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Metronidazole and albendazole susceptibility of 11 clinical isolates of *Giardia duodenalis* from France

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The metronidazole and albendazole susceptibility of 11 clinical isolates of *Giardia duodenalis* from France was determined using a neonatal mouse model and compared with the outcome in patients after standard metronidazole therapy (0.75 g/day for 5 days). All isolates found to be clinically resistant to metronidazole (4/11) exhibited an ID_{50} > 120 mg/kg in the mouse model. This therefore appears to be a suitable animal model in which to explore drug failures in human giardiasis.

Introduction

In human giardiasis, therapeutic failure is occurring more and more frequently, due to low compliance with drug therapy, reinfestation or parasite resistance to metronidazole and/or the nitroimidazole-related compounds secnidazole, tinidazole and ornidazole.[1] In such cases, albendazole has been proposed as an alternative to metronidazole but is not always effective. The aim of this study was to evaluate the use of a neonatal mouse model to establish the cause of therapeutic failures.

Materials and methods

Giardia isolates

Faecal samples containing *Giardia* cysts were obtained from three French University Hospitals over a 6 month period (Amiens University Hospital, 13 samples; Lyon University Hospital Edouard Herriot, seven samples and Rouen University hospital, 10 samples). Patients received standard metronidazole therapy (0.75 g/day for 5 days) (with the exception of two HIV-infected patients who received 1.5 g/day for 7 days) and the outcome of treatment was noted. If treatment failure occurred, patients received a standard albendazole therapy (0.4 g/day for 5 days). One patient dropped out of the study.

Faeces were diluted in water and cysts were isolated using sucrose gradients.[2] Parasites were stored in liquid nitrogen.[3]

Giardia excystation in gerbils

All animals were managed according to the regulations of the French Ministry for Agriculture. Mongolian gerbils (*Meriones inguiculatus*) were used as the animal model for *Giardia* excystation.[4] Briefly, animals were killed 7 days after oral infection (10^4–10^5 cysts/animal) and the intestinal contents were examined for the presence of trophozoites. This procedure resulted in an apparent excystation in 11 of 30 isolates that were used in the present work. Failure was due to parasite death [as identified by means of propidium iodide (PI) staining] in six out of 19 isolates but the cause of failure was not established in the remainder.

Evaluation of drug susceptibility in a mouse model

Drugs were purchased as pure powders (Sigma, San Quentin Fallavier, France). Drug susceptibility was determined using the NMRI neonatal mouse model described previously.[5,6] Briefly, 6-day-old suckling mice were infected with 10^5 trophozoites in 100 μL of MHSP3 medium via an intragastic animal feeding biomedical needle (Poppers and Sons, Inc., New York, NY, USA) attached to a 1 mL syringe.[7] On day 6 post-infection, half of each litter was treated by gavage with 100 μL of albendazole dispersed in water containing 0.5% of methyl cellulose, or metronidazole diluted in a 0.9% NaCl solution in water. The other half of the litter served as the control and received methyl cellulose or NaCl alone. Forty eight hours after treatment (i.e. day 8 post-infection) the mice were killed and the entire

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small intestine was removed, opened longitudinally and placed in 3 mL cold NCTC 135 medium (Life Technologies, Cergy-Pontoise, France) for at least 10 min. Tubes were vortexed to ensure complete detachment of parasites. *Giardia* trophozoites were separated from the mucosa by gauze filtration and filtrates were mixed with 100 μL of an aqueous solution of formaldehyde. Parasites were counted in a haemocytometer. For each isolate, four doses (10, 50, 100 and 200 mg/kg) of each drug were tested twice, using one litter for each dose (i.e. a total of 12–32 animals/dose).

Expression of results

A log transformation was applied to the percentage of surviving trophozoites and the dose required to eliminate 50% of the trophozoites (ID_{50}) was computed from a plot value against the logarithm of drug concentration. From these data, it was observed that one standard deviation accounted for less than 10% of the mean value.

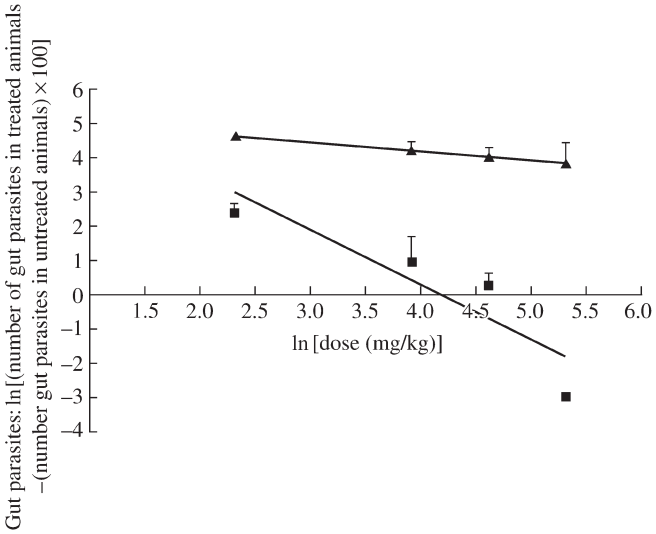

Figure 1. Dose–response for one albendazole-sensitive (Rouen/99/lpe/3, ■) and one albendazole-resistant (Rouen/99/lpe/6, ▲) *Giardia* isolate. Rouen/99/lpe/3: $y = -1.631 \times +6.761$; Rouen/99/lpe/6: $y = -0.271 \times +5.29$.

Results

As shown in the Table, isolates were distributed in three groups according to their ID_{50} of metronidazole: three isolates with ID_{50} from 31.0 to 32.9 mg/kg, three isolates with ID_{50} from 71.5 to 81.5 mg/kg and five with $ID_{50} > 120$ mg/kg. In this latter group, three out of five isolates were found to be clinically resistant to standard metronidazole therapy. Figures 1 and 2 illustrate dose–response examples for one drug-sensitive and one drug-resistant *Giardia* isolate. In one of the two HIV-infected patients, treatment with metronidazole 1.5 g/day for 7 days was successful. With albendazole, the isolates were distributed in three groups: five isolates with $ID_{50} < 10$ mg/kg, 5 with ID_{50} from 30 to 53 mg/kg and one very resistant isolate in which the ID_{50} was 161 mg/kg.

Clinically, 7/11 isolates responded to metronidazole therapy (0.75 g/day for 5 days for six patients and 1.5 g/day for 7 days for one patient), one was clinically resistant to metronidazole (0.75g/day for 5 days) but responded to albendazole (0.4 g/day for 5 days) while two isolates were clinically resistant to both drugs: metronidazole 0.75 g/day for 5 days for one patient, 1.5 g/day 7 days for the other patient and albendazole 0.4 g/day for 5 days for both (Table).

Table. ID_{50} results for metronidazole and albendazole against 11 clinical *Giardia* isolates in a neonatal mouse model

Isolate	Mean trophozoite no. in untreated animals ($\times 10^4$ parasites/animal)	ID_{50} (mg/kg) (no. of mice used)[a]		Clinical resistance to a standard therapy	
		metronidazole	albendazole	metronidazole 0.75 g/day 5 days	albendazole 0.4 g/day 5 days
Rouen/98/lpe/5	473.7 ± 32.7	31.0 (66)	40.6 (54)	no	–
Rouen/98/lpe/2	380.1 ± 39.9	32.9 (67)	9.2 (56)	no	–
Rouen/98/lpe/3	92.4 ± 10.8	31.8 (81)	5.7 (60)	no	–
Rouen/98/lpe/7	373.8 ± 57.9	71.5 (78)	6.8 (67)	no	–
Rouen/98/lpe/1	578.7 ± 72.6	76.1 (66)	30.5 (65)	no	–
Rouen/98/lpe/10	257.7 ± 67.8	81.5 (52)	9.9 (55)	no	–
Rouen/98/lpe/11	97.8 ± 11.1	125.2 (57)	44.2 (59)	yes[b]	yes
Rouen/98/lpe/9	638.4 ± 56.1	150 (82)	53 (57)	no[b]	–
Rouen/98/lpe/4	190.5 ± 17.1	175.8 (55)	9.7 (56)	yes	no
Rouen/98/lpe/8	175.5 ± 27.5	181.8 (51)	40.5 (56)	unknown	unknown[c]
Rouen/98/lpe/6	755.7 ± 84.9	149.5 (62)	161.3 (71)	yes	yes

[a]Results are ID_{50} values extrapolated from experimental data.
[b]These patients received metronidazole 1.5 g/day for 7 days.
[c]This patient withdrew from the study.

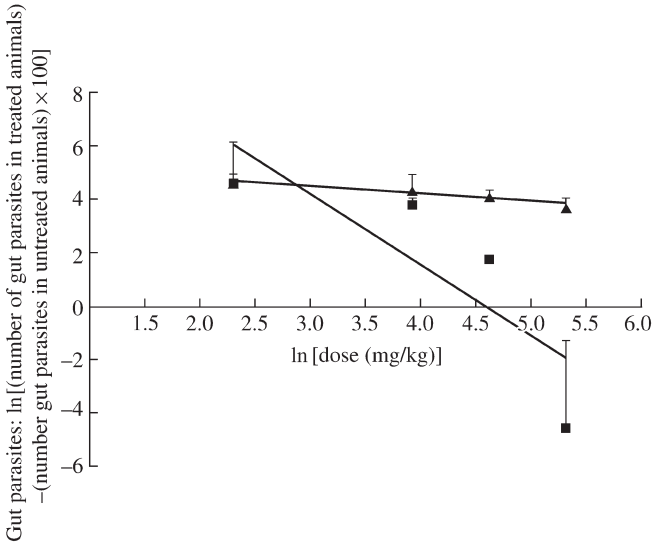

Figure 2. Dose–response for one metronidazole-sensitive (Rouen/99/lpe/5, ■) and one metronidazole-resistant (Rouen/99/lpe/6, ▲) *Giardia* isolate. Rouen/99/lpe/5: $y = -2.45 \times +12.2$; Rouen/99/lpe/6: $y = -0.259 \times +5.202$.

Discussion

ID_{50} values obtained for metronidazole-sensitive isolates in this study were similar to those reported previously.[8] All isolates associated with metronidazole treatment failure exhibited an ID_{50} greater than 120 mg/kg, which suggests that an ID_{50} breakpoint of 120 mg/kg may be useful in distinguishing drug resistance as a cause of treatment failure from other causes. In this context, the metronidazole treatment failure that occurred in this study appears to be due to true drug resistance. Interestingly, the patient infected by the isolate Rouen/98/lpe/9 (ID_{50} 150 mg/kg) was cured by a higher dose of metronidazole (1.5 g/day for 7 days instead of 0.75 g/day for 5 days) whereas the patient infected by isolate Rouen/98/lpe/11 (ID_{50} = 125.2 mg/kg) was not. An increase in drug dose regimen may therefore be helpful in the management of some cases of clinically resistant human giardiasis.

A standard albendazole therapy was used to treat only the three patients infected with isolates that were resistant to metronidazole. Two of these isolates, which were less sensitive to albendazole in the neonatal mouse model, were clinically resistant to albendazole (0.4 g/day for 5 days). This is in agreement with a previous clinical study that reported treatment failures with both metronidazole and albendazole.[9] In spite of the difficulties linked to the use of experimental animals, our results demonstrate the value of a neonatal mouse model to explore drug failures in human giardiasis.

Clinically, therapeutic failure with standard metronidazole therapy occurred in three of 11 patients. Although the high number of *Giardia* metronidazole-resistant strains found in this study may be explained in part by the referral of patients with therapeutic failure to the University hospital, clinical resistance to metronidazole and/or to albendazole does not appear to be uncommon.[9] This underlines the need for new antigiardial drugs such as lactone-substituted imidazoles or nitazoxanide.[10,11]

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