RESEARCH NOTE

Influence of rifaximin treatment on the susceptibility of intestinal Gram-negative flora and enterococci

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ABSTRACT

The development of rifaximin- and rifampicin-resistant intestinal coliforms was studied in 27 subjects receiving rifaximin for 3 days by plating stool samples on media containing rifaximin 200 mg/L or rifampicin 64 mg/L before treatment (day 0), after treatment was completed (day 3), and after a further 2 days (day 5). The susceptibility of enterococci grown on day 0 and day 3 was also studied in 71 subjects. Significant increases in antimicrobial-resistant coliform flora were not seen in either the rifaximin-treated or the placebo-treated subjects. Enterococci recovered pre- and post-treatment showed similar susceptibilities. Rifaximin did not select for significant resistance in the Gram-negative and Gram-positive intestinal flora during therapy.

Keywords Coliforms, enterococci, resistance, rifampicin, rifaximin

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The aim of this study was to determine whether rifaximin, used for treatment of acute travellers' diarrhoea, encouraged the development of rifaximin- and rifampicin-resistant Gram-negative and Gram-positive faecal flora. College students from the USA who suffered from diarrhoea in Guadalajara, Mexico during the summer of 2000 were included in a placebo-controlled trial evaluating rifaximin treatment [1]. Stool samples were collected from subjects receiving rifaximin 200 mg three times daily (600 mg/day) for 3 days, rifaximin 400 mg three times daily (1200 mg/day) for 3 days, or a placebo for 3 days. Samples were collected immediately before therapy (day 0), immediately after therapy (day 3), and after a further 2 days (day 5), and were investigated for resistant flora as described previously [2].

Ten-fold dilutions (to 10^{-5}) of samples from 27 patients were plated (100 μL) on to Mueller–Hinton agar (Becton Dickinson, Sparks, MA, USA) containing rifaximin (Salix Pharmaceuticals, Raleigh, NC, USA) 200 mg/L or rifampicin (Sigma, St Louis, MO, USA) 64 mg/L. Rifaximin 200 mg/L was selected because this concentration is eight-fold higher than the median MIC_{90} for enteric pathogens, and is at the upper range of susceptibility for any of the enteric bacterial pathogens studied [3]. Rifampicin 64 mg/L was selected because this is the upper range of susceptibility for Gram-positive and Gram-negative bacteria [4,5]. Following incubation overnight at 37°C, one colony of a Gram-negative bacterium isolated on rifaximin- or rifampicin-containing agar from each of three stool samples (days 0, 3 and 5)/patient (three colonies/patient) was identified with the API 20E system (bioMérieux, Marcy L'Etoile, France). MICs of rifaximin were determined [3] in the Houston laboratory for one colony of Gram-negative bacteria/stool sample growing on either rifaximin or rifampicin.

Enterococci were recovered from the day 0 and day 3 samples taken from 71 patients by plating on to *Streptococcus faecalis* medium (Bacto SF Medium; Difco Laboratories, Detroit, MI, USA) and Bacto Bile Esculin Agar (Difco), and incubating at 45.5°C for 72 h. Putative colonies of enterococci were transferred to storage media stabs (BHI agar; Difco) and transported to Houston for further testing. Colonies isolated from both pre- and post-treatment samples were tested for susceptibility to rifaximin [3]. Differences in numbers of rifaximin- or rifampicin-resistant coliforms, and differences in post-treatment vs. pre-treatment susceptibilities of enterococci, were compared between groups with Student's *t*-test.

The numbers of rifaximin- and rifampicin-resistant coliforms seen in the pre-treatment stool sample and following treatment with rifaximin or

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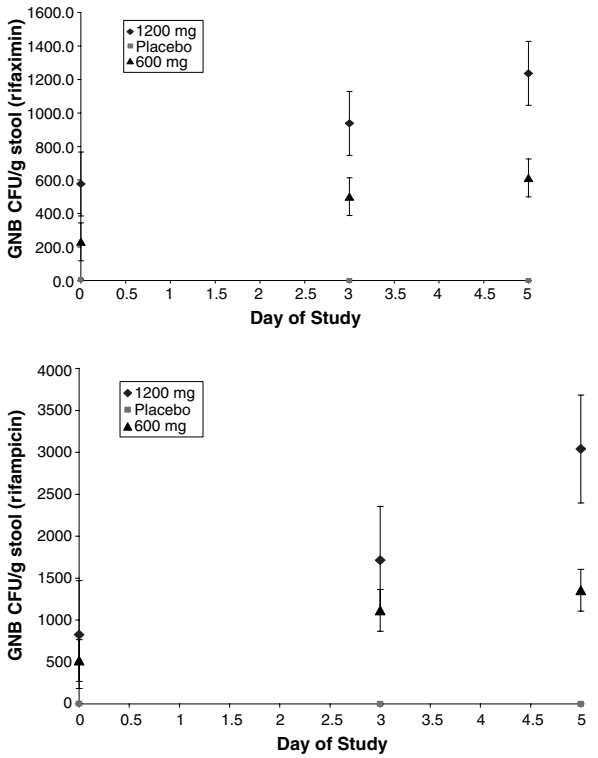

Fig. 1. Numbers of faecal rifaximin- and rifampicin-resistant coliforms (GNB) isolated pre-treatment (day 0), immediately post-treatment (day 3) and 2 days post-treatment (day 5) from patients treated for 3 days with either rifaximin 1200 mg/day, rifaximin 600 mg/day or placebo.

placebo are shown in Fig. 1. Low numbers of rifaximin- and rifampicin-resistant coliforms were isolated from the initial stool sample in all groups before treatment. Baseline pre-treatment numbers of antimicrobial-resistant coliforms were higher for the groups randomised to receive 1200 mg/day and 600 mg/day, compared with the group randomised to receive a placebo, but these differences were not significant (p 0.0683; Wilcoxon Mann–Whitney test). Significant increases in the numbers of rifaximin- or rifampicin-resistant coliforms were not found in the day 3 and day 5 post-treatment samples, compared with the pre-treatment baseline samples for the three groups. Similar values were seen for the same subjects when stool samples were plated on to rifampicin-containing medium.

Twenty-seven coliforms growing on rifaximin-containing agar were identified biochemically as *Escherichia coli*. These isolates did not show increased resistance when pre-treatment stool isolates were compared with post-treatment isolates in the three groups. The MIC_{90} for coliforms

Table 1. Rifaximin and rifampicin MICs (mg/L) for enterococci recovered before and after therapy for 3 days with one of two doses of rifaximin or a placebo

	Treatment groups		
	Rifaximin 600 mg	**Rifaximin 1200 mg**	**Placebo**
Number of patients	23	24	24
Enterococci from paired faecal samples	9 (39%)	10 (42%)	8 (33%)
Rifaximin MIC range			
Day 0	8–64	8–64	8–64
Day 3	8–64	8–64	4–64
Rifaximin MIC_{50}			
Day 0	16	32	16
Day 3	16	32	32
Rifaximin MIC_{90}			
Day 0	64	64	64
Day 3	64	64	64
Rifampicin MIC range			
Day 0	1–16	0.25–8	0.25–8
Day 3	1–16	0.5–8	0.25–8
Rifampicin MIC_{50}			
Day 0	2	2	4
Day 3	2	2	4
Rifampicin MIC_{90}			
Day 0	16	2	8
Day 3	16	2	8

growing on rifaximin- or rifampicin-containing media from pre-treatment (day 0) and post-treatment (days 3 and 5) stools was 64 mg/L for subjects randomised to receive either of the two doses of rifaximin (range 1–128 mg/L) or for subjects in the placebo group (range 2–128 mg/L).

Enterococci were isolated from pre-treatment and post-treatment samples at similar frequencies (10/24 vs. 20/24 for the 1200 mg/day group; 9/23 vs. 18/23 for the 600 mg/day group; 8/24 vs. 17/24 for the placebo group). In total, enterococci were recovered from both pre- and post-treatment stool samples for 27 (38%) of 71 patients. MICs of enterococci were ≤64 mg/L for rifaximin and ≤16 mg/L for rifampicin. No significant changes in susceptibility were seen between the pre-treatment and post-treatment samples.

Rifaximin is a poorly absorbed rifamycin derivative showing bactericidal activity against a broad range of enteric pathogens [3,6]. Rifaximin is available in several countries in Europe, Asia and Latin America for the treatment of bacterial diarrhoea [7]. It has also been used for the treatment of small-bowel bacterial overgrowth syndrome [8], hepatic encephalopathy [9], and diverticular disease of the colon, as well as for preventing infectious complications in colorectal surgery [7]. A paediatric suspension of this drug has been developed for children with bacterial diarrhoea [10].

Although the development of resistance is a major concern when rifamycins are used for

therapy, rifaximin did not select for significant resistance among gut flora when given for 3 days. It is unlikely that rifaximin would stimulate the development of rifampicin-resistant *Mycobacterium tuberculosis*, since the drug remains largely in the gut during short-term use for the treatment of diarrhoea. Extra-intestinal tissues infected with *M. tuberculosis* should not be exposed to significant concentrations of the drug, and growth of *M. tuberculosis* on media containing varying concentrations of rifaximin does not lead to the selection of rifampicin-resistant strains [11].

In summary, rifaximin appears to be a suitable drug for the management of travellers' diarrhoea [12] and other non-systemic, non-dysenteric enteric bacterial infections. This drug has now been licensed for use in the USA.

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RESEARCH NOTE

Production by *Escherichia coli* isolates of siderophore and other virulence factors and their pathogenic role in a cutaneous infection model

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ABSTRACT

Escherichia coli isolates from urinary tract infections (UTIs) (n = 124), extra-urinary sites (n = 37) and normal faecal samples (n = 51) were examined for the presence of virulence factors, including siderophores (aerobactin and enterobactin). The proportion of aerobactin producers was significantly higher in UTI (69.4%; p 0.001) and extra-urinary samples (70.3%; p 0.007) than in controls (41.2%), while the proportion of enterobactin producers was significantly lower in the UTI samples than in the controls (p 0.027). In a cutaneous infection model, aerobactin-positive *E. coli* showed more growth than non-aerobactin and non-enterobactin isolates, even when other virulence factors were identical.

Keywords Aerobactin, cutaneous infection model, enterobactin, *Escherichia coli*, siderophore, virulence factors

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