Effects of Biofermin-R Administered in Combination with Antibiotics on the Fecal Flora

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For the purpose of investigating the effects of Biofermin-R (BF-R) on the bacterial flora, BF-R was administered in combination with antibiotics, and the fecal flora of children treated with antibiotics alone was compared with that of children treated with both BF-R and antibiotics. Three types of effects were investigated: 1) the inhibitory effect on antibiotic-induced changes of the bacterial flora in patients without diarrhea, 2) the bacterial flora-maintaining and normalizing effect in patients with gastrointestinal symptoms, and 3) the process of normalizing in the fecal bacterial flora of mice administered with antibiotics. The results indicated that the concurrent use of BF-R and an antibiotic inhibited the changes of the intestinal flora that usually occur during antibiotic therapy alone, by preventing a decrease in Bifidobacterium, and restored disturbed flora to normal.

Key words: Biofermin-R; fecal flora; ABPC; CEX

Preparations of multiple drug resistant lactic acid bacteria are used to improve symptoms resulting from antibiotic-induced changes in the intestinal flora. Few reports elucidate the effects of these preparations on the bacterial flora. Therefore, we investigated the effects of Biofermin-R, a multiple drug resistant streptococcal preparation, in combination with antibiotics on the bacterial flora.

1. Materials and Methods

   Experiment 1: Biofermin-R in Combination with Ampicillin: Thirty-four patients (5 months to 7 years old) with upper respiratory tract infections and no gastrointestinal symptoms were treated with ampicillin (ABPC: Solcillin dry syrup, Takeda Chemical Industries, Ltd.) at a daily dose of 30 mg/kg divided by four. Seventeen of these patients were administered Biofermin-R (BF-R: Biofermin-R powder, Biofermin Pharmaceutical Co., Ltd.) at a daily dose of 1.5 g divided by three to be taken after each meal. Both drugs were administered for more than three consecutive days. Feces were examined before treatment and on day 3 of treatment.

   Experiment 2: BF-R in Combination with Cephalexin: Ten patients (10 months to 12 years old) with upper respiratory tract infections and gastrointestinal symptoms were treated with cephalexin (CEX: Cepole dry syrup, Torii & Co., Ltd.) at a daily dose of 30-50 mg/kg, and to five of these patients, BF-R at a daily dose of 1.5 g was given concurrently. Both drugs were administered throughout the entire period of study. Fecal examinations were carried out once before treatment and on day 2 or 3 and day 4 or 5.
Experiment 3: **BF-R Administered to Mice in Combination with ABPC**: Mice in groups of five (Jcl-ICR, male, at 5–6 weeks of age) were treated with ABPC at a dose of 100 mg/kg/day and BF-R at a dose of $10^8$ cells/animal/day for four days. Feces were examined once before treatment, three times during treatment and on day 1, 3, 6 and 10 after treatment.

The intestinal flora was examined according to the method of Mitsuoka (4). In experiments 1 and 3, the number of bacteria of each bacterial group was counted, and in experiment 2, the bacterial count of species was determined according to the Manual for the Identification of Medical Bacteria (2) and Anaerobe Laboratory Manual (3).

The number of the administered bacteria, *S. faecalis* 129 B10 3B-R, in feces was calculated as to its colonies grown on TATAC medium (4) containing 1,000 $\mu$g/ml of streptomycin, and its identification was carried out by testing its resistant value against several kinds of other antibiotics and its bacterial characteristics.

2. Results and Discussion

**Experiment 1**: Table 1 shows the fecal flora before treatment. There was no significant difference between the two groups of patients in both bacterial counts and detection rates of each bacterial group.

After ABPC treatment, the fecal flora was changed with a difference in bacterial counts in each bacterial group. ABPC-induced changes in each bacterial group were divided into five grades, A–E, for convenience. Table 2 shows the number of cases in each grade. The group treated with ABPC alone showed large decreases in Gram-positive organisms in the fecal flora, *Bifidobacterium* decreasing by more than 2 orders of magnitude in bacterial count in 11 of the 17 patients. *Eubacterium* and *Peptococcus* showed a considerable decrease. In contrast, the counts of Gram-negative organisms such as *Bacteroides* and Enterobacteriaceae, decreased in response to ABPC in 3 cases, but 8 cases showed an increase in the latter bacterial group. Few of the patients treated with both ABPC and BF-R showed a large decrease in the count of each bacterial group by more than 2 orders of magnitude, with the exception of *Clostridium*, as compared with the ABPC-treated group. In particular, decrease in the count of *Streptococcus* was significantly inhibited ($p<0.05$), and that of *Bifidobacterium* tended to be inhibited ($p<0.1$).

In each case, the effect of ABPC on the
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Table 2. Change of fecal flora caused by administration of drug

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>ABPC treated group</th>
<th>ABPC+BF-R treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Bacteroidaceae</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Peptococcaceae</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Bifidobacterium**</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus*</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>C. perfringens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium others</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Yeasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

A: The number of cases decreased in bacterial counts more than 2 orders of magnitude. B: Those decreased more than 1 order and less than 2 orders. C: Those increased or decreased less than 1 order. D: Those increased more than 1 order and less than 2 orders. E: Those increased more than 2 orders.

* $\chi^2$ test ($m \times n$); $\rho < 0.05$.  ** $\chi^2$ test ($m \times n$); $\rho < 0.1$.

Fig. 1. Fecal flora of a patient treated with ABPC.

Fig. 2. Fecal flora of a patient treated with ABPC+BF-R.

Fecal flora was variable, ranging from a high to no appreciable degree. Figure 1 shows the fecal floral changes in one of the patients with diarrhea caused by ABPC administered alone. This patient was most greatly affected by ABPC among the entire series. Most of the bacterial groups, except Candida, which had existed before treatment were no longer detected after treatment.

Figure 2 shows the results in one of the patients treated with both BF-R and ABPC. In this case, the account of every bacterial group showed no differences between pre- and post-treatment. All other cases ranked between these two cases, as shown in Figs. 1 and 2, with respect to the extent of changes in the fecal flora and were roughly classified according to the patterns shown in Table 3. The bacterial flora is generally stable in healthy individuals over a long period of...
Table 3. Changes of fecal flora and the patterns

<table>
<thead>
<tr>
<th>Type of fecal flora</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABPC treated</td>
</tr>
<tr>
<td>Total bacterial count decreased(a)</td>
<td>6/17</td>
</tr>
<tr>
<td>Total bacterial count with almost no decrease(b)</td>
<td>11/17</td>
</tr>
<tr>
<td>Fecal flora</td>
<td></td>
</tr>
<tr>
<td>Distinctly changed(c)</td>
<td>8/11</td>
</tr>
<tr>
<td>Not distinctly changed(d)</td>
<td>3/11</td>
</tr>
</tbody>
</table>

\(a\) Log value of total bacterial count decreased more than 0.5.
\(b\) The value decreased less than 0.5.
\(c\) The ratio of Gram-negative bacteria to Gram-positive bacteria increased more than ten times after ABPC administration.
\(d\) The ratio increased less than ten times.

\(\chi^2\) test; \(p<0.05\).

Time (T), the standard deviation of total count being about 0.2 order of magnitude. The upper limit of decrease in the total count was set at 0.5 order of magnitude, i.e., more than two times that of 0.2 order of magnitude, and the decrease greater than 0.5 order of magnitude was regarded as reflecting an effect of ABPC. This evaluation revealed that while the total bacterial count decreased in six of the 17 children treated with ABPC alone, only one of the 17 individuals given both BF-R and ABPC showed such a decrease, and a significant difference was noted between the two groups (\(p<0.05\)). Then, 11 patients in the ABPC-treated group and 16 in the concomitant BF-R group showed no decreases in the total count. Since ABPC caused a decrease in Gram-positive organisms, with no large decrease in Gram-negative organisms, the ratio of Gram-negative to Gram-positive organisms increased. A ratio more than 10 times higher than the pretreatment ratio was regarded as reflecting a large change in the bacterial flora, and a ratio less than 10 times lower than that of the pretreatment was regarded as a small change. According to this classification, eight (73\%) of the 11 patients in the ABPC-alone group showed a large change in the bacterial flora, whereas only five (31\%) of the 16 treated concurrently with BF-R showed a large change, and a significant difference was observed between the two groups (\(p<0.05\)).

These results indicated that BF-R administered in combination with ABPC inhibited ABPC-induced changes in the bacterial flora.

Experiment 2: The patients studied showed some gastrointestinal symptoms and various bacterial floras before treatment. After antibiotic treatment, each patient gave a different result without consistent tendency. Table 4 shows the changes of the bacterial flora in one contrast case. This child showed no *Bifidobacterium* before treatment, the bacterial flora containing a large number of facultative anaerobes (abbreviated as aerobes hereafter) with few anaerobes. After CEX administration, the aerobes decreased, and the anaerobes showed a slight increase with no *Bifidobacterium* detected throughout the period of study. This patient showed diarrhea even at the end of the study.

Table 5 shows the results in a patient treated with both BF-R and CEX. This patient had increased numbers of aerobic bacteria as compared with low numbers of a few anaerobic species, before treatment. However, after treatment, the number of anaerobes increased, and in particular, *Bifidobacterium* increased ten times more than the pretreatment value and became the dominant organism. Diarrhea and abdominal pain which had been observed before treatment promptly improved.
Mitsuoka (5) maintained that the decrease in Bifidobacterium and increase in aerobes are common changes observed in an abnormal flora, and Shimoyama (7) argued that Bifidobacterium decreases most sharply in diarrheal stools. Therefore, changes that occurred in the bacterial flora of all patients were summarized on the basis of the following two indicators—detection of Bifidobacterium and an increase in aerobes. Detection of Bifidobacterium is shown in Fig. 3. In the control group, the percentage of Bifidobacterium remarkably decreased in two of the three patients in whom this organism was detected. In contrast, patients treated with both CEX and BF-R showed an increase of this organism in four of five patients. The second indicator, the extent of increase in aerobes, was referred to as log (anaerobes/aerobes) proposed by Ozawa (6). The
log value for healthy individuals was thought to be more than 2.0 for their fecal flora generally contains anaerobes in an amount more than 100 times as high as that of aerobes. Three control patients with log value of more than 2.0 before treatment showed a decrease after CEX administration. In contrast, the log value showed a slight decrease in only one of the five concomitant BF-R treated patients, and the other four showed an increase.

As to the gastrointestinal symptoms, one patient in the control group treated with CEX alone showed delayed improvement. However, all patients in the test group treated with both CEX and BF-R showed rapid improvement.

The above results indicate that CEX did not induce greater changes in the bacterial flora than ABPC, but caused a decrease in Bifidobacterium in some cases and an increase in aerobes in others, resulting in delayed normalization of the fecal flora, and that when BF-R was administered concurrently with CEX, the bacterial flora rapidly returned to normal.

**Experiment 3:** Mice treated with ABPC showed large changes from pretreatment values in the fecal flora, and practically no bacteria was detected. After completion of treatment, the count for each bacterial group resumed its increase, with different patterns for each bacterial group. Figure 5 shows a comparison between the fecal flora of the ABPC-treated group and that of the group treated with both ABPC and BF-R.

1) **Administered organism (Streptococcus faecalis 129 BIO 3B-R):** This organism was detected at counts of $10^6$-$10^7$ cells/g of feces during treatment with ABPC. After completion of treatment, the count increased once to $10^9$, indicating bacterial growth in the intestine. Subsequently, the bacterial count decreased rapidly to the point where the organism was no longer detected in any mouse on day 6 after completion of treatment.

2) **Other Streptococcus:** Other Streptococcus showed a large decrease during treatment with ABPC. After completion of treatment, the bacterial count once showed a sharp increase, followed by a decrease. The count
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Fig. 5. Effect of ABPC or ABPC+BF-R administration on the fecal flora of mice. ○: ABPC-treated group, ●: ABPC+BF-R-treated group. ( ) : Frequency of occurrence.

decreased to pretreatment level in the group treated with both ABPC and BF-R more promptly than in the group treated with ABPC alone.

3) Lactobacillus: Before treatment, this organism was detected at a count of about $10^{10}$ as the dominant organism in both groups. Although the count decreased during treatment with ABPC, it increased again to the pretreatment level after completion of treatment.

4) Bifidobacterium: This organism was observed earlier in the group treated with both BF-R and ABPC than that treated with ABPC alone.

5) Enterobacteriaceae: Enterobacteriaceae was no longer detected in either group during ABPC treatment. After completion of treatment, Klebsiella showed a drastic increase to the level as high as $10^{10}$. However, in the group treated with both ABPC and BF-R, the counts decreased rapidly to the pretreatment level, whereas it did not decrease promptly in the group treated with ABPC alone.

6) Bacteroides: Bacteroides was no longer detected in either group given ABPC treatment. After completion of treatment, the ABPC-treated group showed a count of $10^{10}$ on day 3, which was subsequently maintained. In contrast, this organism was not detected in the group treated with both
BF-R and ABPC on day 3 after completion of treatment, but the count became close to the pretreatment level on and after day 6.

7) Clostridium: No great difference of the counts was observed between the two groups, but *C. perfringens* was detected only in the group treated with ABPC alone.

The above results revealed that ABPC significantly disturbed the balance of the fecal flora. The dominant organisms changed from *Lactobacillus* to domination by Gram-negative organisms (*Bacteroides, Enterobacteriaceae*), and that a changed flora did not readily regain its original balance. When BF-R was used concurrently with ABPC, the fecal flora showed similar transient changes and subsequently rapidly reversed to the previous state.

These three experiments revealed that concomitantly administered BF-R inhibited antibiotic-induced changes in the intestinal flora and allowed the disturbed bacterial flora to regain its previous balance and that multiple drug resistant lactic acid bacteria contained in BF-R proliferated in the intestine. Furthermore, BF-R seemed to prevent *Bifidobacterium* from decreasing, and in some cases helped increase in number. However, the mechanisms of such an action of multiple drug resistant lactic acid bacteria belonging to the genus *Streptococcus*, and the relationship between the growth in the intestine of this organism and its effect on normalization of the flora remains to be elucidated. We intend to investigate these issues in the future.

**References**


