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Antidiarrheal and Antinociceptive Effects of a Probiotic Mixture in Rats

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Abstract

Probiotics have been shown to have preventive and therapeutic effects on diarrhea. Because effects tend to be strain specific, benefit of a strain or mixture has to be substantiated by experimental evidence. The aim of this study was to investigate the antidiarrheal and antinociceptive effects of a probiotic mixture (Lactibiane Imedia®, PiLeJe). Castor oil-induced diarrhea test was performed in Wistar rats following oral administration of probiotics (20 × 10^9, 30 × 10^9 or 40 × 10^9 CFU/kg), loperamide (5 mg/kg) or vehicle (water; 10 mL/kg). Time to initial evacuation, number of feces and diarrheal feces, fresh weight and water content of the feces and body weight loss were monitored. Behavioral parameters (eye closing, abnormal posture, activity, fur aspect) were used as pain indices. Probable mechanisms of action were evaluated by using the castor oil-induced enteropooling and charcoal meal transit tests. Probiotics significantly and dose-dependently delayed onset time to first feces and had a beneficial effect on all other parameters ($p<0.05$ versus vehicle). This effect was lower than loperamide on most of parameters evaluated. Loperamide totally stopped diarrhea (100%) but also blocked defecation (by 98.5%) whereas probiotics strongly inhibited diarrhea (>90%) at the two highest doses tested (30 × 10^9 or 40 × 10^9 CFU/kg) without completely blocking defecation (65.7% at 30 × 10^9 CFU/kg). Behavioral parameters were improved with probiotics compared to vehicle, improvement that was not observed with loperamide. Probiotics significantly and dose-dependently decreased the volume of intestinal fluid ($p<0.05$ versus vehicle) in the enteropooling test and transit time of charcoal meal. These results indicate that the probiotic mixture tested is strongly antidiarrheic through the combination of antimotility and antisecretory properties. Observations are also in favor of an antinociceptive effect. Agents that can decrease both intestinal hypersecretion and motility are very useful in the management of diarrhea therefore, our probiotic mixture could be an effective alternative to standard drugs.

Keywords: Probiotic; Diarrhea; Castor oil; Rats

Abbreviations: CFU: Colony-forming unit; IBS: Irritable bowel syndrome.

Introduction

Diarrhea is diagnosed in millions of people per year in both developed and developing countries and is the second leading cause of death in children under five years of age [1]. This common and impactful symptom is characterized by an alteration in bowel movement and an imbalance between secretory and absorptive activities in the intestine, which results in an increase in the frequency, fluidity and/or volume of stools, and may be accompanied with tenesmus, fever and abdominal pain [1,2]. Diarrhea is usually a symptom of an infection, which can be caused by various types of bacteria, viruses, and parasites, but can have a multitude of other origins such as gastro-intestinal disorders and certain medications like antibiotics [2].

Treatment of diarrhea is generally nonspecific and aimed at reducing dehydration and the discomfort and inconvenience of frequent bowel movements [3,4]. Major drugs currently available for the treatment of acute diarrhea, such as loperamide and diphenoxylate, are not completely free from adverse events. Post-treatment constipation is frequent with loperamide and these anti-motility drugs are not recommended for children due to potential respiratory and central nervous system side effects [3]. Treatment with antibiotics is controversial unless diarrhea is severe or due to cholera [3] or in cases of traveler's diarrhea where the likelihood of bacterial pathogens is high [5]. As a consequence, the search for safe and effective agents is still ongoing.

Probiotics have been shown to have preventive and therapeutic effects on diarrhea, in particular in children with acute diarrhea caused by rotaviruses [6,7]. In adults, the use of probiotics is recommended in cases of postantibiotic-associated illness [5]. The mechanisms underlying the beneficial effects of probiotics include lowering the intestinal pH, production of antimicrobial substances such as organic acids, competitive adherence to the mucosa and epithelium, strengthening of the gut epithelial barrier and modulation of the immune system [6].

The purpose of our study was to investigate the antidiarrheal effect of a probiotic mixture of three strains of lactic acid bacteria and one strain of bifidobacteria. Lactic acid bacteria are along with bifidobacteria, microorganisms the most commonly used as probiotics. However, generalization with regards to their potential health benefits cannot be made because probiotic effects tend to be strain specific as shown in numerous studies (for example [8-12]). That means that the health benefit attributed to one strain is not necessarily applicable to another strain even within the same species and that consequently, efficacy of a specific strain or mixture has to be substantiated by experimental evidence. Therefore, the antidiarrheal effect of our probiotic mixture was evaluated in comparison with the standard drug loperamide using the rat model of castor oil-induced diarrhea, a model extensively used

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to assess anti-diarrheal substances [13-18]. The antinociceptive effect of the probiotic mixture was also assessed in this model. We also investigated the possible mechanisms of action by evaluating the effect of the probiotic mixture on intestinal fluid accumulation and motility in the castor oil-induced enteropooling model and the charcoal meal transit test in rats.

Materials and Methods

Probiotic mixture

The probiotic dietary supplement (Lactibiane Imedia®, PiLeJe, France) tested is a mixture of four viable bacterial strains: *Bifidobacterium longum* LA 101, *Lactobacillus helveticus* LA 102, *Lactococcus lactis* LA 103 and *Streptococcus thermophilus* LA 104.

Drugs and chemicals

Loperamide hydrochloride, atropine sulfate, castor oil, active charcoal and methylcellulose (Tylose® MH300) used in this study were all from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Animals

Male Wistar rats (Charles River Laboratories, L'Arbresle, France) weighing 225–250 g were housed in groups of two per cage in a room with 12-hour inverted light/dark cycle (9:00 pm/9:00 am) and controlled temperature (22 ± 2°C) and hygrometry (50 ± 20%). Food (M20, SDS Dietex, Argenteuil, France) and water were available *ad libidum*. Animal care protocols were used in accordance with guidelines of the European Communities Directive 2010/63/EU and the ASAB Ethical Committee. The study received the approval by the French Ministry of Higher Education and Research (agreements no. APAFIS#1012 from September 2015 and no. APAFIS#1807 from November 2015).

Treatment

Prior to every tests, rats were fasted for 16-18 hours but allowed free access to drinking water. In all cases, the probiotic mixture, all drugs and chemicals, as well as the charcoal meal in the motility test were administered once, by gavage, at a volume of 10 mL/kg. The probiotic mixture was given at the doses of 20×10^9, 30×10^9 or 40×10^9 CFU/kg as specified. Loperamide hydrochloride and atropine sulfate were administered at the usual dose of 5 mg/kg [15,19]. Vehicle was spring water. The investigator was blind to the experimental status of the rats.

Anti-diarrheal and -nociceptive effects in rats with castor oil-induced diarrhea

Castor oil-induced diarrhea was performed according to the method described by Karim and colleagues [20]. After 12 days of acclimatization, rats were randomized into five groups (n=8 each). Rats received the probiotic mixture at the doses of 20×10^9, 30×10^9 or 40×10^9 CFU/kg in the three test groups, loperamide in the positive control group and vehicle in the negative control group. Diarrhea was induced with castor oil administered one hour later. Each animal was thereafter placed in an individual cage and observed for 4 hours (with no access to food or water). The following parameters were monitored: time to initial evacuation (onset time; min), total number of feces (diarrheal and non-diarrheal) and diarrheal feces, fresh weight and water content of the feces (g) and body weight loss (g). Percentage of inhibition of defecation was calculated as follows:

$$\% \text{ inhibition defecation} = [(A-B)/A] \times 100$$

Where A represents the mean number of feces with vehicle and B with loperamide or probiotics. The same equation was used to calculate the percentage of inhibition of diarrhea with A the mean number of diarrheal feces with vehicle and B with loperamide and probiotics.

Behavioral parameters i.e., eye closing, abnormal posture, activity and fur aspect were used as pain indices. These parameters were adapted from published studies [21,22]. Eye closing was scored 0 for complete opening (normal eyes), 1 for half-closed eyes and 2 for complete closing. Animal posture was scored 0 for normal posture, 1 for slightly arched back, 2 for arched back and 3 for very arched back. Animal activity was scored 0 for intense activity, 1 for normal activity, 2 for moderate activity, 3 for little activity and 4 for very little activity. Fur aspect was scored 0 for normal fur, 1 for slightly ruffled fur, 2 for ruffled fur and 3 for very ruffled fur. The global behavioral score was calculated by adding the scores obtained for each behavioral parameter.

Assessment of enteropooling induced with castor oil

Enteropooling was determined by the method of Robert and colleagues [23]. Rats, randomized into four groups (n=8 each), received the probiotic mixture at the doses of 20×10^9 or 40×10^9 CFU/kg, loperamide or the vehicle. One hour after treatment, diarrhea was induced by the administration of castor oil. One hour later again, the rats were sacrificed by cervical dislocation under anesthesia. The small intestine was ligated both at the pyloric sphincter and the ileocecal junction. Its content was expelled into a graduated measuring cylinder. The activity of each treatment was expressed as the percentage of inhibition of intraluminal fluid accumulation calculated as follows:

$$\% \text{ Inhibition of intraluminal fluid accumulation} = [(A-B)/A] \times 100$$

Where A represents the volume of intestinal fluid with vehicle and B represents the volume of intestinal fluid after treatment with loperamide or probiotics.

Assessment of gastrointestinal motility

Gastrointestinal motility was evaluated using the charcoal transit method [19]. Rats were randomized into four groups (n=8 each) and given the probiotic mixture at the doses of 20×10^9 or 40×10^9 CFU/kg, atropine sulfate or the vehicle. One hour after treatment, each rat received a freshly prepared charcoal meal (10% active charcoal in 100 mL of 5% aqueous methylcellulose). One hour later, the rats were sacrificed by cervical dislocation under anesthesia. The small intestine from the pylorus to the cecum was isolated and measured (cm). The distance traveled by the charcoal meal from the pylorus was also measured (cm) and expressed as a percentage of the length of the small intestine according to the following equation:

$$\text{Distance travelled by the charcoal meal (\%)} = (\text{Distance travelled by charcoal meal/Length of small intestine}) \text{ (cm)} \times 100$$

Statistical analysis

The results are presented as mean ± SEM. The significance of differences between groups was evaluated using the Kruskal-Wallis one-way analysis of variance followed when significant, by the Mann-Whitney *post hoc* test. For all statistical analyses, the level of significance was set at $p<0.05$. All statistical analyses were carried out using the software StatView®5 statistical package (SAS, Institute Inc., USA).

Results

Treatment with a single dose of probiotic mixture one hour before the administration of castor oil in rats afforded protection against diarrhea. In comparison with the vehicle, the probiotic mixture

significantly and dose-dependently delayed the onset time to first feces, reduced the total number of both feces and diarrheal feces, and decreased fresh weight and water content of stools (p values ranged between <0.05 and ≤ 0.001 for the three doses; Table 1). In addition, the loss of body weight in the three groups treated with the probiotic mixture was approximately half that observed in the vehicle group (p ≤ 0.001 for the three doses). Comparison between the three doses of probiotics showed a significant greater antidiarrheal effect with the two highest doses (30×10^9 and 40×10^9 CFU/kg) for all parameters (p values ranged between <0.05 and ≤ 0.001 for comparisons versus 20×10^9 CFU/kg; Table 1).

Treatment with the probiotic mixture, at the three doses, had a significant lower effect than loperamide on the onset time to first feces, total number of feces, fresh weight and water content of stools (p values ranged between <0.05 and ≤ 0.001; Table 1). Body weight loss and number of diarrheal feces observed with the highest dose of probiotics (40×10^9 CFU/kg) were not statistically different from the observations made with loperamide; the effect of the 20×10^9 and 30×10^9 CFU/kg doses of probiotics on these two parameters was significantly lower than that of loperamide (p≤0.001 for 20×10^9 CFU/kg and p<0.05 for 30×10^9 CFU/kg for both parameters).

Half of the rats treated with the two highest doses of probiotics (30×10^9 and 40×10^9 CFU/kg) and 7 of the 8 rats treated with the lowest dose (20×10^9 CFU/kg) had diarrhea whereas none with loperamide (Table 1). Loperamide totally stopped diarrhea (100%) but also blocked defecation (by 98.5%). In contrast, the probiotic mixture strongly inhibited diarrhea (>90%) at 30×10^9 and 40×10^9 CFU/kg without completely blocking defecation: maximum was 65.7% at 30×10^9 CFU/kg (Table 1).

An improvement of all behavioral parameters used as indicators of pain (except for fur aspect) was observed with the probiotic mixture compared to the vehicle (Table 2), an observation that was not done with loperamide: the scores reported with the standard drug were not significantly different from vehicle. Specifically, rats treated with the probiotic mixture were significantly more active (lower scores) than rats treated with the vehicle (p<0.05 for 20×10^9 CFU/kg, p<0.005 for 30×10^9 and 40×10^9 CFU/kg) or loperamide (p<0.001 for the three doses). They also had a better posture (slightly arched back) in comparison with vehicle and loperamide (p<0.05 for 20×10^9, p<0.005 for 30×10^9 and p<0.001 for 40×10^9 CFU/kg versus vehicle or loperamide). Fur aspect of rats treated with the probiotic mixture (slightly ruffled) was similar to that of rats in the vehicle group but had a significant better look than the fur of rats in the loperamide group (ruffled fur; p<0.05 for 20×10^9, p<0.005 for 30×10^9 and p=0.001 for 40×10^9 CFU/kg versus loperamide). Eyes of rats treated with the probiotic mixture at the three doses were completely opened whereas those of rats in the vehicle and loperamide groups were between half-closed and closed (p<0.001 for the three doses versus vehicle and loperamide). Behavioral scores were not significantly different between the three test groups except for activity with a significant lower score at 30×10^9 and 40×10^9 CFU/kg (p<0.05 versus 20×10^9 CFU/kg).

The global behavioral score in the probiotic mixture groups was significantly and in a dose-dependent manner lower than with vehicle (p<0.01 at 20×10^9, p=0.001 at 30×10^9 and p<0.001 at 40×10^9 CFU/kg) and loperamide (p<0.001 in all cases). This score was significantly lower with the two highest doses of probiotics (30×10^9 and 40×10^9 CFU/kg; p<0.05 versus 20×10^9 CFU/kg). The global behavioral score in the loperamide group was significantly higher than in the vehicle group (p<0.05).

As shown in the castor oil-induced enteropooling test, the probiotic mixture significantly and dose-dependently decreased the volume of intestinal fluid (p<0.05 at 20×10^9 and p<0.005 at 40×10^9 CFU/kg versus vehicle; Table 3). The inhibition of intraluminal fluid accumulation with the probiotic mixture at 40×10^9 CFU/kg (61.11%; 2.66 ± 0.48 mL) was not different from that reported with loperamide (69.44%; 2.09 ± 0.34 mL). Furthermore, the probiotic mixture dose-dependently reduced the transit time of the charcoal meal during the 1-hour exposure period (Table 4). The charcoal meal travelled

	Vehicle (n=8)	Loperamide (n=8)	Probiotic mixture (n=8 × 3)		
Doses	-	5 mg/kg	20×10^9 CFU/kg	30×10^9 CFU/kg	40×10^9 CFU/kg
Onset time (min)	58.9 ± 3.8	229.0 ± 11.0^{a}	98.0 ± 12.3^{b,*}	154.5 ± 13.3$^{a,*,\#}$	156.5 ± 7.6$^{a,*,\#}$
Total number of feces	16.8 ± 1.3	0.3 ± 0.3^{a}	11.0 ± 1.5^{d,*}	5.8 ± 1.2$^{a,*,\#}$	6.4 ± 0.8$^{a,*,\#}$
Total number of diarrheal feces	9.1 ± 1.5	0^{a}	2.9 ± 0.6^{c,*}	0.8 ± 0.3$^{b,***,\#}$	0.5 ± 0.3^{a,‡}
Fresh weight of feces (g)	8.47 ± 0.53	0.03 ± 0.03^{a}	4.31 ± 0.74^{a,*}	1.66 ± 0.38^{a,*,‡}	2.20 ± 0.34$^{a,*,\#}$
Water content of feces (g)	6.68 ± 0.40	0.01 ± 0.01 ^{a}	2.75 ± 0.44^{a,*}	1.05 ± 0.27^{a,*,‡}	1.21 ± 0.22$^{a,*,\#}$
Body weight loss (g)	13.6 ± 0.8	5.3 ± 0.5^{a}	9.8 ± 0.3^{a,*}	6.6 ± 0.4^{a,***,†}	6.1 ± 0.5^{a,†}
Incidence of diarrhea	8/8	0/8	7/8	4/8	3/8
Inhibition of diarrhea (%)	-	100.0	65.8	91.8	94.5
Inhibition of defecation (%)	-	98.5	34.3	65.7	61.9

Values are means ± SEM; ^{a}p≤0.001, ^{b}p<0.005, ^{c}p<0.01, ^{d}p<0.05 versus vehicle; *p≤0.001, **p<0.005, ***p<0.05 versus loperamide; †p≤0.001, ‡p<0.01, $^{\#}$p<0.05 versus 20×10^9 CFU/kg. All substances administered at a volume of 10 mL/kg.

Table 1: Antidiarrheal activity of a mixture of four bacterial strains in rats with castor oil-induced diarrhea.

	Vehicle (n=8)	Loperamide (n=8)	Probiotic mixture (n=8 × 3)		
Doses	-	5 mg/kg	20×10^9 CFU/kg	30×10^9 CFU/kg	40×10^9 CFU/kg
Activity (0-4)	3.5 ± 0.4	4.4 ± 0.2	2.5 ± 0.2^{d,*}	1.8 ± 0.2$^{b,*,\#}$	1.8 ± 0.2$^{b,*,\#}$
Posture (0-3)	2.0 ± 0.2	2.3 ± 0.2	1.4 ± 0.2^{c,***}	1.3 ± 0.2^{b,**}	1.1 ± 0.1^{a,*}
Fur (0-3)	1.4 ± 0.2	2.1 ± 0.2^{d}	1.3 ± 0.2***	0.9 ± 0.2**	1.0 ± 0.0*
Eye (0-2)	1.1 ± 0.2	1.5 ± 0.2	0.0 ± 0.0^{a,*}	0.0 ± 0.0^{a,*}	0.0 ± 0.0^{a,*}
Global behavioral score (0-12)	8.0 ± 0.6	10.3 ± 0.7^{d}	5.1 ± 0.4^{c,*}	3.9 ± 0.4$^{a,*,\#}$	3.9 ± 0.2$^{a,*,\#}$

Values are means ± SEM; ^{a}p≤0.001, ^{b}p<0.005, ^{c}p<0.01, ^{d}p<0.05 versus vehicle; *p<0.001, **p<0.005, ***p<0.05 versus loperamide; $^{\#}$p<0.05 versus 20×10^9 CFU/kg. All substances administered at a volume of 10 mL/kg.

Table 2: Effect of a mixture of four bacterial strains on behavioral parameters used as indicators of pain in rats with diarrhea.

approximately 85% and 75% of the length of the small intestine after treatment with the probiotic mixture at 20×10^9 and 40×10^9 CFU/kg, respectively compared to more than 90% with vehicle ($p<0.005$ at 20×10^9 and $p=0.001$ at 40×10^9 CFU/kg). A significantly greater effect was observed with the highest dose of probiotics ($p<0.005$ for 40×10^9 versus 20×10^9 CFU/kg). With atropine, a significant shorter distance was travelled by the charcoal meal compared to both vehicle and probiotic mixture (~60%; $p=0.001$ versus vehicle and 20×10^9 CFU/kg; $p<0.005$ versus 40×10^9 CFU/kg).

Discussion

Evidence obtained from the different animal models used in this study substantiate the use of the mixture of probiotics tested in the management of diarrhea and associated pain. To our knowledge this is the first study performed in these experimental models with a mixture of bacteria. The only studies found in the literature that were performed with probiotics in these experimental models assessed the effects of *Sacchamomyces boulardii*, a yeast; in these studies, significant antidiarrheal effects were observed at the dose of 12×10^{10} CFU/kg, i.e., more than four times the dose of bacteria administered in our study [13,24,25].

We observed a strong dose-dependent antidiarrheal effect of the probiotic mixture in the castor oil-induced diarrhea model. Interestingly, the probiotic mixture at the two highest doses tested (30×10^9 and 40×10^9 CFU/kg) strongly inhibited diarrhea (over 90%) without completely blocking defecation (maximum 65%) whereas loperamide, the reference drug, both stopped diarrhea (100%) and defecation (by 98.5%). The blockage of defecation could, in part, explain the pain we observed in rats treated with loperamide. Indeed, the global behavioral score observed in our study with loperamide suggests pain sensation in rats treated with the reference drug. In contrast, the results we observed with the probiotic mixture are in favor of an antinociceptive effect. An analgesic effect of the same probiotic mixture was previously reported by us in a clinical study [26]; treatment of patients with irritable bowel syndrome (IBS) with the probiotic mixture at a daily dose of 1×10^{10} CFU for four weeks significantly decreased the abdominal pain score assessed on a visual analogue scale between the first and fourth week of treatment. Data from the literature suggest that probiotics can alleviate visceral pain through diverse mechanisms. Direct contact of a specific *Lactobacillus* strain (L-NCFM) with epithelial cells was shown to induce the expression of opioid (MOR1) and cannabinoid (CB2) receptors, which exert analgesic effects, and to contribute to the modulation and restoration of normal perception of visceral pain in mice and rats [27]. This strain was also shown to increase MOR expression in humans [28]. It is also well known that probiotics enhance intestinal barrier function and that by reducing hyperpermeability, probiotics decrease visceral hypersensitivity, the effect being strain specific [29]. For instance, *Lactobacillus farciminis* was observed to prevent stress-induced hyperpermeability and hypersensitivity through a decrease of the myosin light chain phosphorylation, responsible for epithelial cells cytoskeleton contraction and subsequent tight junction opening [30]. The antinociceptive effect of a strain of *Bifidobacterium infantis* in patients with IBS was shown to be linked to a decrease in the inflammatory tone characterizing the disease [31]. Whether similar mechanisms apply to the analgesic effect of our probiotic mixture remains to be determined.

It is well known that castor oil induces diarrhea through its active metabolite, ricinoleic acid [14,15,18]. The production of ricinoleic acid in the intestine results in inflammation and irritation of the mucosa and the release of mediators like prostaglandins that prevent fluid and electrolyte absorption and increase the intestinal peristaltic movements, this series of events ultimately leading to diarrhea [14,15,18]. By using the castor oil-induced enteropooling model and the charcoal transit method, we showed that the probiotic mixture decreased both the accumulation of fluid into the lumen and intestinal motility. These effects were dose-dependent. Our results obtained in these two models suggest that the bacterial strains contained in the mixture tested are strongly antidiarrheic through the combination of antimotility and antisecretory properties. Further research will be needed to unravel the actual mechanisms of action of our probiotic mixture as an antidiarrheal agent.

Conclusion

Agents that can decrease both intestinal hypersecretion and motility are very useful in the management of diarrhea, because diarrhea involves an increase in the motility of the gastrointestinal tract along with increased secretion and decreased absorption of fluid, and thus a loss of electrolytes particularly sodium and water. Therefore, with antimotility and antisecretory properties as well as analgesic effects, the probiotic mixture tested in this study could be an interesting alternative to standard drugs for the management of diarrhea.

	Vehicle (n=8)	Loperamide (n=8)	Probiotic mixture (n=8 × 2)	
Doses	-	5 mg/kg	20×10^9 CFU/kg	40×10^9 CFU/kg
Volume of intestinal fluid (mL)	6.84 ± 0.69	2.09 ± 0.34[a]	4.25 ± 0.70[c,*]	2.66 ± 0.48[b]
Inhibition of intraluminal fluid accumulation (%)		69.44	37.87	61.11

Values are means ± SEM (n=8); [a]p≤0.001, [b]p<0.005, [c]p<0.05 versus vehicle; *p<0.01 versus loperamide. All substances administered at a volume of 10 mL/kg.

Table 3: Effect of a mixture of four bacterial strains on castor oil-induced enteropooling in rats.

	Vehicle (n=8)	Atropine (n=8)	Probiotic mixture (n=8 × 2)	
Doses	-	5 mg/kg	20×10^9 CFU/kg	40×10^9 CFU/kg
Total length of the small intestine (cm)	110.5 ± 1.2	110.9 ± 0.9	111.8 ± 1.6	112.3 ± 1.1
Distance travelled by the charcoal meal (cm)	102.0 ± 1.7	69.6 ± 3.5[a]	95.8 ± 2.1[*]	84.5 ± 2.0[a,***,‡]
Distance travelled by the charcoal meal (%)	92.4 ± 1.6	62.7 ± 2.9[a]	85.7 ± 1.5[b,*]	75.3 ± 1.7[a,**,†]

Values are means ± SEM; [a]p=0.001, [b]p<0.005 versus vehicle; *p=0.001, **p<0.005, ***p<0.05 versus atropine; †p=0.001, ‡p<0.005 versus 20×10^9 CFU/kg. All substances administered at a volume of 10 mL/kg.

Table 4: Effect of a mixture of four bacterial strains on the transit of a charcoal meal in rats.

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