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EXPERIMENTAL PROVOCATION OF MUCOID ENTEROPATHY SYMPTOMS IN RABBITS VIA LIGATION OF CAECAL SEGMENTS

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Summary

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The symptoms of mucoid enteropathy in rabbits were reproduced via ligation of separate caecal segments in order to study the role of obstruction in the aetiopathogenesis of the disease. The experiment was performed in 20 rabbits from the same breed and age, housed and fed under uniform conditions. They were divided into 2 groups with 5 male and 5 female animals in each, used in 2 experimental models.

The pathoanatomical and histopathological findings were described.

The results of the experiment showed that the symptoms of mucoid enteropathy in rabbits could be reproduced via ligation of caecal segments. The symptoms were provoked in 50% of rabbits with ligation against *Ampulla ilei* (S*acculus rotundus)* and in 70% of rabbits with ligation between the caecal body and the appendix. The number of diseased animals, the mortality rate and the severity of enteropathy increased paralelly to the distance between the ligature and the ileocaecal valve. Our results confirmed the hypothesis that the ligation of large intestinal segments resulted in release of toxins in ligated segment, that stimulated the local production of mucus and damaged the other organs after absorption through the injuried mucosa. Therefore, the symptoms of mucoid enteropathy could be considered as toxin-induced secretory disease, that probably developed consequently to direct or vegetative irritation of the caecum.

Key words: mucoid enteropathy, rabbit disease

INTRODUCTION

Mucoid enteropathy is a disease that most commonly affects weaned rabbits at the age of 4–12 weeks (Hagen, 1959; Harkness and Wagner, 1995) and is one of the major cause for the high mortality rate in industrial rabbit farms. The disease was firstly discovered in Los Angeles in 1928 but was described in detail in the UK by Hurt (1949). In Bulgaria, the disease was reported by Simeonov *et al.* (2002). The aetiology of the disease in unknown. According to some investigators, the disease occurred following inappropriate feeding (Cheeke and Patton, 1978; Patton and Cheeke, 1981), had a microbial aetiology (Vetesi, 1974; Whitney, 1976; Targowski and Targowski, 1979) or was caused by neonatal hypoamilasaemia (McCuiston, 1964). Some authors (Sincovich, 1976; Toofanian and Targowski, 1983; Lelkes and Chang, 1987) support the thesis that gastrointestinal obstipation (obstruction) has a primary role in the development of the disease.

The principal clinical manifestations of the disease are abdominal distention, bristled hair, anorexia and perianal staining with mucous or brownish-mucoid matter (Van Kruiningen and Williams, 1972; Harkness and Wagner, 1995).

The course of mucoid enteropathy is acute or subacute with survival of only a small percentage of affected animals (Flatt *et al.*, 1974). The incidence of the disease is higher in spring and affects rabbits from both genders. In acute cases, intestines are distended and contain gases and aqueous fluid while in subacute cases the colon and rectum are filled with mucus often forming mucoid plugs (Flatt *et al.*, 1974; Harkness and Wagner, 1995; Simeonov *et al.*, 2002). The disease is to be histologically confirmed, the principal sign being the increase in both number and size of goblet cells especially in the ileum and the caecum (Van Kruiningen and Williams, 1972; Flatt *et al.*, 1974). Hyperplasia of goblet cells is also observed in urinary bladder, biliary ducts, pancreas and tracheal mucosa (Van Kruiningen and Wagner, 1972).

The aim of the present study was to study the role of intestinal impaction in the aetiopathogenesis of mucoid enteropathy symptoms in rabbits, experimentally provoked via two operative caecal ligation techniques.

MATERIALS AND METHODS

The experiment was performed with 20 rabbits, weighing 1.1 ± 0.3 kg, Californian breed, aged 8 weeks, from both genders. All animals were bought from a disease-free rabbit farm. The rabbits were fed with commercial granulated fodder according to nutrition standards of the age. Water was given *ad libitum*. The rabbits were vaccinated against viral haemorrhagic disease (Hebovac 88T®, Vetbiopharm, Bulgaria). They were examined coproscopically after Füleborn for detection of *Eimeria* oocysts. Prior to the experiment all animals were clinically healthy, in a good condition and submitted to 12-hour fasting. They were divided into 2 groups with an equal number of rabbits from each gender within.

The symptoms of the disease were experimentally provoked by ligation of dif-

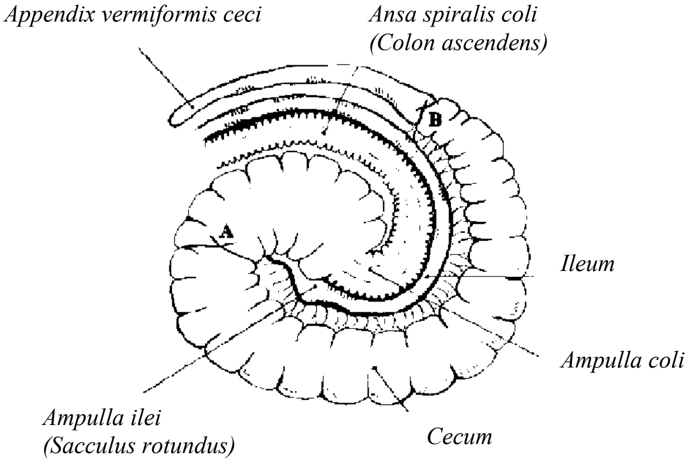

Fig. 1. Placement of ligatures. A. Caecal ligature placed against *Ampulla ilei (Sacculus rotundus)*; B. Caecal ligature placed at the boundary between the caecal body and the appendix.

ferent caecal segments (Fig. 1). The animals from both groups were submitted to standard anaesthesia with 25 mg/kg body weight i.m. ketamine hydrochloride (Ketamin 10%®–Alfasan, Holland), followed by 3 mg/kg. body weight i.m. xylazine hydrochloride (Xylazin 2%® – Alfasan, Holland) 10 min later. The ventral abdominal wall was aseptically prepared.

The model used in the first experimental group consisted in ligation of caecum against *Ampulla ilei* (S*acculus rotundus)* (Fig. 2).

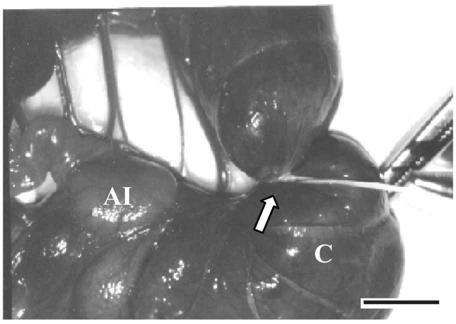

Fig. 2. Ligation of caecum (C) against *Ampulla ilei (AI)* (arrow). Bar = 2 cm.

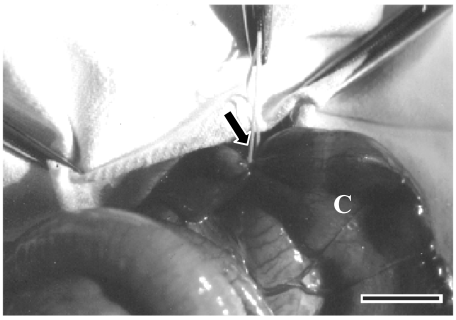

Fig. 3. Ligation at the boundary between caecum (C) and appendix (arrow). Bar = 2 cm.

Following a cranial median laparotomy, the caecum was taken away from the abdominal cavity and *Ampulla ilei* – identified. The ligature was put against it, on caecal body, sparing the blood vessels. The ligature did not impair the free passage of nutritive masses from the caecum to the colon. A non-absorbable polycaproamide suture material was used (Polycon®, Pharmachim, Bulgaria). The caecum was put back into the abdominal cavity and the latter – closed routinely with a two-layer suture.

In the second experimental group, the ligation was performed on a caecal segment, located immediately near the appendix (Fig. 3). The approach was created by cranial median laparotomy. After taking away of caecum and identification of the boundary between caecum and appendix, a Polycon® ligature was put, the caecum – replaced into the abdominal cavity and the operative wound – closed as in group I.

Prior to, during and following the operation the animals were not treated with chemotherapeutics. During the postoperative period the rabbits were put into individual cages. The onset and the development of clinical signs of the disease, the time of occurrence of death, the pathoanatomical and histopathological findigs were followed out. Immediately after a lethal issue, specimens from the different segments of gastrointestinal tract were obtained for histopathological study. The material was fixed in 10% neutral formalin and processed through an ethanol series. The samples were embedded in paraffine blocks, cut on a microtome (5 μm cross-sections) and stained with haematoxylin-eosin. The presence of the characteristic pathoanatomical and histopathological findings (mucoid substance within large intestines, increase in the number and size of large intestine goblet cells, lack of inflammation) were considered as evidence for experimental reproduction of the disease symptoms.

RESULTS

Rabbits with ligation of the caecum against Ampulla ilei

Five out of 10 rabbits (50%) developed signs of mucoid enteropathy by days 3–5 and died within 10 days after the operation. The release of mucus with faeces began between post ligation days 3–5. During the first few days, the decrease in both appetite and thirst was slight but then became significant. The hair was bristled and rabbits grinded their teeth. The anal region was stained with mucoid matter. The consistency of faeces was initially normal but then they were mixed with mucus. The pathoanatomical examination revealed a distention of the abdomen and a moderate dehydratation. The stomach was enlarged, the stomach content – pulpy and covered with mucus. Small intestine was filled with semi-liquid masses. The caecum was balloon-like distended, filled with pulpy material, that was mixed with mucus near the colon. Both the colon and the rectum were filled with mucus, whose quantity varied between animals.

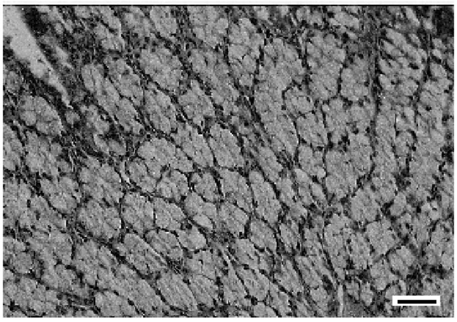

Fig. 4. Increase in the number and the size of caecal goblet cells. H/E, bar = 50 µm.

The histology of intestines revealed an increased number and size of goblet cells, especially caecal ones (Fig. 4). A moderate atrophy of caecal absorptive epithelium and shortening of small intestine villi were also observed.

From the other 5 rabbits, two died without signs of mucoid enteropathy and three survived. Initially, they manifested anorexia and grinded their teeth, but then their appetite and condition improved. In rabbits without signs of mucoid enteropathy there were no changes in the gastrointestinal tract.

Rabbits with ligation of the end of the caecum (at the boundary between caecal body and the appendix)

Seven rabbits from that group (70%) died as early as the 24th post operation hour. A mucoid matter was present in faeces and in the perianal region. The other 3 rabbits (30%) were depressed and lost their appetite. Afterwards the hair became bristled, the abdomen – distended and a grinding the teeth was observed. The condition of those rabbits improved gradually and later, they recovered completely. The pathoanatomical finding in that group was similar to that of group I, although manifested in a lesser extent. The stomach was filled with food and the gastric mucosa – covered with mucus. The small intestine content was aqueous. The caecum was balloon-like distended, but its content was with a normal colour and consistency. There was a mucoid exudate into the colon, especially in the distal colon and the rectum, that was partly out of the anus and mixed with faeces. The histopathological findings were similar to that in group I.

DISCUSSION

The results of our experiment showed that signs of mucoid enteropathy in rabbits could be reproduced via ligation of different caecal segments. On the basis of the most important features of the disease it could be assumed that after ligation against *Ampulla ilei,* its symptoms were provoked in 50% and after ligation between the caecal body and the appendix – in 70% of the animals. In another study, the disease was experimentally reproduced in 45% of rabbits with ligature of the colon, i.e. complete impaction (Targowski and Toofanian, 1982; Toofanian and Targowski, 1983). Our data showed that the bigger the distance between the ligature from the ileocaecal valve in the direction of the appendix, the bigger the number of diseased animals, the morbidity rate and the severity of the disease. Simultaneously the time between the onset of clinical signs and the lethal issue was shorter, although the amount of mucus was significantly lesser. It must be also emphasized that in both experimental groups we did not found out thick gelatinous plugs inside the colon and the rectum, but only semi-liquid mucoid material.

From all hitherto existing studies it became clear that large intestine played a primary role in the manifestation of diseases's symptoms. Hotchkiss and Merrit (1996a) reproduced the signs of the diseases in 70% of rabbits with ligated caecum, preliminary shown to be free from pathogenic pasteurellae and coccidiae. The role of bacteria in the aetiopathogenesis of the disease is not definitely proven. Toofanian and Targowski (1983) isolated 34 microbial species from the large intestine of rabbits with caecal ligatures but observed the same composition of large intestinal microflora in untreated control rabbits. The results from the studies of Sincovich (1976) revealed that the inoculation of *E. coli* and clostridiae in caudal intestinal sections did not result in mucin accumulation into the colon, whereas the symptoms of experimental illness were induced via colon ligation without the involvement of microorganisms. The author concluded that *E. coli* had not a primary role in the onset of the disease. Mucoid enteropathy could therefore be regarded as an intestinal obstruction syndrome in rabbits (Sincovich, 1976; Targowski and Toofanian, 1982; Toofanian and Targowski, 1983). From the other side, there are studies showing an epizootic course of the disease (Licois *et al.*, 1999) and sometimes, classical bacterial pathogens as enteropathogenic *E. coli* (EPEC) and *Clostridium spiroforme* are ioslated (Rossi *et al.*, 1999). The treatment with antibiotics however gives no satisfactory results (Licois *et al.*, 1999). According to other investigators (Legal *et al.*, 1998), viruses could also play an important role in the aetiology of the disease. In the most recent studies, neither EPEC nor *Cl. spiroforme* (Vandekerchove *et al.*, 2000) were isolated.

Others support the hypothesis for the toxic origin of the disease (Bang and Bang, 1979). The application of blood serum from diseased rabbits to invertebrates from the *Sipunculus nudus* species, an overproduction of mucus was observed and the presence of mucus-secretagogue substance, passing through the caecal mucosa into the blood of ill animals was suggested. Hotchkiss and Merrit (1996b) proved that caecal filtrates from rabbits with caecal ligatures provoked *in vitro* a goblet cell hyperplasia in intestinal explants unlike the caecal filtrates of healthy animals. Our results supported the conclusion that the ligation of large intestinal segments resulted in release of toxic products into the ligated segment, stimulating locally the production of mucus and damaging the other organs via absorption through the impaired mucosa, i.e. mucoid enteropathy could be considered as a toxin-induced secretory disease, most probably developing following a direct or vegetative irritation of the caecum.

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