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The Effect of Multi-Spicies Probiotic on Antibiotic-Induced Changes in Colonic Ion and Water Transport.

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ABSTRACT

The aim of our study was to investigate the effect of probiotic "Symbiter®" acidophilic concentrated ($1,6x10^9$ KUO/kg, *per os*) in prevention of ceftriaxone-induced (50 mg/kg, i/m) changes in water and ion transport through the colonic epithelium in male Wistar rats (180-250 g) by perfusion of isolated colonic loop *in vivo*. Short-term administration (5 days) evoked pro-secretory changes, may be due to a general shift in the composition of the colonic microbiota. Prolonged multiprobiotic administration (14 days) on the contrary induced insignificant pro-absorptive changes. Multiprobiotic monotherapy-induced changes in water and ion transport is dependent on the duration of therapy. It is allowed us to speculate that organism needs time to adapt to the antigenic load of exogenously introduced bacteria. Combined administration of multiprobiotic with antibiotic ceftriaxone prevented clinical signs of antibiotic-induced diarrhea. This effect was due to increased absorption of water, which was associated with changes in the net potassium transport and were not dependent on the net transport of sodium and chlorine.

Keywords: colon, ceftriaxone, probiotic, diarrhea.

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INTRODUCTION

The composition of the microbiota of the human gastrointestinal tract consists of more than 1,000 species of bacteria, which set of genes is the second genome of the human body and prevail for more than a 100 times. It has been shown that increasing biodiversity of intestinal microflora provides metabolic homeostasis and structural stability, while its decline that occurs with aging, diseases or after antibiotic therapy, reduces resistance to infections [1, 2]. The microflora is very sensitive to different factors: age, gender, climate, diet, microbiocenosis environment, personal hygiene skills, drugs and so on. Epidemiological and experimental studies have shown that intestinal microbiota disturbance (dysbiosis) might be involved in the occurrence of diseases such as chronic ulcerative colitis and Crohn's disease [3]. Widespread use of antibiotics induces adversely affect on the body functions, and especially in the colon [4]. Colon is lower part of the digestive tract, where the absorption of water, vitamins and electrolytes occurs. Treatment with antibiotics leads to imbalance of intestinal microflora, which may result in colonic mucosa inflammation and disruption of intestinal epithelium function [5]. In previous studies we have shown that 5- and 14-das antibiotic ceftriaxone treatment induced diarrhea, which was associated with a decrease in net water and electrolytes (Na, K, Cl) absorption through the colonic epithelium [6, 7].

Taking into account, that antibiotics causes serious disturbances in the composition of colonic microflora, as well as the data on the direct effect of bifidobacteria [8] and lactobacilli [9] on ion transport by epithelial cells, logically suggest a positive effect of probiotics in prevention antibiotic-induced colonic epithelial transport disturbance. Furthermore, considering the complex microflora interrelationship, it is more promising to use multi-species vs. single-species probiotic.

The aim of our study was to investigate the effect of multi-species probiotics "Symbiter®" acidophilic concentrated (multiprobiotic) on net water and electrolytes transport through the rat colonic epithelium in norm and against the background of antibiotic ceftriaxone.

MATERIALS AND METHODS

Animals

Male Wistar rats (180-230 g) were bred and housed in the conventional animal facility of the ESC “Institute of Biology” Taras Shevchenko National University of Kyiv (Kyiv, Ukraine) under standard environmental conditions (12-h light/dark cycle at a constant temperature of 22°C). All animals had unlimited access to animal chow and tap water throughout the study. To normalize gut microbiota, rats from all groups were kept in the same room and maintained by the same personal.

Experiments conducted according to ethical principles adopted by the First National Congress on Bioethics Ukraine, international agreements, national legislation in this field [10] and committee of ESC “Institute of Biology” Shevchenko National University of Kyiv.

Ceftriaxone 1g powder for solution for injection (“Arterium”, Ukraine) was dissolved in sterile water immediately prior injection and administered intramuscularly once a day in dose 50 mg/kg for 5 or 14 consecutive days.

Multiprobiotic was administered orally at a dose $1{,}6x10^9$ CUO/kg 15 minutes before administration of ceftriaxone. One sachet/dose of non-lyophilized multiprobiotic “Symbiter® acidophilus concentrated” consists of active substance (symbiosis of live cells of (CFU/cm^3): *Lactobacilli* and *Lactococci*: $1.0x10^9$; *Bifidobacterium*: $1.0x10^8$; propionate-oxidising bacteria: $3.0x10^7$; acetic acid bacteria: $1.0x10^5$) and additive (skimmed fermented milk). Symbiter is manufactured by Research and Production Company “OD Prolisok” (Kyiv, Ukraine) and has been registered in Ukraine as a pharmaceutical product (Certificate of Registration No. UA/10146/01/01 if 22.10.2009, No. 763).

Animal grouping

Rats were randomly divided into control and ceftriaxone-treated group: 1 control group (n=19) was administered orally with 1 ml of water for 5 days; 2 - control group (n=9) was administered orally with

multiprobiotic for 5 days; 3 - rats (n=14) were administered with ceftriaxone for 5 days; 4 - rats (n=7) were administered with ceftriaxone and multiprobiotic for 5 days; 5 - control group (n=9) was administered orally with 1 ml of water for 14 days; 6 - control group (n=6) was administered orally with multiprobiotic for 14 days; 7 - rats (n=7) were administered with ceftriaxone for 14 days; 8 - rats (n=6) were administered with ceftriaxone and multiprobiotic for 14 days. On the 6th (groups 1-4) and 15th (groups 5-8) day after the start of the experiment (the first day of the injection of test substances) measurement of net water and electrolytes transport through the epithelium of the colon was performed by perfusion of isolated colonic loop *in situ* [11].

Rat's body weight was measured before treatment and in day after treatment withdrawal.

Perfusion of isolated colonic loop *in vivo*

The rate of the net fluid and electrolytes' movement were measured by perfusion of isolated colonic loop *in vivo* [6, 7]. After an overnight fast, the animals were anesthetized by urethane (110 mg/100 g, i.p.; Sigma, Aldrich). A tracheotomy was performed immediately after anesthetize to prevent respiratory insuffiency caused by glotteric edema. The colonic loop was prepared and perfused as modified from previously described methods [6, 7].

The abdomen was opened along the midline and a 15-cm segment of the colon was gently pulled out from the abdominal cavity and cannulated with polyethylene tube about 0.5 cm below the cecum where it was secured with a nylon ligature. The distal colon (2 cm from anus) was ligated before the colonic loop was filled with saline prewarmed at 37°C. The distal colon was then incised, through which another polyethylene tube was inserted and sutured into place to collect the aspirated perfuse solution. The resultant closed colonic loop (perfused length, 8-7 cm) was perfused with pre-warmed modified Krebs-Henseleit solution (mM per L: NaCl – 117; KCl – 5.9; $NaHCO_3$ – 24.8; NaH_2PO_4 – 1.2; $MgCl_2$ – 1.2; $CaCl_2$ – 2.5; glucose – 5.5; T 37°C; pH 7.3-7.4) contained unabsorbed marker phenol red (20 mg/L) [12], using a perfusion pump (Gilson ® Minipuls 3, France) at 0,2 ml/min.

After 60 min stabilization, the perfusate was collected for each subsequent 20-min period of perfusion, overall 180 min; the data obtained for each period were then averaged and expressed as mean of the 20 min. The rectal temperature was kept at 37˚C by table lamps adequately positioned. At the end of the experiment, the animal was killed by overdose of anesthesia and the colon segment was immediately excised, opened longitudinally, gently wiped by filter paper. After that, it was dried out for 20 h (T=60°C) to get the dry weight of colon segment.

The rate of the net water transport was estimated by the level of the unabsorbed marker phenol red in fluid effluent by spectrophotometry. C_{PRF}/C_{PRE} calculated from phenol red absorbencies measured at 520, 560, and 600 nm by the formula: corrected absorbency = 560 -1/2(520 + 600). This corrects for linear background absorbencies in this region of the spectrum. The concentrations of Na^+, K^+ were determined by flaming spectrophotometry and the concentration of Cl^- was defined by chloridometer. Then these indices were calculated by formulas [13].

Positive results indicate net absorption, and negative results indicate net secretion into the lumen.

Statistical analysis

Statistical analysis of data was performed using the program Statistica 8.0. For each of the samples tested is normal or distribution of the studied parameters using the Shapiro-Wilk test. For comparison, samples of total data flow of water and electrolytes used the t-Student test. A statistically significant for all parameters considered difference of $p<0.05$.

RESULTS AND DISCUSSION

We found that ceftriaxone treatment for 5 days decreased net water absorption by 47.5 % ($p<0.001$), sodium ions absorption by 28.8% ($p<0.01$) and net potassium secretion by 25.4% ($p<0.001$). Net chloride ions absorption was not changed (Fig. 1). Thus, the effect of ceftriaxone coincides with the known data on the occurrence of AAD [14].

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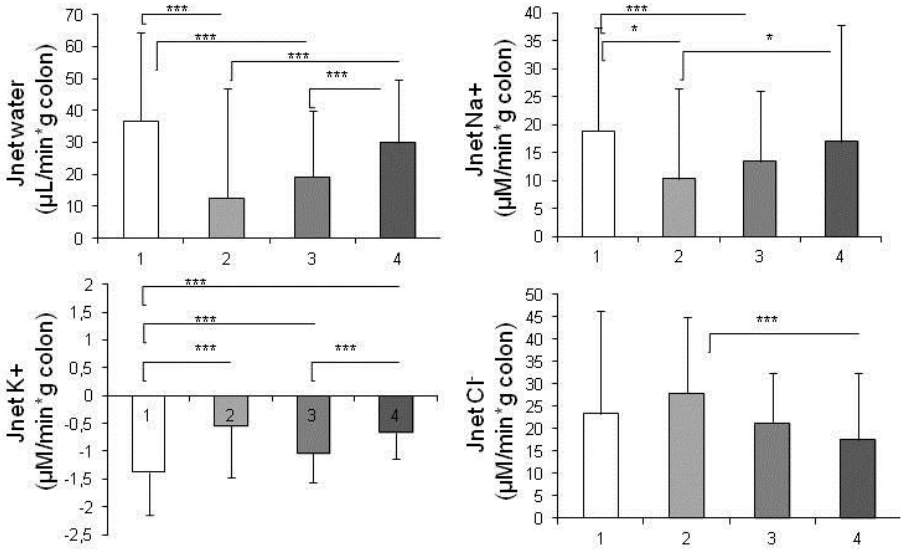
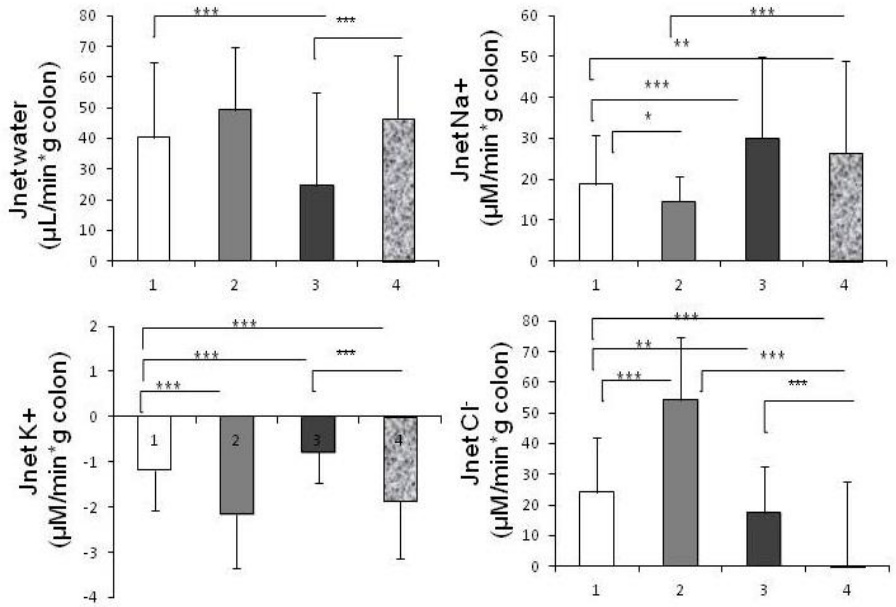
Figure 1: Changes in water and ion transport (Na, K, Cl) through the rat colonic epithelium after 5 days administration of multiprobiotic (1,6x10^9 KUO/kg, *per os*) or its combination with cephalosporin antibiotic ceftriaxone (50 mg/kg, intramuscularly).



Note: 1 - control water; 2 - multiprobiotic; 3 - ceftriaxone; 4 - multiprobiotic+ceftriaxone.
* - P<0,05, *** - P<0,001 - *vs* control (1).

Figure 2: Changes in water and ion transport (Na, K, Cl) through the rat colonic epithelium after 14 days administration of multiprobiotic (1,6x10^9 KUO/kg, *per os*) or its combination with cephalosporin antibiotic ceftriaxone (50 mg/kg, intramuscularly).



Note: 1 - control water; 2 - multiprobiotic; 3 - ceftriaxone; 4 - multiprobiotic+ceftriaxone.
* - P<0,05, ** - P<0,01, *** - P<0,001 – *vs.* control (1)

Administration of multiprobiotic for 5 days lowered the absorption of water by 34.4% (p<0.001), sodium ions by 54.6% (p<0.001), potassium secretion by 39.1% (p<0.001) *vs.* control group. Total transport of

chloride ions remained unchanged (Fig. 1). Thus, short duration of multiprobiotic administration may cause pro-secretory effect on the transport of water and electrolytes, similar to antibiotics.

Contrary to the single effect of antibiotic ceftriaxone and multiprobiotic on the colonic net water and ion transport, their combined administration prevented the decline in net water and sodium absorption. While, net potassium transport was similar to single effect of multiprobiotic. Net transport of chloride ions remained at baseline (control) (Fig. 1).

During severe infectious diseases (meningitis [17], sepsis, endocarditis [18]) duration of antibiotic treatment can be extended to 10-14 days. Therefore, it is important to investigate the effectiveness of multiprobiotic treatment for prevention of disturbance in epithelium function under prolonged administration of ceftriaxone.

We showed that ceftriaxone treatment for 14 days decreased net water absorption by 61.2% ($p<0.001$), chloride ions absorption by 72.5% ($p<0.05$) and net potassium secretion by 65.2% ($p<0.05$). Net sodium ions absorption was significantly increased (159.7%, $p<0.001$) (Fig. 2).

Unlike, after 5 days of single administration of multiprobiotic, 14 days therapy did not cause changes in the colonic net water transport, but reduced levels of sodium absorption to 77.8% ($p<0.05$), increased potassium secretion by 183.0% ($p<0.01$) and chlorine absorption by 224.4% ($p<0.001$) (Fig. 2).

Similar to 5 days therapy, co-administration of antibiotic with multiprobiotic for 14 days prevented the ceftriaxone-induced decline in net water absorption. The level of sodium absorption and potassium secretion were even higher vs. control, rspectively 140.4% ($p<0.01$) and 157.6% ($p<0,001$). A chlorine level of absorption compared with the control group decreased 50 times ($p<0.001$) (Fig. 2).

So, administration either antibiotics ceftriaxone or multiprobiotic for 5 days leads to pro-secretory changes in net water, sodium and potassium transport through the rat colonic epithelium. This effect disappeared after prolongation of multiprobiotic administration, while the effect of the antibiotic was similar to the results after 5-days therapy. Multiprobiotic prevented antibiotic-induced changes in water transport via colonic epithelium independently on therapy duration.

Analyzing our findings, we can assume that the initial shift in the balance of intestinal flora, whether as a result of the use of antibiotics or probiotics, is seen as a shift in body homeostasis. Therefore, diarrhea occurs as a defensive reaction and may have an inflammatory nature, caused by the rapid release of pro-secretory mast cell mediators (e.g. histamine). Another situation after 14-days therapy, probiotic bacteria colonize the intestine and the body comes to a balanced coexistence with administered microorganisms, whereas antibiotic continues to shift this balance even leads to excessive growth of opportunistic bacteria.

In favor of our assumption, there is evidence that *Lactobacillus plantarum 299v* and *Lactobacillus rhamnosus GG* stimulated the production of MUC2 MUC3 enterocytes [19]. That, in turn, plays a protective role and prevents adhesion of pathogens to the epithelial cells of the colonic mucous membrane by increased mucus secretion. As known, this process is accompanied by a parallel increased in chlorine absorption via electroneutral exchange with bicarbonate ions on apical membrane of epithelial cells [20]. A similar effect we observed after 14 days treatment of multiprobiotic, but not after 5 days treatment (Fig. 1 and 2). In addition, it was found that *Lactobacillus rhamnosus*10893 and *Bifidobacterium breve* C50 can inhibit forskolin- and carbachol-stimulated secretion of chlorine by IEC epithelial cells [8].

In support on the possible pro-inflammatory mechanism of antibiotic-induced diarrhea, in our previous study we observed mast cells degranulation in colonic mucous after 5-days, but not 14-days of ceftriaxone treatment [21].

According literature, diarrhea may be the result either changes in the transport function of the epithelium or enhance motor function of the intestine. It has been shown that multiprobiotic “Symbiter” was effective in the prevention of constipation in older rats inducing pro-secretory changes in colonic epithelium [15]. Also, this multiprobiotic “Symbiter”, similar to multiprobiotic “Apibact”, intensified colonic motor activity in rats with long-term gastric hypoacidity [16].

Thus, the administration of multiprobiotic for 5 days is useful to prevent negative effects of antibiotic therapy on epithelial transport function. In case of longer antibiotic use, multiprobiotic is able to prevent water secretion, but is not effective for the correction of electrolyte transport. So we can hypothesize, to prevent changes in epithelial function after long term of antibiotic therapy longer course of multiprobiotic might be recommended.

CONCLUSIONS

Multiprobiotic monotherapy induced changes in water and ion transport *per se*. This effect was dependent on the duration of therapy. Short-term administration (5 days) evoked pro-secretory changes, may be due to a general shift in the composition of the colonic microbiota. Prolonged multiprobiotic administration (14 days) on the contrary induced insignificant pro-absorptive changes. Thus, the organism needs time to adapt to the antigenic load of exogenously introduced bacteria.

Combined administration of multiprobiotic with antibiotic ceftriaxone prevented clinical signs of antibiotic-induced diarrhea. This effect was due to increased absorption of water, which was associated with changes in the net potassium transport and was not dependent on the net transport of sodium and chlorine.

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