Bacteriocinogeny in experimental pigs treated with indomethacin and *Escherichia coli* Nissle

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Abstract

AIM: To evaluate bacteriocinogeny in short-term high-dose indomethacin administration with or without probiotic *Escherichia coli* Nissle 1917 (EcN) in experimental pigs.

METHODS: Twenty-four pigs entered the study: Group A (controls), Group B (probiotics alone), Group C (indomethacin alone) and Group D (probiotics and indomethacin). EcN (3.5×10^{10} bacteria/d for 14 d) and/or indomethacin (15 mg/kg per day for 10 d) were administered orally. Anal smears before and smears from the small and large intestine were taken from all animals. Bacteriocin production was determined with 6 different indicator strains; all strains were polymerase chain reaction tested for the presence of 29 individual bacteriocin-encoding determinants.

RESULTS: The general microbiota profile was rather uniform in all animals but there was a broad diversity in coliform bacteria (parallel genotypes A, B1, B2 and D found). In total, 637 bacterial strains were tested, mostly *Escherichia coli* (*E. coli*). There was a higher incidence of non-*E. coli* strains among samples taken from the jejunum and ileum compared to that of the colon and rectum indicating predominance of *E. coli* strains in the large intestine. Bacteriocinogeny was found in 24/77 (31%) before and in 155/560 (28%) isolated bacteria at the end of the study. Altogether, 13 individual bacteriocin types (out of 29 tested) were identified among investigated strains. Incidence of four *E. coli* genotypes was equally distributed in all groups of *E. coli* strains, with majority of genotype A (ranging from 81% to 88%). The following types of bacteriocins were most commonly revealed: colicins Ia/Ib (44%), microcin V (18%), colicin E1 (16%) and microcin H47 (6%). There was a difference in bacteriocinogeny between control group A (52/149, 35%) and groups with treatment at the end of the study: B: 31/122 (25%, $P = 0.120$); C: 43/155 (28%, $P = 0.222$); D: 29/134 (22%, $P = 0.020$). There was a significantly lower prevalence of colicin Ib, microcins H47 and V (probiotics group, $P < 0.001$), colicin E1 and microcin H47 (indomethacin group, $P < 0.001$) and microcins H47 and V (probiotics and indomethacin group, $P = 0.025$) compared to controls. *Escherichia fergusonii* (*E. fergusonii*) was identi-

fied in 6 animals (6/11 isolates from the rectum). One strain was non-colicinogenic, while all other strains of *E. fergusonii* solely produced colicin E1. All animals started and remained methanogenic despite the fact that EcN is a substantial hydrogen producer. There was an increase in breath methane (after the treatment) in 5/6 pigs from the indomethacin group (C).

CONCLUSION: EcN did not exert long-term liveability in the porcine intestine. All experimental pigs remained methanogenic. Indomethacin and EcN administered together might produce the worst impact on bacteriocinogeny.

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Key words: Bacteriocinogeny; *Escherichia coli* Nissle 1917; Experimental pigs; Indomethacin

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent the group of most commonly used drugs worldwide. NSAIDs may cause severe injury to all parts of the gastrointestinal tract. The pathogenesis of NSAID-induced entero- and colopathy is more multifactorial and complex than formerly assumed but is not yet fully understood. A combination of local and systemic effects plays an important role in pathogenesis. NSAID-induced entero- and colopathy is a stepwise process involving direct mucosal toxicity, mitochondrial damage, breakdown of intercellular integrity, enterohepatic recirculation and neutrophil activation by luminal contents including bacteria. Unlike upper gastrointestinal toxicity, cyclo-oxygenase-mediated mechanisms are probably less important^[1-3]. Intestinal bacteria play a significant role in the pathogenesis of NSAID-induced entero- and colopathy. In experimental studies, NSAIDs cannot induce enteropathy in germ-free rats^[4].

Probiotic bacteria are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host^[5]. Probiotics likely function through enhancement of the barrier function of the gut, immunomodulation, and competitive adherence to the mucus and epithelium^[6]. Probiotic bacteria may exert a systemic anti-inflammatory effect^[7] and modulate apoptosis^[8]. Probiotics have been suggested for amelioration or prevention of

various diseases including antibiotic-associated diarrhoea, irritable bowel syndrome and inflammatory bowel disease. Further possible beneficial effects are being studied (including anti-cancer potential, lowering of serum cholesterol levels and blood pressure reduction, *etc.*)^[9-11]. It has been hypothesised that probiotic bacteria might reduce the adverse effects of NSAIDs on the small and large intestine. However, initial studies provided controversial results, both with ameliorating and deteriorating outcomes^[12-15]. NSAID-induced small intestinal injury is Toll-like receptor 4 dependent^[14]. Probiotic *Escherichia coli* Nissle 1917 (EcN) might ameliorate experimental colitis (induced by dextran sodium sulphate) *via* Toll-like receptor 2 and 4 pathways^[16,17].

Colicins and microcins, members of the bacteriocin family, are produced by bacteriocinogenic strains of *Escherichia coli* (*E. coli*) and some related species of *Enterobacteriaceae*. They are toxic to susceptible bacterial strains of the same family^[18-20]. However, some bacteriocins also exert an inhibitory effect on eukaryotic cells, including observed antineoplastic action *in vitro* and *in vivo*^[21-25]. Bacteriocins might induce apoptosis^[26] as some regulators of apoptosis (e.g. Bcl family with pro- and anti-apoptotic members) share similar structures with pore-forming colicins^[27]. The possible role of bacteriocins was also investigated in clinical studies on bacillary dysentery^[28], inflammatory bowel disease^[29] and colorectal cancer^[30]. Bacteriocins might have a dual role: they may act as both antibiotics and probiotics^[31]. One of the most commonly used probiotic bacterial strains, EcN, is a producer of microcins H47 and M^[32-34].

The aim of this study was to evaluate bacteriocinogeny in short-term high-dose indomethacin administration with or without probiotic bacteria EcN in an experimental porcine model. A small adult pig can be used in experiments as a representative of an omnivore due to its relatively similar gastrointestinal functions in comparison with man^[35-38].

MATERIALS AND METHODS

Ethics

The Project was approved by the Institutional Review Board of Animal Care Committee of the Institute of Experimental Biopharmaceutics, Academy of Sciences of the Czech Republic, Record Number 1492006. Animals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes^[39].

Animals

Twenty-four healthy mature (4-5 mo old) female pigs (*Sus scrofa* f. *domestica*, hybrids of Czech White and Landrace breeds) weighing 33.0 ± 1.7 kg, were included in our study. The animals were divided into four groups: Group A (controls, 6 animals), Group B (probiotics alone, $n = 6$), Group C (indomethacin alone, $n = 6$) and Group D (probiotics and indomethacin, $n = 6$). All animals were fed twice a day (standard assorted food A1 of equal amounts).

Drug and probiotic bacteria administration

EcN (3.5×10^{10} live bacteria/d for 14 d) and/or indomethacin (Indomethacin suppositories, Berlin-Chemie, Germany; 15 mg/kg per day for 10 d) were administered as one-shot dietary bolus to hungry pigs.

Autopsy

Twenty-four hours after the last drug and/or probiotic bacteria administration (groups B to D) or after 14 d of stabling (Group A), the pigs were sacrificed (after 24 h of fasting) by means of pharmacological euthanasia (iv administration of embutramide, mebezonium iodide and tetracaine hydrochloride - T61, Intervet International BV, Boxmeer, the Netherlands; dose of 2 mL per kg) and exsanguinated. Immediate autopsy was performed and smears for bacterial cultures were taken.

Bacterial culture, isolation and identification

Before the experiment anal smears were taken from all animals. At autopsy, smears from mucosa of the jejunum, ileum, caecum, transverse colon and rectum were taken from each animal and immediately inserted into a transport liver-enriched broth. Standard primary cultures were inoculated on blood and MacConkey agars (24 h at 37°C), followed by standard clone isolation. Up to 9 different colonies of coliform bacteria were isolated from each sample (on blood, MacConkey and deoxycholate agars). Particular bacteria were precisely identified by the Vitek2 system (BioMérieux, Marcy l'Etoile, France). All bacterial strains were frozen in cryotube vials at minus 90°C until bacteriocin genotyping.

Analysis of bacteriocin production

The bacteriocin production of all strains was tested in parallel on 4 different agar plates containing (1) TY medium; (2) nutrient broth; (3) TY medium supplemented with mitomycin C; and (4) TY medium supplemented with trypsin. The TY medium consisted of yeast extract (Hi-Media, Mumbai, India) 5 g/L, tryptone (Hi-Media) 8 g/L, sodium chloride 5 g/L; the TY agar consisted of a base layer (1.5%, w/v, solid agar) and a top layer (0.7%, w/v, soft agar). A Difco™ nutrient broth (Difco Laboratories, Sparks, MD) 8 g/L, NaCl 5 g/L, was used for production of relatively unenriched 1.5% (w/v) agar plates. Mitomycin C (0.01%, w/v) and trypsin (0.1%, w/v) were used for induction of colicin production and for protease sensitivity tests, respectively. The previously described set of *E. coli* indicator strains including *E. coli* K12-Row, C6 (φ), B1, P400, and S40 was used to identify the producer strains together with *Shigella sonnei* 17 indicator^[40,41]. To test bacteriocin production, the agar plates were inoculated by needle stab and the plates were incubated at 37°C for 48 h. The tested macrocolonies were then killed with chloroform vapours and each plate was then overlaid with a thin layer of soft agar containing 10^7 cells/mL of an indicator strain and the plates were incubated at 37°C overnight. All investigated *E. coli* strains were tested on four parallel plates against 6 indicator strains stated.

Table 1 DNA primers used for polymerase chain reaction detection of colicin encoding genes

Bacteriocin type	Primer name	5'-sequence-3'	Length of PCR product (nt)
A	ColA-F	CGTGGGAAAAGTCATCATC	475
	ColA-R	GCTTIGCTCTTCCTGATGC	
B	colicinB-F	AAGAAAATGACGAGAAGACG	493
	colicinB-R	GAAAGACCAAAGGCTATAAGG	
D	ColD-F	CTGGACTGCTGCTGGTGATA	420
	ColD-R	GAAGGTGCGCCTACTACTGC	
E1	colicinE1-F	TGTGGCATCGGGCGAGAATA	650
	colicinE1-R	CTGCTTCGTGAAAAGCCTTTT	
E2	ColE2-F	TGATGCTGCTGCAAAAGAG	409
	ColE2-R	TTCAAAGCGTTCCCTACCAC	
E3	ColE3-F	TAAGCAGGCTGCATTGTGATG	413
	ColE3-R	TGGATCTGGACCTTTCAAC	
E4	ColE4-F	GAAGGCTGCATTGTGATGCT	409
	ColE4-R	CGGATCCGGACCTTTAATTT	
E5	ColE3-F	TAAGCAGGCTGCATTGTGATG	430
	ColE5-R	TTGAATTTCTCGAATCGTCCA	
E6	ColE6-F	ACCGAACGTCCAGGTGTT	399
	ColE6-R	TTTAGCCTGTCGCTCCTGAT	
E7	ColE7-F	GCATTCCTGCCATCTGAAAT	431
	ColE7-R	CTTCTGCCCACCTTTCTTTTCG	
E8	ColE3-F	TAAGCAGGCTGCATTGTGATG	449
	ColE8-R	GACTGATTTGGCTTGTCTGTGA	
E9	ColE3-F	TAAGCAGGCTGCATTGTGATG	418
	ColE9-R	GACTTTTCTCCCTCCGACCT	
Ia	ColIa-F	GCATGCAAATGACGCTCTTA	473
	ColIa-R	GAGGACGCCAGTCTCTGTGC	
Ib	ColIb-F	AACGAGTGGGTCGATGATTC	464
	ColIb-R	CCTTTTCTGCGCTCGTATTC	
Js	ColJs-F	TCAAAATGTTTGGGCTCCTC	254
	ColJs-R	TAATCTGCCCTGTCCCACTG	
K	ColK-F	CAGAGGTGCGTGAACATGAA	469
	ColK-R	TCCGCTAAATCCTGAGCAAT	
M	ColM-F	GCTTACCACTTCGCAAAACC	429
	ColM-R	GAGCGACTCTCCGATAATGC	
N	ColN-F	AGCTTGGCGAGTATCTTGGA	401
	ColN-R	CAACACAGCCCCGAATAAAC	
S4	ColS4-F	TATATGGCCCCAAGTCTGGT	456
	ColS4-R	CGTAAGGACGGACACCTGTT	
U	ColU-F	TGATTGCTGCGAGAAAAATG	485
	ColU-R	TCTGACAGCCTCTCCCTGTT	
Y	ColY-F	GCAGGCAGAAAAGAACAAGG	477
	ColY-R	CGGACGTTATTTGCCCTTCAT	
5	Col5-F	CATTGGCAAAAGCGAAATCT	443
	Col5-R	TGCAACTCTGGAAACAATCG	
10	Col10-F	GGTTACCGGATTTCTGGAT	448
	Col10-R	TTCTAGATGCTTGGCCCACT	

PCR: Polymerase chain reaction.

Identification of individual colicin types

All investigated strains were tested with colony polymerase chain reaction (PCR). A bacterial colony was resuspended in 100 µL of sterile water and 1 µL of this suspension was added to the PCR reaction. Individual colicin types (colicins A, D, E2-E9, Ia, Ib, Js, K, M, N, S4, U, Y, 5 and 10) were detected using PCR with primers designed using the Primer3 program^[42]. The list of primer pairs and the corresponding length of PCR products are listed in Table 1. Control bacterial producers stemmed from our stock and comprised *E. coli* BZB2101pColA - CA31, BZB2102 pColB - K260, BZB2103 pColD -

Table 2 Bacteriocinogeny of particular strains isolated at the end of experiment

Parameter	Small intestine			Colon and rectum		
	Bacteriocinogeny	Types of bacteriocin producers (No. of strains)	No. of unique bacteriocin producers	Bacteriocinogeny	Types of bacteriocin producers (No. of strains)	No. of unique bacteriocin producers
Group A	22/55 (40%)	E1 (1); E1, Ia, V (1); E1, V (2); Ia (2); Ia, B, K, M, H47 (2); Ia, H47, V (2); Ia, V (6); Ib (3); J25 (1); S4, U (1); S4, V (1)	11	30/94 (32%)	B (1); B, H47, Ib, K, M (2); B, M (1); C7, E1, Ib, V (1); E1 (1); E1, Ia (1); E1, Ia, V (1); E1, V (2); E7 (1); H47, Ia, V (1); H47, S4 (1); H47, V (2); Ia (6); Ia, V (4); Ib (3); M (1); S4, V (1)	17
Group B	11/43 (26%)	E1 (7); Ia (2); Ia, V, H47 (1); Ib (1)	4	20/79 (25%)	B, H47, K, M, Ia (1); B, M (1); E1 (4); E1, V (1); Ia (11); Ia, V (1); Ib (1)	7
Group C	17/58 (29%)	B, M, V (1); Ia (1); Ia, V (7); Ib (6); S4 (2)	5	26/97 (27%)	B, Ia, (1); E1, Ib (1); J25, Ia (1); Ia (5); Ia, E7, V (1); Ia, H47 (1); Ia, M (1); Ia, V (8); Ib (7)	9
Group D	9/45 (20%)	E1 (3); E1, Ia (1); E1, Ia, V (1); E1, Ib (1); Ia (1); Ia, V (1); Ib (1)	7	20/89 (22%)	E1 (4); E1, Ia (1); E1, Ia, V (1); E1, Ib (2); H47, V (1); Ia (7); Ia, V (1); Ib (2); Ib, V (1)	9

Small intestine: Bacterial strains isolated from mucosa of the jejunum and ileum; Colon and rectum: Bacterial strains isolated from mucosa of the caecum, transverse colon and rectum; Group A: Control animals with no treatment; Group B: Probiotics alone (see text for details); Group C: Indomethacin alone (see text for details); Group D: Probiotics and indomethacin (see text for details); Bacteriocinogeny: Number of bacteriocinogenic strains out of all tested; Types: Particular bacteriocin types found in single isolates; M: Colicin M, not for microcin M.

CA23, BZB2107 pColE4 - CT9, BZB2108 pColE5 - 099, BZB2150 pColE6 - CT14, BZB2120 pColE7 - K317, BZB2279 pColIa - CA53, BZB2202 ColIb - P9, BZB2116 pColK - K235, PAP1 pCol101M - BZBNC22, BZB2123 pColN - 284, *E. coli* 189BM pColE2 - P9, *E. coli* 385/80 pColE1, pColV, *E. coli* 185M4 pColE3 - CA38, *E. coli* W3110 pColE8, W3110 pColE9, *E. coli* K-12 pColS4, *Shigella boydii* M592 (serovar 8) pColU, *E. coli* K339 pColY, *Sh. sonnei* pColJs, *E. coli* pCol5, *E. coli* pCol10, *E. coli* 449/82 pColX (microcin B17), *E. coli* 313/66 pColG (microcin H47), *E. coli* 363/79 pColV (microcin V), *E. coli* TOP10F⁺ pDS601 (microcin C7), *E. coli* D55/1 (microcin J25), and *E. coli* B1239 (microcin L). Sequentially related colicin genes (colicins E2-E9, Ia-Ib, U-Y, and 5-10, respectively) often yielded PCR products with primer pairs of related colicin types and therefore all these PCR products were sequenced. The PCR detection primers for colicins B and E1 and for 6 microcin types including B17, C7, H47, J25, L, and V, were taken from Gordon *et al.*^[43]. The phylogenetic group of each *E. coli* strain was determined using the triplex PCR protocol according to Clermont *et al.*^[44]. Sequence analysis was performed using Lasergene software (DNASTAR, Inc., Madison, WI, USA).

Hydrogen and methane breath testing

Hydrogen and methane breath tests were performed before and the morning following completion of the treatment, carried out under general anaesthesia in spontaneously breathing animals. Alveolar air was aspirated by means of percutaneous puncture of the trachea. Immediate measurement of hydrogen and methane was accomplished in triplicate by means of gas chromatography (Microlyzer DP Plus Quintron, Milwaukee, WI, USA). Results were expressed as parts per million (ppm).

Statistical analysis

Data were statistically analysed with χ^2 with Yates cor-

rection and by Mann-Whitney rank sum test. Statistical software was used for this analysis (SigmaStat version 3.1, Jandel Co., Erkrath, Germany).

RESULTS

The general microbiota profile was rather uniform in all animals but there was a broad diversity in coliform bacteria (parallel genotypes A, B1, B2 and D found). In total, 637 bacterial strains were tested, mostly *E. coli*. The remaining isolates comprised *Salmonella enterica* ssp *Arizonae* (21 isolates), *Pasteurella aerogenes* (20), *Escherichia fergusonii* (*E. fergusonii*) (11), *Aeromonas hydrophila/caviae* (9), *Klebsiella pneumoniae* (8), *Enterobacter cloacae* (4), *Morganella morganii* (4), *Citrobacter braakii* (2), *Citrobacter youngae* (2), *Citrobacter freundii* (1), *Acinetobacter hvoffii* (1) and *Pseudomonas aeruginosa* (1). There was a higher incidence of non-*E. coli* strains among samples taken from the jejunum and ileum compared to that of the colon and rectum indicating predominance of *E. coli* strains in the large intestine (data not shown).

Bacteriocinogeny was found in 24/77 (31%) before and in 155/560 (28%) isolated bacteria at the end of the study. Altogether, 13 individual bacteriocin types (out of 29 tested) were identified among investigated strains. Incidence of four *E. coli* genotypes was equally distributed in all groups of *E. coli* strains, with majority of genotype A (ranging from 81% to 88%). The following types of bacteriocins were most commonly revealed: colicins Ia/Ib (44%), microcin V (18%), colicin E1 (16%) and microcin H47 (6%). There was a difference in bacteriocinogeny between control group A (52/149, 35%) and groups with treatment at the end of the study: B: 31/122 (25%, $P = 0.120$); C: 43/155 (28%, $P = 0.222$); D: 29/134 (22%, $P = 0.020$). See Table 2 for details. There was a significantly lower prevalence of colicin Ib, microcins H47 and V (probiotics group, $P < 0.001$), colicin E1 and microcin H47 (indomethacin group, $P < 0.001$) and microcins H47 and V (probiotics and indomethacin group, $P = 0.025$) com-

Table 3 Analysis of porcine alveolar breath for hydrogen and methane (in ppm - parts per million) before and after the treatment

Group	Hydrogen before	Hydrogen after	Statistical significance	Methane before	Methane after	Statistical significance
A	N/A	3.50 ± 2.81	N/A	N/A	69.33 ± 56.64	N/A
B	6.0 ± 2.82	2.0 ± 0	NS	106.50 ± 94.05	80.00 ± 48.02	NS
C	1.17 ± 0.41	5.0 ± 3.29	NS	34.67 ± 25.65	66.17 ± 38.83	NS
D	2.0 ± 1.16	6.0 ± 6.0	NS	60.75 ± 34.77	62.00 ± 27.71	NS

Group A (controls with no treatment, $n = 6$), Group B (probiotics alone, $n = 6$), Group C (indomethacin alone, $n = 6$) and Group D (probiotics plus indomethacin, $n = 6$). N/A: Not applicable; NS: Not significant.

pared to controls (Table 2). *E. fergusonii* was identified in 6 animals (6/11 isolates from the rectum). One strain was non-colicinogenic, while all other strains of *E. fergusonii* solely produced colicin E1.

Data on porcine alveolar breath analysis of hydrogen and methane are given in Table 3. All animals started and remained methanogenic. Differences between groups were not statistically significant. There was an increase in breath methane (after the treatment) in 5/6 pigs from the indomethacin group (C).

DISCUSSION

Probiotic bacteria might act in three different ways: they are able to modulate the host's defence mechanisms, they have a direct impact on other micro-organisms and finally probiotic effects may be based on actions affecting microbial products like toxins, host products (e.g. bile salts) and food ingredients^[45].

Our hypothesis for this study was that (1) indomethacin would suppress bacteriocin production of *Enterobacteriaceae*; (2) probiotic bacteria EcN would colonise the porcine gastrointestinal tract permanently; (3) they would protect intestinal microbiota from suppressive action of indomethacin; and (4) EcN would convert the starting methanogenic phenotype of pigs to a hydrogenic one. Surprisingly, most of our presumptions were not proved.

There is no simple way to explain this. The first question that should be addressed is a possible role of human probiotic bacteria in the porcine gastrointestinal tract. It is necessary to consider whether human probiotics can be also assumed to act as probiotic microbiota for domestic pigs. Criteria for probiotics of human origin were proposed^[46], however, potential probiotic bacteria isolated from porcine faeces are usually tested *in vitro* to be active against two or three common porcine pathogens only^[47-50].

Genotype B2 and production of microcin H47 were considered as markers of EcN in our study. None of our 637 isolates comprised these bacteria. Viability and sufficient amount of bacteria were ensured before their administration in our project. According to our results, it is unlikely that EcN could exert long-term viability in the porcine intestinal tract. Other swine studies by several authors^[51-54] were able to identify intestinal colonisation by EcN in pigs and piglets but not by all of them^[55]. There is no final proof of long-term colonisation of the gastroin-

testinal tract by EcN in healthy humans. In an interesting study by Schierack *et al.*^[56], probiotic *Enterococcus faecium* supplementation showed no significant effect on the numbers and diversity of *Enterobacteriaceae* species, or on the total counts, diversity and distribution of virulence gene-positive *E. coli* strains in healthy domestic pigs.

Aspirin and some NSAIDs, including indomethacin, influence intestinal bacteria^[57-60]. Indomethacin might exert some impact on intestinal microbiota in our study, as there was an increase in breath methane after the treatment in 5/6 pigs from the indomethacin group. Another interesting result from our current study showed a marked lower prevalence of colicin Ib, microcins H47 and V (probiotics group), colicin E1 and microcin H47 (indomethacin group) and microcins H47 and V (EcN and indomethacin group) compared to controls. We interpret this difference as a sign of adverse effects of probiotics and/or indomethacin on porcine microbiota. Bacteriocinogeny in controls (35%) was higher compared to the indomethacin (28%), probiotic (25%) and indomethacin and probiotic groups (22%). This evident trend did not reach statistical significance for the probiotic group (B) and indomethacin group (C). However, there was a statistically significant difference between controls and indomethacin and probiotic group (D). We can assume that indomethacin and EcN comprise the worst impact on bacteriocinogeny in the porcine gastrointestinal tract. This would be consistent with other studies showing that other probiotics might deteriorate NSAID-induced injury to the intestine^[13].

Composition of food, especially supplements with probiotics, might influence the probiotic effect of intestinal bacteria^[61,62]. This factor is unlikely to play an important role in our study. All animals received identical assorted food with cereals, animal fat, soya oil and a mix of supplements (lysine, threonine, methionine, lactic acid).

In our current study, the microbiota profile was rather uniform in all animals due to identical breed and feed. However, there was a broad diversity in coliform bacteria; the main four genotypes A, B1, B2 and D were identified in parallel. Similar diversity was also found in other porcine studies, with prevailing group A^[56,63]. Dixit *et al.*^[63] showed that differences among individual pigs accounted for 6% of the observed genetic diversity, whilst 27% of the genetic variation could be explained by clonal composition differences among gut regions (isolates obtained from the duodenum, ileum, colon and faeces of 8 pigs). Finally, the absence of virulence genes in these com-

mensals indicates that they may be suitable as a probiotic consortium, particularly if they also display increased adherence to enterocytes and antagonistic activity against pathogenic strains of *E. coli*^[63].

E. fergusonii was identified as a new species of *Enterobacteriaceae* in 1985^[64]. This is considered to be an opportunistic pathogen of farm animals including domestic pigs^[65]. We identified *E. fergusonii* in 6 animals, all pigs were healthy without any sign of infective disease. Interestingly 10/11 isolated bacteria solely produced colicin E1. Colicins produced by *E. fergusonii* strains closely resemble colicins encoded by *E. coli*^[66]. In a previous series of human isolates, only 6/50 (12%) strains were bacteriocinogenic, 3 of which produced colicin E1^[67].

In humans, all intestinal hydrogen and methane are produced by so called “hydrogenic and methanogenic” bacteria^[68-70]. However, most authors do not usually specify which particular bacteria constitute these producers. Hydrogen is produced by bacterial fermentation of saccharides in the intestinal lumen. Concurrently, hydrogen is consumed by other intestinal bacteria to synthesise methane, acetate and hydrogen sulphide. Methane is synthesised solely by bacteria in the intestine (four mols of hydrogen and one mol of carbon dioxide create one mol of methane and water). This reaction reduces the volume of gas that would otherwise be present in the colon^[71-76]. The question of intestinal methane producers has not been definitely solved yet. We hypothesised that common coliform bacteria could also synthesise methane^[77], however, this assumption was not proved by our further studies^[78,79]. McKay *et al.*^[80] found that several anaerobes (*Bacteroides*, *Clostridium* and others) produced hydrogen but rarely methane. Hydrogen is also produced by *Enterobacteriaceae*^[81]. In adult Caucasians, only 30%-50% of people produce methane while hydrogen is produced by 90%-98% of people^[69]. Kien *et al.*^[82] found low breath hydrogen and higher methane in piglets (even in a subgroup supplemented with lactulose). In our current study, all animals revealed a solely methanogenic phenotype (by the analysis of their alveolar breath). This fact could be explained as they came from an identical breed and received the same assorted food. All animals remained methanogenic despite the fact that EcN is a substantial hydrogen producer^[77]. This further supports our finding that EcN 1917 did not have major impact on porcine intestinal microbiota.

In conclusion, it is unlikely that probiotic EcN could exert long-term liveability in the porcine intestine. All experimental pigs remained methanogenic, despite the fact that EcN is a substantial hydrogen producer. The indomethacin and probiotic group had a significantly lower rate of bacteriocinogeny compared to controls with no treatment. These control pigs revealed higher bacteriocinogeny with simultaneous production of up to five different bacteriocins per single strain. Indomethacin and probiotics administered together might provide the worst impact on bacteriocinogeny in the porcine gastrointestinal tract.

COMMENTS

Background

Non-steroidal anti-inflammatory drugs (NSAIDs) represent the group of most commonly used drugs worldwide. NSAIDs may cause severe injury to all parts of the gastrointestinal tract. The pathogenesis of NSAID-induced entero- and colopathy is more multifactorial and complex than formerly assumed but has still not been fully understood. A combination of local and systemic effects plays an important role in pathogenesis. NSAID-induced entero- and colopathy is a stepwise process involving direct mucosal toxicity, mitochondrial damage, breakdown of intercellular integrity, enterohepatic recirculation and neutrophil activation by luminal contents including bacteria. Unlike upper gastrointestinal toxicity, cyclo-oxygenase-mediated mechanisms are probably less important. Intestinal bacteria play a significant role in the pathogenesis of NSAID-induced entero- and colopathy. In experimental studies, NSAIDs cannot induce enteropathy in germ-free rats.

Research frontiers

Probiotic bacteria are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host. Probiotics likely function through enhancement of the barrier function of the gut, immunomodulation, and competitive adherence to the mucus and epithelium. Probiotic bacteria might exert a systemic anti-inflammatory effect and modulate apoptosis. Probiotics have been suggested for amelioration or prevention of various diseases including antibiotic-associated diarrhoea, irritable bowel syndrome and inflammatory bowel disease. Further possible beneficial effects are being studied (including anti-cancer potential, lowering serum cholesterol levels and blood pressure reduction, *etc.*). It has been hypothesised that probiotic bacteria might reduce the adverse effects of NSAIDs on the small and large intestine. However, initial studies provided controversial results, both with ameliorating and deteriorating outcomes.

Innovations and breakthroughs

Based on the current study, it is unlikely that probiotic *Escherichia coli* Nissle 1917 (EcN) could exert long-term liveability in the porcine intestine. Genotype B2 and production of microcin H47 were considered as markers of EcN in the study. The authors did not find such bacteria among any of the 637 isolates. All experimental pigs remained methanogenic, despite the fact that EcN is a substantial hydrogen producer. The indomethacin and probiotic group had a significantly lower rate of bacteriocinogeny compared to controls with no treatment. These control pigs revealed higher bacteriocinogeny with simultaneous production of up to five different bacteriocins per single strain. Indomethacin and probiotics administered together might produce the worst impact on bacteriocinogeny in the porcine gastrointestinal tract.

Applications

Bacteriocins might induce apoptosis as some regulators of apoptosis (e.g. Bcl family with pro- and anti-apoptotic members) share similar structures with pore-forming colicins. Bacteriocins might have a dual role: they may act as both antibiotics and probiotics. One of the most commonly used probiotic bacterial strains, EcN, is a producer of microcins H47 and M.

Terminology

Colicins and microcins, members of the bacteriocin family, are produced by bacteriocinogenic strains of *Escherichia coli* and some related species of *Enterobacteriaceae*. They are toxic to susceptible bacterial strains of the same family. However, some bacteriocins also exert an inhibitory effect on eukaryotic cells, including observed antineoplastic action *in vitro* and *in vivo*.

Peer review

This is an innovative manuscript in basic research, which adequately addresses the ethics of the experiment. Its presentation is accurate but very complex in the reading and interpretation of many variables.

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