Efficacy of nitazoxanide and paromomycin in biliary tract cryptosporidiosis in an immunosuppressed gerbil model

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Objectives: To evaluate the efficacy of nitazoxanide and paromomycin in biliary tract cryptosporidiosis in an immunosuppressed Mongolian gerbil (Meriones unguiculatus) model.

Methods: Gerbils (1-month-old) were dexamethasone-immunosuppressed for 10 days and challenged orally with 10⁵ Cryptosporidium parvum oocysts. From day 0 to day 12 post-infection, one group (n = 14) was treated with 200 mg/kg/day nitazoxanide and another (n = 15) with 100 mg/kg/day paromomycin. Infection and efficacy of nitazoxanide and paromomycin were assessed by measuring oocyst shedding in faeces, biliary tract and ileum histological examination.

Results: In nitazoxanide-treated and paromomycin-treated groups as compared with untreated animals (P < 0.05), oocyst shedding was partially suppressed in a similar manner (P > 0.05). Parasites were present in histological sections of the ileal mucosa of 16/16 infected untreated animals versus 3/14 and 6/15 in the nitazoxanide-treated and the paromomycin-treated groups, respectively (P < 0.05). In addition, gall bladder infection was less frequent in nitazoxanide-treated (2/14, P < 0.01) and paromomycin-treated (5/15, P = 0.07) animals than in untreated controls (9/16). No histological alteration of biliary mucosa was observed in both treated and untreated infected gerbils.

Conclusions: Present data support the efficacy of nitazoxanide and, to a lesser extent, paromomycin on biliary C. parvum infection in gerbils, and prompt further investigation of the potential clinical benefits of nitazoxanide in treating human biliary cryptosporidiosis.

Keywords: Cryptosporidium parvum, Meriones unguiculatus, gall bladders, cryptosporidial cholangitis

Introduction

In cryptosporidiosis patients, cholangiopathy may result from biliary Cryptosporidium sp. infection, the most common extra-intestinal site.¹ In immunodeficient subjects with persistent cryptosporidiosis, including patients with the X-linked immunodeficiency with hyper-IgM, acalculous cholecystitis, sclerosing cholangitis or both may develop.²³ In one study, 70% of children with X-linked immunodeficiency with hyper-IgM who were systematically screened microscopically for infection exhibited Cryptosporidium parvum infection and all had clinically significant chronic liver disease.⁴ In patients with primary immunodeficiency, PCR procedures were found to be more sensitive than microscopy to detect Cryptosporidium infection.⁵ Cryptosporidium sp. was identified in the bile or intestine of 20–65% of patients with AIDS-associated cholangiopathy with sclerosing cholangitis leading to biliary cirrhosis and ultimately to liver failure and/or cholangiocarcinoma.¹³ In AIDS cholangiopathy patients, the presence of cryptosporidial infection at the time of diagnosis was found to be an indicator of poor prognosis.⁶ Recently, chronic gastrointestinal and extra-intestinal cryptosporidiosis with secondary sclerosing cholangitis has been reported in a child in the absence of an associated immunodeficiency.⁷ Biliary carriage represents a reservoir for intestinal cryptosporidiosis relapse which is not prevented by luminal agents such as paromomycin.⁸ The aim of this study was to evaluate the efficacy of nitazoxanide, a 2-acetyloxy-N-[(5-nitro-2-thiazolyl)] benzamide recently approved in the United States for treatment

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of cryptosporidiosis in immunocompetent children under 12 years of age, as compared with paromomycin on biliary tract \textit{C. parvum} infection in an immunosuppressed Mongolian gerbil (\textit{Meriones unguiculatus}) model.\textsuperscript{9}

Materials and methods

\textit{C. parvum} oocysts

Oocysts were purified from faeces obtained from calves experimentally infected by an isolate maintained by Drs Mancassola and Naciri, Laboratoire de Pathologie Aviaire, Institut National de Recherche Agronomique, Nouzilly, France, and confirmed as \textit{C. parvum}.\textsuperscript{10} Viable (>80% excluding propidium iodide) oocysts were purified from faecal samples as earlier described, and stored in phosphate-buffered saline (PBS), pH 7.2, at 4°C for <3 months before use.\textsuperscript{11}

\textbf{Agents}

A powdered form of nitazoxanide was obtained from Romark Laboratories, Tampa, FL, USA. Paromomycin was purchased from Sigma, St Louis, MO, USA. Agents were suspended in a 5% carboxymethylcellulose solution in water.

\textbf{Immunosuppressed gerbils}

Gerbils (4–5 weeks old; 22–27 g) free of Cryptosporidium sp. oocysts before the study were individually housed in plastic cages providing heat-sterilized rodent food and water \textit{ad libitum} and protected by a top filter. Animals were handled according to the technical and ethical regulations of the French Ministry of Agriculture and this project was approved by the ethics committee of ADEN. From 10 days before the day of oocyst ingestion (day 0) to day 12, gerbils were intraperitoneally injected with 0.80 mg/animal dexamethasone (Qualimed, Puteaux, France) every second day.

\textbf{Assessment of nitazoxanide and paromomycin anticryptosporidial activities in immunosuppressed gerbils}

On day 0, gerbils were gavaged with \textsuperscript{10} \textit{C. parvum} oocysts and divided into groups administered orally on the same day 10 mL/kg of a 5% carboxymethylcellulose water solution with nitazoxanide (100 mg/kg) (\textit{n} = 14), paromomycin (30 mg/kg) (\textit{n} = 15) or no agent (control group, \textit{n} = 16) twice daily for 12 days. These regimens were adapted from previous studies in immunodeficient rats.\textsuperscript{12,13} Faeces were collected on days 4, 8 and 12 post-infection from each gerbil, and pooled. Faecal smears were microscopically examined using Heine staining and 24 h shedding was expressed as mean blind oocyst count per 10 phase contrast microscopic field (MF) (x400 magnification) from 30 MF by two independent investigators. All animals were killed 12 days post-infection by deep pentothal anaesthesia and necropsied. Distal ileal segments and biliary tracts fixed in 10% formalin were cut, embedded in paraffin for blind histological examination of 4 μm sections stained with haematoxylin–eosin–safron and Giemsa, and considered infected if at least one cryptosporidial developmental form was microscopically observed within an epithelial cell by two independent investigators.

\textbf{Statistical analysis}

Comparison of intestinal oocyst shedding between groups was performed using the Wilcoxon’s rank sum test. Comparisons of the effects on ileal and gall bladder mucosa infection were performed using Fisher’s exact tests.

\begin{table}[h]
\centering
\caption{Effect of nitazoxanide and paromomycin on intestinal oocyst shedding in \textit{C. parvum} infected immunosuppressed Mongolian gerbils}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Agent (dose)} & \textbf{Number of gerbils} & \textbf{Oocyst number per 10 microscopic fields [median (maximum and minimum values)]} \\
\hline
Nitazoxanide (200 mg/kg/day) & 14 & 16.5 (7–29) & 10 (0–21) & 0\textsuperscript{b} (0–21) \\
Paromomycin (100 mg/kg/day) & 15 & 18 (8–28) & 12 (0–28) & 8\textsuperscript{b} (0–28) \\
None (control) & 16 & 12 (6–23) & 18 (9–27) & 23 (6–29) \\
\hline
\end{tabular}
\end{table}

Pooled results from two independent experiments.
\textsuperscript{a}Day post-infection.
\textsuperscript{b}P < 0.01 versus control.

\begin{table}[h]
\centering
\caption{Effect of nitazoxanide and paromomycin on ileal and gall bladder mucosa \textit{C. parvum} infection in immunosuppressed Mongolian gerbils}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Agent (dose)} & \textbf{Ratio of animals with }\textit{C. parvum} ileal infection & \textbf{Ratio of animals with }\textit{C. parvum} gall bladder infection \\
\textbf{P-value versus control} & \textbf{P-value versus control} & \\
\hline
Nitazoxanide (200 mg/kg/day) & 3/14 & <0.01 & 2/14 & <0.03 \\
Paromomycin (100 mg/kg/day) & 6/15 & 0.01 & 5/15 & 0.07 \\
None (control) & 16/16 & 9/16 & & \\
\hline
\end{tabular}
\end{table}

Results

As shown in Table 1, a significant decrease in oocyst shedding was observed in treated animals with the same magnitude for nitazoxanide and paromomycin (\textit{P} > 0.05). As shown in Table 2, the number of animals with infected ileum and/or gall bladder was lower in the nitazoxanide group than in the untreated group. In contrast, no significant decrease in the number of animals with infected gall bladder was found in the paromycin-treated group (\textit{P} > 0.05).

\textbf{Discussion}

In humans, biliary involvement due to cryptosporidiosis was documented in immunodeficient patients including HIV-infected individuals and patients with the X-linked hyper-IgM syndrome.\textsuperscript{14} In most animal models, infection was limited to the intestine during acute cryptosporidiosis.\textsuperscript{11,12} The biliary system was found to be involved in immunosuppressed chronically infected animals and usually not established before 4–5 weeks post-intestinal oocyst challenge. In the present protocol, Mongolian gerbils infected
Nitazoxanide and paromomycin in biliary gerbil cryptosporidiosis

10 days after the initiation of immunosuppression exhibited biliary cryptosporidiosis as assessed by gall bladder histological examination on day 12 post-infection.

When administered 4 h after infection, nitazoxanide and paromomycin at doses previously shown to be effective on gut infection in rats and neonatal mice prevented the establishment of developmental stages in the intestinal tract. Nitazoxanide exerted a more significant anticryptosporidial activity in the gall bladder than paromomycin, consistent with previous studies in other rodent models in which paromomycin exhibited limited effectiveness against biliary tract infection. Similarly, AIDS patients developed biliary tract infections during their treatment with paromomycin. Paromomycin was not absorbed by the human gut, in contrast with orally administered nitazoxanide whose metabolite tizoxanide glucuronide was found to be excreted in the biliary tract. Tizoxanide glucuronide was previously shown to exhibit predominantly its in vitro anticryptosporidial activity on intracellular parasitic forms, which may relate to the activity of nitazoxanide in vivo. Present data prompt further studies to determine if nitazoxanide has clinical activity in human cryptosporidial cholangitis.

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Transparency declarations

Jean-François Rossignol owns equity interest in Romark Laboratories, the pharmaceutical company that owns the patent for nitazoxanide.

References