EFFECT OF ENTEROCIN A ON THE INTESTINAL EPITHELIUM OF JAPANESE QUAILS INFECTED BY SALMONELLA DUESSELDORF

VIERA CIGÁNKOVÁ, ANDREA LAUKOVÁ*, PETER GUBA** AND RADOMÍRA NEMCOVÁ**

University of Veterinary Medicine, Department of Anatomy, Histology and Physiology, 041 81 Košice, Slovakia
*Institute of Animal Physiology, Slovak Academy of Sciences, 041 01 Košice, Slovakia
**University of Veterinary Medicine-Institute of Experimental Veterinary Medicine, 040 01 Košice, Slovakia

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Abstract

The preventive and therapeutic effects of enterocin A produced by the Enterococcus faecium EK13 strain (own isolate) on the structure of intestinal epithelium of gnotobiotic Japanese quails. Three days old quails were infected with Salmonella Duesseldorf SA 31 strain. Prominent marked damage to enterocytes and their necrotization was observed in Salmonella infected quails. Application of enterocin A before and after the Salmonella-infection had a protective effect on the duodenal epithelium. The damage to microscopic and submicroscopic structure of enterocytes and goblet cells of the intestinal epithelium and its subsequent necrosis was less intensive after the treatment with enterocin A.

Key words: Japanese quails, Salmonella Duesseldorf, enterocin, intestines, pathology.

Bacteriocins are antimicrobial substances of proteinaceous character, produced by some microorganisms with antagonistic effect against bacteria from more or less related bacterial genera (6, 15). Enterocins are in fact bacteriocins produced by several members of the genus Enterococcus. Enterocin A is produced by the Enterococcus faecium EK13 strain. It belongs to Gram-positive, lactic-acid producing bacteria (5, 7, 8). The majority of these bacteria are known to produce small, ribosomally synthesized antimicrobial peptides called bacteriocins (5). Enterocin A is a thermostable bacteriocin-peptide of molecular weight of 4829.98 Da with a wide antimicrobial spectrum. By the use of sequential analysis N-terminal (T-T-H-S-G) was detected in this bacteriocin which proves the identity of enterocin A (12) as it was described for the first time by Aymerich et al. (1). Moreover, Ent. faecium EK13 is a promising probiotic candidate (12). The study of enterocins has attracted increased attention not only in the field of veterinary medicine (4, 8) but also with regard to their potential use in the food industry (10, 11). There are few studies with experimental application of enterocins to prevent or to treat some digestive tract disorders (13). However, they were directed to check reducing bacterial effect (13). The histological aspect in association with enterocin and/or bacteriocin addition to animals was never investigated. Therefore, the aim of this study was to observe microscopical and submicroscopical pictures of the epithelium of the duodenum, jejunum, and caeca of gnotobiotic Japanese quails before and after the infection with Salmonella Duesseldorf SA31 strain.

Material and Methods

The experiment was conducted on gnotobiotic Japanese quails. Their eggs were disinfected with 2% formaldehyde vapours and placed to sterile polyethylene bags ventilated through microbial filters. This way treated eggs were subsequently transported to and incubated under sterile gnotobiotic conditions. The quails were fed sterilized standard feeding mixture BR1 (Tatrat, Huncovce, Slovakia) and had access to water ad libitum. Three days old quails were divided into three groups, seven birds in each.

Enterocin A, produced by the Enterococcus faecium EK13, strain was prepared according to Mareková et al. (12). The strain was cultivated in MRS broth (Becton & Dickinson, Cockeysville, USA) for 18
h at 37°C. Bacteriocidal activity of enterocin A (crude extract-ent A, i.e. concentrated supernatant of the EK13 strain) was tested according to De Vuyst et al. (3) by the so-called “agar spot test” on BHI agar (Becton & Dickinson) and expressed in arbitrary units (AU/ml) which correspond to the highest dilution of bacteriocin that still inhibits the indicator strain. The Salmonella Duesseldorf SA 31 strain was also used as an indicator strain to check the activity of enterocin A. The indicator strain was inhibited by enterocin A at the activity of 800 AU/ml, using an in vitro test.

All the groups, i.e. experimental group 1 (EG1), experimental group 2 (EG2) and control group (CG) were infected with the Salmonella Duesseldorf SA31 strain (200 µl; 10⁷ CFU.ml⁻¹), kindly supplied by Dr Zuzana Vasilková from the Parasitological Institute of the Slovak Academy of Sciences in Košice. The bacteria were applied per os. To observe the preventive effect of enterocin A, the bacteriocin was administered to the CG1 quails before infecting them with the SA31. The therapeutic effect of enterocin A was observed in Japanese quails from the group EG2 to which enterocin A was administered 8 h after SA31 infection.

On day 7 of the experiment, the quails were sacrificed and samples from the duodenum, jejunum and caecum were taken for electron microscopic examination. The specimens were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% OsO₄, dehydrated with acetone and propylene oxide, and embedded in Durcupan. Ultrathin sections were contrasted according to Reynolds (17) and examined under a transmission microscope JEOL 1200 EX. Semithin sections were stained with toluidine blue and examined and photographed under a light microscope.

Results

The intestinal epithelium consists of enterocytes, goblet cells, Paneth cells, and endocrine cells. Enterocytes are tall, slender cells of cylindrical shape. They rest on a basal membrane and their free apical surface is covered with microvilli. Their cytoplasm is polarised and they participate in the uptake of nutrients. Goblet cells are distributed in varying numbers among enterocytes and produce mucus that covers the surface of the intestinal epithelium.

Fig. 1. A portion of intestinal villi of a Japanese quail from CG (semithin section). Necrotizing enterocytes and the void spaces left behind them (star), and normal enterocytes (e). 400x.

Fig. 2. Electronograph of a duodenal epithelium of CG. Necrotizing enterocytes have pycnotic nuclei (n) and their cellular organelles are damaged. Goblet cells (gc) and endocrine cells (ec) have normal structure. 8 000x.

Fig. 3. A portion of intestinal villi of a Japanese quail from EG1 (semithin section). Enterocytes (e) and goblet cells (gc) are not damaged. 400x.

Fig. 4. Electronograph of enterocytes from EG1. Microvilli (mv) are preserved and nuclei (n) and cellular organelles are not damaged. 12 000x.
The control group (CG) showed the most pronounced signs of damage to intestinal epithelium. Multiple necrosis of enterocytes was observed (Fig. 1), particularly in the apical zones of intestinal villi. Some cells were necrotized totally, leaving behind only cell remnants or empty spaces. Goblet cells were not damaged. Electron microscopic observations showed changes in both nucleus and cytoplasm of necrotizing enterocytes (Fig. 2). These cells had markedly darker cytoplasm and the microvilli were absent or low. The nucleus was pyknotic and the nuclear membrane formed deep invaginations. The dense cytoplasm contained damaged mitochondria and dilated cisternae of the endoplasmatic reticulum and Golgi complex. The morphological signs of damage to enterocytes described above indicated their necrotization.

Both the first experimental group (EG1) that was given enterocin preventively and the second one (EG2) that was treated with enterocin after the infection showed considerably lower damage to the intestinal epithelium than the untreated control group (CG), or no damage at all (Fig. 3). The submicroscopical picture of enterocytes and goblet cells was normal. There were usually 1 or 2 necrotizing enterocytes, which corresponds to normal physiological regeneration processes (Fig. 4).

Discussion

Although the studies on the level of transmission electron microscopy allowed us to carry out qualitative rather than quantitative observations of changes in the intestinal epithelium, the morphological lesions observed allowed us to state that toxins of Salmonella Dueseldorf SA 31 cause damage to the cells of the intestinal epithelium leading to their necrotization. Similar changes in enterocytes in Japanese quails due to insufficient uptake of food under conditions of space - flights were observed by Cigánková et al. (2). Preventive and therapeutic administration of enterocin A to Japanese quails affected positively the structure of the epithelial cells. This protective influence can partially be ascribed to the probiotic properties of the Enterococcus faecium EK13 strain. It is well known that some probiotic strains with bacteriocidal influence affect favourably the resistance of the body not excluding the resistance to toxins (16, 18).

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References
