an increase of the bile duct diameter related to worm expulsion as seen ultrasonographically. Primary criteria for therapy failure were persistent fecal egg excretion and/or persistence of spontaneously moving parasites in the biliary system detected by ultrasonography. Additional criteria for therapy failure were an increase in anti-Fasciola antibodies, persistence or increase of FES, and/or of eosinophilia 60 days post-therapy, accompanied by the persistence of typical symptoms, such as nausea, abdominal pain, urticaria, and pruritus. A minor criterion was the absence of clinical signs (biliary colic) and ultrasonographic signs (bile duct dilatation) of worm expulsion after therapy.

Measurement of parasite eggs in the stool. Patient stool specimens were examined for the presence of parasite eggs by microscopy, at baseline, and on Days 3, 7, 30, and 60 post-therapy. Examinations were simultaneously carried out by the method described by Ritchie,26 rapid sedimentation technique,27 and by the Kato-Katz method.28

Immunological measurements of efficacy. The level of Fasciola-specific antibody in patient serum was monitored at baseline and throughout the study on Days 30, and 60 post-therapy. A soluble extract of somatic antigen of mature Fasciola hepatica with a protein concentration of 5 mg/L was used for the enzyme-linked immunosorbent assay (ELISA) test, where an optical density of ≥ 0.368 was considered positive.

The levels of FES in patient feces were measured using a sandwich ELISA method as described by Espino and Finlay.24,25 An optical density of ≥ 0.24 was considered positive.

Ultrasonographic examination. Ultrasound examinations were carried out throughout the study at baseline, daily on Days 1–7, and on Days 15, 30, and 60 post-therapy. Patients were examined using a portable US device equipped with a 3.5 Mhz convex and a 5 Mhz parallel scanner (Fukuda FF sonic 4500, Tokyo, Japan). Details on ultrasonographic methodology are published elsewhere.28

Clinical evaluation and safety assessment. For close monitoring, patients were hospitalized at the Institute of Tropical Medicine ‘Pedro Kouri’ (IPK) during the first 7 days after treatment in order to allow prompt therapy if adverse events occurred. Routine hematology and biochemistry parameters including erythrocyte sedimentation rate; red, white, and differential blood cell count; alkaline phosphatase; alanine amino transferase, aspartate amino transferase; bilirubine; serum urea; and creatinine; as well as urine sed-
Clinical signs and symptoms. The number of patients reporting clinical signs and symptoms of fascioliasis at baseline and at Day 60 is shown in Table 2. At study entry, 36 of 82 (44%) patients reported clinical signs and symptoms. On Day 60, 8 of 79 (10%) patients had signs and symptoms of fascioliasis, including the 6 patients deemed to have failed therapy by the continued presence of Fasciola hepatica eggs in the stool.

**Measurements of parasite eggs in the stool.** All patients (100%) were positive for the presence of Fasciola hepatica eggs in the stool at baseline. In this respect, repeated examinations by the Kato-Katz method were more sensitive than sedimentation. Average egg excretion, as compared to other settings, was moderate.\(^2\) By Day 60, all but 6 of 77 (8%) were egg-negative, resulting in a final cure rate of 92% (71 of 77 patients).

**Ultrasonography results.** Ultrasonography was found to be less sensitive in detecting parasites at baseline than expected. In three patients who also continued to excrete Fasciola eggs, parasites were detected at Day 60; in two of them parasites continued to move spontaneously, thus confirming therapy failure.

**Immunological results.** The number of patients where FES was detected decreased from 66 of 80 (83%) patients at baseline, to 28 of 78 (36%) at Day 7, and to 12 of 79 (15%) at Day 60: Six of these patients had no ova in stools by Day 60. Although FES was still positive, the level had dropped significantly and eventually decreased below the cut-off value by Day 90. These patients were, therefore, also classified as cured. In the remaining 6 (8%) patients, parasites were detected by other means (parasitology and ultrasonography).
and they were therefore classified as true treatment failures. As expected, anti-\textit{Fasciola} antibodies were still detected by Day 60 post-therapy, but in most patients antibody concentration had decreased (Table 2). Safety evaluations. Liver enzymes. Liver enzymes increased moderately between Days 7 and 15 post-therapy. Primarily alkaline phosphatase (AP) increased consistent with biliary congestion (Table 3).

\textbf{Adverse events.} A total of 74 adverse events were experienced by 54 of 82 (66\%) patients during the course of the study (Table 4). The adverse events deemed by the investigator to be possibly, probably, or highly probably related to therapy are shown in Table 4. Forty of 82 patients (49\%) experienced biliary colic, and 41 of 82 (50\%) had elevated alkaline phosphatase levels consistent with parasite expulsion from the hepatobiliary system confirmed by ultrasonography on Days 4–7. Three of the patients with biliary colic had associated obstructive jaundice. In 24 of 74 of the reported adverse events spasmolytic therapy was given. Transient fever of low to moderate degree occurred in 7 cases. In only one febrile patient antibiotic coverage was administered to prevent bacterial superinfection of the biliary tract. Most adverse events (53) were graded as mild and 20 as moderate; only one patient reported severe abdominal pain on Day 6 of the study and the pain resolved within 2 hr after a single 20 mg intramuscular dose of hyoscine butylbromide. Analysis of treatment failures. Four treatment failures continued to excrete parasite ova throughout the follow-up

\begin{table}[h]
\centering
\caption{Liver enzymes pre- and post-therapy}
\begin{tabular}{lcccccc}
\hline
Day & 3 & 7 & 15 & 30 & 60 & \\
\hline
Alkaline phosphatase (AP)\textsuperscript{a} & \\
No. of patients & 14/79 & 18/82 & 41/81 & 19/78 & 8/79 & 6/80 \\
% & 17.72 & 21.95 & 50.62 & 24.35 & 10.12 & 7.50 \\
Mean & 142.89 & 156.09 & 183.25 & 146.76 & 113.97 & 108.39 \\
Alanine aminotransferase (ALT)\textsuperscript{b} & \\
No. of patients & 33/82 & 34/82 & 54/81 & 41/79 & 28/79 & 29/80 \\
% & 40.24 & 41.46 & 66.67 & 51.89 & 35.44 & 36.25 \\
Mean & 30.15 & 37.28 & 118.79 & 48.33 & 22.06 & 22.40 \\
Range & 6–315 & 6–452 & 6–837 & 8–711 & 5–100 & 6–109 \\
Aspartate aminotransferase (AST)\textsuperscript{c} & \\
No. of patients & 41/82 & 40/81 & 54/81 & 36/79 & 36/79 & 37/80 \\
% & 50.00 & 51.25 & 66.67 & 45.57 & 45.57 & 46.25 \\
Mean & 28.21 & 34.65 & 67.06 & 34.28 & 23.48 & 22.86 \\
Bilirubin\textsuperscript{d} & \\
% & 5.00 & 4.94 & 7.32 & 11.39 & 6.33 & 7.50 \\
Mean & 0.59 & 0.51 & 0.72 & 0.66 & 0.61 & 0.56 \\
Range & 0.15–2.51 & 0.1–4.59 & 0.14–7.98 & 0.11–1.82 & 0.16–3.00 & 0.05–1.77 \\
\hline
\end{tabular}
\textsuperscript{a} Percentage of patients with AP > 170 IU/mL. \textsuperscript{b} Percentage of patients with ALT > 21 IU/mL. \textsuperscript{c} Percentage of patients with AST > 21 IU/mL. \textsuperscript{d} Percentage of patients with bilirubin > 1.2 mg/dL.
\end{table}
Table 4
Adverse experiences possibly, probably, and highly probably related to therapy

<table>
<thead>
<tr>
<th>Event</th>
<th>No.</th>
<th>Day of onset post-therapy median (range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biliary colic</td>
<td>40</td>
<td>5 (2–30)</td>
</tr>
<tr>
<td>Associated with jaundice</td>
<td>3</td>
<td>5, 9</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td>35</td>
<td>4 (1–60)</td>
</tr>
<tr>
<td>Nausea, anorexia, vomiting</td>
<td>7</td>
<td>4 (2–30)</td>
</tr>
<tr>
<td>Fever</td>
<td>7</td>
<td>3 (2–60)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>5</td>
<td>5 (1–60)</td>
</tr>
<tr>
<td>Associated with urticaria</td>
<td>2</td>
<td>1, 60</td>
</tr>
<tr>
<td>Associated with jaundice</td>
<td>3</td>
<td>5, 9</td>
</tr>
<tr>
<td>Intensity of events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Severe*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total events‡</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Total patients‡</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>

* When events occurred sporadically, the single days of onset in each patient are given.
† Biliary colic responding to spasmolytics within two hours.
‡ More than one event may have occurred in a single patient.

period. In two patients ova were no longer seen initially after therapy but reappeared at Day 60. This could also be explained by very early reinfection. However, TCZ is also active on immature flukes and the time between infection and appearance of ova (prepatency) is long (70 days), making true failure more likely. These patients may have lost some of their parasites and/or the fecundity of the parasites might have been affected transiently by TCZ.

Validity of statistical analysis was limited by the small number of therapy failures. No significant difference between therapy failures and cured patients was found for: 1) quantitative egg excretion, 2) FES-concentrations in feces at baseline, 3) absorption of TCZ and plasma concentrations of TCZ metabolites, and 4) frequency of biliary colic accompanied by bile duct dilatation and parasite expulsion after TCZ therapy as visualized by US.

All therapy failures responded to another dose of TCZ of 10 mg/kg, after the evaluation period ended at Day 60.

**Discussion**

Current therapy options for human fascioliasis are unsatisfactory. Antihelminthic drugs applied so far either require parenteral administration and hospitalization for surveillance of drug toxicity or are not sufficiently efficacious.4

The results of this study show that TCZ given at a double dosage of 10 mg/kg postprandially 12 hr apart is highly effective—the cure rate achieved was higher than any other known fasciocide. These results may be limited, however, by the open, non-comparative design of the study. This design was chosen because a placebo-control was ethically not acceptable since fascioliasis is life-threatening. However, although spontaneous self-healing of fascioliasis may sometimes occur,29 it is unlikely to have happened in such a high proportion of patients within the short observation period. More frequently, untreated fascioliasis may persist in humans for more than ten years due to the longevity of the parasite.7 The high efficacy of TCZ is underlined by the fact that all patients cured by TCZ had previously failed to respond to at least one other antihelminthic drug, in particular, praziquantel.2,20 The conceivable synergic effect of TCZ with a previously-taken antihelminthic is unlikely because the patients had completed these therapies more than 30 days before enrollment. It is safe to assume that synergism of their pharmacokinetic characteristics did not exist because previous antihelminthics had been completely metabolized and excreted before the study. The few patients who did not respond to the first course of TCZ were readily cured by a second dose of 10 mg/kg, two months later.

Moreover, TCZ is the only drug administered as a one-day therapy and is therefore suitable not only for chemotherapy but also as an operational component for control.5 Triclabendazole is also well tolerated: most adverse events possibly related to TCZ were mild, and only in some patients graded as moderate. In only one patient was a severe adverse event observed, a biliary colic, which resolved promptly after spasmolytic therapy. That the most important adverse event, i.e., biliary colic, is not related to drug toxicity but to the expulsion of dead or damaged parasites induced by TCZ has been supported by a number of observers. Firstly, biliary colics occurred between Days 3–7 post-therapy, whereas the maximum serum concentration of TCZ is achieved 4–10 hr post-ingestion, with elimination from the body occurring 48 hr post-ingestion.18 Secondly, hepatic enzymes increased significantly only from Day 7 forward but not on Day 3. This means that liver enzymes usually did not increase before but only after biliary colics had begun. Thirdly, biliary colic and increase of liver enzymes do not occur in patients treated with TCZ for other indications, such as paragonimiasis.29,30

And fourthly, ultrasonography observations showed that the adult *F. hepatica* stopped moving after 3 days post-therapy, and parasites and/or parasite fragments were seen to be eliminated through the bile ducts accompanied by a transient increase in bile duct diameter.28 Biliary colic is effectively controlled by the use of spasmolytic agents.

To date most therapies for fascioliasis have been relatively ineffective, and thus are unlikely to have induced expulsion of the parasite to the same degree as observed with TCZ. The occurrence of biliary colic in patients with fascioliasis can also be seen as an early indication of the effectiveness of the treatment. Whereas this effect is easy to manage in a hospital, it may limit the use of TCZ in community-based management. In this circumstance, a single dose regimen of 10 mg/kg may be preferable as first-step therapy.2,4,5,16,18,23,31 Nonetheless, further community-based studies require close surveillance of patients. Another alternative which should be investigated is the administration of TCZ in divided, increasing dosages or with the addition of prophylaxis with an oral spasmolytic drug to prevent biliary colic, especially during Days 3–7.

In conclusion, triclabendazole is the most effective drug for treating human fascioliasis. Adverse effects are related to its high efficacy in inducing parasite expulsion through the bile ducts. The results of the present study are supported by the results obtained in a total number of 622 patients in Egypt,51 Iran, South America, and Europe (Ripert C, unpublished data). Furthermore, TCZ can be given as a single-day therapy, a particular advantage in regions with limited healthcare facilities. The efficacy and convenience of drug administration prompted the World Health Organization Expert Committee on the Use of Essential Drugs to put TCZ
on the list of essential drugs. Triclabendazole has recently been registered for human use by the Egyptian Ministry of Health, which will make it widely available.

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