PROBIOTICS AND INTESTINAL COLONIZATION BY VANCOMYCIN RESISTANT ENTEROCOCCI IN MICE AND HUMANS

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ABSTRACT

We investigated the impact of probiotics on the intestinal carriage of vancomycin-resistant Enterococcus. Administration of Lactobacillus rhamnosus Lcr35 but not Escherichia coli Nissle reduced, although not significantly, the density of VRE colonization in a murine model. No effect of Lcr35 was observed in a double-blind placebo randomized study, involving nine patients.
Vancomycin-resistant *Enterococcus* (VRE) is a widespread nosocomial pathogen, whose main site of colonisation is the intestinal tract, leading to asymptomatic digestive carriage and hence to the formation of a reservoir of VRE (4). Once colonized, patients are at greater risk of developing subsequent infections or of transmitting VRE to other patients. While reducing VRE intestinal carriage is a major step in limiting dissemination and infection, there still exists no effective VRE decolonization treatment. Because VRE persistence in the gut may be due both to intestinal flora disruption and to inhibition of the intestinal immune function, we hypothesize that probiotics are good candidates for achieving VRE clearance.

We developed a murine model of digestive VRE colonization and investigated the effects of two probiotic bacterial strains, one Gram-positive, *Lactobacillus rhamnosus* Lcr35 (Lcr35) and the other Gram-negative, *Escherichia coli* Nissle 1917 (EcN), on established VRE intestinal colonization. We used a resistant VanA-type *Enterococcus faecium* strain isolated from a bacteriaemic patient (2). Female IOPS OF1A mice, none of which being initially colonized, were individually caged and screened for VRE prior to any treatment.; They were assigned to receive either sterile drinking water (control) or water containing vancomycin (250 µg/ml) for 14 days (D0 to D14). Gastric gavage of VRE (10^9 CFU in 0.2 ml) was performed on D7, and seven days after VRE inoculation (D14) the mice received either Lcr 35 (10^8 CFU) once a day for three or eight consecutive days or EcN (10^9 CFU) for eight consecutive days. The density (CFU/g) of VRE in the animal faeces was determined three times a week over a 40-day period. Eight out of eight mice who had received prior oral vancomycin became colonized with VRE, with counts ranging from 3.2.10^6 to 8.3.10^9 CFU/g of faeces on D9 (48h after VRE inoculation) (Fig 1), similar to the observations of Donskey *et al.* (1). These levels of VRE faecal concentration persisted for nine days, including two days...
after vancomycin was stopped, and then rapidly declined to reach a level of $10^3$ to $10^4$ CFU/g of faeces on D26, which was maintained in five mice up to D80 (Fig. 1). Only one mouse out of five in the control group (receiving neither Lcr35 nor EcN) had detectable VRE over a 21-day period of time.

Mean faecal counts of VRE were similar in 3-day Lcr35 treated mice and in the control group (data not shown). In the group with 8-day treatment (n=8), the density of VRE was lower, although not statistically different, than in the control group (n=7) from D30 (i.e. 9 days after probiotic discontinuation) until D41 (Fig. 2). With EcN, there was no difference in VRE density between the test (n=9) and the control groups throughout the 26-day period (Fig. 2).

The dominant faecal microbiota was analysed by temporal temperature gradient gel electrophoresis (TTGE), as previously described (5, 7). Faeces samples were collected before treatment and three times a week from D0 to D40 from Lcr35 8 day-treated (n=5) and control mice (n=5). Total DNA was extracted from 150-200 mg faecal samples. Primers U968-GC and L1401 (7) were used to amplify the V6 to V8 regions of bacterial 16S rDNA, and PCR-TTGE profiles were compared using GelCompar software (QUANTITY ONE, Bio-Rad). The TTGE profiles obtained were composed of 11 to 32 bands representing the dominant flora. Figure 3 shows an example of a TTGE profile and its relevant dendogram. Dice’s coefficient was used to calculate distances between the samples. As from 48 hours of vancomycin administration (D2), a few major bands appeared, with TTGE patterns exhibiting a similarity varying from 44.3 to 72.9 % on D2 when compared to D0. These new bands were detected throughout the antibiotic treatment (Fig 3A). VRE administration on D7 did not induce any major modification in the TTGE profiles, whereas main changes occurred after discontinuation of vancomycin. Comparison of the TTGE profiles of Lcr35 treated mice with those of control mice showed no major difference, indicating that administration of this
probiotic did not drastically modify the evolution of faecal microbiota in the animals.

Lcr35 was also tested in VRE cross-transmission among mice. Twenty-one VRE-free mice were placed in communal cages (three mice per cage) and 12 were supplied with $10^8$ CFU Lcr35 via daily gastric gavage from days D0 to D9. At the same time, all mice were supplied with vancomycin in drinking water (250 µg/ml). On D7, one VRE-colonized mouse was placed in each communal cage. Four hours after co-housing, 3 of the 12 separately housed naïve mice were colonized (mean VRE density: $5.2 \times 10^8$ CFU/g) versus 5 of the 9 control mice (mean VRE density: $1.6 \times 10^8$ CFU/g). No statistical difference was observed either in the number of colonized mice or in the mean VRE density. At 8 and 12 hours of co-housing, all 21 mice were colonized.

In addition, we looked at the effectiveness of Lcr35 in clearing VRE colonization in a limited number of VRE-carrying patients. After written informed consent, patients were included in a double-blind randomized pilot study (ClinicalTrials.gov Identifier: NCT00437580), conducted over a 2-year period (2007-2008). Inclusion criteria were age ≥ 18 and positive stool screening for VRE. Exclusion criteria were inability to take pills, neutropenia <1000/mm3 and immunosuppressive therapy. Patients were randomly assigned to orally receive a 5-week course of Lcr 35 ($10^9$ active cells daily) or a placebo. The patients were asked to provide stool samples every week for five weeks then every two weeks until three consecutive negative VRE cultures were obtained. Results are summarized in table 1 and figure 4. No significant effect of Lcr35 was observed on VRE intestinal colonization.

Our study validated a mouse model of VRE colonization remarkably similar to that seen in humans, with VRE carriage sustained for 66 days after discontinuation of vancomycin and spontaneous clearance in some animals. Vancomycin treatment prior to VRE inoculation
seems to play a key role in VRE colonization of the gastro-intestinal tract since it was associated with high levels of VRE intestinal colonization (Fig 1). When vancomycin was discontinued, the VRE was quickly cleared from the gut and sustained colonization was not achieved (data not shown). We hypothesize that administration of vancomycin promotes VRE colonization by disrupting the barrier formed by the intestinal microbiota, and hence provides a selective advantage for resistant bacteria such as VRE to the detriment of the normal flora.

TTGE analysis showed that vancomycin administration induces changes in the intestinal microbiota that could corroborate this hypothesis. Whether the influence of antibiotic-linked microbiota changes on VRE colonization is direct or not remains to be determined. It has been shown that Gram-negative probiotic bacteria are potentially able to regulate intestinal homeostasis (6). However, in our study, the administration of *E. coli* Nissle to VRE colonized mice failed to decrease VRE density in the gut, whereas administration of *Lcr35* lowered VRE density, albeit not to a level of significance.

Several studies performed either in murine models or in humans have suggested that the administration of probiotics improve intestinal microbial balance and the modulation of immune functions and may be interesting agents to eradicate or prevent VRE intestinal colonization (3). Our study showed that the two probiotics tested had little or no effect. Since the action of probiotics in VRE-colonized humans is likely to be influenced by host-, VRE and probiotic-related factors, further investigations are required to determine if probiotics have any effect on VRE clearance.

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FIGURE 1. Persistence and density of vancomycin-resistant Enterococcus faecium (VRE) intestinal colonization in mice. Mice received either vancomycin (■) or saline (○) seven days before and after VRE gastric inoculation (from D0 to D14). Stool VRE levels CFU/ g of faeces were quantified every two or three days from D8 until D80. In the control group, the number of VRE CFU was under the limit of detection (1.4.10^2 CFU/g) from D41.

FIGURE 2. Effect of an 8-day course administration of either Lactobacillus rhamnosus Lcr35 or Escherichia coli Nissle on intestinal carriage of vancomycin-resistant Enterococcus faecium (VRE). All mice received vancomycin (250µg/ml) in drinking water for 14 days and VRE gastric inoculation was performed on D7. On D14, vancomycin was stopped and mice received once daily either probiotic (●, Lcr35, 10^8 CFU; ○, E. coli Nissle, 10^9 CFU) or saline (▲), for eight days. Stool VRE levels were quantified over a period of four weeks.

FIGURE 3. TTGE analysis of 16S rRNA (V6-V8) amplification products from faeces samples collected over time (40 days) of mice receiving vancomycin (D0-D14) followed (test group) or not (control group) by daily administration of Lcr35 (D15 – D21). Representative example of TTGE community profile (A) and its phylogenetic tree constructed using Dice’s coefficient (B) of an animal from the test group. The periods of vancomycin and probiotic administration are indicated by horizontal double arrows, and VRE gastric inoculation by a vertical arrow. MW, molecular weight markers.
FIGURE 4: Outcome of VRE colonization in patients receiving Lcr35 (plain arrow, n=6) versus placebo (hatched arrow, n=2) for a 5-week period (W1 to W5). VRE was detected in patient stools from W1 to W11; ■, positive VRE culture and □, negative VRE culture. Nine VRE-positive patients were recruited. One patient died two days after inclusion and was excluded from the analysis. Of the eight remaining patients, six received Lcr35 and two placebos. Patients 1 and 2 completed the treatment with placebo but stopped the study after W7. Patient 5 stopped the treatment with Lcr35 after three weeks. The Charlson Index of comorbidity was 3-4 for two patients and higher than 5 for six of them.
Table 1: Characteristics of VRE-positive patients included in the study to compare a 5-week course of oral administration of *Lactobacillus casei rhamnosus* Lcr 35 with a placebo on VRE intestinal carriage clearance

<table>
<thead>
<tr>
<th>Patients Nb°</th>
<th>Arm</th>
<th>Age (years)</th>
<th>Other VRE positive colonization site</th>
<th>Previous antibiotic therapy</th>
<th>Antibiotic therapy during treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Placebo</td>
<td>82</td>
<td>None</td>
<td>Amox-clavulanic acid None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2) Placebo</td>
<td>84</td>
<td>Urine</td>
<td>Ofloxacin</td>
<td>Ofloxacin (9)</td>
<td></td>
</tr>
<tr>
<td>(3) Lcr35</td>
<td>75</td>
<td>Urine</td>
<td>Ciprofloxacin Vancomycin Metronidazol</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(4) Lcr35</td>
<td>52</td>
<td>Urine</td>
<td>Cefotaxim</td>
<td>Norfloxacin (77)</td>
<td></td>
</tr>
<tr>
<td>(5) Lcr35</td>
<td>75</td>
<td>None</td>
<td>Imipenem</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(6) Lcr35</td>
<td>92</td>
<td>Urine</td>
<td>Vancomycin Ceftriaxone Amoxicillin Metronidazol</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(7) Lcr35</td>
<td>66</td>
<td>Urine</td>
<td>Linezolid Teicoplanin Ofloxacin Ticarcillin</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(8) Lcr35</td>
<td>89</td>
<td>None</td>
<td>Amox-clavulanic acid Ofloxacin Amoxicillin</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES


Fig. 1

![Graph showing the effect of vancomycin treatment on VRE CFU/gram of faeces over time.](image)

- **Vancomycin**: Black squares, (n=8)
- **Control mice**: Diamond symbols, (n=5)

**Y-axis**: VRE CFU/gram of faeces

**X-axis**: Time (days)

- **Vancomycin administration on D7**
- **Saline**

Limit of detection at 10^2 CFU/gram of faeces.
Fig. 3
Fig. 4

- **VRE culture negative**
- **VRE culture positive**
- **Lcr35 administration**
- **Placebo**
- **Died**