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EFFECTS OF *SACCHAROMYCES BOULARDII* ON ANTIBIOTIC INDUCED OROCECAL TRANSIT IN RATS

D.G. Duman[a]*, H. Akin[a], M. Deniz[b], G. Can[c] and B.C. Yegen[d]

[a]Department of Gastroenterology, School of Medicine, Marmara University, T.C. Saglik Bakanligi Marmara Universitesi Pendik E.A.H., Gastroenteroloji Bilim Dalı,
Mimar Sinan Cad. 41, Üst Kaynarca Pendik, 34899 Istanbul. Turkey
[b]Department of Physiology, School of Medicine, Çanakkale Onsekiz Mart University, Terzioğlu Yerleşkesi, 17100 Çanakkale. Turkey
[c]Department of Gastroenterology, School of Medicine, Trakya University, Balkan Yerleşkesi 22030 Edirne. Turkey
[d]Department of Physiology, School of Medicine, Marmara University, Selimiye mah. Tıbbiye Cad. No:38 34668 Haydarpaşa Istanbul. Turkey

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Clarithromycin is an antibiotic widely used for *Helicobacter pylori (H. pylori*) eradication and together with amoxicillin and proton pump inhibitors they constitute the first line triple treatment regimen against *H. pylori*. Diarrhoea is one of the major drawbacks during *H. pylori* eradication and is majorly attributed to clarithromycin, while *Saccharomyces boulardii* is a probiotic and is shown to be effective in the treatment of antibiotic associated diarrhoea. We aimed to evaluate the effect of clarithromycin on orocecal transit in rats and to identify whether the supplementation with *S. boulardii* has a role on orocecal transit index. Adult rats of both sexes were divided into two groups to determine immediate or chronic effects of *S. boulardii* and clarithromycin on orocecal transit. The first group was given single dose of the test drug, while the second group received the test drugs for one week through orogastric intubation. Both groups were randomly distributed into four subgroups; the placebo group (group A), the *S. boulardii* group (group B), the clarithromycin group (group C), and the co-administration that is clarithromycin plus *S. boulardii* group (group D). Rats were given 20 mg kg^{-1} clarithromycin and 500 mg kg^{-1} *S. boulardii*. We did not find any difference among the subgroups in group 1, where only single dose of the test drugs was administered. In chronic administration group, that is group 2, significant differences among the subgroups were observed (P=0.004). Post-hoc comparisons of orocecal transit index between group "2A and 2C" and "2C and 2D" were significantly different (P=0.013 and P=0.005, respectively). Our results show that long term clarithromycin administration leads to rapid orocecal transit index and *S. boulardii* supplementation to clarithromycin can abolish this adverse effect in rats. Those findings suggest the beneficial use of *S. boulardii* in *H. pylori* eradication regimens.

Keywords: *Saccharomyces boulardii,* probiotics, clarithromycin, orocecal transit, antibiotic associated diarrhoea

Diarrhoea is one of the side effects of the broad spectrum antibiotics that limit their use and its incidence varies from 5–25% of patients who receive antibiotics (Bergogne-Bérézin, 2000). *Helicobacter pylori (H. pylori*) is a Gram-negative bacterium that leads to type B gastritis and peptic ulcers and may be a risk factor for gastric cancer, thus antibiotics are used for the treatment. Clarithromycin and amoxicillin are the antibiotics widely used for *H. pylori* eradication and together with proton pump inhibitors they constitute the first line triple treatment regimen against *H. pylori*. Antibiotic associated gastrointestinal side effects are considered amongst the major drawbacks of the therapies that hinder the efficacy of *H. pylori* eradication. It was shown that oral clarithromycin stimulated the cyclic gastroduodenal motility, while amoxicillin did not in patients with functional dyspepsia (Bortolotti et al.,

* To whom correspondence should be addressed.
Phone: +90 216 6254684; fax: +90 216 6570695; e-mail: gduman@marmara.edu.tr

1998). Therefore, the prokinetic effect of clarithromycin rather than amoxicillin was suggested to be responsible for the gastrointestinal side effects of the triple therapy for *H. pylori* eradication including diarrhoea (Bortolotti et al., 1998).

Moreover, *Saccharomyces boulardii* (*S. boulardii*), which is a probiotic yeast, has been proved to be effective in preventing the antibiotic associated diarrhoea (AAD) (Duman et al., 2005; McFarland, 2006). Probiotics are live micro-organisms that confer a health benefit to the host when administered in adequate amounts. *S. boulardii* exerts trophic effects both in the mucosa and the endoluminal fluid of the small intestine that are likely mediated by the endoluminal release of polyamines. The increased release of polyamines, namely spermine and spermidine, stimulate the disaccharidases in the enterocytes and have positive impact on the humoral immune defence by secreting the IgA and the polymeric immunoglobulin receptors into the lumen of the small intestine (Buts et al., 1986; 1990; 1994). Thus, the attachment of the microorganisms and external antigens to intestinal epithelial cells is impaired and pathogenic proliferation in gut lumen is prevented. These changes may contribute to the explanation of the beneficial effects of the *S. boulardii* in the antibiotic induced gastrointestinal disturbances. However, the physiological effects of the yeast therapy on orocecal motility have not been described before in comparison to an antibiotic having marked prokinetic features, such as clarithromycin.

We aimed to investigate the effects of clarithromycin on orocecal intestinal transit in rats and whether *S. boulardii* can abolish the untoward effects of the clarithromycin on intestinal motility.

1. Materials and methods

Adult Sprague Dawley rats of both sexes weighing 215–415 mg were housed in cages and acclimatized in an air-conditioned room at 21±1 °C and relative humidity was kept constant (65–70%) with a 12-h light-dark cycle. The animals were fed ad libitum standard pellet lab chow and water. Food was withdrawn overnight before the experiment day but the rats were allowed free access to water. We used a dose of 500 mg kg^{-1} body weight *S. boulardii* per day (corresponding to 2.5×10^9 viable yeast cells per 250 mg sachet as provided by the manufacturer) through the orogastric tube and clarithromycin was given in a dose of 20 mg kg^{-1} daily. Clarithromycin and *S. boulardii* were dissolved in saline. The dose selection of the test drugs were according to the previous in vivo rat studies (Buts et al., 1990; Kim et al., 2005).

Equal numbers of male and female rats were divided into two groups. Both groups were randomly distributed into four subgroups, each one consisting of six to seven rats: the placebo group (group A), the *S. boulardii* group (group B), the clarithromycin group (group C), and the co-administration that is clarithromycin plus *S. boulardii* group (group D). Rats in the placebo group received saline in identical volume by orogastric intubation.

The first group (group 1) received standard diet for one week and at the end of this period the immediate effects of the test drugs were evaluated by administering single dose of placebo (group 1A), *S. boulardii* (group 1B), clarithromycin (group 1C), or *S. boulardii* plus clarithromycin (group 1D) by orogastric intubation. The second group of rats (group 2) received the identical test drugs or placebo from day 1 to 7 aiming to evaluate the long term effects of the drugs.

The study was approved by the Marmara University, Animal Care and Use Committee. Rats were obtained from the Marmara University Animal Center.

1.1. Experimental protocol

In the first experiment, Group 1 was given single dose of placebo (group 1A), *S. boulardii* (group 1B), clarithromycin (group 1C), or *S. boulardii* plus clarithromycin (group 1D) by orogastric intubation after the rats had fasted for 16 h. Thirty minutes after the test drug was given, gum arabic was administered by orogastric intubation as described in detail in the "Measurement of orocecal transit" section.

The second experiment was conducted on group 2, which were given the identical drugs as group 1 for 7 days, twice daily by orogastric intubation. Group 2 underwent the same protocol as group 1 on day 8.

1.2. Measurement of orocecal transit

Experimental studies were performed by giving 1 ml of a mixture of gum arabic (gum arabic from Acacia tree, Sigma Chemical, St Louis, MO) and activated charcoal and saline via the orogastric intubation. Thirty minutes later, rats were killed by decapitation under brief ether anaesthesia, the abdomen was opened, and the ligatures were made around the pylorus and ileocecal valve. The small intestine was dissected and freed from its mesentery, with the continuity retained. Total length of the intestine and the length of small bowel filled with the black meal were then measured by laying it longitudinally. To avoid movement of intraluminal contents, the intestine was not stretched. Intestinal transit index (%) was expressed by the fraction of the total length of the small bowel filled with the black material.

1.3. Statistical analysis

For multiple comparisons in single dose or chronic administration groups, one-way analysis of variance (ANOVA) was used and for post-hoc analysis Tukey test was used. Subgroup comparisons between the single dose and chronic administration groups were tested by Mann Whitney U test or Student's *t* test. The results are expressed as means and standard error of the mean (SEM). Differences were considered statistically significant if the P value was less than 0.05.

2. Results and discussion

A total of 50 rats (25 male and 25 female) were taken. They were divided into two groups to assess the immediate and the long term effects (group 1 and 2, respectively) of placebo, *S. boulardii*, clarithromycin, and combination of *S. boulardii* and clarithromycin. When all the female and male rats in this study were compared, male rats were significantly heavier than the females (349±8 g vs. 261±5.05 g, respectively, P=0.048). There was no difference in between the groups or subgroups with regard to the distribution of the sex and the body weight of the animals.

The immediate effects of clarithromycin, *S. boulardii,* and co-administration compared with the control group on orocecal transit are shown in Fig. 1. When the acute effects of the treatment with *S. boulardii*, clarithromycin, or combination of *S. boulardii* and clarithromycin on orocecal transit were compared, there was no difference among those subgroups. Although orocecal transit was more rapid in clarithromycin group compared to placebo or *S. boulardii*, the difference was insignificant.

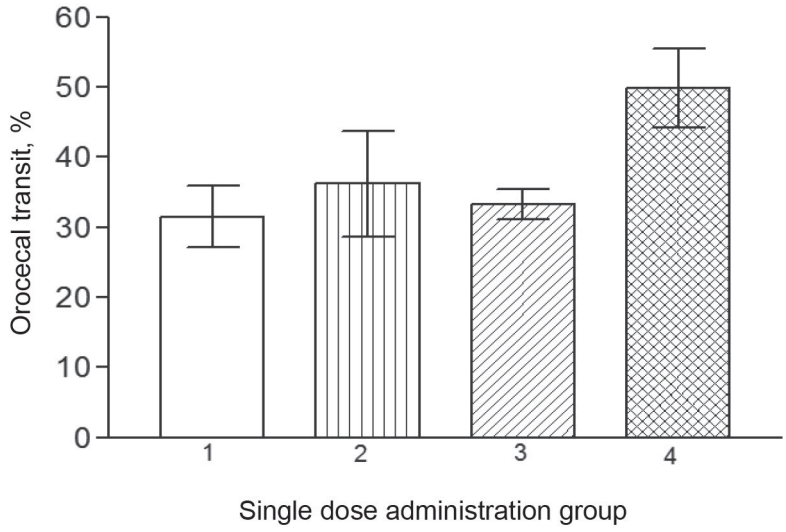

Fig. 1. The immediate effects of clarithromycin, *S. boulardii,* and co-administration of both clarithromycin and *S. boulardii* compared with the control group on orocecal transit index. (ANOVA, P>0.05).
□: Control, ▥: *S. Boulardii;* ▨: Clarythromycin, ▩: co-administration

In group 2, long term administration of clarithromycin (group 2C) significantly increased the orocecal transit index compared to the control group (group 2A) [41.01+3.35% vs. 24.03+3.7%, respectively, (P=0.013)]. Moreover, combined administration of *S. boulardii* and clarithromycin (group 2D) resulted in significant decrease in orocecal transit index compared to clarithromycin administration alone (group 2C) [22.6+3.57% vs. 41.01+3.35%, respectively, (P=0.005)]. Effects of the chronic administration of the test drugs are illustrated in Fig. 2.

Comparison of the single dose and chronic administration experiments with regard to the test drugs given revealed significant difference between the single dose and chronic administration of co-administration drugs that are *S. boulardii* plus clarithromycin (P=0.005).

2.1. Discussion

Our results show that long term oral clarithromycin administration leads to rapid orocecal transit and *S. boulardii* supplementation to clarithromycin can abolish this adverse effect in rats. Lack of any apparent effect on orocecal transit index with the single dose administration of clarithromycin compared to the control group shows that the rapid intestinal transit with clarithromycin develops in response to several days of treatment.

Clarithromycin is a macrolide antibiotic with a chemical structure similar to that of erythromycin, the prokinetic effect of which is well known (Peeters, 1993). The stimulatory effect of clarithromycin on gut motility was shown to be due to its structural relation with erythromycin and not a local irritation of the gut mucosa as the motor stimulant effect was also manifested after intravenous clarithromycin administration in patients with *H. pylori* gastritis (Bortolotti et al., 1999). Similarly, our results confirmed significant increase in intestinal transit in rats receiving oral clarithromycin.

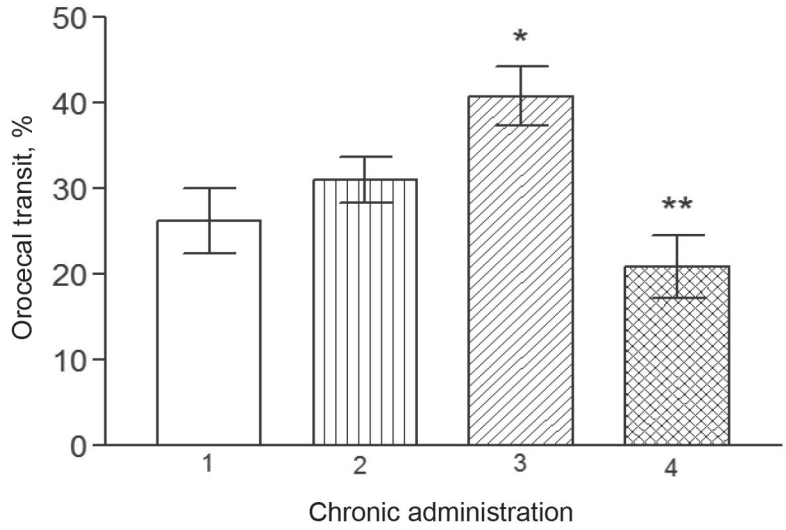

Fig. 2. Significant differences were observed among the groups with chronic administration of the test drugs (P=0.004). *Significant difference between control (1) and clarithromycin (3) group; P=0.013. ** Significant difference between clarithromycin (3) and co-administration that is the combination of clarithromycin and *S. boulardii* (4) group; P=0.005. □: Control, ▥: *S. Boulardii;* ▨: Clarythromycin, ▩: co-administration

On the basis of our findings two possible mechanisms may be suggested for the ability of *S. boulardii* to counteract the increased orocecal transit index induced by claritromycin: i) a direct effect of *S. boulardii* on the intestinal motility antagonizing the prokinetic properties of clarithromycin, ii) an indirect antagonism by *S. boulardii* against diarrhoea due to its trophic effects on the small intestinal mucosa and the immunomodulating potential as mentioned above. The former possibility has not yet been studied until now, while the latter has been investigated in several animal and human studies in the past (Buts et al., 1986; 1990; 1994).

Most human studies evaluating the effect of concomitant *S. boulardii* use in preventing AAD typically were conducted in patients receiving broad-spectrum antibiotics (Duman et al., 2005; McFarland, 2006). They compared the diarrhoea development rate in patients who took the yeast or the placebo concomitantly. Therefore, the primary end-point of the studies was the diarrhoea incidence. In accordance with these, we could have designed a similar study in healthy volunteers using pellets, dyes, capsule endoscopy, or radionuclide studies to measure small intestinal transit, but the results would have been affected by many variables, such as the ingested food, exercise, menstrual cycle, or drugs/treatments. In the present study none of the rats taking the antibiotic had diarrhoea, but single dose *S. boulardii* + Clarithromycin treatment resulted in the highest orocecal transit index. When considering these results, we can speculate that the single dose of concentrated bolus might induce a relatively more rapid intestinal transit due to its osmotic effect, since all groups received different test drugs in identical volumes. Eventually, our results may suggest that long term oral administration of *S. boulardii* together with an antibiotic that is known to have prokinetic properties in rats can counteract the side effects due to the increased intestinal transit.

Furthermore, our findings may be extrapolated to the improved intestinal absorption of the antibiotics when given along with *S. boulardii,* since the rapid intestinal transit may hinder the antibiotic efficacy. Hence, improved antibiotic absorption may result in better treatment efficacy, which can be taken as better *H. pylori* eradication rate in the scope of the current trial. This hypothesis deserves to be studied by bioavailability tests of the antibiotics. In fact, clinical evidence from a recent meta-analysis indicates that *S. boulardii* supplementation to standard triple therapy on *H. pylori* eradication significantly increased the *H. pylori* eradication rate (Szajewska et al., 2010). In addition, there are numerous clinical studies showing improved compliance to treatment with less side effects in patients receiving standard triple therapy supplemented with *S. boulardii* compared to those taking placebo and triple therapy (Cremonini et al., 2002; Hurduc et al., 2009).

3. Conclusion

The current study shows that *S. boulardii* supplementation to clarithromycin can counteract the stimulated intestinal motility by clarithromycin in rats. Those findings suggest the beneficial use of *S. boulardii* in *H. pylori* eradication regimens and one of the underlying mechanisms for that may be the direct effects of the yeast on motility.

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