The Effect of *Lactobacillus casei* on Experimental Porcine Inflammatory Bowel Disease Induced by Dextran Sodium Sulphate

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ABSTRACT

Background: Gastrointestinal injury caused by dextran sodium sulphate (DSS) is a reliable porcine experimental model of inflammatory bowel disease (IBD). The purpose of this study was to evaluate the effect of probiotic *Lactobacillus casei* DN 114001 (LC) on DSS-induced experimental IBD.

Results: Eighteen female pigs (Sus scrofa f. domestica, weight 33–36 kg, age 4–5 months) were divided into 3 groups (6 animals per group): controls with no treatment, DSS, and DSS + LC. LC was administered to overnight fasting animals in a dietary bolus in the morning on days 1–7 (4.5×10^{10} live bacteria/day). DSS was applied simultaneously on days 3–7 (0.25 g/kg/day). On day 8, the pigs were sacrificed. Histopathological score and length of crypts/glands (stomach, jejunum, ileum, transverse colon), length and width of villi (jejunum, ileum), and mitotic and apoptotic indices (jejunum, ileum, transverse colon) were assessed.

DSS increased the length of glands in the stomach, length of crypts and villi in the jejunum and ileum, and the histopathological score of gastrointestinal damage, length of crypts and mitotic activity in the transverse colon. Other changes did not achieve any statistical significance. Administration of LC reduced the length of villi in the jejunum and ileum to control levels and decreased the length of crypts in the jejunum.

Conclusions: Treatment with a probiotic strain of LC significantly accelerated regeneration of the small intestine in a DSS-induced experimental porcine model of IBD.

KEYWORDS

dextran sodium sulphate; experimental inflammatory bowel disease; *Lactobacillus casei* DN 114001; pigs

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FOOTNOTE

Preliminary results of this study were presented as a poster at the 26th United European Gastroenterology Week, Vienna, October 20–24, 2018 (abstract published in UEG J 2018; 6, Suppl 1: A654) and Digestive Disease Week, San Diego, CA, USA, May 18–21, 2019 (abstract published in Gastroenterology 2019; 156, No 6 Suppl 1: S623–S624).

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BACKGROUND

The aetiology and pathogenesis of inflammatory bowel disease (IBD) comprise genetic susceptibility, various environmental factors (including infectious agents and xenobiotics), and abnormal immune response to intestinal microbiota (1). Both ulcerative colitis (UC) and Crohn's disease (CD) are associated with a reduced microbial diversity (2). Thus, the use of probiotics could be beneficial as they increase microbial diversity, which may subsequently improve the balance and function of intestinal microbiota (3, 4). The possible therapeutic role of probiotics and/or synbiotics has been evaluated in several studies; however, no indisputable final conclusions were achieved (5, 6).

The European Crohn's and Colitis Organisation has stated that there is no evidence to suggest that probiotics are beneficial for the maintenance of remission in CD (7). According to the Cochrane Database, there is insufficient evidence currently to draw any conclusion regarding the efficacy of probiotics for induction and maintenance of remission and prevention of post-operative recurrence of CD (8–10). In contrast, probiotic VSL#3 (a mixture of eight strains, including bifidobacteria, lactobacilli and Streptococcus thermophilus) can improve therapeutic response and maintenance of remission in UC patients (11). The probiotic VSL#3 has been shown to prevent pouchitis within the first year after surgery. According to Magro et al., after achieving remission in chronic pouchitis by treatment, VSL#3 can maintain the remission (12). Another probiotic strain that was found possibly beneficial to maintain the remission in UC is *Escherichia coli Nissle*. However there is no evidence on the efficacy of other probiotics regarding UC (13). The Cochrane Database reviews stated that conventional therapy combined with a probiotic does not improve overall remission rates in patients with mild to moderate UC (14–15). The effects of antibiotics, probiotics and other interventions for treating and preventing pouchitis are uncertain (16). Further studies are indispensable so that conclusive inference on the efficacy of probiotics in UC and CD can be made (4).

The experimental model of colitis induced by dextran sodium sulphate (DSS) in mice was proposed in mid 90s (17–19). DSS-induced mucosal injury also represents a suitable and reliable experimental porcine model of IBD (20–22). Pigs can be used in various preclinical experiments due to their relatively similar gastrointestinal physiology compared to that of humans (23–24), including the porcine intestinal microbiome (25–27). In our previous projects, we studied the effect of probiotic *Escherichia coli Nissle* on bacteriocin production and indomethacin-induced gastrointestinal injury in experimental pigs (28, 29). *Escherichia coli Nissle* alone provided a significantly favourable trophic effect on the colonic mucosa. By contrast, indomethacin and probiotics administered together led to the worst outcome on the porcine stomach, small and large bowel ("anti-synbiotic" effect), and bacteriocin production (28, 29). On the other hand, lactobacilli can ameliorate indomethacin-induced intestinal injury (30). Additionally, lactobacilli possess a protective effect against DSS-induced experimental colitis in mice (31–34). The purpose of this study was to evaluate the effect of probiotic *Lactobacillus casei* DN 114001 (LC) on a DSS-induced experimental porcine model of IBD.

METHODS

ANIMALS

Eighteen experimental adult female pigs (Sus scrofa f. domestica, hybrids of Czech White and Landrace breeds; weight: 33–36 kg, mean 34.3 ± 1.0; age 4–5 months) were enrolled into the study. The animals were purchased from a certified breeder (Stepanek, Dolni Redice, Czech Republic; SHR MUHO 2050/2008/41). The pigs were housed in an accredited vivarium (temperature 21 ± 1 °C, 12 hour light/dark cycle; Faculty of Military Health Sciences, Hradec Kralove, Czech Republic). All animals were fed with standard assorted A1 food (Ryhos, Novy Rychnov, Czech Republic) of equal amounts twice a day and had free access to drinking water. The acclimatization period was 21 days before the experiment.

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (35). Animals were held and treated in conformity with the European Convention for the Protection of Vertebrate Animals (36) and in accordance with the ARRIVE Guidelines (37).

STUDY DESIGN

The animals were divided into 3 groups: controls with no treatment (n = 6), DSS (n = 6) and DSS + LC (n = 6). LC was administrated to overnight fasting animals in a single dietary bolus in the morning on days 1–7 (4.5×10^{10} live bacteria/day). DSS (molecular weight 40 kDa; purchased from Sigma-Aldrich, St. Louis, MO, USA) was applied simultaneously in another dietary bolus on days 3–7 (0.25 g/kg/day). On day 8 (after 24 hours of fasting), the pigs were anaesthetized (intramuscular ketamine, Narkamon, Bioveta, Ivanovice na Hane, Czech Republic, dose 20 mg/kg; and azaperone, Stresnil, Jansen Pharmaceutica, Beerse, Belgium, dose 2 mg/kg), and sacrificed by exsanguination. Immediate autopsy was performed and specimens for structural and morphometric analysis were collected, including the stomach (middle part of the gastric body), and the middle parts of the jejunum, ileum and transverse colon. The samples were immediately fixed with 10% neutral buffered formalin (Bamed, Ceske Budejovice, Czech Republic). There were no adverse events in any experimental group.

STAINING OF SAMPLES

The formalin-fixed samples were routinely processed. This included dehydration, embedding into paraffin (Paramix, Holice, Czech Republic), preparation of 5 µm thick tissue sections using microtome model SM2000 R (Leica, Wetzlar, Germany), rehydration, staining with haematoxylin-eosin (Sigma-Aldrich), final dehydration, and mounting into an aqueous-free mounting medium DPX (Sigma-Aldrich).

HISTOPATHOLOGICAL SCORE

Stained samples were evaluated using a BX-51 microscope (Olympus, Tokyo, Japan). The histopathology score (from 0 to 11) was measured according to Appleyard and Wallace by summation of scores for loss of mucosal architecture, cellular infiltration, muscle thickening, crypt abscess formation, and goblet cell depletion (Table 1) (38). Evaluation of all samples was performed by one person.

Tab. 1 Histopathology score of gastrointestinal damage (ref. 38).

Parameter	Score
loss of mucosal architecture	0, 1, 2, 3 (absent, mild, moderate, severe)
cellular infiltration	0, 1, 2, 3 (absent, mild, moderate, extensive)
muscle thickening	0, 1, 2, 3 (absent, mild, moderate, extensive)
crypt abscess formation	0 or 1 (absent or present)
goblet cell depletion	0 or 1 (absent or present)

LENGTH OF CRYPTS AND GLANDS AND LENGTH AND WIDTH OF VILLI

The length of crypts/glands (all segments) and length and width of villi (small intestine only) were assessed by BX-51 microscope equipped with image analysis software ImagePro plus 7 (Media Cybernetics, Rockville, MD, USA). For this analysis, 20 randomly selected glands and 20 randomly selected crypts and villi per animal were measured under 80× and 200× magnification, respectively.

EVALUATION OF MITOTIC AND APOPTOTIC ACTIVITIES

In crypts, mitotic and apoptotic activity were measured under 400× magnification and published as apoptotic and mitotic indices. The definition of an apoptotic cell and calculation of both indices were according to previous work done by Pejchal et al. (39).

STATISTICS

Kruskal-Wallis test with multiple pairwise comparisons was used for statistical analysis (IBM SPSS Statistics, version 24; IBM Corp., Armonk, NY, USA). Differences were considered significant when $p < 0.05$.

ETHICS APPROVAL

The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence (Record Number 14922006), Faculty of Military Health Services, Hradec Králové, Czech Republic.

RESULTS

DSS treatment increased the length of gastric glands by 12% ($p < 0.001$), the length of villi and crypts in the jejunum by 10% ($p = 0.023$) and 41% ($p < 0.001$) respectively, the length of villi and crypts in the ileum by 16% ($p = 0.047$) and 23% ($p < 0.001$) respectively, and the histopathological score in the colon from 0 (controls) to 3.80 ± 1.3 ($p = 0.007$), which was associated with increased length of crypts and mitotic activity by 57% and 158% respectively (Table 2). Administration of LC reduced the length of villi in the jejunum and ileum to control levels. It also decreased the length of crypts in the jejunum by 13% when compared with DSS-treated animals (Table 2). Minor to moderate inflammatory changes were found over the small and large intestine (Figures 1–3).

Tab. 2 Average values of histopathological score, morphometric parameters and apoptotic and mitotic indices in the stomach, jejunum, ileum, and transverse colon (mean ± SEM).

		Controls	DSS	DSS + LC
	Stomach			
Histopathological score		0 ± 0	0 ± 0	0 ± 0
glands	length (μm)	1000 ± 28	1118 ± 19 †	1102 ± 17 †
	Jejunum			
Histopathological score		0 ± 0	0.6 ± 0.4	0.4 ± 0.4
villi	length (μm)	293 ± 14	308 ± 13 †	282 ± 16 ‡
	width (μm)	196 ± 11	191 ± 10	189 ± 10
crypts	length (μm)	312 ± 9	440 ± 13 †	385 ± 15 †‡
	apoptotic index (%)	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
	mitotic index (%)	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.3
	Ileum			
Histopathological score		0 ± 0	0.2 ± 0.4	0.6 ± 0.7
villi	length (μm)	251 ± 13	292 ± 19 †	245 ± 14 ‡
	width (μm)	186 ± 9	187 ± 12	195 ± 12
crypts	length (μm)	282 ± 9	347 ± 12 †	323 ± 11 †
	apoptotic index (%)	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
	mitotic index (%)	1.3 ± 0.6	1.0 ± 0.3	1.1 ± 0.3
	Transverse colon			
Histopathological score		0 ± 0	3.8 ± 1.3 †	3.6 ± 1.2 †
crypts	length (μm)	421 ± 10	660 ± 19 †	660 ± 18 †
	apoptotic index (%)	1.0 ± 0.4	0.8 ± 0.3	0.7 ± 0.4
	mitotic index (%)	1.2 ± 0.3	3.1 ± 1.6 †	2.4 ± 1.0 †

† Significant differences between control and DSS or control and DSS + LC groups: $p \leq 0.05$.

‡ Significant differences between DSS and DSS + LC groups: $p \leq 0.05$.

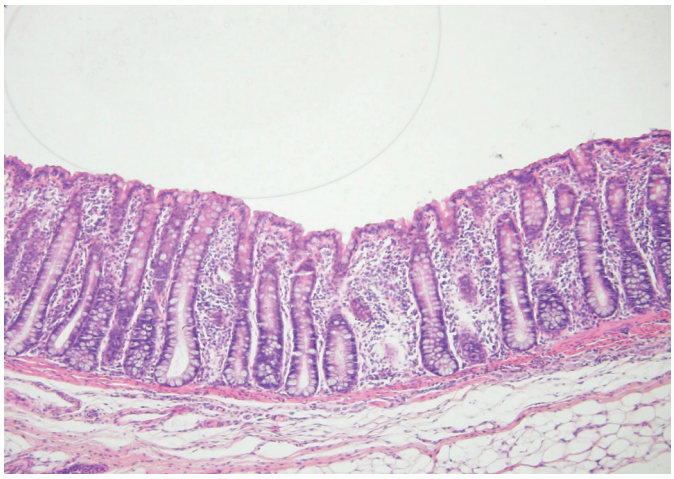

Fig. 1 Control sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. No pathology can be observed.

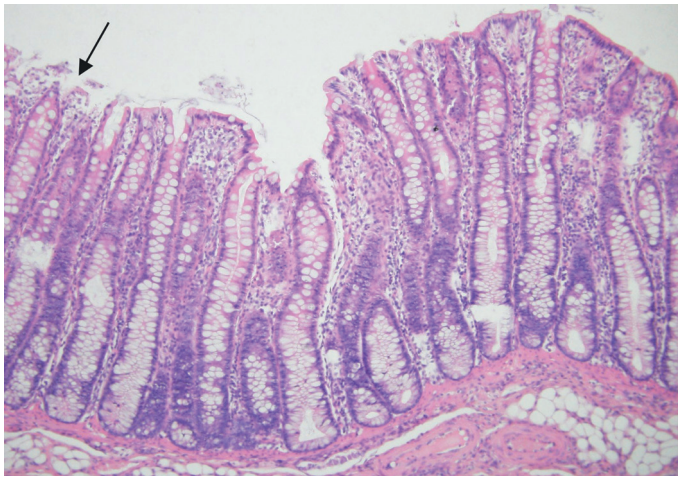

Fig. 2 DSS-treated sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. Slight mucosal oedema with acute inflammatory infiltrate and prolonged crypts can be observed. Arrow indicates mucosal erosion.

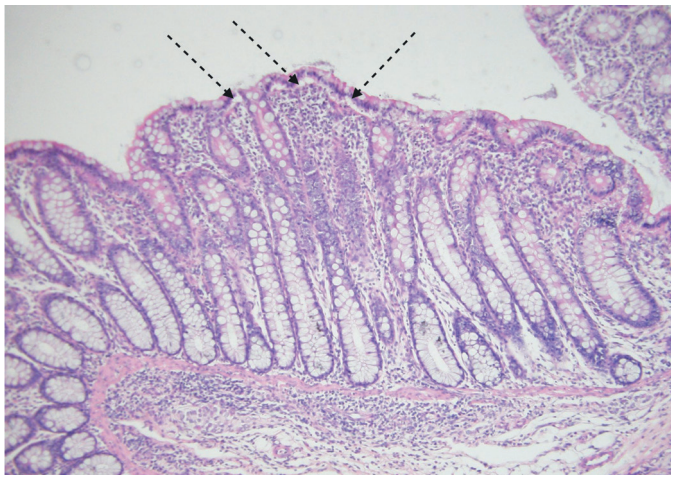

Fig. 3 DSS and Lactobacillus casei treated sample of the porcine transverse colon stained with haematoxylin-eosin at 100fold original magnification. Slight subepithelial (dashed arrows) and mucosal oedema with an in inflammatory infiltrate and prolonged crypts can be observed.

DISCUSSION

Our current study brought new important insight into experimental IBD. To our best knowledge, this is the first study of LC in a DSS-induced porcine experimental model of IBD. DSS is able to induce not only colonic but also small intestinal injury. The lengths of jejunal villi and small intestinal and colonic crypts were significantly taller in the DSS group compared to controls and the DSS+LC group. The histopathology score and mitotic index were increased significantly only in the porcine colon.

Knowledge on a number of species of the genus *Lactobacillus* has broadened considerably during the past 15 years. More than two hundred species are currently recognized (40). Some probiotic lactobacilli have been used for decades, and several species are clearly characterized by their anti-inflammatory effect (41–43). Nonetheless molecular mechanisms underlying the probiotic impact have as yet not been fully understood (40). An ameliorating and/or preventive impact of lactobacilli in murine DDS-induced colitis has been found in several studies (44–47). This effect may be explained by inhibition of excessive activation of the NF-κB pathway (44, 45), suppression of TNF-α-mediated apoptosis of intestinal epithelial cells (48), by activation of epidermal growth factor receptor (49), down-regulation of neutrophilic infiltration (in the case of incomplete toll-like receptor 4 complex signalling) (46), or by down-regulation of T follicular helper cells (50).

Even a lysate of non-living probiotic lactobacilli can prevent severe inflammation by improving the integrity of the intestinal barrier, and/or by modulation of the murine gut microenvironment (51–53). *Lactobacillus casei* decreases caecal and colonic inflammatory scores (41, 47). It can also prevent body weight loss in experimental animals in DSS-induced murine colitis (47, 54).

Vetuschi et al. (55) and Araki et al. (56) found increased apoptosis and decreased proliferation of epithelial cells that might lead to a breakdown of the epithelial barrier function. The authors concluded that this could facilitate the mucosal invasion of intraluminal microorganisms in DSS-induced murine colitis (56). Chae et al. found that lactic acid bacteria can reduce both colitis-induced and NF-κB-mediated apoptosis of intestinal epithelial cells in mice (48). We did not find any significant difference in apoptosis in our current porcine study. However, the mitotic index of the colonic mucosa was significantly higher in the DSS group. It is surprising that the apoptotic index did not change in any segment of the investigated gastrointestinal tract. However, apoptosis is a very complex event which is regulated by both pro-apoptotic and anti-apoptotic components. Survivin, an anti-apoptotic protein has been studied extensively in cancer patients, but little knowledge exists about this inhibitor of apoptosis in IBD patients. It has been reported that levels of survivin are increased in lamina propria T-cells in patients with CD, which leads to an anti-apoptotic effect of the T cells (57). Mennigen et al. found that the probiotic mixture VSL#3 (also containing lactobacilli) protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis (58). Other studies with VSL#3

in murine DSS-induced colitis found also a beneficial effect of probiotics improving ileal microbiota composition (59, 60). The impact of DSS on the entire gastrointestinal tract depends on three variables: molecular weight of DSS, daily dose and cumulative dose of DSS. In our current study, only minor to moderate inflammatory changes were found over the small and large intestine. Differing doses of DSS have been recommended (from 0.25 to 1 g/kg/day) to induce experimental IBD. We intentionally decided for a lower dose. Experimental animals (mouse, rat, pig) may express different sensitivity to DSS. In addition, particular batches of DSS may differ in their grade of toxicity. That is why we recommend conducting preliminary testing of a particular batch of DSS on control animals so that the dose can be adjusted accordingly (our unpublished data).

We are aware of possible limits of our current study. The project was designed as an acute one, lasting eight days only. Longer duration could reveal additional findings, especially possible apoptotic changes of the intestinal epithelial cells.

Probiotics may have a positive impact on intestinal inflammatory changes through their interaction directly with the immune system or indirectly through the modulation of gut microbiota (61). Further studies, both experimental and clinical, are needed to understand this process in detail. Only thus, possible clinical applications may be possible.

CONCLUSIONS

Treatment with the probiotic strain LC significantly accelerated regeneration of the small intestine in a DSS-induced experimental porcine model of IBD.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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