ORIGINAL ARTICLE

Absorption kinetics of 5-aminosalicylic acid in rat: influence of indomethacin-induced gastrointestinal lesions and *Escherichia Coli* Nissle 1917 medication

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Abstract

OBJECTIVES: The therapeutic effect of probiotics has been studied in many clinical and experimental studies but no data exist concerning the influence of probiotics on pharmacokinetics of contemporary administered drugs. In this paper, we describe the influence of indomethacin-induced gastrointestinal lesions and *Escherichia Coli* Nissle 1917 medication on absorption of 5-aminosalicylic acid and its metabolite N-acetyl-5-aminosalicylic acid in rat.
METHODS: 5-aminosalicylic acid (5-ASA) was given orally to rat using gastric probe as a suspension (25 mg/kg). The plasma time profiles of 5-ASA and its metabolite were compared between Group A (animals medicated with a suspension of *Escherichia coli* Nissle 1917 [EcN] in dose of 5×10^8 CFUs/day for 14 consecutive days), Group B (animals with indomethacin [IND]-induced gastrointestinal lesions; single dose of 25 mg/kg of IND), Group C (simultaneous administration of EcN and IND), and Group D (control animals without any medication). The blood samples for HPLC analysis has been taken from incannulated vena jugularis in time 30, 60, 90, 120, 180, 240, 360 min after 5-ASA administration to rat.
RESULTS: The pharmacokinetics of 5-ASA was not significantly changed by EcN medication (Group A) in comparison to control animals (Group D). The significantly elevated absorption (AUC and c_{max}) of 5-ASA was found in animals with induced gastro-enteropathy with concurrently medicated with EcN (Group C) when compred to controls. In the case of metabolite N-acetyl-5-ASA, statistically no-significant differences were found between groups.
CONCLUSIONS: Simultaneous probiotics (EcN) medication did not affect absorption 5-ASA from intestinal tract (the main site of ASAs action).

Abreviations:

ASAs	- aminosalicylates
AUC	- area under the curve
5-ASA	- 5-aminosalicylic acid
CFU	- colony-forming unit
c_{max}	- peak concentration
EcN	- *Escherichia coli* Nissle 1917
GI	- gastrointestinal
IBD	- inflammatory bowel disease
HPLC	- high-performance liquid chromatography
IFN-γ	- interferon-gamma
IL-2	- interleukin-2
IND	- indomethacin
LLOQ	- lower limit of quantification
LPS	- lipopolysaccharide
N-acetyl-5-ASA	- N-acetyl-5-aminosalicylic acid
N-propionyl-5-ASA	- N-propionyl-5-aminosalicylic acid
NSAIDs	- non-steroidal anti-inflammatory drugs
T_{max}	- time to peak concentration
TLRs	- toll-like receptors
TNF-α	- tumor necrosis factor-alpha
UC	- ulcerative colitis
UV	- ultraviolet

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used agents in clinical practice today. Indometahacin, besides its antiphlogistic effect, is known to produce erosions, ulcerative lesions, and petechial bleeding in the mucosa of all parts of the gastrointestinal tract, both in humans and in animal experiments (Kuneš *et al.* 2009; Kim *et al.* 2011; Heeba *et al.* 2009; Kamil *et al.* 2007; Tachecí *et al.* 2010; Hawkey & Langman 2003). Recent evidence has suggested the potential therapeutic role of probiotics in the prevention or treatment of gastrointestinal (GI) disorders (Mach 2006). Efficacy of EcN against inflammatory states in GI tract has been shown in numerous trials (Kruis *et al.* 1997, 2004). There is also an evidence for the efficacy of EcN in Crohn's disease (Malchow 1997), pouchitis (Kuzela *et al.* 2001), collagenous colitis (Tromm *et al.* 2004), antibiotic-associated colitis (Goerg & Schlorer 1998), irritable bowel syndrome (Krammer *et al.* 2006) and diverticular disease of the colon (Fric & Zavoral 2003). Supportive probiotic therapy has seen the biggest progress in inflammatory bowel disease in the last twenty years although there are not currently regulated. In 2004, based on the clinical efficacy and documented low-side effect profile the guidelines for diagnosis and treatment of ulcerative colitis as issued by the German Society of Gastroenterology and Digestive Diseases. EcN was recommended as an alternative to standard mesalazine treatment to maintain remission (Hoffmann *et al.* 2004).

Currently, no studies exist addressing the issue of influence of probiotics on pharmacokinetics of concomitant conventional drug administration.

In our study, we aimed to evaluate the pharmacokinetics 5-aminosalicylic acid (5-ASA) and its metabolite N-acetyl-5-aminosalicylic acid (N-acetyl-5-ASA) in rats medicated with probiotic strain *Escherichia coli* Nissle 1917 (EcN) and in rats with experimentally indomethacin-induced gastrointestinal lesions.

MATERIAL & METHODS

Animals

21 males of laboratory rat (Wistar Han II from breeding facility Konárovice nad Labem), weighing 287 ± 21 g, entered the study. They were kept in plastic breeding containers in air-conditioned room allowed access to water and food ad libitum. The animals were fasted 12 hours before pharmacokinetic study.

Study design

The rats were divided into four groups. Group A –the animals were medicated with a suspension of probiotic strain *Escherichia coli* Nissle 1917 (obtained from laboratories of Microbiological Institute of the Czech Academy of Sciences, Prague), serotype O6:K5:H1 (5×10^8 CFUs/day) for 14 consecutive days (using gastric probe). Group B – the rats were probed for 14 days with a saline (as a "sham manipulation"). Fourteenth day, indomethacin was administered (25 mg/kg as a single dose using gastric probe) to rat to induce of gastrointestinal lesions. Group C – rats were administered with *Escherichia coli* Nissle 1917 (as in group A) and indomethacin (as in group B). Group D (control group of animals) – animals probed with a saline (see group B) only.

Pharmacokinetics

The pharmacokinetic study of 5-aminosalicylic acid (5-ASA) was made next day (15^{th} day) after the last dose of medication according to the scheme of study design. The cannulation of vena jugularis (in general inhalation anaesthesia; mixture of nitrous oxide, oxygen and halothane) was performed in order to blood samples taken. The cannula was led out subcutaneously on the dorsal side of neck. The blood sampling was done in time 30, 60, 90, 120, 180, 240, 360 min after 5-ASA (mesalazine substance obtained from PRO.MED.CS Praha a.s. in dose of 25 mg/kg in 40% polyethylene glycol using gastric probe) administration from animals with free movement in breeding container. Blood samples were centrifuged (3 000 t./min, 10 min). The blood plasma was frozen at –30 °C until analysis.

Analytical procedure

HPLC bioanalytical method for the determination of 5-ASA and its metabolites in blood plasma was developed and validated in our laboratory (Nobilis *et al.* 2006). The sample preparation step consists of the deproteination of plasma by $HClO_4$ and the derivatization of ASAs followed by liquid-liquid extraction of all N-acyl-ASA-derivatives. Chromatographic analyses were performed on a 250-4 mm column containing Purospher RP-18 e, 5 microm (Merck, Darmstadt,

Germany) with a precolumn (4-4 mm). The column effluent was monitored using both UV photodiode-array (lambda = 313 nm) and fluorescence detectors (lambda(exc.) = 300 nm/lambda(emiss.) = 406 nm) in tandem. The identity of individual N-acyl-ASAs in the extracts from biomatrices was verified by characteristic UV-spectra and by HPLC/MS experiments. The whole analysis lasted 23 min at the flow rate of 1 ml.min^{-1}. LLOQ (LOD) was estimated 126(20) pmol.ml^{-1} of plasma for N-acetyl-5-ASA and 318(50) pmol.ml^{-1} of plasma for N-propionyl-5-ASA.

Statistical analysis

All data were compared using analysis of variance (ANOVA) followed by multiple-comparison tests as post hoc analysis or a Student's *t*-test for group comparison of parametric data. The differences were considered significant when $p<0.05$.

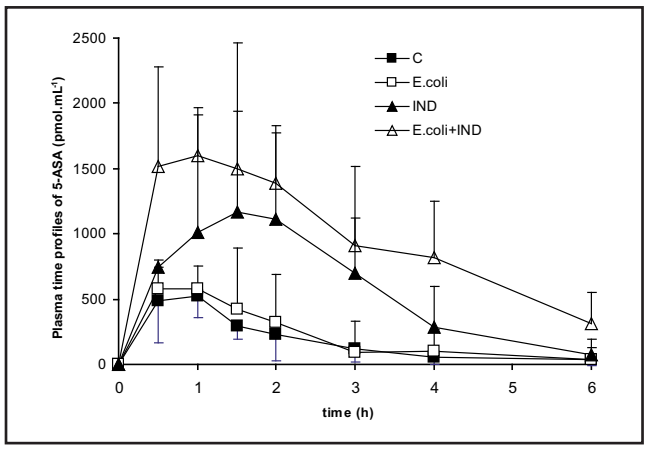

Fig. 1. Pharmacokinetics of 5–ASA in particular groups of rats after its intragastric administration (25 mg/kg). No differences were found in rats pre-medicated with probiotics E.coli in comparison to controls. Significantly higher absorption was found in animals with the combinatory treatment (E. coli + IND). Higher absorption, but statistically no-significant was in animals given indomethacin (IND). Average values ± standard deviation.

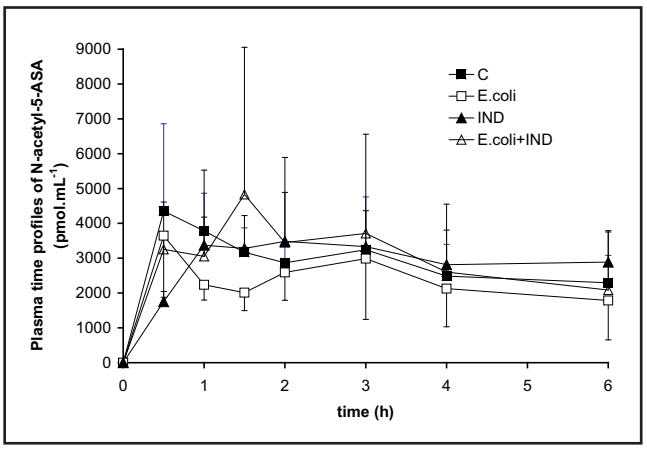

Fig. 2. Pharmacokinetics of N-acetyl-5-ASA in particular groups of rats after intragastric administration of 5-ASA (25 mg/kg). Statistically no-significant differences were found between particular groups, Average values ± standard deviation.

Ethics

The study was approved by the Institutional Review Board of the Animal Care Committee from the Institute of Experimental Biopharmaceutics, Czech Academy of Sciences. Animals were held and treated in accordance with the European Convention for The Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe 1986).

RESULTS

The pharmacokinetics of 5-ASA was not significantly changed by EcN medication (Group A) in comparison to control animals (Group D) as seen from plasma time profiles (Figure 1) and evaluated basic pharmacokinetic parameters (Figures 3–5). The elevated (but no statistically significant) absorption (AUC and c_{max}) of 5-ASA was found in animals after indomethacin (Group B), whereas the levels of 5-ASA were significantly higher in rats medicated with EcN and with indomethacin (Group C) in comparison to controls (Group D) (Figures 1, 3 and 5).

The concentrations of metabolite N-acetyl-5-ASA in blood were lowest in EcN medicated rats (Group A), Overall, however, plasma time profiles did not differ significantly between groups (Figure 2) as well as seen from parameters AUC, C_{max} and T_{max} (Figures 6–8).

DISCUSSION

The therapeutic effect of probiotics has been studied in many clinical and experimental studies. Selective probiotics such as *Lactobacillus GG* (Kalliomäki *et al.* 2001, 2003), *Saccharomyces boulardi* (McFarland *et al.*, 1995) and *Escherichia coli* Nissle 1917 (Kruis *et al.* 1997, 2004; Rembacken *et al.* 1999) have been proven to be clinically effective, the mode of action by which they achieve their beneficial effects remained unclear. Particular probiotic strains have been successfully used for prophylaxis of intestinal infection also in livestock animals (Vanbelle *et al.* 1990; Alexopoulos *et al.* 2004). In piglets, an efficient prophylactic effect of orally administered EcN strain against the epidemic pathogenic action of the porcine enterotoxigenic *E. coli* strain – fatal in pork livestocks – was found (Schroeder *et al.* 2006). The probiotic strain *E. coli* Nissle 1917 used in this study is of the serotype O6:K5:H1 and was isolated for the first time in 1916 by the German physician Alfred Nissle (Loew 2000). Since then this bacterial strain has been used as a probiotic drug and is considered to be safe (Blum *et al.* 1995; Grozdanov *et al.* 2002, 2004; Westendorf *et al.* 2005; Duncker *et al.* 2006). EcN has been characterized extensively at the phenotypic level as well as the molecular genetic level (Blum *et al.* 1995; Blum-Oehler *et al.* 2003; Grozdanov *et al.* 2004; Sun *et al.* 2005).

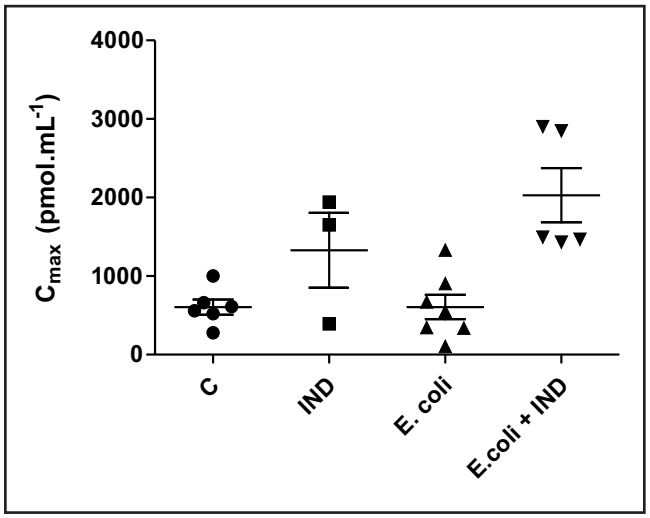

Fig. 3. 5-ASA: parameter C_{max} in each experimental group of animals (horizontal lines means average value ± SEM). Statistically significant differences were found in animals with combinatory treatment (E. coli + IND) when compared to controls (C) and to probiotics medicated rats (E. coli) – $p<0.05$ – Tukey's Multiple Comparison Test.

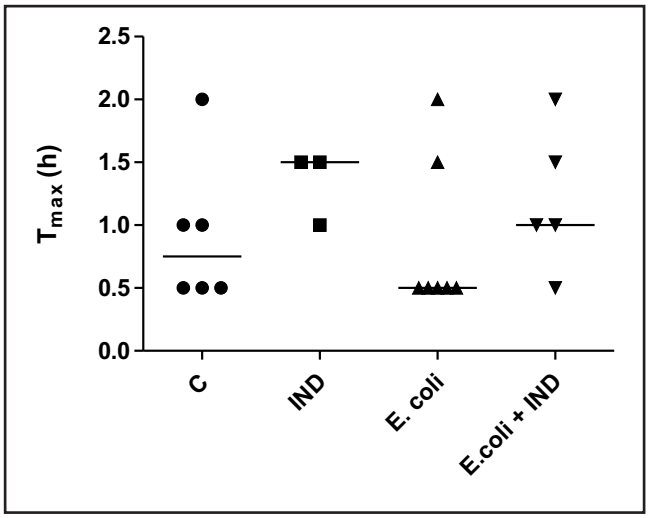

Fig. 4. 5-ASA: parameter T_{max} in each experimental group of animals (horizontal lines are medians). Statistically no-significant differences were found between groups – Dunn's Multiple Comparison Test.

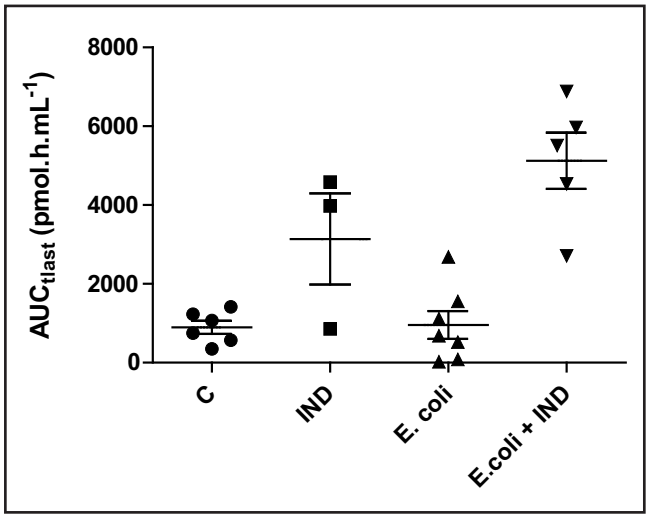

Fig. 5. 5-ASA: parameter AUC in each experimental group of animals (horizontal lines means average value ± SEM). Statistically significant differences were found in animals with combinatory treatment (E. coli + IND) when compared to controls (C) and probiotics medicated rats (E. coli) – $p<0.05$ – Tukey's Multiple Comparison Test.

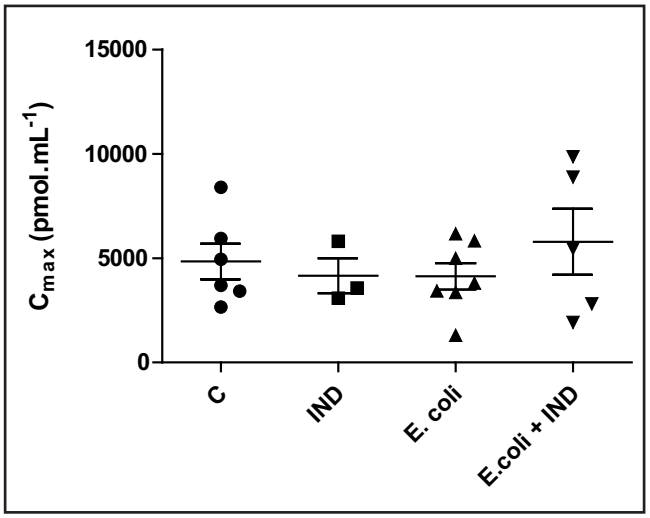

Fig. 6. N-acetyl-5-ASA: parameter C_{max} in each experimental group of animals (horizontal lines means average value ± SEM). Statistically no-significant differences were found between groups – $p<0.05$ – Tukey's Multiple Comparison Test.

EcN, an active component of Mutaflor®, have been evaluated in the last few years as an alternative and safe treatment modality for inflammatory bowel diseases (IBD). Several randomized, placebo controlled studies have clearly demonstarted the beneficial effects of probiotics in the treatment of ulcerative colitis and pouchitis (Gionchetti *et al.* 2000, 2002; Lammers *et al.* 2005), and showing equivalent effectiveness as 5-aminosalicylic acid in maintaining remission in ulcerative colitis (UC) in humans (Malchow *et al.* 1997; Kruis *et al.* 1997, 2004; Rembacken *et al.* 1999). Furthermore, antibiotic as well as probiotic therapy attenuates both experimental colitis and human IBD (Greenberg *et al.* 2004; Kruis 2004; Sartor 2004; Schultz *et al.* 2003).

Despite the demonstrated benefit, the underlying modes of action in intestinal inflammation have yet to be elucidated at the cellular and molecular level (Grabig *et al.*, 2006). Although the probiotic medication is highly recomended as a supportive therapy in various gastrointestinal inflammatory disorders no data exist concerning the influence of probiotics on pharmacokinetics of contemporary administered drugs in literature.

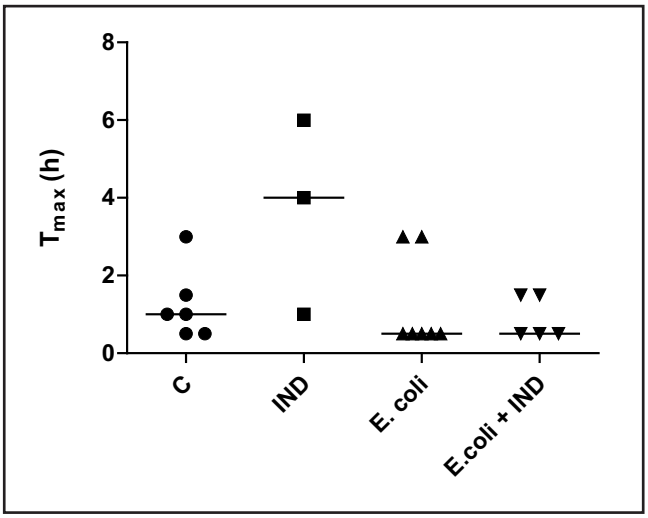

Fig. 7. N-acetyl-5-ASA: parameter T_{max} in each experimental group of animals (horizontal lines are medians). Statistically no-significant differences were found between groups – Dunn's Multiple Comparison Test.

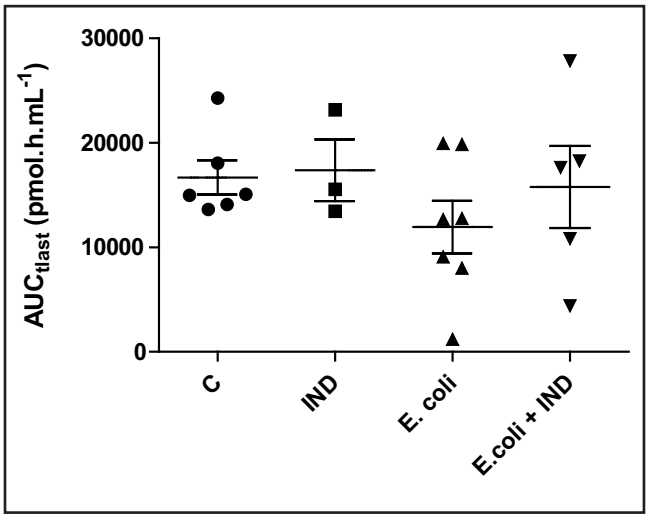

Fig. 8. N-acetyl-5-ASA: parameter AUC in each experimental group of animals (horizontal lines means average value ± SEM). Statistically no-significant differences were found between groups – $p<0.05$ – Tukey's Multiple Comparison Test.

In this study we evaluated the effect of EcN pre-medication on pharmacokinetics of 5-ASA in rat. At the same time, we studied the effect of EcN under the pathological condition (after the induction of gastro-intestinal lesions). Indomethacin, a representative of NSAIDs family, was used as an inducer of gastrointetsinal lesions. It is a model drug commonly used to induce gastroenteropathy in the experimenatal animals, in the rats (Suleyman *et al.* 2009; Obadasoglu *et al.* 2006; Mehrabani *et al.* 2009), mice (Ettarh & Carr 1993, 1996) and pigs (Kvetina *et al.* 2008; Bures *et al.* 2011, Rainsford *et al.* 2003). Also in our previous experiments (Kunes *et al., 2009*) we demonstrated its effect in the creation of lesions in various parts of rat's gastrointestinal tract.

These results document that the pre-medication (simultaneous medication) with probiotic strain *Escherichia coli* Nissle 1917 (EcN) did not affect the absorption of 5-aminosalicylic acid from gastrointestinal tract under the physiological conditions (Group A vs D) and slightly elevated in animals with induced GI lesions (Group B vs C). On the other side, the absorption of 5-ASA (without medication with EcN) was elevated in animals with indomethacin-induced gastro-enteropathy in comparison to controls. This increase in transintestinal transport of 5-ASA may indicate the predominance of its transport via mechanism of diffusion. Its elevation can be interpretated by changes (reducing of cellularity) in intestinal barrier after indomethacin-induction of GI-lesions, which are also documented by inducing other intestinal malabsorption syndroms (by methotrexat, irradiation, etc.) (Kvetina & Parizek 1966; Kunes *et al.* 2005).

The mechanism by which EcN might ameliorates the indomethacin-induced injury can be explained via TLRs signaling. EcN demonstrates potent immunomodulatory properties. In different cell culture models a differential effect on distinct T-cell populations by EcN was observed that might be the basis for immunoregulatory properties, allowing a potent but limited inflammatory response on the mucosal level. These results in reduced secretion of proinflammatory cytokines (IL-2, IFN-γ, and TNF-α) and an up-regulation of the secretion of regulatory IL-10, IL-8, and IL-1β (Sturm *et al.* 2005; Helwig *et al.* 2006; Otte & Podolsky 2004). These effects are mediated by Toll-like receptor-2 (TLR-2) signaling, expressed on activated T-cells (Sturm *et al.* 2005). The concept of recognition of EcN by TLRs was tested in TLR-2 and TLR-4 knockout mice with a significantly ameliorated dextran sulphate sodium-induced colitis in wild-type animals but no effect in either knockout (Grabig *et al.* 2006). Further study of Watanabe *et al.* (2009) describe that the inflammatory responses triggered by activation of the lipopolysaccharide (LPS)/TLR-4 signaling pathway are a key mechanism in non-steroidal anti-inflammatory drug-induced enteropathy. Earlier literature data also note that the generation of oxygen free radicals and lipid peroxidation play an important role in the development of gastric mucosal lesions (Del Soldato *et al.* 1985; Takeuchi *et al.* 1991; Vaananen *et al.* 1991).

On the base of above mentioned facts we hypothetised that EcN medication will reduce (or do not affect) the elevated absorption of 5-ASA in animals with induced GI lesions and not that it will be further increased. The statistically significantly higher absorption of 5-ASA was found in the animals with gastro-intestinal lesions and concurrently pre-treated with probiotic EcN (Group C) when compared to control animals without any medication (Group D). This unex-

pected result is not easy to interpret. On the other hand, these findings are consistent with our previous experiments in pigs. The morphometric analysis of gastrointestinal tract proved deteriorating conjunctive effect of indomethacin and EcN combinatory medication (Bures *et al.* 2011a). Another experiments also documented that indomethacin and EcN administered together comprised the worst impact on bacteriocinogeny in the porcine gastrointestinal tract (compared to indomethacin alone or probiotics alone) (Bures *et al.* 2011b).

It is also interesting to compare the kinetics of 5-ASA and its metabolite N-acetyl-5-ASA in animals treated with EcN. It seems that EcN medication has certain (but no statistically significant) effect on N-acetylation process of 5-ASA in the intestine.

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