Long-lasting anticryptosporidial activity of nitazoxanide in an immunosuppressed rat model

Xunde Li¹, Philippe Brasseur³, Patrice Agnaye¹, Denis Leméteil¹, Loïc Favennec¹, Jean-Jacques Ballet² and Jean-François Rossignol¹

¹Laboratoire de Parasitologie, Faculté de Médecine-Pharmacie, and ADEN, UPRES-EA 3234, 76138 Rouen, France;
²Laboratoire d’Immunologie et Immunopathologie, CHU Clemenceau, 14033 Caen Cedex, France;
³Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Key words: Cryptosporidium parvum, nitazoxanide, immunosuppressed rat model

Abstract. Cryptosporidium parvum, Tyzzer, 1912 is identified as a common cause of diarrhoea in immunocompetent individuals. While in immunocompetent individuals, cryptosporidiosis may be responsible for mild and self-limiting diarrhoea, infection may be prolonged and life-threatening in immunocompromised individuals, especially in AIDS patients (Griffiths 1998). Among the numerous compounds tested for specific treatment of cryptosporidiosis, none has been found consistently efficient (Blagburn et al. 1998, Tzipori 1998). Nitazoxanide (NTZ, 2-acetolylxylo-N-5-nitro 2-thiazolyl benzamide), a nitrothiazole salicylilate derivative partially absorbed (NTZ, 2-acetolylxylo-N-5-nitro 2-thiazolyl benzamide), a nitrothiazole salicylilate derivative partially absorbed from the gastro-intestinal tract, was found active on bacteria such as Helicobacter pylori, Clastodiscus difficile, Bacteroides fragilis, and a broad spectrum of parasites such as the helminths Taenia saginata, Hymenolepis nana, Fasciola hepatica and protozoans such as Isospora belli, Entamoeba histolytica, Giardia lamblia and Enterocytozoon bienuesi (Rossignol and Maison-Filliatre 1997, Bourek et al. 1998, Rossignol et al. 1998a, Bicart-See et al. 2000, McVay and Rolfe 2000). In clinical studies conducted with NTZ in AIDS patients with cryptosporidiosis, NTZ treatment reduced the duration of diarrhoea and oocyst shedding (Rossignol et al. 1998b, Davis et al. 2000, Rossignol et al. 2001) The aim of the present study was to document in vivo activity of NTZ in an immunosuppressed rat cryptosporidiosis model, compared to sinefungin (SNF) and paromomycin (PRM), which were previously found effective (Brasseur et al. 1994, Verdon et al. 1995).

MATERIALS AND METHODS

A previously described immunosuppressed rat model was used in this study Brasseur et al. (1988). Briefly, male Sprague Dawley rats from SPF breeding (Janvier, Saint Berthevin, France) weighting 200–250 g and free of C. parvum oocysts in faeces before experiment were used. Immunosuppression of animals was obtained by a regimen of 25 mg hydrocortisone acetate (Roussel, Paris, France) injected subcutaneously twice a week for 5 weeks before, and 3 weeks after challenge by C. parvum oocysts. Controls were immunosuppressed rats without challenge with C. parvum oocysts. Animals were fed a regular low protein (7%) diet (white bread exclusively), and housed one per cage. Each cage was sterilized twice a week to avoid possible reinfections. Drinking bottles were heat-sterilized every day. Cryptosporidium parvum oocysts were obtained from the faeces of a calf infected with an isolate of human origin maintained by serial passage in calves (a kind gift of Dr. Naciri, INRA, Nouzilly, France). Faeces were stored in a 2.5% (wt/vol) solution of potassium dichromate at 4°C for less than 3 months before use and oocysts were purified using a sucrose density gradient (d = 1.044 and d = 1.088). Each rat was infected by oral gavage at day 0 with 10³ C. parvum oocysts. From day 7 to day 14 after infection, 3 groups of 20 rats were administered nitazoxanide (NTZ, Romark Laboratories, Tampa, Florida, USA) at doses of 50, 100 and 200 mg/kg/day, respectively. NTZ was diluted in 5% (vol/vol) DMSO in distilled water and administered in 3 parts daily. A control group consisted of 20 rats receiving DMSO in distilled water without drug three times a day. During the same period, two groups of 4 rats were treated with 10 mg/kg/day of sinefungin (SNF, kindly provided by Aventis, Vitré sur Seine, France) or 100 mg/kg/day paromomycin...
Curative activities of NTZ, SNF and PRM were evaluated by measuring oocyst shedding. For each animal, faeces were collected at two days intervals between day 7 and day 21 post-infection, resuspended in a 10% (vol/vol) formalin solution, and homogenized. Oocysts were counted by phase-contrast microscopy examination of smears prepared by mixing faeces suspensions with a carbolfuchsine solution (Heine 1982). Results were expressed as the mean oocyst number per 10 microscopic fields (×400). Percentages of inhibition were calculated as : [(mean number of oocysts shed in the control group) minus (mean number of oocysts shed in the treated group)] ×100. In addition, the number of oocyst-shedder rats was noted in each group at day 21 and expressed as the ratio: (number of shedder rats) : (total number of rats).

Since a correlation was previously established in this model between oocyst shedding and ileal infection, control histological examinations were performed in 3 rats from each group at the end of experiment (Brasseur et al. 1994). A portion of ileum was collected, fixed in a 10% (wt/vol) formalin solution, embedded in paraffin and stained by haematoxylin-eosin. Developmental stages of *C. parvum* were counted in the epithelial cells of 10 ileal villi.

Statistical comparisons between groups were performed using the Chi-square test or the non-parametric Mann and Whitney test, depending on the distribution of data.

All experiments were performed in agreement with the standards for animal experimentation of the French Ministry of Agriculture.

**RESULTS AND DISCUSSION**

One day after treatment was initiated, NTZ at all doses exerted significant inhibition of oocyst excretion (*p* < 0.05, Fig. 1). From day 9 to day 21 after infection, NTZ inhibition (25–70%) was dose-dependent (*p* < 0.05) and peaked between days 13 and 21. Significant SNF and PRM inhibition was detected 1 and 2 days after initiation of therapy, respectively, and reached 90% at day 13 (Fig. 2). At all doses, NTZ inhibition was maintained for seven days (day 15 to day 21) after cessation of therapy on day 15. In contrast, cessation of PRM or SNF resulted in relapse of oocyst shedding 3 days later and lack of inhibition at day 21. At day 21, rats from all groups shed oocysts (except one in the group receiving 200 mg/kg/day NTZ). Mean numbers of developmental stages per 10 villi were 22, 17 and 10 (mean standard deviation: 11% of the mean value) in rats receiving 50, 100 or 200 mg/kg/day NTZ respectively, and were correlated with oocyst shedding (*r* = 0.82, *p* < 0.01). No histological alteration of mucosa was observed in NTZ-treated rats.

Data suggest that NTZ is efficient in reducing oocyst shedding in an immunosuppressed rat model of cryptosporidiosis, in which more than 30 agents have been previously tested with reproducible and consistent results (Leméteil et al. 1993). In contrast with SNF and PRM, NTZ activity lasted at least 7 days after discontinuation of therapy. Both SNF and PRM were used as positive controls since their activities had been previously demonstrated in reducing oocyst shedding and parasite density in histological sections (Leméteil et al. 1993, Brasseur et al. 1994, Tzipori et al. 1994, Healey et al. 1995). NTZ has been previously tested in a
REFERENCES


number of different animal models with varying degrees of activity depending on the model used, the dose and the method of administration. In a gnotobiotic piglet model, 11-day treatment with 250 mg/kg/day was effective in reducing oocyst shedding and mucosal infection (Theodos et al. 1998). However, using an anti-IFN-gamma conditioned SCID mouse model, it was concluded that NTZ administered in 100% DMSO was ineffective in reducing oocyst shedding and mucosal infection, although partial reduction of oocyst shedding was noted in some experiments (Theodos et al. 1998). In a neonatal mouse model, oral administration of NTZ was noted in some experiments (Theodos et al. 1998). These discrepancies may be at least partially explained by differences in anti-cryptosporidial activities due to drug formulation. Fast absorption and elimination of NTZ administered in 100% DMSO may be responsible for lack of efficiency reported in a SCID mouse model, and using 5% DMSO may avoid some of the “clumping” of the chemical in the intestine (Blagburn et al. 1998, Theodos et al. 1998).

Although SNF and PRM were highly active during the period of treatment, oocyst shedding resumed after discontinuation of therapy. In contrast, in animals treated with NTZ, a dose-related oocyst shedding inhibition lasted at least 7 days after cessation of treatment. A likely explanation is that intestinal NTZ absorption results in metabolite concentrations active against C. parvum biliary sequestration, and data prompt further investigation of intestinal and extraintestinal anti-cryptosporidial activities of NTZ and/or its metabolites (Roussel et al. 1996).


Received 10 June 2002

Accepted 2 October 2002