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Effect of Supplements with \textit{Bifidobacterium longum} and \textit{Lactobacillus acidophilus} on the Intestinal Microbiota during Administration of Clindamycin

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Thirty healthy volunteers in three groups participated in a study of the influence of supplements containing \textit{Bifidobacterium longum} and \textit{Lactobacillus acidophilus} on the intestinal microbiota during administration of clindamycin. All groups received clindamycin perorally q.d.s. for 7 d. Group I also received a supplement with \textit{B. longum} and \textit{L. acidophilus}, group II received a supplement with \textit{B. longum} and group III received a placebo, for 21 d. The numbers of anaerobic microorganisms decreased in all groups, but the reduction of bacteroides was significantly smaller in group I than in group III (\(P<0.05\)). No subject in group III had any intestinal bifidobacteria on day 7. Significant decreases of volatile fatty acids in faecal specimens were seen (\(P<0.05\)). There was a smaller incidence of gastrointestinal discomfort in group I than in group III (\(P<0.05\)).

KEY WORDS — \textit{Bifidobacterium longum}; \textit{Lactobacillus acidophilus}; Probiotics; Clindamycin; Supplementation; Intestinal microbiota (microflora); Volatile fatty acids.

INTRODUCTION

The human gastrointestinal microbiota is, under normal circumstances, a stable ecosystem where the microorganisms remain relatively constant. The delicate balance of the ecosystem can however be altered by some dietary and environmental factors. The most common and significant cause of disturbances in the gastrointestinal microbiota is the administration of antimicrobial agents.\textsuperscript{28} Suppression of the endogenous microorganisms during therapy reduces colonisation resistance and this may lead to several unwanted effects. One effect is overgrowth of microorganisms present resistant to the antimicrobial agent, such as yeasts, which may cause systemic infections in immunocompromised patients.\textsuperscript{17} \textit{Clostridium difficile} may also overgrow and cause diarrhoea or most seriously pseudomembranous colitis.\textsuperscript{2} Another consequence is development of new antimicrobial-resistant bacterial strains in the normal microbiota.\textsuperscript{27}

Clindamycin is an antimicrobial agent used in the treatment of anaerobic infections, especially those caused by \textit{Bacteroides fragilis} or other penicillin-resistant anaerobic bacteria. One disadvantage of the agent is the high concentrations in the colon when administered either perorally or parenterally, which leads to pronounced changes in the intestinal microbiota.\textsuperscript{16} Clindamycin has been reported to be a potent agent inducing \textit{C. difficile}-associated diarrhoea.\textsuperscript{13}

One way to re-establish the ecological balance of the intestinal microbiota is to use supplements of intestinal microorganisms during the antibiotic treatment. Fermented dairy products with viable lactic-acid-producing bacteria have been used during or after antibiotic therapy.\textsuperscript{23}

\textit{Bifidobacteria} constitute a major part of the normal intestinal bacteria in humans and probably are of great importance in colonisation resistance of the large bowel to exogenous microorganisms. \textit{Bifidobacterium longum} and other species of \textit{bifidobacteria} have been used as dietary supplements after antibiotic therapy.\textsuperscript{5,7,18} \textit{Lactobacillus acidophilus} is also a part of the normal intestinal ecosystem in humans. The effect of giving \textit{L. acidophilus} to humans has been studied for many years.\textsuperscript{12,22,25} \textit{Lactobacilli} have also been used as dietary supplements after antibiotic therapy.\textsuperscript{14,21,34}
It is of great clinical interest to re-establish, or even better to maintain, the ecological balance in the intestine during and after antibiotic therapy. The aim of the present investigation was to study the intestinal microbiota, volatile fatty acids and pH in faeces before, during and after administration of clindamycin with or without supplements of \textit{B. longum} and \textit{L. acidophilus}.

\textbf{MATERIALS AND METHODS}

\textbf{Subjects}

Thirty healthy subjects with no history of gastrointestinal, hepatic or renal diseases participated in the study. They were divided into three groups, each consisting of seven or eight women and two or three men, yielding a total of ten per group. The mean age was 37 yr (range 21–54 yr). None of the volunteers had been treated with antibiotics during the 3 mth immediately prior to the study. No other medication except clindamycin was allowed during the investigation period. The investigation was performed as a double-blind study and it was approved by the Local Ethics Committee of Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden.

\textit{Administration of clindamycin}

The subjects received perorally 150 mg clindamycin capsules (Dalacin, Upjohn, Kalamazoo, Michigan, USA) q.d.s. for 7 d.

\textit{Administration of supplements with B. longum and L. acidophilus}

Ten subjects (group I) received a fermented milk product containing $5 \times 10^7$ to $2 \times 10^8$ colony forming units (c.f.u.) per ml of \textit{B. longum BB 536} (Nutritional Science Laboratory, Morinaga, Tokyo, Japan) and $2 \times 10^8$ to $3 \times 10^8$ c.f.u. per ml of \textit{L. acidophilus NCFB 1748} (Arla, Stockholm, Sweden). Ten subjects (group II) received a fermented milk product containing $5 \times 10^7$ to $2 \times 10^8$ c.f.u. per ml of \textit{B. longum BB 536} and ten subjects (group III) received a fermented milk product, as placebo, without any of the above-mentioned strains. All milk products contained the yoghurt culture bacteria \textit{L. delbrueckii} subsp. \textit{bulgaricus} LBU 108 and \textit{Streptococcus salivarius} subsp. \textit{thermophilus} STH 482. Neither of these two latter species is an intestinal microorganism. A 250-ml portion of the fermented milk was given twice daily for 21 d starting at the same time as the clindamycin administration.

\textit{Collection and processing of specimens}

Faecal samples were collected before taking the supplement and on the 2nd, 4th and 7th days during the clindamycin administration period and again 2, 4, 7, 14 and 21 d after withdrawal of the antimicrobial agent. The specimens were collected into sterile plastic containers, placed on ice chests and immediately sent to the laboratory where they were stored at $-70^\circ$C until analysed. The freezing procedure was validated to ensure that the results of the study were not influenced by this technique.$^{15}$

\textit{Microbiological procedures}

The samples were inoculated on non-selective and selective media and processed as described by Heimdahl and Nord.$^{16}$ Bifidobacteria were cultured on media developed for isolation of bifidobacteria, BL-agar without addition of horse blood$^{33}$ and Beerens agar.$^{3}$ Lactobacilli were cultured on Rogosa SL-agar (Difco, MI, USA). Aerobic and anaerobic microorganisms were identified using morphological, serological and biochemical tests and gas–liquid chromatography.$^{16}$ The lower limit of detection was $10^2$ microorganisms/g faeces.

\textit{Clostridium difficile cytotoxin test}

The cytotoxin test was performed according to Aronsson \textit{et al.}$^3$ The faecal specimens were diluted 1:10 in phosphate buffer (pH 7.2), homogenised and centrifuged at 5000 g for 15 min and passed through a sterile Millipore filter ($0.2 \mu$m). Strains of \textit{Clostridium difficile}, isolated from the samples on selective \textit{C. difficile} agar plates,$^{10}$ were grown under anaerobic conditions in chopped meat medium, centrifuged and filter sterilised analogous to the stool samples. As the positive control strain \textit{C. difficile} ATCC 9689 was used. Twenty microlitres of the supernatants were incubated for 20 h at 37$^\circ$C with mouse fibroblast cells in microtitre plates. The cytopathogenic activity was observed by light microscopy. A positive finding was confirmed by neutralisation of a 20-µl sample with 20 µl antiserum against \textit{C. difficile} produced in goats$^8$ (TechLab, Blacksburg, Virginia, USA).

\textit{Minimum inhibitory concentrations}

The minimum inhibitory concentrations (MIC) of clindamycin for \textit{B. longum BB 536} and \textit{L. acidophilus}
NCFB 1748 were determined by using an agar dilution method.\textsuperscript{16}

**Analyses of volatile fatty acids in faecal samples**

The quantities of volatile fatty acids in faeces were analysed by gas–liquid chromatography. Analyses were made on samples from days 0, 7, 21 and 28. The analyses were performed as described by Borthen et al.\textsuperscript{6}

**Measurement of pH in faecal samples**

The faecal samples were diluted 1:1 in distilled water\textsuperscript{24} and the pH of the mixture was measured with a pH glass electrode (Gallenkamp pH stick PHK-120-B).
Table 1. Logarithms of numbers of total anaerobic intestinal microorganisms during intake of clindamycin and supplements

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I (mean and range of log number)</th>
<th>Group II (mean and range of log number)</th>
<th>Group III (mean and range of log number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.6 (7.6-9.7)</td>
<td>8.0 (7.1-9.0)</td>
<td>7.9 (5.7-8.7)</td>
</tr>
<tr>
<td>2</td>
<td>7.5 (4.1-8.6)</td>
<td>6.9 (4.6-8.6)</td>
<td>6.9 (ND-8.6)</td>
</tr>
<tr>
<td>4</td>
<td>7.1 (ND-8.9)</td>
<td>5.9 (ND-8.5)</td>
<td>4.7 (ND-8.3)</td>
</tr>
<tr>
<td>7</td>
<td>7.0 (ND-8.3)</td>
<td>4.8 (ND-8.6)</td>
<td>4.7 (ND-8.0)</td>
</tr>
<tr>
<td>9</td>
<td>7.0 (ND-8.6)</td>
<td>5.3 (ND-7.8)</td>
<td>5.2 (ND-8.3)</td>
</tr>
<tr>
<td>11</td>
<td>7.5 (5.0-9.3)</td>
<td>6.2 (ND-7.8)</td>
<td>5.2 (ND-7.6)</td>
</tr>
<tr>
<td>14</td>
<td>7.9 (6.1-8.9)</td>
<td>6.9 (4.3-8.9)</td>
<td>6.8 (5.4-8.8)</td>
</tr>
<tr>
<td>21</td>
<td>8.2 (7.2-9.4)</td>
<td>7.1 (6.0-8.7)</td>
<td>7.8 (6.4-9.0)</td>
</tr>
<tr>
<td>28</td>
<td>8.1 (7.4-9.3)</td>
<td>7.2 (5.3-8.8)</td>
<td>7.9 (6.2-9.3)</td>
</tr>
</tbody>
</table>

Results given are mean values and ranges.
ND = not detected.

Statistical analysis

Statistical analysis was performed by computing the areas under the curves via the trapezoidal rule and comparing the areas by Kruskal-Wallis one-way analysis of variance by ranks. One-way analysis of covariance was used to test the equality of adjusted means for analysis of volatile fatty acids. Fisher's exact test was used for the evaluation of clinical findings.

RESULTS

Effect of bacterial supplements on aerobic intestinal microorganisms

Figure 1 shows components of the aerobic intestinal microbiota before, during and after administration of clindamycin and bacterial preparations or placebo. In the aerobic gram-positive microorganisms there was an increase in entero-cocci from day 4 in all three groups. The numbers of *Escherichia coli* were basically stable, with a small decrease on day 9, but the numbers of other aerobic clindamycin-resistant gram-negative rods—*Klebsiella, Enterobacter* and *Citrobacter* spp.—increased during the administration of clindamycin in all groups and reached a maximum on day 14. The levels of intestinal yeasts did not change during the period. There were no significant differences between the three groups.

Effect of bacterial supplements on the anaerobic intestinal bacteria

Table 1 shows the mean values and ranges of the logarithms of total anaerobic bacteria in the three groups during the investigation period. There was a marked decrease in total numbers during administration of clindamycin, which was most pronounced in group III on day 4 and 7. The numbers of members of the genera of lactobacilli, bifidobacteria, clostridia and bacteroides are shown in Figure 2. The reduction in bifidobacteria was smallest in the group receiving *B. longum* and *L. acidophilus* (I), larger in the group who only got *B. longum* (II) and largest in the placebo group (III). On the 7th day of clindamycin administration no subjects in group III had detectable numbers of bifidobacteria. The number of total bacteroides during the investigation period was also highest in group I, lower in group II and lowest in group III. The difference between groups I and III was statistically significant ($P<0.05$). After the end of clindamycin treatment the numbers of clostridia quickly exceeded the initial numbers and new species appeared in all of the groups. No significant differences were observed between the three groups in the alterations of lactobacilli and clostridia. *C. difficile* was isolated from six (group I), three (group II) and seven (group III) subjects. None of the faecal samples were positive in the cytotoxin test. The isolated *C. difficile* strains were positive in three (group I), two (group II) and three (group III) of the cases.

Minimum inhibitory concentrations

The susceptibility for clindamycin was 0.25 mg/l for *B. longum* BB 536 and 1 mg/l for *L. acidophilus* NCFB 1748.

Volatile fatty acids in faecal samples

Acetic, propionic and butyric acids were detected in all specimens. Iso-propylic, iso-valeric and valeric acids were also detected in most samples. Table 2 shows the mean concentrations of total volatile fatty acids in each group. Changes in the total amounts of volatile fatty acids were observed during the treatment period. In Figure 3 the concentration of each fatty acid expressed as a percentage of the level found on day 0 in each group is shown. The mean values of acetic acid decreased to about 50 per
cent of the pretreatment values on day 7 of the administration of clindamycin. The mean values for the other detected acids decreased even more. Almost 80 per cent of the acids was acetic acid on day 7 compared with about 60 per cent before intake of clindamycin. On the seventh day of administration, group I had the highest relative concentrations of acetic, propionic, iso-butyric and valeric acids. Butyric and iso-valeric were highest in group II. The lowest concentrations of propionic, butyric and iso-valeric acids on day 7 were registered in group III. Acetic, iso-butyric and valeric acids were equally low in groups II and III. Group I had significantly higher total levels of acetic acid, iso-butyric acid and total fatty acids than group II, during the whole administration period ($P < 0.05$).

**pH in faecal samples**

A small increase in mean pH during the administration period was seen in all groups (Figure 4). When the mean values at the starting point on day 0 were adjusted to the same initial level in the three groups, there was a tendency to smaller increase in pH in group I.

**Clinical findings**

All volunteers completed the trial without any serious adverse effects. The most commonly reported side-effects were softer consistency of stools/diarrhoea and abdominal pain. In group I two subjects were affected, in group II four subjects and in the placebo group seven persons reported
Table 2. Concentrations of total volatile fatty acids (μmol/g wet weight faeces) during intake of clindamycin and supplements in the three groups respectively

<table>
<thead>
<tr>
<th>Group</th>
<th>D0</th>
<th>D7</th>
<th>D21</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>93.0</td>
<td>38.5*</td>
<td>73.0</td>
<td>76.4</td>
</tr>
<tr>
<td></td>
<td>(60.3–142.0)</td>
<td>(20.1–89.7)</td>
<td>(50.1–102.2)</td>
<td>(43.9–112.4)</td>
</tr>
<tr>
<td>II</td>
<td>79.1</td>
<td>28.1</td>
<td>48.2</td>
<td>66.9</td>
</tr>
<tr>
<td></td>
<td>(50.8–116.8)</td>
<td>(15.8–44.1)</td>
<td>(30.9–75.6)</td>
<td>(32.2–112.4)</td>
</tr>
<tr>
<td>III</td>
<td>79.2</td>
<td>25.4*</td>
<td>66.0</td>
<td>67.6</td>
</tr>
<tr>
<td></td>
<td>(41.4–117.6)</td>
<td>(16.6–34.6)</td>
<td>(39.7–98.6)</td>
<td>(41.2–94.5)</td>
</tr>
</tbody>
</table>

Results given are mean values and ranges.
*Significant differences before and during clindamycin intake (P<0.05).

Figure 3. Mean concentrations of acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids in faeces of 10 subjects in the three groups respectively. The concentrations are expressed as the percentage of those found on day 0 in each group. — , Group I; — — — , Group II; — — — , Group III.
PROBIOTICS AND CLINDAMYCIN TREATMENT

Figure 4. Faecal mean pH of the three groups respectively. ▲, Group I; ■, Group II; ●, Group III. Means and standard errors (SE) are shown.

these side-effects. The difference between group I and III was statistically significant (P < 0.05).

DISCUSSION

Treatment with certain antimicrobial agents often leads to dramatic changes in the intestinal microbiota.26 Pronounced changes have earlier been observed in the oral and intestinal microbiota in patients receiving clindamycin.27 The aim of the present study was to investigate if it was possible to decrease these disturbances with supplements of *B. longum* and *L. acidophilus*.

In the aerobic component of the microbiota a proliferation of clindamycin-resistant enterococci and enterobacteria was observed, which has earlier been reported during clindamycin treatment.27 There were no differences between the three groups.

The differences in the anaerobic bacteria were most pronounced between groups I and III. The concentrations of bacteroides were significantly higher in group I. Also the number of bifidobacteria decreased more in group III than in groups I or II, but the difference was not statistically significant.

Other investigations have been performed with supplements containing lactic acid bacteria with and without administration of antimicrobial agents. When the same supplement which group II in the present study received (*B. longum* in fermented milk) was given to healthy, untreated volunteers for three weeks, no major changes were seen in the faecal microbiota.29 Black et al.5 gave 10 healthy subjects capsules containing *L. acidophilus* and *B. bifidum* for seven days during ampicillin administration and 14 days thereafter. There were only minor effects on the intestinal microbiota. Lidbeck et al.21 gave five healthy volunteers *L. acidophilus* as a fermented milk product after clindamycin administration. There was a partial restoration of the intestinal microbiota due to re-establishment of lactobacilli, but the normalisation of most other strongly suppressed anaerobic microorganisms was not accelerated by the supplement. Zoppi et al.34 administered a preparation of *L. acidophilus* and *B. bifidum* to infants treated with ampicillin. The administration resulted in a significant increase in aerobic and anaerobic lactobacilli and cocci. These results indicate that during normal circumstances, there are usually only minor effects on the numbers of intestinal microorganisms after administration of oral supplements with intestinal bacteria, but when parts of the microbiota are suppressed by antibiotic treatment, simultaneously given supplements can promote microbial preservation.

The risk of developing *C. difficile* diarrhoeal disease associated with the use of clindamycin is well established.2 *C. difficile* has been shown to be the aetiological agent in about 30 per cent of cases of antimicrobial-associated non-specific colitis and in about 20 per cent of cases of antimicrobial-associated diarrhoea without colitis.9 Some strains of *Bifidobacterium* spp. have been
shown to inhibit the multiplication of *Clostridium difficile* in vitro.\textsuperscript{31} In a recent study, simultaneous intake of yoghurt containing *B. longum* with erythromycin decreased the level of faecal clostridial spores and reduced the frequency of gastrointestinal disorders.\textsuperscript{7} A report by Gorbach et al. indicated that *Lactobacillus* strain GG was useful in the treatment of relapsing colitis secondary to *C. difficile* infection.\textsuperscript{11} A recent animal study showed that yoghurt containing dairy cultures of *L. bulgaricus* and *Streptococcus thermophilus* was bactericidal to *C. difficile* in vitro but did not prevent the development of *C. difficile* colitis or reduce the mortality rate in clindamycin-treated hamsters.\textsuperscript{20} In the present investigation no differences between the three groups in the occurrence of *C. difficile* or total clostridia were observed.

Rolfe\textsuperscript{31} has reported that the production of volatile fatty acids by intestinal bacteria can play an important role in the development of colonisation resistance against *C. difficile* in hamsters. Several studies\textsuperscript{6,10,29} have shown that clindamycin treatment causes a dramatic decrease of volatile fatty acids in faeces. The changes in fatty acids in the present study coincided with the changes in numbers of acid producing anaerobic microorganisms. On day seven the concentrations of total acids were about 35 per cent of the initial values, which is higher than earlier reported.\textsuperscript{19} Group I in the present study had the smallest reduction of anaerobic bacteria and also the smallest decrease of volatile fatty acids, indicating a preservation of the anaerobic bacteria.

There was a small increase in faecal pH in connection with the clindamycin intake and this finding correlated with the decreased fatty acid concentrations. The increase in pH tended to be smaller in group I than in the other two groups. A decrease in faecal pH has earlier been reported in a group of 10 healthy volunteers given *B. longum* for 21 days.\textsuperscript{29} Biasco et al.\textsuperscript{4} demonstrated a significantly reduced pH in faecal samples in a group of 20 patients with colonic adenomas who had been treated with *L. acidophilus* and *B. bifidum* capsules for 3 months.

The preparation with only *B. longum* tended to have a preservative effect on the microbiota, but a better effect was achieved with both *B. longum* and *L. acidophilus* together. This indicates that for re-establishing or preserving intestinal microorganisms during disturbances caused by antibiotic treatment, the best effect is obtained with supplements of several normal intestinal microorganisms such as bifidobacteria and lactobacilli.

This study shows that supplementation with a combination of two normal intestinal microorganisms, *B. longum* and *L. acidophilus*, reduced the ecological changes in the intestinal microbiota caused by administration of clindamycin.

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**REFERENCES**


