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Moorthy G
Murali MR
Devaraj SN
Basic nutritional investigation

Lactobacilli facilitate maintenance of intestinal membrane integrity during Shigella dysenteriae 1 infection in rats

Guhapriya Moorthy, M.Phil., Malliga Raman Murali, M.Sc., and S. Niranjali Devaraj, Ph.D.*

Department of Biochemistry, University of Madras, Guindy Campus, Chennai, India

Abstract

Objective: Lactobacilli are used in various dairy products and fermented foods for their potential health beneficial effects. Recently we reported the protective role of Lactobacillus rhamnosus and Lactobacillus acidophilus during Shigella dysenteriae 1 infection. Nevertheless, investigations on the membrane-stabilizing effect of L. rhamnosus and L. acidophilus have not been done. Hence, the present study evaluated the effect of L. rhamnosus and L. acidophilus on the maintenance of intestinal membrane integrity during S. dysenteriae 1-induced diarrhea in rats.

Methods: Rats were divided into eight groups (n = 6 in each group). Induced rats received single oral dose of S. dysenteriae (12 × 10^8 colony-forming units [cfu]/mL). Treated rats received L. rhamnosus (1 × 10^7 cfu/mL) or L. acidophilus (1 × 10^7 cfu/mL) orally for 4 d, alone or in combination, followed by Shigella administration. At the end of the experimental period, animals were sacrificed and the assay of membrane-bound adenosine triphosphatases (Na^+/K^+-ATPase, Ca^{2+}-ATPase, and total ATPase), immunoblot analysis of tight junctional proteins (claudin-1 and occludin), and transmission electron microscopic studies were performed.

Results: Induced rats showed a significant (P < 0.05) reduction in the membrane-bound ATPases and reduced expression of tight junction proteins in the membrane, coupled with their increased expression in the cytosol, indicating membrane damage. Transmission electron microscopic studies correlated with biochemical parameters. Pretreatment with combination of L. rhamnosus and L. acidophilus significantly prevented these changes.

Conclusion: Lactobacillus rhamnosus and L. acidophilus synergistically offered better protection to the intestinal membrane when compared with individual treatments with these strains during S. dysenteriae 1 infection. © 2009 Published by Elsevier Inc.

Keywords: Lactobacillus acidophilus; Lactobacillus rhamnosus; Tight junction; Membrane-bound adenosine triphosphatase; Shigella dysenteriae 1

Introduction

Lactobacilli are used extensively in the food industry and traditional dairy products contain various lactobacilli strains in different combinations. A myriad of fermented dairy foods containing lactic acid bacteria has been consumed for centuries in Western countries. These products are also becoming popular with consumers in other parts of the world. Flavored milk containing lactobacilli strains are also widely consumed for their beneficial effects. Lactic cultures have been reported to have health-promoting characteristics that make these micro-organisms desirable for use as probiotics in the production of dairy and other food products [1]. Possible mechanisms of the health benefits provided by lactobacilli include upregulation of mucus production, improvement in epithelial barrier function [2], increase in immunoglobulin A production [3], increased competition for adhesion sites on intestinal epithelia [4,5], and the production of organic acids, ammonia, hydrogen peroxide, and bacteriocins that inhibit growth of pathogenic bacteria [6]. Beneficial effects of Lactobacillus in in vitro models have been implicated in many studies; nevertheless, the effect of...
*Lactobacillus* during *Shigella* infection in vivo has not been thoroughly investigated.

*Shigella* spp. is a group of gram-negative enteric bacilli that causes acute bacillary dysentery in humans. Among *Shigella* species, *Shigella dysenteriae* 1, a major food-borne pathogen, has been associated with epidemic outbreaks that pose major public health problems in developing countries. The syndrome caused by *Shigella* consists of painful abdominal cramps, nausea, fever, and blood and mucus in the stool. In its most severe forms, shigellosis is associated with an intense inflammatory reaction that leads to the destruction of the colonic mucosa [7]. Shigellosis is primarily a Third-World disease, with approximately 150 million cases every year, and it is mostly a pediatric disease, with more than 60% of cases occurring in children 1 to 5 y of age [8].

In multicellular organisms, epithelial and endothelial cellular sheets function not only as diffusion barriers to establish compositionally distinct fluid compartments but are also involved in active transport of materials across the barrier to dynamically maintain the internal environment of each component [9]. The intestinal epithelium, besides regulating the uptake of fluid, electrolytes, and nutrients, has the task of establishing a barrier to the penetration of pathogens. In addition to the possibility of traversing the cell after invasion, the most vulnerable point of penetration is the paracellular space between epithelial cells. The human gut has developed a sophisticated network of tight junction proteins that seal this route to prevent microbial translocation but allow the transport of electrolytes, etc. The development of intestinal barrier function is determined by the assembly of tight junction and adherens junction proteins [10,11]. These macromolecular complexes of proteins form contiguous rings at the apices of epithelial cells. Claudin [12] and occludin [13,14] have been identified as tight junction–specific integral membrane proteins. A limited number of pathogens and virulence factors has been reported to affect paracellular permeability of intestinal epithelial cells. Breakdown of the tight junction integrity occurs in response to bacterial products such as *Clostridium perfringens* enterotoxin [9], cytotoxic necrotizing factor 1 from *Escherichia coli* [15], and Bordetella dermonecrotic toxin [16]. It is feasible that pathogens aim to destroy the integrity of the epithelial barrier to gain easy access to the gut interstitium, which can further give access to the bloodstream and thereby allow systemic spreading of the organism [17].

One mechanism by which *Shigella* translocates the epithelial layer before entering the epithelial cell is through specialized microfold cells or follicle-associated epithelium [18]. There is growing evidence that *Shigella* can also gain access to the basolateral cell surface by traversing the paracellular space after manipulating tight junctions. It has been reported that *Shigella flexneri* can induce alterations in intestinal membrane stability by removal of claudin from the tight junctions and dephosphorylates occludin and directly interferes with tight junction integrity [19].

It has been demonstrated that *Lactobacillus plantarum* 299v can prevent the *E. coli*–induced increase in intestinal permeability in a rat model [20]. In addition, Madsen et al. [21] reported that a commercial mixture of probiotic bacteria (VSL#3) containing *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbruckii*, *L. plantarum*, and *Streptococcus salivarius* can enhance the epithelial barrier in INTERLUKE10 knockout mice which serves as a model for inflammatory bowel disease. However, although the epithelial barrier integrity was determined using physiologic parameters, individual tight junction proteins were not investigated [17]. Recently we reported a synergistic protective role of *Lactobacillus rhamnosus* and *L. acidophilus* during *S. dysenteriae* 1 infection, but investigations on the effect of *L. rhamnosus* and *L. acidophilus* on the intestinal membrane during *S. dysenteriae* 1 infection have not been done.

Given these premises, it was of interest to analyze whether synergistic interactions of *L. rhamnosus* and *L. acidophilus* facilitate the maintenance of intestinal membrane integrity by assessing the levels of membrane bound adenosine triphosphatases (Na+/K+-ATPase, Ca2+-ATPase, and total ATPase), individual tight junctional protein expression (claudin-1 and occludin), and transmission electron microscopic studies during *S. dysenteriae* 1 infection in rats.

**Materials and methods**

**Bacterial strains**

The strain used in this study, *S. dysenteriae* 1, was isolated from the stool of a patient with dysentery and was provided by the Department of Microbiology, Christian Medical College, Vellore, India. The bacterium was grown in Luria-Bertani broth at 37°C for 18–20 h before use. The two lactobacilli studied were *L. rhamnosus* (MTCC 1408/ATCC 7469) and *L. acidophilus* (MTCC 447/ATCC 4356) and were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. These bacteria were grown in MRS broth at 37°C for 18–20 h before use.

**Animals**

Adult male albino Wistar rats, weighing approximately 120–140 g, were obtained from Tamilnadu Veterinary and Animal Science University, Chennai, India. They were acclimatized to animal house conditions and fed commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India) and water ad libitum. The study was conducted according to the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and by the animal ethics committee guidelines of our institution (IAEC no. 01/019/06).
Experimental design

The rats were divided into eight groups (n = 6 in each group). Group 1 served as the control; group 2, *S. dysenteriae* 1 induced; group 3, *L. rhamnosus* alone; group 4, *L. acidophilus* alone; group 5, *L. rhamnosus* + *L. acidophilus*; group 6, *L. rhamnosus* pretreatment followed by infection with *S. dysenteriae* 1; group 7, *L. acidophilus* pretreatment followed by infection with *S. dysenteriae* 1; and group 8, *L. rhamnosus* + *L. acidophilus* pretreatment followed by infection with *S. dysenteriae* 1. Control rats received saline orally for 4 d. A dosage of $1 \times 10^7$ colony-forming units (cfu)/mL of *L. rhamnosus* or *L. acidophilus* was administered orally [21] for 4 d, alone or in combination, to rats in the treatment groups. This particular dosage was fixed based on the protection offered by *L. rhamnosus* and *L. acidophilus* against *Shigella* infection after trying out different doses ($1 \times 10^5$, $1 \times 10^7$, $1 \times 10^8$, or $1 \times 10^9$ cfu/mL) for 2, 4, 6, and 8 d before *S. dysenteriae* 1 induction. After pretreatment of rats with lactobacilli for 4 d, $12 \times 10^8$ cfu/mL of *S. dysenteriae* 1 was orally administered to rats [22]. On the second day after induction, animals were sacrificed; the ileocecal portion of the intestine was removed, slit longitudinally, weighed, and rinsed with ice-cold saline. Mucosae were scraped off, homogenized in 10 mmol/L of sodium phosphate buffer (1:10, w/v), pH 7.4, and centrifuged at 3000 × g for 10 min at 4°C. The homogenate was used for the estimation of protein [23] assay of the activities of total ATPase, Na$^+$/K$^+$-ATPase, and Ca$^{2+}$-ATPase [24]. A portion of intestinal tissue was washed with isotonic saline and used for membrane and cytosol separation. Membrane and cytosol fractions were used for immunoblot analysis of tight junctional proteins.

**Assay of Na$^+$/K$^+$-ATPase**

The incubation mixture contained 1.0 mL of Tris-HCl buffer (90 mmol/L, pH 7.5), 0.2 mL each of magnesium sulfate (50 mmol/L), potassium chloride (50 mmol/L), sodium chloride (600 mmol/L), ethylene-diaminetetra-acetic acid (1 mmol/L), ATP (40 mmol/L), and homogenate. The mixture was incubated at 37°C for 15 min. The reaction was arrested by the addition of 1.0 mL of 10% trichloroacetic acid (TCA), mixed well, and centrifuged at 105 000 g for 15 min to remove nuclei and cellular debris. The supernatant was then centrifuged at 105 000 × g for 1 h, resulting in the sedimentation of a membrane fraction and soluble cell fluid. The membrane pellet was suspended in the same buffer used for homogenization, aliquoted, frozen, and stored at −80°C or used immediately. Occludin and claudin-1 expressions were assessed in membrane and cytosol fractions.

**Immunoblot (western blot) analysis**

Immunoblotting was performed according to the standard method of Towbin et al. [29] to detect the expression patterns of claudin-1 and occludin in the membrane and cytosol fractions. Denatured proteins (50 μg) were loaded onto polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose membranes followed by blocking with phosphate buffered saline containing 0.1% Tween and 5% skimmed milk powder for 1 h at room temperature and later incubated overnight at 4°C with a rabbit antibody for claudin-1 and occludin. Subsequently, membranes were incubated with a horseradish peroxidase–linked goat anti-rabbit immunoglobulin G antibody for 1 h at room temperature. The membrane was washed and diaminobenzidine substrate solution was added and incubated at room temperature in the dark for 5–10 min for color development. The specific protein was detected as a band in the nitrocellulose membrane. Immunoblot for β-actin was also performed to check for equal loading.

**Transmission electron microscopy**

A portion of the tissue was instantaneously immersed in 25 g/L of glutaraldehyde fixation solution and buffered with 0.1 mol/L of sodium cacodylate (pH 7.4). The specimen was...
then placed in the buffer fixative medium, followed by washing with sodium cacodylate and fixation in 20 g/L of osmium tetroxide buffered with 0.1 mol/L of sodium cacodylate. After dehydration in a graded series of alcohol and propylene oxide, the tissues were transferred to the propylene oxide:ethanol mixture (1:1) and embedded in resin. The specimens were mounted on epoxy resin blocks and left in the oven at 65°C for 72 h. Ultrapathin sections were cut, stained with uranyl acetate for 15 min and lead citrate for 7 min, and then examined under an electron microscope.

**Statistical analysis**

All grouped data were evaluated with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Hypothesis testing methods included one-way analysis of variance followed by least significant difference test. $P < 0.05$ was considered to indicate statistical significance. All these results were expressed as mean ± SD for six animals in each group.

**Results**

**Membrane ATPase levels**

The levels of Na\(^+\)/K\(^+\)-ATPase, Ca\(^{2+}\)-ATPase, and total ATPase are presented in Figures 1, 2, and 3, respectively. A significant ($P < 0.05$) decrease in the activities of membrane-bound ATPases was observed in group 2 (S. dysenteriae 1 induced); cstatistically significant ($P < 0.05$) compared with group 7 (S. acidophilus + S. dysenteriae 1). Activity is expressed as phosphorus liberated per milligram of protein. L.a, Lactobacillus acidophilus; L.rh, Lactobacillus rhamnosus; Pi, phosphorus; Sd1, Shigella dysenteriae 1.

![Fig. 1. Effect of Lactobacillus rhamnosus and Lactobacillus acidophilus pretreatment on the activity of Na\(^+\)/K\(^+\)-ATPase in the intestinal mucosa of control and experimental groups of rats. Results are expressed as mean ± SD (n = 6). *Statistically significant (P < 0.05) compared with group 1 (control); †statistically significant (P < 0.05) compared with group 2 (Shigella dysenteriae 1 induced); ‡statistically significant (P < 0.05) compared with group 6 (L. rhamnosus + S. dysenteriae 1); ‡‡statistically significant (P < 0.05) compared with group 7 (L. acidophilus + S. dysenteriae 1). Activity is expressed as phosphorus liberated per milligram of protein. L.a, Lactobacillus acidophilus; L.rh, Lactobacillus rhamnosus; Pi, phosphorus; Sd1, Shigella dysenteriae 1.](Image 47x162 to 284x310)

![Fig. 2. Effect of Lactobacillus rhamnosus and Lactobacillus acidophilus pretreatment on the activity of Ca\(^{2+}\)-ATPase in the intestinal mucosa of control and experimental groups of rats. Results are expressed as mean ± SD (n = 6). *Statistically significant (P < 0.05) compared with group 1 (control); †statistically significant (P < 0.05) compared with group 2 (Shigella dysenteriae 1 induced); ‡statistically significant (P < 0.05) compared with group 6 (L. rhamnosus + S. dysenteriae 1); ‡‡statistically significant (P < 0.05) compared with group 7 (L. acidophilus + S. dysenteriae 1). Activity is expressed as phosphorus liberated per milligram of protein. L.a, Lactobacillus acidophilus; L.rh, Lactobacillus rhamnosus; Pi, phosphorus; Sd1, Shigella dysenteriae 1.](Image 307x162 to 544x325)

![Fig. 3. Effect of Lactobacillus rhamnosus and Lactobacillus acidophilus pretreatment on the activity of total ATPase in the intestinal mucosa of control and experimental groups of rats. Results are expressed as mean ± SD (n = 6). *Statistically significant (P < 0.05) compared with group 1 (control); †statistically significant (P < 0.05) compared with group 2 (Shigella dysenteriae 1 induced); ‡statistically significant (P < 0.05) compared with group 6 (L. rhamnosus + S. dysenteriae 1); ‡‡statistically significant (P < 0.05) compared with group 7 (L. acidophilus + S. dysenteriae 1). Activity is expressed as phosphorus liberated per milligram of protein. L.a, Lactobacillus acidophilus; L.rh, Lactobacillus rhamnosus; Pi, phosphorus; Sd1, Shigella dysenteriae 1.](Image 307x548 to 544x713)
Immunoblot analysis for claudin-1 and occludin expressions

Claudin-1 and occludin dissociate from the membrane and are translocated to cytosol in response to Shigella infection [19]. To confirm the distribution of tight junction-associated proteins during S. dysenteriae infection, western blot analysis of cytosol and membrane protein fractions of intestinal tissue was carried out with specific antibodies.

Figure 4a shows the pattern of claudin-1 expression in the membrane fraction of the control and induced rats. Figure 5a shows the pattern of claudin-1 expression in the cytosol fraction of the control and induced rats. It is clearly seen that, in the membrane fraction of S. dysenteriae-induced rats (Fig. 4a, lane 2), claudin-1 expression was decreased markedly compared with control (Fig. 4a, lane 1). In the cytosol fraction of S. dysenteriae–induced rats (Fig. 5a, lane 2), claudin-1 expression was increased markedly compared with control (Fig. 5a, lane 1). This pattern indicates that claudin-1 has dissociated from the membrane and has translocated to cytosol in the case of S. dysenteriae infection. In the case of rats pretreated with a combination of L. rhamnosus and L. acidophilus followed by S. dysenteriae 1 administration (group 8; Fig. 4a, lane 6), claudin-1 expression in the membrane was almost similar to that of control (Fig. 4a, lane 1) compared with group 6 (L. rhamnosus + S. dysenteriae 1; Fig. 4a, lane 3), group 7 (L. acidophilus + S. dysenteriae 1; Fig. 4a, lane 4), and group 2 (S. dysenteriae 1 induced; Fig. 4a, lane 2).

Figure 4b shows the pattern of occludin expression in the membrane fraction of the control and induced rats. Figure 5b shows occludin expression analysis by western blot in the cytosol fraction of the control and induced rats. It is clearly seen that the expression of occludin in the membrane fraction of the S. dysenteriae–induced rats (Fig. 4b, lane 2) was decreased markedly compared with control (Fig. 4b, lane 1). In the cytosol fraction, occludin expression in S. dysenteriae–induced rats (Fig. 5b, lane 2) was increased markedly compared with control (Fig. 5b, lane 1), indicating the translocation of the tight junctional protein occludin from the membrane to the cytosol. In the case of rats pretreated with a combination of L. rhamnosus and L. acidophilus followed by S. dysenteriae 1 administration (group 8; Fig. 4b, lane 6), occludin expression in the membrane was almost similar to that of control (Fig. 4b, lane 1) compared with group 6 (L. rhamnosus + S. dysenteriae 1; Fig. 4b, lane 3), group 7 (L. acidophilus + S. dysenteriae 1; Fig. 4b, lane 4), and group 2 (S. dysenteriae 1 induced; Fig. 4b, lane 2).

Figures 4c and 5c show β-actin expression in the membrane and cytosol fractions, respectively.

Transmission electron microscopic studies

Control uninfected tissue section showed a normal brush border with an intact membrane (Fig. 6a). Shigella dysenteriae 1–induced rats showed extensive membrane disruption and internalization of Shigella (Fig. 6b). Rats pretreated with L. rhamnosus followed by S. dysenteriae 1 administration showed membrane disruption to a lesser extent compared with the intestinal section of induced rats (Fig. 6d). Rats pretreated with L. acidophilus followed by S. dysenteriae 1 administration showed a slightly disturbed membrane (Fig. 6e). Rats with combined pretreatment with L. rhamnosus and L. acidophilus presented a normal brush border and unperturbed membrane (Fig. 6f), which demonstrates the protection offered by the combination treatment.

Discussion

Interest in the role of lactic acid bacteria in promoting human health goes back at least as far as 1908, when
Metchnikoff suggested that consumption of milk fermented with lactobacilli would prolong life [30]. However, there are not much recent data about the effect of lactobacilli in S. dysenteriae 1 infection available. Shigella dysenteriae 1 infection results in decreased endogenous intestinal protection by activation of protein kinase C [31], leading to the enterocyte membrane damage, changes in membrane permeability, and fluid secretion [32]. Different mechanisms are involved in regulating transport of electrolytes and water in the enterocyte. Increased chloride (Cl\(^-\)) secretion (in crypts) and decreased sodium (Na\(^+\)) absorption (in villus tips) lead to net fluid loss from the small intestine. Cl\(^-\) secretion and Na\(^+\) absorption are dependent on the function of Na\(^+\)/K\(^+\)-ATPase in the basolateral membrane. Sodium absorption is abolished and Cl\(^-\) secretion is reduced when Na\(^+\)/K\(^+\)-ATPase is inhibited [33].

In our study we found that Na\(^+\)/K\(^+\)-ATPase, Ca\(^{2+}\)-ATPase, and total ATPase were significantly reduced in the S. dysenteriae 1–induced rat intestine, consistent with a previous report [34]. It has been reported that the activity of Na\(^+\)/K\(^+\)-ATPase was reduced significantly in calves with diarrhea. Considering the functional role of Na\(^+\)/K\(^+\)-ATPase, it is suggested that weakening of its activity in pathology leads to loss of sodium from the body. Those well-known diarrhea-inducing factors including laxatives [35] and cholera toxin [36] depress Na\(^+\)/K\(^+\)-ATPase activity in the mucous epithelium of the intestine. Biochemical changes connected with inhibition of Na\(^+\)/K\(^+\)-ATPase result in the loss of Na\(^+\) by the body during diarrhea [34]. Decreased Na\(^+\)/K\(^+\)-ATPase activity also has been observed in inflamed intestine [37].

The Ca\(^{2+}\)-ATPase is another important ATPase whose functional role in the membranes of the brush border and basal membrane differs. In the brush border it takes part in the work of the contractile apparatus of the microvilli of the enterocytes; in the basal membrane it is involved in active Ca\(^{2+}\) transport [38]. In the present study, a significant decrease in the activity of Ca\(^{2+}\)-ATPase is in agreement with a previous report [38] stating that, in calves with diarrhea, Ca\(^{2+}\)-ATPase activity was lower than in healthy neonates. In calves with diarrhea the total serum calcium level is depressed. This has been explained on the basis of data showing increased excretion of calcium phosphates and vitamin D with the feces [34].

Parallel to these alterations in the membrane-bound ATPases, we have found that the expression of tight junctional proteins claudin and occludin were altered in the intestinal tissue of S. dysenteriae 1–induced rats. This might be explained by a study on a canine kidney cell line, which suggested that Na\(^+\)/K\(^+\)-ATPase activity is required for acute maintenance of tight junctional permeability [39]. The relation among pump activity, tight junctional proteins, and permeability may be influenced by the amount of cell swelling that occurs after pump inhibition and by how “tight” the occluding junctions are in a particular epithelium [40].

Enteric pathogens can increase epithelial permeability by altering the protein composition of the tight junction. For example, enteropathogenic E. coli increases epithelial permeability by dephosphorylating occludin, a process associated with the shift of occludin away from the tight junction and into the cytosol [41].

Consistent with this, in the present study during S. dysenteriae infection, tight junctional protein occludin expression was greater in the cytosol (Fig. 5b, lane 2) and less in the membrane (Fig. 4b, lane 2). Claudin expression was also found to be greater in the cytosol (Fig. 5a, lane 2) and less in the membrane (Fig. 4a, lane 2), confirming their translocation from intestinal membrane tight junctions into the cytosol. Dephosphorylation of occludin is correlated with its removal from the tight junction and redistribution into the cytoplasm [19]. Ocludin in its phosphorylated form is 72–79 kDa, and when it is dephosphorylated its molecular weight is approximately 65–71 kDa. Because the functional association of occludin with a tight junction requires phosphorylation, when it is dephosphorylated the tight junction barrier breaks down [41]. Shigella can induce dephosphorylation of occludin within a tight junction independent of its invasion machinery [19].
Shigella can regulate a variety of proteins involved in the tight junctions of the epithelial barrier [19]. Shigella flexneri rapidly decreases the transepithelial resistance of T84 cell monolayers after apical and basolateral contacts, indicating the physical disruption of tight junctions. Furthermore, S. flexneri has the capacity to translocate across the tight junctional seal into the paracellular space between intestinal epithelial cells. This observation strongly raises the possibility that Shigella can penetrate the intestinal epithelium directly and by a microfold-cell–dependent invasion route, thereby gaining access to the basolateral side of the epithelium [42].

It has been reported that only direct interaction of Shigella with the epithelial cell will cause disruption of the tight junctional barrier [19]. Lactobacilli have been shown to possess surface adhesins similar to those on bacterial pathogens [43]. Competition for adhesion on the surface of mucin was observed when Lactobacillus was co-incubated with enteropathogens in vitro [5]. It has also been reported that L. rhamnosus can modify the host cell response through interacting intimately and diminish the pathogen–internalizing reaction of the host cell [44]. In our previous report, we reported that in the rats pretreated with L. rhamnosus and L. acidophilus followed by S. dysenteriae 1 administration, Shigella density in feces was significantly reduced when compared with rats induced with S. dysenteriae 1 alone, indicating that the pathogen interaction and internalization was reduced significantly during combination pretreatment [45]. Such reported competitive exclusions and interactions of lactobacilli with host epithelium would have led to minimal Shigella interactions with the epithelial cell and therefore the maintenance of intestinal tight junctions because only direct interactions of Shigella with membrane can disrupt tight junctions [19]. This could justify the observed normal expression of tight junctional proteins and near-normal levels of the membrane-bound ATPases in the combined L. rhamnosus and L. acidophilus pretreated group (group 8), reiterating the presence of intact membrane, which was further confirmed in the transmission electron microscopic study. We previously reported that during combination pretreatment, peroxidation of membrane lipids is significantly attenuated [45], which indicates that the membrane is stable.

A preceding study showed that colons from interleukin-10 gene–deficient mice had increased permeability of the intestinal membrane as measured by in vivo perfusion [46]. A probiotic compound VSL#3 containing Lactobacillus and Bifidobacteria when administered to interleukin-10 gene–deficient mice resulted in complete normalization of mannitol fluxes. It was proved that microflora present in the colon can directly alter membrane permeability [2]. Such an effect could have resulted in the observed membrane-stabilizing activity of the tested strains in our study.

Inhibitory activity of lactobacilli against enteropathogens has been explained in two ways. One is by acidification with lactic acid as observed in L. casei against enterohemorrhagic E. coli growth [47]. Another is by the secreted non-acidic products as exemplified by L. acidophilus, which inhibited the growth of Salmonella typhimurium, enterohemorrhagic E. coli, enteropathogenic E. coli, or Shigella [44].

In a previous study, the combination of L. casei and L. acidophilus was reported to provide protection against S. typhimurium, whereas the individual strains alone did not [48]. Lactobacillus rhamnosus (ATCC 7469) used in this study is the equivalent of L. casei (Orla-Jensen). In the present study, pretreatment with the combination of L. rhamnosus and L. acidophilus synergistically offered better protection to the intestine during Shigella infection when compared with pretreatment with the individual strains. It has been reported that L. acidophilus has significant antioxidative activity as assessed by its free radical scavenging activity [49,50]. Metabolites of L. acidophilus have been shown to inhibit ileal ulcer formation and lipid peroxidation in rats treated with a non-steroidal anti-inflammatory drug, 5-bromo-2-(4-fluorophenyl)-3-(4-methyl sulfonyl phenyl) thiophene [51,52].

Lactobacillus rhamnosus has been reported to interact with intestinal epithelium and prevent the internalization of enteropathogenic bacteria [44]. When these beneficial lactobacilli are given in combination, the ability of L. rhamnosus to prevent the internalization of enterobacteria coupled with the potential of L. acidophilus in the impediment of ileal ulcer formation and membrane lipid peroxidation may lead to the observed synergistic protective effect to facilitate the maintenance of intestinal membrane integrity.

Enterobacterial infections predominantly affect children, leading to morbidity and mortality. Lactobacilli are used mainly in the dairy industry. Dairy products are consumed by and large by children and, hence, the use of these specific strains in combination in such products of nutritive value will provide health benefits to the intended target group.

Conclusion

The present study provides biochemical and molecular evidence for the enhanced protective efficacy of the combination of L. rhamnosus and L. acidophilus in the maintenance of intestinal membrane integrity when compared with pretreatment with individual strains during Shigella infection in rats.

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