

In-vitro evaluation of nitrofurantoin as an alternative agent for metronidazole in combination antimicrobial therapy against *Helicobacter pylori*

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Increasing metronidazole resistance suggests the need for alternative antibiotics for combination therapy of *Helicobacter pylori* infections. We evaluated a metronidazole-resistant and a clarithromycin-resistant strain of *H. pylori* under stationary growth phase conditions that favoured physiological conditions in order to determine if nitrofurantoin might be a suitable alternative for metronidazole in combination therapy. The results demonstrated that the triple combination of bismuth, tetracycline and nitrofurantoin achieved greater bactericidal activity against these two strains than did the combination of bismuth, tetracycline and metronidazole. These results suggest that further evaluation is warranted.

Introduction

Triple-drug therapy with bismuth, metronidazole and tetracycline (or amoxicillin) is widely used for *Helicobacter pylori* therapy although metronidazole resistance in certain patient populations has raised concerns about the continued efficacy of this regimen.^{1–3} The use of newer macrolides, such as clarithromycin, for *H. pylori* therapy has increased. As a result, resistance to clarithromycin has now been reported in up to 10% and 60% of *H. pylori* isolates recovered from patients before and after treatment with clarithromycin, respectively.⁴ Strains resistant to both metronidazole and clarithromycin have been reported.^{1,2}

Because of increasing resistance to metronidazole and clarithromycin, we addressed the following questions: (i) What are the effects of selected antimicrobial agents on a metronidazole-resistant and a clarithromycin-resistant strain of *H. pylori* when tested in stationary growth phase under conditions that favour slow metabolism and in a medium that enhances the formation of glycocalyx-encased microorganisms;⁵ (ii) Could nitrofurantoin, an antimicrobial agent in the same class as metronidazole and with a similar bactericidal mechanism of action involving electrophilic radical formation, be an alternative for metronidazole in combination therapy where metronidazole resistance has become a clinical problem?

Materials and methods

Microorganisms

A metronidazole-resistant strain, *H. pylori* ATCC 43504, and a clarithromycin-resistant clinical strain, Hp 110-2, were selected for testing. The MIC of metronidazole against the ATCC strain and the MIC of clarithromycin against the Hp 110-2 strain have been determined previously as 128 mg/L⁶ and 8 mg/L,⁷ respectively. Inocula were prepared from overnight growth on serum-supplemented agar medium and contained motile cells with typical curved morphology when viewed by phase microscopy.

Susceptibility testing

Bactericidal studies were performed as described previously⁶ with several modifications. Bottles contained 5 mL of agar medium on the bottom and 20 mL of broth medium. The latter consisted of trypticase soy broth (Becton Dickinson and Co., Sparks, MD, USA) supplemented with 0.5% starch (Difco Laboratories, Detroit, MI, USA), 3 mg/L amphotericin B, and final Ca²⁺ and Mg²⁺ concentrations of 25 mg/L and 12.5 mg/L respectively (Sigma Chemical Co., St Louis, MO, USA). The agar medium consisted of trypticase soy broth (base),

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4.5% Bacto agar (Difco), 0.5% starch, 20 mg/mL mucin (Sigma) and 3 mg/L amphotericin B. Sealed bottles containing medium were inoculated, using a syringe, with approximately 8×10^6 cfu/mL. The bottles were flushed with anaerobic gas (99.995% CO₂) and incubated at 37°C without shaking. After 5 days, samples were removed and colony counts were determined.⁶ Bottles contained approximately 2×10^5 cfu/mL; these cells were assumed to be in sessile form.

After the sampling on day 5, the following antimicrobial agents were added (mg/L): metronidazole (0.1, 8.0 and 12.0), tetracycline (0.25 and 3.0), nitrofurantoin (0.25 and 1.0) (Sigma), and colloidal bismuth subcitrate (4.0 and 8.0; Brocades Pharma bv, Leiderdorp, The Netherlands). Omeprazole (8 mg/L; Astra Hassle AB, Molndal, Sweden) was also added to selected bottles. To determine the effect of combined agents, drug concentrations were intentionally selected such that bactericidal effects of singular agents were minimal. The effect of combination agents at presumably achievable drug levels in the antrum was also tested. After addition of drugs, bottles were reincubated at 37°C for two additional days at which time the viable cell count was again determined.⁶ The change in the log₁₀ cfu/mL after the last 2 days of incubation relative to the log₁₀ cfu/mL at the time of the addition of antimicrobial agents was determined. Maximal killing was defined as <30 cfu recovered from a 1.0 mL aliquot.

For comparative purposes, combination agents were also tested against both strains when grown under conditions that favour cells in planktonic form.⁶ The same biphasic medium was used as described above, but after inoculation bottles were flushed with a microaerophilic gas mixture and were shaken continuously at 37°C. Cell counts were determined at 24 h.

Results

In a preliminary experiment, we tested the survival of *H. pylori* cells incubated for an extended time period in biphasic medium and under conditions that favour formation of cells in sessile form. Unlike strain Hp 110-2, the ATCC strain remained viable for 20 months, indicating that some strains of *H. pylori* can remain viable *in vitro* and presumably *in vivo* for considerable time periods.

Results of antimicrobial agents tested against *H. pylori* cells maintained in sessile form are shown in the Table. The lack of growth of both strains in bottles with no drugs maintained in sessile mode relative to the exponential growth of both strains maintained in planktonic mode demonstrates, that cells in sessile mode were indeed not replicating. Although none of the combination agents at low concentrations demonstrated synergic activity for the ATCC strain, combination agents at achievable levels which contained nitrofurantoin achieved complete bactericidal effect. At low concentrations, combination agents

containing nitrofurantoin also demonstrated greater activity against the metronidazole-sensitive strain, Hp 110-2, than did metronidazole-containing combinations. Combination agents tested against *H. pylori* cells in planktonic form generally demonstrated greater killing than did cells in sessile form. Surprisingly, the addition of omeprazole to the metronidazole-containing combination at achievable levels and to the nitrofurantoin-containing combination at the lower concentration for the ATCC (sessile form) and the Hp 110-2 (planktonic form) strains, respectively, actually decreased the bactericidal effects.

Discussion

We have previously described modified time-kill kinetic methodology that uses broth medium to evaluate antimicrobial agents against rapidly replicating *H. pylori* cells.⁶ In this study, we further modified this method in order to provide conditions that more accurately reflect the *in-vivo* state of *H. pylori*.

The results clearly demonstrate that combination antimicrobial therapy is more active against *H. pylori* cells in planktonic form than against cells in sessile form. Our data also suggest that the addition of omeprazole adds little to the bactericidal activity of the combinations tested, and in some instances it appeared antagonistic. The beneficial effects of omeprazole seen clinically may be a consequence more of its ability to alter the pH of gastric secretions, than to any direct effect against *H. pylori*. The results indicate that combinations that include nitrofurantoin achieved a greater bactericidal effect against a metronidazole-resistant as well as a metronidazole-susceptible strain of *H. pylori* than did those with metronidazole.

Nitrofurantoin has been evaluated as monotherapy as well as in limited combination therapy to treat *H. pylori*-related gastroduodenal disease. In one study, 24 adults received nitrofurantoin as a single agent.⁸ *H. pylori* was markedly reduced, and in some cases cleared, from the antrum although most patients later experienced relapse. In another study, nitrofurantoin was combined with bismuth subsalicylate; this combination was found to be ineffective.⁹ Nitrofurantoin was selected in the present study because it is in the same drug class as metronidazole and has a similar mechanism for its bactericidal effect. In addition, nitrofurantoin appears to have additional modes of action which include interfering with mRNA translation¹⁰ and decreasing the likelihood of a microbial stringent response.¹¹ This study demonstrates that when used *in vitro* as a part of combination therapy, nitrofurantoin is more effective against *H. pylori* than is metronidazole. Therefore, nitrofurantoin, although having potential side effects, might be a suitable alternative for metronidazole in combination therapy.

Nitrofurantoin versus metronidazole for *H. pylori*

Table. Bactericidal effects of antimicrobial agents alone and in combination against a metronidazole-resistant strain (ATCC 43504) and a clarithromycin-resistant strain (Hp 110-2) of *H. pylori* maintained in either sessile or planktonic mode. The values listed represent the change in log₁₀ cfu/mL after 48 h (sessile) or 24 h incubation (planktonic) with agents relative to the log₁₀ cfu/mL before addition of drugs.

Drug(s) (concentration, mg/L)	<i>H. pylori</i> strain (growth phase) ^a			
	ATCC 43504 (sessile)	ATCC 43504 (planktonic)	Hp 110-2 (sessile)	Hp 110-2 (planktonic)
None	0.0	+1.6	0.0	+1.3
Bismuth (4)	ND	ND	0.0	ND
Bismuth (8)	0.0	ND	ND	ND
Metronidazole (0.1)	ND	ND	0.0	ND
Metronidazole (8)	0.0	ND	ND	ND
Tetracycline (0.25)	0.0	ND	0.0	ND
Omeprazole (8)	-0.5	ND	-0.5	ND
Nitrofurantoin (0.25)	0.0	ND	0.0	ND
Bismuth (4), metronidazole (0.1) and tetracycline (0.25)	ND	ND	-1.3	-4.9
Bismuth (8), metronidazole (8) and tetracycline (0.25)	-0.3	-2.8	ND	ND
Bismuth (8), metronidazole (12) and tetracycline (3) ^b	-2.0	≥-5.3	≥-3.5	≥-5.4
Bismuth (4), nitrofurantoin (0.25) and tetracycline (0.25)	ND	ND	≥-3.5	≥-5.4
Bismuth (8), nitrofurantoin (0.25) and tetracycline (0.25)	-0.8	-2.2	ND	ND
Bismuth (8), nitrofurantoin (1) and tetracycline (3) ^b	≥-4.0	≥-5.3	≥-3.5	≥-5.4
Bismuth (4), metronidazole (0.1), tetracycline (0.25) and omeprazole(8)	ND	ND	-2.0	-4.9
Bismuth (8), metronidazole (8), tetracycline (0.25) and omeprazole(8)	-1.8	-2.8	ND	ND
Bismuth (8), metronidazole (12), tetracycline (3) and omeprazole(8) ^b	-1.8	-3.8	≥-3.5	ND
Bismuth (4), nitrofurantoin (0.25), tetracycline (0.25) and omeprazole(8)	ND	ND	≥-3.5	-3.4
Bismuth (8), nitrofurantoin (0.25), tetracycline (0.25) and omeprazole(8)	-2.3	-2.9	ND	ND
Bismuth (8), nitrofurantoin (1), tetracycline (3) and omeprazole(8) ^b	≥-4.0	≥-5.3	≥-3.5	ND

ND = not done.

^a ≥ means greatest change measurable (i.e. complete killing).

^bAchievable drug levels in human serum.

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