Effects of Oral Saccharomyces boulardii on Bacterial Overgrowth, Translocation, and Intestinal Adaptation after Small-Bowel Resection in Rats

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Hôpital Robert Debré and Laboratoire Biocodex, Paris, France


Background: Small-bowel resection in animals results in alterations of the morphology and functional adaptation in the remaining intestine. The aim of our study was to study the effect of Saccharomyces boulardii versus placebo in rats after 50% small-bowel resection.

Methods: Sixty-three rats were assigned to one of three groups: small-bowel resection (n = 31), transected surgery controls (n = 16), or non-surgical controls (n = 16). Of the 31 rats with small-bowel resection, 15 were given S. boulardii (140 mg/dl), and 16 were given placebo. Intestinal markers measured included bacterial overgrowth (BO) on days 4 and 8 and translocation into mesenteric lymph nodes, liver, and spleen. Markers of small-bowel adaptation included histomorphology of the mucosa, protein content, and various brush-border enzymes (sucrase, glucoamylase, n-aminopeptidase).

Results: In the jejunal mucosal samples on day 8, S. boulardii-treated rats showed a significant increase in protein content (58.3 ± 12 mg/10 cm) compared with placebo-treated rats (29.2 ± 1.8) or non-surgery controls (18.3 ± 1.2; P < 0.001). S. boulardii-treated rats also had significantly higher levels of all three brush-border enzymes. A significant increase of enzyme-specific activities was observed in the ileum of S. boulardii resected rats compared with the placebo resected group on day 4, and no significant differences were seen in the remnant ileum except an increase in protein content in S. boulardii-treated rats on day 8. Histomorphometric studies showed no differences in ileal villus height or translocation frequencies by day 8 in S. boulardii or placebo resected rats.

Conclusions: These data indicate that, after resection, S. boulardii does not modify bacterial overgrowth or translocation frequency but does significantly enhance the functional adaptation of the remaining intestinal segments.

Key words: Bacterial overgrowth; intestinal resection; Saccharomyces boulardii; small-bowel adaptation

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After partial resection of the small bowel the intestinal remnant undergoes morphologic and functional adaptation to maintain digestive and absorptive processes (1–6). Nutrients and hormones play an important role in these changes (7–11). Extensive resection of the small bowel may also be followed by alterations in motor activity, which may lead, first, to bacterial overgrowth and, secondarily, to intestinal translocation (12–14).

Saccharomyces boulardii is a non-pathogenic yeast used in the treatment of acute and chronic diarrheal diseases. Controlled clinical trials have shown that S. boulardii is effective in the prevention and treatment of antibiotic-associated-diarrhea (15, 16). In gnotobiotic mice continuous oral administration of S. boulardii has been shown to reduce Candida albicans intestinal proliferation (17). In immuno-suppressed mice orally administered S. boulardii has been shown to decrease the incidence of C. albicans translocation to the mesenteric lymph nodes, liver, and kidneys (18). It was also shown that S. boulardii exerts trophic effects on the small-intestinal mucosa and that oral treatment of humans and animals results in a marked increase of duodenal and jejunal levels of intestinal disaccharidases and secretory IgA (19, 20).

The purpose of this work was to analyse the potential benefit of oral administration of S. boulardii on bacterial overgrowth and translocation and functional adaptation in the intestinal remnant after partial small-intestinal resection in a rat model.

Materials and Methods

Sixty-three male Sprague–Dawley rats, weighing 137 ± 2 g were used. The rats were housed individually in stainless steel
cages in an animal room maintained at 22°C with a 12-h light period and were fed the same amounts of a laboratory pelleted diet (UAR 113, Villemoisson S/Orge, France). They were divided into several groups: a) partial small-bowel resection, b) intestinal transection, and c) controls.

Forty-seven rats were subjected to laparotomy, and 16 were used as non-surgery controls. Before surgery all animals were starved for 24 h with free access to water. They were anaesthetized with an intraperitoneal injection of sodium pentobarbital. Before surgery their abdomens were shaved, and the skin carefully cleaned with polyvidone iodine.

Thirty-one rats underwent a 50% midjejunoileal resection leaving the proximal 25% of the jejunum and the distal 25% of the ileum. Sixteen rats were sham-operated (transected), consisting in enterotomy and reanastomosis of the ileum 15 cm from the ileocaecal junction. For both resected and sham-operated rats, food was withheld for 24 h. Then, all the rats were fed 20 g daily of the same standard diet.

The 31 resected rats were randomly assigned to receive orally either S. boulardii (n = 15) or placebo (n = 16). S. boulardii was prepared in a lyophilized form (140 mg per flask) by the manufacturer (Laboratoires Biocodex Montrouge/France). This corresponded to a dose of 1 mg of yeast cells per gram body weight per day. The S. boulardii preparation was dissolved in 15 ml water and administered orally on day 0 immediately after surgery and then every evening until the rats were killed. Placebo-treated resected rats were submitted to the same schedule and received equal volumes of liquid. Transected rats (n = 16) were given the same amounts of water but no S. boulardii. Sixteen rats matched for age and weight served as non-surgery controls.

The rats were killed at two time periods, 4 and 8 days after surgery, and assays were performed in each of the four groups of rats. The animals were killed at the same hour to minimize the influence of circadian rhythm on the brush-border enzyme activity (21, 22).

### Bacteriologic studies

Two 5-cm loops of the remaining jejunum and ileum just beyond the anastomosis and samples of liver, spleen, and mesenteric lymph nodes were aseptically collected. Tissue samples were homogenized and diluted in sterile saline solution and then cultured on different selective media under aerobic conditions. Quantitative counts of bacteria were made, using a Spiral plater (Spiral Systems Inc., Cincinnati, Ohio, USA). Results are expressed as log 10 of colony-forming units (CFU)/g of wet tissues.

### Histomorphometry

Contiguous to the tissue removed for bacteriologic studies, two 5-mm segments were taken for histomorphometry. The samples were fixed in 10% formaldehyde and embedded in Paraplast (Prolabo Co., Paris, France). Longitudinal sections (5 μm) were cut and stained with haematoxylin and by the periodic acid-Schiff (PAS) procedure. Villus heights were measured in at least 10 sites per section, using a micrometer eye piece (Leitz Co, Wetzlar, Germany).

### Small-intestinal protein and enzyme contents

Two additional 5-cm loops of jejunum and ileum were excised and cleared of vascular and peritoneal connections. Each segment was washed with cold 0.9% saline solution and opened. Then the mucosa was scraped with a glass slide, weighed, homogenized, and frozen. The protein and DNA concentrations were determined with the Bradford method (23) and the Burton method (24), respectively. Sucrase and glucoseamylase activities were assayed with a modification of the Dahlquist method (25). Neutral brush-border aminopeptidase (NAP) was measured as previously described (26). Results are expressed per 100 mm length of intestine and per milligram of protein (specific activity).

### Statistical analysis

Numerical data are expressed as means ± standard error of the mean. The differences between the different groups were analysed with analysis of variance (ANOVA) and the Student unpaired test.

### Results

In the jejunum

Changes in intestinal flora and translocation. Compared...
with non-surgery controls and transected controls, resected rats showed bacterial overgrowth of Enterobacteriaceae and enterococci on day 4 and day 8; however, there were no differences between S. boulardii and placebo-treated groups (Table I).

On day 4 the resected group had a modest increase in intestinal translocation levels compared with other groups. Translocation was significantly higher in placebo-treated resected rats. On day 8 the translocation levels were lower in all groups, and no significant differences were observed as a result of S. boulardii or placebo treatment (Table II).

Morphologic adaptation. DNA content was significantly increased by day 4 in the S. boulardii-treated resected group compared with the placebo-treated resected group, but there were no statistically significant differences in mucosal weight, protein content, or villus height between the two groups. However, on day 8, when compared with placebo-treated resected rats, S. boulardii treatment resulted in a significant increase in mucosal weight and protein content ($P < 0.001$) but not in mucosal DNA content and villus height (Table III).

Functional adaptation. On day 4 there were no differences in total and specific activities of sucrase, glucoamylase, or NAP between the different resected groups (Table IV).

On day 8 oral S. boulardii treatment in the resected group resulted in a significant increase of sucrase ($+95\%$, $P < 0.001$ versus placebo), NAP ($+96\%$, $P < 0.001$), and glucoamylase ($+47\%$, $P < 0.05$) total activities (Table IV), compared with the placebo resected group. Specific activities were similar on day 8 for S. boulardii and placebo-treated resected rats, except for glucoamylase-specific activity, which was significantly lower for S. boulardii-treated resected rats.

In the ileum

Changes in intestinal flora. Compared with non-surgery controls on days 4 and 8 and transected controls on day 8, resected rats had a significant increase in levels of Enterobacteriaceae, but there were no significant differences between S. boulardii- and placebo-treated groups (Table V).

Morphologic adaptation. S. boulardii-treated resected rats did not show an increased mucosal weight, protein content, or villus height compared with placebo on day 4. DNA mucosal content was significantly higher after placebo treatment ($P < 0.01$), but the protein/DNA ratio increased significantly in the S. boulardii-treated groups.

On day 8 S. boulardii treatment resulted in a modest but significant increase in protein content ($P < 0.02$), whereas mucosal weight, DNA content, and villus height did not differ from placebo resected rats (Table VI).

Functional adaptation. On day 4 oral administration of S. boulardii produced significant increases in specific activities of sucrase ($+111\%$, $P < 0.001$ versus placebo), glucoamylase ($+219\%$, $P < 0.001$), and NAP ($+59\%$, $P < 0.001$) (Table VII).

On day 8 only NAP total activity ($+57\%$) remained significantly increased in S. boulardii resected rats compared with placebo (Table VII).

Table II. Bacterial translocation

<table>
<thead>
<tr>
<th></th>
<th>Non-surgery controls</th>
<th>Transected controls</th>
<th>Placebo resected</th>
<th>S. boulardii resected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Spleen</td>
<td>25</td>
<td>12</td>
<td>75*†</td>
<td>62</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>37</td>
<td>37</td>
<td>87</td>
<td>75</td>
</tr>
</tbody>
</table>

Results are expressed as percentage of positive translocation/organ. * $P < 0.05$ versus transected; † $P < 0.05$ versus control.

Table III. Morphologic adaptation in the jejunum remnant on days 4 and 8

<table>
<thead>
<tr>
<th></th>
<th>Non-surgery controls</th>
<th>Transected controls</th>
<th>Placebo resected</th>
<th>S. boulardii resected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Mucosal weight (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>266 ± 15</td>
<td>300 ± 19</td>
<td>475 ± 72*</td>
<td>525 ± 28*</td>
</tr>
<tr>
<td>Protein (mg/100mm)</td>
<td>18.3 ± 1.2</td>
<td>21.6 ± 1.3</td>
<td>26.2 ± 3.2</td>
<td>27.7 ± 1.8</td>
</tr>
<tr>
<td>DNA (μg/100mm)</td>
<td>382 ± 23</td>
<td>350 ± 17</td>
<td>330 ± 30</td>
<td>421 ± 302</td>
</tr>
<tr>
<td>Villus height (μm)</td>
<td>527 ± 38</td>
<td>564 ± 30</td>
<td>552 ± 60</td>
<td>590 ± 16</td>
</tr>
</tbody>
</table>

Saccharomyces boulardii resected versus placebo resected: * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$. Resected versus non-surgery controls: § $P < 0.01$; resected versus transected: ¶ $P < 0.01$.
Table IV. Functional adaptation in jejunal remnant on days 4 and 8

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-surgery controls</td>
<td>Transected controls</td>
</tr>
<tr>
<td>Total activity (mU/10cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. boulardii</td>
<td></td>
<td></td>
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<tr>
<td>Glucoamylase</td>
<td></td>
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<tr>
<td>NAP</td>
<td></td>
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<tr>
<td>Enterococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
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<tr>
<td>Gram-positive bacteria</td>
<td></td>
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</tr>
</tbody>
</table>
| Data are expressed as log 10 of colony-forming units (CFU)/g of tissues. Resected versus surgery controls: * P < 0.05; resected versus placebo resected: † P < 0.05. Resected versus non-surgery controls: § P < 0.01; resected versus transected: ¶ P < 0.01.

Table V. Changes in ileal flora on days 4 and 8

<table>
<thead>
<tr>
<th></th>
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<tr>
<td></td>
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Table VI. Morphologic adaptation in the remnant ileum on days 4 and 8

<table>
<thead>
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<th></th>
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<th>Day 8</th>
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<tbody>
<tr>
<td></td>
<td>Non-surgery controls</td>
<td>Transected controls</td>
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<tr>
<td>Mucosal weight (g)</td>
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<tr>
<td>Protein (mg/100mm)</td>
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<tr>
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<tr>
<td>villus height (μm)</td>
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</tbody>
</table>
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Table VII. Functional adaptation in ileal remnant on days 4 and 8

<table>
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<tr>
<th></th>
<th>Day 4</th>
<th>Day 8</th>
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<tbody>
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Discussion

After small-bowel resection important morphologic and functional changes occur in the remnant intestine (1–6). They have been studied mainly in animals and seldom in human subjects (2–5). The usual types of morphologic change observed are an increase in the length and structure of the intestine. It is associated, notably in animals, with mucosal hyperplasia, which depends on the length of the resection and is more pronounced if the remnant intestine is the ileum. This occurs quickly (within a few days) in animals. In humans the mucosal hyperplasia may not be observed (4, 5).

These morphologic changes are accompanied by a functional increase of digestive and absorption capacities, notably in the remnant ileum. These functional modifications have been observed in both animals and humans (1–6). The mechanisms are not well identified but may involve nutrient and hormone changes (7–11, 27–30).

After small-bowel resection pathologic events occur, such as gastric hypersecretion (3), functional pancreatic insufficiency (3), and motor disorders responsible for bacterial overgrowth and possible translocation (12–14).

Saccharomyces boulardii, a non-pathogenic yeast, has been shown to have beneficial effects in the intestine, such as an increase in disaccharidase activities in animals and humans (19) and a stimulation of secretory IgA production (20) and prevention or treatment of infectious diarrhoea, notably due to Clostridium difficile (15, 16, 31).

Our study confirms that small-bowel resection produces luminal bacterial overgrowth in the remaining intestine, with an increase of bacterial translocation in the spleen on day 4. S. boulardii treatment did not modify this bacterial overgrowth or translocation pattern, but S. boulardii did not translocate to the lymph nodes, liver, or spleen compared with in control animals. Many factors could be implicated in intestinal translocation, such as intestinal permeability, hormonal aggressive factors, and mucosal defence mechanisms. These factors, and notably intestinal permeability, should be further studied to confirm the potential increase of translocation after small-bowel resection (32, 33).

Furthermore, a spectacular effect of S. boulardii treatment on the functional adaptation of the remnant intestine was observed after resection. Changes were progressive and differed in the remnant jejunum and ileum.

A significant increase in the histomorphometric data between the various resected groups was observed after resection, but S. boulardii treatment did not result in significant histomorphometric changes. However, it was shown that S. boulardii treatment, after small-bowel resection, increased the functional adaptation of the remnant small bowel by a rapid increase of the disaccharidases and NAP-specific activities on day 4 in the ileum, followed in the jejunum and ileum by an increase of the total protein content of the mucosa on day 8. The amount of proteins and carbohydrates ingested probably also modulates these changes, as shown in normal animals and humans (34).

The mechanisms of action of S. boulardii on the small intestine are currently unknown. However, previous studies have suggested that an increase in intestinal enzyme levels could be a consequence of an exogenous supply of the enzymes provided by the yeast itself (19). This is not the case in our study, as S. boulardii does not have any glycoamylase and NAP activities, and the amount of proteins or sucrose ingested with S. boulardii by rats/day was minor compared with the amount of the increase of sucrase-specific activity and protein content (sucrase activity of S. boulardii ingested/ day = 10 mU; protein content of S. boulardii ingested/ day = 6.7 mg).

The main mechanism of S. boulardii action is probably related to the large amount of polyamines contained in S. boulardii (673 nmol/100 µg of S. boulardii). The trophic action of oral polyamines is known and corresponds to the amount of polyamines ingested with S. boulardii, as shown previously in rats and humans by Buts et al. (35).

In conclusion, this study showed that S. boulardii treatment after small-bowel resection in rats does not directly modify the small-intestinal microflora and translocation but does significantly increase the functional adaptation of the remaining jejunum and ileum. This recovery results initially from an increase of the specific activity of brush-border enzymes, followed by an increase of the protein content of the mucosa. Further studies should be done to determine whether the role of S. boulardii is only to accelerate the functional adaptation or also to permanently increase the functional digestive capacity of the remnant intestine.

Acknowledgement

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