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CHANGES IN THE MUCOPOLYSACCHARIDE COMPOSITION OF MUCUS IN ILEAL MUCOSAL GOBLET CELLS FROM CATTLE INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*[*]

ZMĚNY MUKOPOLYSACHARIDOVÉHO SLOŽENÍ HLENŮ V POHÁRKOVITÝCH BUŇKÁCH SLIZNICE ILEA SKOTU INFIKOVANÉHO *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

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ABSTRACT: Samples of ileum from 34 Holstein and Jersey cows from 5 herds naturally infected with paratuberculosis were examined using bacterial cultures and histochemical methods. The mucopolysaccharide composition of mucus formed in goblet cells of ileal mucosa was determined by staining samples of the mucosa with alcian blue at pH 2.5 and PAS reaction. This staining allowed us to distinguish neutral mucins and glycoproteins from sialomucins and mixed mucins. In the ileal goblet cells from 13 cows in which no *Mycobacterium avium* subspecies *paratuberculosis* was detected by culture and histological methods, neutral mucins and glycoproteins formed the major component of mucus. Mixed mucins were present less frequently and sialomucins least frequently. In 13 cows with subclinical paratuberculosis the number of goblet cells with neutral mucins and glycoproteins increased and the number of goblet cells with sialomucins and mixed mucins decreased. In 8 cows with clinical paratuberculosis sialomucins were never present, the level of mixed mucins decreased and neutral mucins and glycoproteins prevailed. Flattening and diminution of the goblet cells of ileal mucosa were observed in cows with clinical paratuberculosis.

small intestine; mucins; glycoproteins; paratuberculosis; Johne's disease

ABSTRAKT: Vzorky ilea 34 krav plemene holštýnského a jerseyského z pěti chovů s výskytem paratuberkulózy byly vyšetřovány kultivačními a histochemickými metodami. Mukopolysacharidové složení hlenu tvořeného pohárkovitými buňkami sliznice ilea bylo zkoumáno barvením alciánovou modří při pH 2,5 a reakcí PAS. Tímto barvením byly rozlišeny neutrální muciny a glykoproteiny, sialomuciny a smíšené muciny. V pohárkovitých buňkách ilea 13 krav, u nichž nebyla kultivačně ani histologicky prokázána přítomnost *Mycobacterium avium* subspecies *paratuberculosis*, tvořily hlavní složku hlenu neutrální muciny a glykoproteiny. Méně bylo smíšených mucinů a nejméně sialomucinů. U 13 krav se subklinickou paratuberkulózou se zvýšil počet pohárkovitých buněk s neutrálními muciny a glykoproteiny a ubylo pohárkovitých buněk se sialomuciny a smíšenými muciny. U osmi krav s klinickou paratuberkulózou zcela vymizely sialomuciny, snížil se obsah smíšených mucinů a převládly neutrální muciny a glykoproteiny. U krav s klinickou paratuberkulózou bylo pozorováno zploštění a zmenšení pohárkovitých buněk sliznice ilea.

tenké střevo; muciny; glykoproteiny; paratuberkulóza; Johnova choroba

INTRODUCTION

In clinically normal ruminants, small intestine mucosa is covered with single-layer columnar epithelium interspersed with mucus producing goblet cells. This slick mucus covers the surface of the intestinal mucosa (Novotný et al., 1966), influences the acidity of the intestinal content and acts as a barrier between intestinal epithelium and infectious agents (Schmidt-Wittig et al., 1994). It also protects the mucosa against allergens and toxins (Corfield et al., 1988). It is known that the number and composition of mucopolysaccharides of in-

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testinal mucus varies with different types of gastrointestinal tract pathology, e.g. enteritis (Gad, 1969) or formation of tumors (Schmidt-Wittig et al., 1994; Bara et al., 1995).

These changes can be investigated using histochemical methods based on different staining of individual types of mucopolysaccharides with alcian blue (AB) and PAS (Periodic acid-Schiff) reaction. Methods have been developed for staining mucus with alcian blue (Steedman, 1950) at various pH (Spicer, 1960, 1965; Johnson and Helwig, 1963; Lev and Spicer, 1964; Mowry and Winkler, 1965). The most frequently used staining method is alcian blue at pH 2.5 followed by PAS (AB pH 2.5 – PAS). Alcian blue stains sialomucins (SM) blue at low pH, whereas PAS reaction stains neutral mucins and glycoproteins (NMG) red. Mixed mucins (MM) containing both SM and NMG stain purple. Mucus staining with AB and PAS has been used, for example, in the study of human esophageal glands (Hopwood et al., 1986), goblet cells of ovine duodenum (Carvalho et al., 1988) and respiratory organs in goats (Kahwa and Purton, 1996).

Paratuberculosis is a chronic intestinal infection of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis*. Animals with clinical paratuberculosis have chronic diarrhea and are emaciated. Necropsy of animals showing clinical signs reveals macroscopic thickening of small intestine, especially of ileum, caused by granulomatous inflammation within the submucosa. Animals with subclinical paratuberculosis may show no changes of the intestinal mucosa at macroscopic examination, may not have diarrhea and are not emaciated, but are able to shed large quantities of *M. a. paratuberculosis* organisms in their feces (Chiodini et al., 1984; Pavlík et al., 1994). The chronic inflammation resulting from this infection impairs both the intestinal structure and function (Buergelt et al., 1978; Clarke and Little, 1996). Changes in the polysaccharide composition of intestinal mucus, confirmed by means of binding of some lectins to intestinal mucosa, also occur (Massone et al., 1991). The goal of our investigation was to assess any changes in mucopolysaccharide composition of intestinal mucus in subclinical and clinical cases of bovine paratuberculosis using staining with alcian blue at pH 2.5 followed by the PAS reaction.

MATERIAL AND METHODS

Examined animals and sampling of ileum

Samples were collected from cows slaughtered from 1989 to 1996. The breed of all animals was confirmed by the Breeding Service. Thirty-four Holstein and Jersey cows aged 3–6 years originated from 5 herds naturally infected by paratuberculosis as confirmed by culture method were examined. Fifteen Jersey cows were from one herd and 19 Holstein cows from 4 herds (Tab. I). Twenty-six cows (11 Holstein and 15 Jersey) were clinically normal (Tab. I, group A and B), whereas clinical signs of paratuberculosis (emaciation and chronic diarrhea) were observed in 8 Holstein cows (Tab. I, group C). Macroscopic changes in the intestine were assessed immediately after slaughter. Samples for culture examination of *M. a. paratuberculosis* and for histological examination were taken from the same section of ileum as examined for goblet cell composition (approx. 20 cm from the outlet of ileum into the caecum). Samples for histological examination were fixed immediately after sampling with 10% formalin.

Bacterial cultures and identification of isolated mycobacterial strains

About 1 g of intestinal mucosa was homogenized (Lab Blender, Stomacher) and decontamination carried out overnight in 0.75% hexadecylpyridium chloride – HPC (Whipple and Merkal, 1985). The sample was then centrifuged at 3 000 rpm for 10 min, resuspended in sterile saline and pipetted in a 0.2 ml onto three Herrold's egg yolk media with mycobactin J (own product according Merkal and McCullough, 1982) and incubated at 37 °C for 14–16 weeks. The isolation of an average of 1 to 10 colonies on one medium was assessed as +, isolation of 11–50 colonies as ++, and isolation of 51 and more colonies as +++. The isolated mycobacterial strains were identified based on growth characteristics during subcultivation (dependence test) and by the PCR method for detection of specific insertion sequence *IS900* (Kunze et al., 1992).

Histological examination

Samples of ileum were processed using a standard paraffin technique. Six µm thick sections were prepared from the paraffin blocks. Sections were attached to the slides with a mixture of glycerol and egg white and allowed to dry overnight at 37 °C (Vacek, 1972), and were stained in three different ways.

1. Harris' haematoxyline and eosin (Vacek, 1972).
2. Ziehl-Neelsen (Vacek, 1972).
3. Alcian blue at pH 2.5 with subsequent staining by PAS (Lev and Spicer, 1964).

The first two staining methods were performed exactly according to the methodology of Vacek (1972), but the third method was partly modified. Sections removed from paraffin were first stained with a 1% solution of alcian blue 8GX (Serva) in 3% acetic acid at pH 2.5. After 30 min of staining at room temperature the sections were washed with distilled water and stained by PAS. Instead of using the reduction bath (Vacek, 1972) sections were rinsed for 10 min. in tap water. The rinsed preparations were dried for at least 3 h at 37 °C and 1 h at 56 °C and then mounted into Canada balm. The preparations were assessed using light microscopy at a magnification of 79x. The proportion of individual types of mucus in the goblet cells was assessed in each

I. Changes in the mucopolysaccharide composition of mucus in ileal mucosal goblet cells of 34 cows naturally infected with *Mycobacterium avium* subspecies *paratuberculosis*

Group No. of cows and their clinical status at slaughter	Breed	Corrugation of ileal mucosa	Culture detection of mycobacteria	Histological detection of mycobacteria	Mucopolysaccharide composition of mucus in ileal goblet cells			
					n	NMG (%)	SM (%)	MM (%)
A 13 cows uninfected with paratuberculosis, clinically normal, without diarrhoea	Holstein	–	–	–	112	23.2	9.8	67.0
	Holstein	–	–	–	118	31.4	54.2	14.4
	Holstein	–	–	–	217	39.2	7.8	53.0
	Holstein	–	–	–	170	50.6	47.6	1.8
	Holstein	–	–	–	213	57.7	5.2	37.1
	Jersey	–	–	–	123	18.7	71.5	9.8
	Jersey	–	–	–	214	33.6	45.8	20.6
	Jersey	–	–	–	159	45.3	0	54.7
	Jersey	–	–	–	189	55.0	33.4	11.6
	Jersey	–	–	–	156	56.4	0	43.6
	Jersey	–	–	–	121	64.5	0	35.5
	Jersey	–	–	–	82	64.6	6.1	29.3
	Jersey	–	–	–	111	84.7	0	15.3
B 13 cows with subclinical paratuberculosis, no clinical signs, no diarrhoea	Holstein	–	+++	–	176	15.9	1.1	83.0
	Holstein	–	+++	–	135	63.0	0	37.0
	Holstein	–	+++	–	177	63.3	1.7	35.0
	Holstein	–	+++	–	120	75.0	0	25.0
	Holstein	–	+++	–	126	92.9	0	7.1
	Holstein	–	+++	–	89	100.0	0	0
	Jersey	–	+++	–	245	41.6	40.8	17.6
	Jersey	–	+++	–	172	47.7	12.2	40.1
	Jersey	–	+++	–	109	49.5	4.6	45.9
	Jersey	–	+	–	155	66.5	0	33.5
	Jersey	–	+++	–	76	100	0	0
	Jersey	–	+++	–	105	100	0	0
	Jersey	–	+++	–	126	100	0	0
C 8 cows with clinical paratuberculosis, emaciated, with chronic diarrhoea	Holstein	+	+++	+	138	79.7	0	20.3
	Holstein	+	+++	+	156	81.4	0	18.6
	Holstein	+	+++	+	157	91.7	0	8.3
	Holstein	+	+++	+	141	93.6	0	6.4
	Holstein	+	+++	+	176	93.8	0	6.2
	Holstein	+	+++	+	130	94.6	0	5.4
	Holstein	+	+++	+	99	94.9	0	5.1
	Holstein	+	+++	+	121	98.3	0	1.7

Notes:
n – number of examined goblet cells
NMG – neutral mucins and glycoproteins
SM – sialomucins
MM – mixed mucins
Corrugation of ileal mucosa: – (no), + (yes)
Histological detection of mycobacteria: – (no), + yes

Culture detection of mycobacteria:
– negative culture examination (no colonies)
+ isolation of 1–10 colonies on 1 medium
++ isolation of 11–50 colonies on 1 medium
+++ isolation of 51 and more colonies on one medium

of the preparations in five to ten randomly selected intestinal villi.

Statistical evaluation

The results were statistically evaluated by test of multifactorial analysis of variance and Schefe's method of contrasts (Dufek and Stávková, 1985) using the programs STAT Plus and Statgraphics (Matoušková et al., 1992).

RESULTS

Macroscopic changes of ileal mucosa

While ileal mucosa of 26 cows without clinical signs of paratuberculosis (Tab. I, group A and B) was smooth, shiny and light rose, in all the 8 cows with signs of advanced emaciation and diarrhea (Tab. I, group C) the ileal mucosa was also light rose but distinctly swollen and corrugated.

Demonstration of mycobacteria by bacterial culture

In 13 of 26 clinically normal cows (5 Holstein, 8 Jersey) no mycobacterial isolations were made (Tab. I, group A). In the remaining 13 clinically normal cows (6 Holstein, 7 Jersey) *M. a. paratuberculosis* was isolated through bacterial culture of ileal mucosa. Minimal infection (+) was detected in one Jersey cow and extensive infection (+++) in 6 Holstein and 6 Jersey cows (group B). A +++ infection with *M. a. paratuberculosis* was detected in 8 Holstein cows showing clinical signs of diarrhea and emaciation with gross pathology (group C). None of the animals were categorized as a grade ++ infection. Growth of all isolated *M. a. paratuberculosis* strains was dependent on Mycobactin J and contained insertion sequence *IS900* as confirmed by PCR.

Histological examination

Staining with Harris' haematoxylin and eosin

In sections stained with Harris' haematoxylin and eosin differences were found in the morphology of intestinal mucosa in 26 clinically healthy cows (Tab. I, group A and B) and 8 cows with clinical signs of paratuberculosis (Tab. I, group C). While in clinically healthy cows ileal villi were slender and goblet cells on villi and Lieberkühn's crypts were regularly oval, in cows with clinical signs of paratuberculosis the ileal villi were reduced to one third of the usual lenght and width. Infiltrates with prevailing mononuclear cells were observed in the submucosa of cows showing clinical signs. Multinuclear Langhans' cells were also sporadically observed in the submucosa. Goblet ileal cells of clinically healthy cows and subclinically infected cows were oval, while goblet ileal cells of cows showing clinical signs were often more smaller and slender.

Staining by Ziehl-Neelsen

No mycobacteria were detected in samples of ileum of clinically healthy cows using the Ziehl-Neelsen staining. Numerous clusters of intensively red mycobacteria were detected in the submucosa of ileal samples of all cows with clinical signs of paratuberculosis (Tab. I).

Staining of mucus in goblet cells

Goblet cells containing neutral mucins and glycoproteins (NMG) stained red by PAS reaction, whereas goblet cells containing sialomucins (SM) stained blue by alcian blue. Goblet cells containing mixed mucins (MM) stained purple by boths staining methods. No significant differences were determined in the proportion of goblet cells with NMG, SM and MM between the Holstein and Jersey breeds in the groups A and B (Tab. I).

Group A

In uninfected, clinically normal cows (Tab. I, group A) NMG exceeded SM and MM. No significant difference ($P > 0.05$) was found between the numbers of goblet cells containing NMG and SM, NMG and MM, and SM and MM in 5 Holstein cows (group A). In 8 Jersey cows (group A) significant differences in the numbers of goblet cells with NMG and SM ($P < 0.01$) were observed, but not with NMG and MM, and SM and MM ($P > 0.05$).

Group B

In 6 Holstein cows and 7 Jersey cows with subclinical paratuberculosis (Tab. I, group B) the number of goblet cells with NMG was significantly higher than those with SM and MM ($P < 0.01$). The difference between SM and MM were not significant ($P > 0.05$)

Group C

In 8 Holstein cows with clinical paratuberculosis (Tab. I, group C) the goblet cells with SM were missing. Cells with NMG markedly prevailed over cells with MM which was confirmed statistically ($P < 0.01$).

Differences in the numbers of goblet cells between the groups A, B and C

Highly significant differences ($P < 0.01$) were found in Holstein cows in the numbers of goblet cells with NMG between the groups A and C and in the numbers of goblet cells with SM between the groups A and B. No significant differences ($P > 0.05$) were found in Jersey cows in the number of goblet cells with NMG, SM and MM between the groups A and B.

DISCUSSION

Buergelt et al. (1978), who studied paratuberculosis in Holstein and Angus breeds found that histological findings in the small intestine did not differ in individual breeds affected with paratuberculosis. Similarly, we did not find any differences in histological findings between the Holstein and Jersey breeds.

Staining by Ziehl-Neelsen for the detection of mycobacteria allows the identification of changes caused by paratuberculosis in contrast with other chronic intestinal inflammations (Groch and Pivník, 1989). Chronic diarrhea in cattle can be caused not only by paratuberculosis but also by helminthiases, chronic salmonelloses, bovine virus diarrhea-mucosal disease, pyelonefritis, renal amyloidosis and lymphosarcomas (Hines et al., 1987). We observed mycobacteria in macrophages, as noted by Hines et al. (1987).

Condron et al. (1994) demonstrated histological changes in 78.2% of 220 animals with subclinical signs. However, we did not find histology changes in cows with subclinical signs. Differences in the results may be explained by the fact that Condron et al. (1994) examined samples only from animals which had produced antibody in response to infection (as determined by ELISA). These animals were likely to have been close to exhibiting clinical signs and therefore in a rela-

tively advanced stage of infection, as antibody production is a late stage event in this disease. While we did not assess the infection status of the animals serologically, we evaluated the body condition and clinical signs of the cows during sample collection in the slaughterhouse. Absence of mycobacteria in histological intestinal sections of cows with subclinical paratuberculosis can be explained either by the animals being uninfected or at an early stage of the disease.

Histological examination can be a less sensitive method of *M. a. paratuberculosis* infection than bacterial culture. Chiodini (1989) states that a limit mycobacterial detection by for histology examination is 10^6 per gram of tissue. The probability of finding mycobacteria in 6 μm slices and areas of 1–2 cm^2 is much lower than in cultures from 1 g of tissue. Tissues fixed in formalin, in which mycobacteria do not propagate, are used for histological examinations. Since mycobacteria propagate within the media used for cultures the probability of detection is higher.

Pathological and histological changes were found in all cows with clinical signs of paratuberculosis. Condron et al. (1994) found histological changes only in 80.7% of 62 cows showing clinical signs.

Staining of mucus in ileal goblet cells from cows with paratuberculosis revealed significant differences in mucopolysaccharide composition of the mucus. The variability included a decrease of goblet cells with SM in infected animals without clinical signs and their complete absence in infected animals with clinical signs. Nonspecific changes in the mucopolysaccharide composition of intestinal mucus has also been observed in patients with inflammatory bowel disease. The number of goblet cells with sialomucins and sulfomucins decreases in such cases, whereas the number of goblet cells with neutral mucins and glycoproteins increased (Gad, 1969). However, in patients with colon tumor the content of sialomucins is increased (Bara et al., 1995). Also in rats affected with tumors of the colon the quantity of sialomucins increased after application of dimethylhydrazine (Schmidt-Wittig et al., 1994).

Study of the changes in mucopolysaccharide composition of mucus in goblet cells of ileum under paratuberculosis conditions is important, because in subclinical paratuberculosis the changes occur prior to histologically detected alteration of intestinal morphology.

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REFERENCES

Bara J., Decaens C., Forgue-Lafitte M. E., Gespach C. (1995): Changes in mucin expression as early markers of colonic carcinogenesis: a survey of human and animal models. Israel J. Vet. Med., *50*, 117–122.

Buergelt C. D., Hall C., McEntee K., Duncan J. R. (1978): Pathological evaluation of paratuberculosis in naturally infected cattle. Vet. Pathol., *15*, 196–207.

Carvalho A. D. V. de, Magalhães M. J., Ribeiro J. E. (1988): Histoquímica de mucinas das glândulas e células caliciformes duodenais do carneiro. Arq. Bras. Med. Vet. Zoot., *40*, 369–375.

Chiodini R. J., Kruiningen H. J. van, Merkal R. S. (1984): Ruminant paratuberculosis (Johne's disease) the current status and future prospects. Cornell Vet., *74*, 218–262.

Chiodini R. J. (1989): Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clin. Microbiol. Rev., *2*, 90–117.

Clarke C. J., Little D. (1996): The pathology of ovine paratuberculosis: gross and histological changes in the intestine and other tissues. J. Comp. Pathol., *114*, 419–437.

Condron R. J., Schroen C. J., Black C. A., Ridge S. E., Hope A. F. (1994): Histological confirmation of subclinical infection with *M. paratuberculosis* in cattle. In: Proc. 4th Int. Coll. Paratuberculosis, ISBN 0-9633043-2-1, July 17–21, Cambridge, U. K., 37–40.

Corfield A. P., Williams A. J. K., Clamp J. R., Wagner S. A., Mountford R. A. (1988): Degradation by bacterial enzymes of colonic mucus from normal subjects and patients with inflammatory bowel disease: the role of sialic acid metabolism and the detection of a novel O-acetylsialic acid esterase. Clin. Sci., *74*, 71–78.

Dufek J., Stávková J. (1985): Biometrics. 2nd ed. Brno, Vysoká škola zemědělská. 228 s. (In Czech).

Gad A. (1969): A histochemical study of human alimentary tract mucosubstances in health and disease. II. Inflammatory conditions. Brit. J. Cancer, *23*, 64–68.

Groch L., Pivník L. (1989): From the album of pathological morphology: paratuberculosis (In Czech). Veterinářství, *39*.

Hines S. A., Buergelt C. D., Wilson J. H., Bliss E. L. (1987): Disseminated *Mycobacterium paratuberculosis* infection in a cow. J. Am. Vet. Med. Assoc., *190*, 681–683.

Hopwood D., Coghill G., Sanders D. S. A. (1986): Human oesophageal submucosal glands. Their detection of mucin, enzyme and secretory protein content. Histochemistry, *86*, 107–112.

Johnson W. C., Helwig E. B. (1963): Histochemistry of the acid mucopolysaccharides of skin in normal and certain pathologic conditions. Am. J. Clin. Pathol., *40*, 123–131.

Kahwa C. K. B., Purton M. (1996): Histological and histochemical study of epithelial lining of the respiratory tract in adult goats. Small Ruminant Res., *20*, 181–186.

Kunze Z. M., Portaels F., McFadden J. J. (1992): Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence *IS901*. J. Clin. Microbiol., *30*, 2366–2372.

Lev R., Spicer S. S. (1964): Specific staining of sulphate groups with alcian blue at low pH. J. Histochem. Cytochem., *12*, 309.

Massone, A. R., Itadaki S. I., Doi K., Gimeno E. J. (1991): Lectin histochemical study on normal and paratuberculosis-affected bovine ileum. J. Vet. Med. Sci., *53*, 761–763.

Matoušková O., Chalupa J., Cígler M., Hruška K. (1992): Manual STAT Plus. 1st ed. Brno, Výzkumný ústav veterinárního lékařství. 168 s. (In Czech).

Merkal R. S., McCullough W. G. (1982): A new mycobactin, mycobactin J, from *Mycobacterium paratuberculosis*. Curr. Microbiol., *7*, 333–335.

Mowry R., Winkler C. H. (1965): The coloration of acidic carbohydrates of bacteria and fungi in tissue sections with special reference to capsules of *Cryptococcus neoformans*, pneumococci, and staphylococci. Am. J. Pathol., 32, 628–629.

Novotný E., Böhm R., Geissel V., Holman J. (1966): Veterinary Histology. 1st ed. Praha, Státní zemědělské nakladatelství. 637 s. (In Czech).

Pavlík I., Pavlas M., Bejčková L. (1994): Incidence, economic importance and diagnosis of paratuberculosis (in Czech). Vet. Med. – Czech, *39*, 451–496.

Schmidt-Wittig U., Enns M. L., Gärtner K. (1994): Veränderungen von Menge und Zusammensetzung isolierter Colonmuzine GF- und SPF-Ratten als Folge einer mechanischen Stimulation. Dtsch. Tierärztl. Wschr., *101*, 170–171.

Spicer S. S. (1960): A correlative study of the histochemical properties of rodent acid mucopolysaccharides. J. Histochem. Cytochem., *8*, 18–35.

Spicer S. S. (1965): Diamine methods for differentiating mucosubstances histochemically. J. Histochem. Cytochem., *13*, 211–234.

Steedman H. F. (1950): Alcian blue 8GS: a new stain for mucin. Q. J. Microsc. Sci., *91*, 477–479.

Vacek Z. (1972): Histology and Histological Methods. 3rd ed. Praha, Avicenum. 320 s. (In Czech).

Whipple D. L., Merkal R. S. (1985): Procedures for the field and laboratory processing of fecal specimens for the isolation of *Mycobacterium paratuberculosis*. In: Proc. 28th Annu. Meet. Am. Assoc. Vet. Lab. Diagnost., 239–245.

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LEUKOCYTE COUNTS AND LYMPHOCYTE SUBPOPULATIONS IN THE PERIPHERAL BLOOD OF PYGMY GOATS FROM HERD INFECTED WITH *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*[*]

POČTY LEUKOCYTŮ A SUBPOPULACE LYMFOCYTŮ V PERIFERNÍ KRVI KOZ KAMERUNSKÝCH ZE STÁDA INFIKOVANÉHO *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

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ABSTRACT: The aim of this paper was to study the total and differential leukocyte counts and lymphocyte subpopulations in the peripheral blood of pygmy goats. Lymphocyte subpopulations were enumerated by flow cytometry using monoclonal antibodies against surface antigens of bovine or ovine leukocytes, but not all of these were found to cross-react. Therefore, only CD2, 5, 8, 11a/18, 18, 14 and B-cells were calculated. Goats were divided into two groups according to age. Three of them were 4–6 months old and eight were adult (3–6 years). Difference in some parameters between age-related groups were found. The group of adult goats had highly statistically significant ($p < 0.01$) more neutrophils and less lymphocytes than the young ones. Adult goats had also statistically highly significant ($p < 0.01$) more T-lymphocytes and less B-lymphocytes. In addition, each goat in both groups was identified to be infected with *Mycobacterium avium* subsp. *paratuberculosis*, but without clinical signs of the disease. Results of both goats did not differ from the other animals in the group.

ruminants; age; flow cytometry; cross-reactivity; monoclonal antibody; Johne's disease

ABSTRAKT: Celkový počet leukocytů a diferenciální rozpočet a subpopulace lymfocytů v periferní krvi byly sledovány u jedenácti koz kamerunských. Subpopulace lymfocytů byly sledovány pomocí průtokové cytometrie za použití monoklonálních protilátek proti povrchovým antigenům leukocytů skotu nebo ovcí. Ne všechny použité protilátky křížově reagovaly s vyšetřovanými leukocyty. Proto byla sledována exprese antigenů CD2, 5, 8, 11a/18, 18, 14 a zastoupení B-lymfocytů. Kozy byly rozděleny podle věku na dvě skupiny: tři kozy ve věku čtyř až šesti měsíců a osm dospělých koz ve věku tří až šesti let. Byly nalezeny rozdíly ve sledovaných parametrech u skupin s rozdílným věkem. Skupina dospělých koz měla statisticky vysoce významně více ($p < 0,01$) neutrofilních granulocytů a méně lymfocytů. Dospělé kozy měly dále statisticky vysoce významně více ($p < 0,01$) T-lymfocytů a méně B-lymfocytů. Navíc u jedné kozy v každé skupině byla kultivačně zjištěna infekce *Mycobacterium avium* subsp. *paratuberculosis* bez klinických příznaků onemocnění. Obě kozy se ve sledovaných parametrech neodlišovaly od ostatních zvířat v příslušné skupině.

přežvýkavci; věk; průtoková cytometrie; křížová reaktivita; monoklonální protilátka; Johnova choroba

INTRODUCTION

Pygmy goats are ruminants sometimes used for different experiments and observations. At the beginning of 1999, according to the Medline-database, there had been more than 80 papers published since 1966. Pygmies and domestic goats have common phylogenetic ancestors but different place of domestication. There is little information about hematological parameters and no information about the possibility of lymphocyte subpopulations determination.

Determination of cross-reactivity of the defined monoclonal antibodies against leukocyte surface antigens is one of the possiblities to collect large numbers of antibodies for the identification of surface antigens of different animal species. A lot of papers about cross-reactivity in ruminants were published during the past years (Naessens et al., 1993; Navarro et al., 1996; Sopp and Howard, 1997).

The aim of our study was to determine the possibility of lymphocyte subsets enumeration and establish physiological leukocyte counts in order to investigate

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changes in these parameters during infection with *Mycobacterium avium* subsp. *paratuberculosis*. However, after the study infection of two goats with *M. a. paratuberculosis* was identified.

MATERIAL AND METHODS

Animals

Eleven clinically healthy pygmy goats (from one ZOO in the Czech Republic) of both sexes were studied. Three of them were 4–6 months old and eight were adults (3–6 years). One goat from the young group and one from the adult group were diagnosed to be shedder of *M. a. paratuberculosis*. Blood was collected from the jugular vein. No pharmaceutical tranquillizer was applied. Heparin (15 i.u./ml) was used as an anticoagulant. The samples were processed on the day of withdrawal.

Total and differential leukocyte counts

Total leukocyte counts were determined using the Digicell 500 cell counter (Contraves AG, Switzerland). Differential leukocyte counts were calculated from blood smears stained with May-Grünwald and Giemsa-Romanowski.

Lymphocyte subsets enumeration

Lymphocyte subsets were enumerated by flow cytometry using the indirect whole-blood-lysis technique as described previously (Faldyna and Toman, 1998). As primary were used monoclonal antibodies shown in Tab. I. As secondary antibody, fluorescein isothiocyanate-labelled swine anti-mouse immunoglobulin (Sevac, Prague, Czech Republic) was used. The enumeration was done using the flow cytometer FACSCalibur (Becton Dickinson, Mountain View, CA).

Microbiological methods

Fecal samples were processed as described by Whipple et al. (1991). Approx. 1 g of tissue was homogenized (Lab Blender, Stomacher), treated overnight with 0.75%HPC (hexadecylpyridium chloride), inoculum (0.2 ml) was applied onto three Herrold's egg yolk media with growth stimulant mycobactin J (own product according Merkal and McCullough, 1982) and incubated at 37 °C for 14–16 weeks. All the isolated strains of mycobacteria were identified by subculture in media containing mycobactin J and free of it (dependence test), by PCR using primers for the detection of *IS900* (Kunze et al., 1992), and DNA fingerprinting (Pavlik et al., 1999).

RESULTS AND DISCUSSION

Hematological parameters in the peripheral blood of eleven pygmy goats are shown in Tab. II. Goats were divided into two groups according to their age. Total and differential leukocyte counts were similar to the data known generally in goats (Jain, 1993). Age-related changes in differential counts (increase in neutrophils and decrease in lymphocytes) have been also described in pashima goats (Somvanshi et al., 1987).

Our results showed the possibility of lymphocyte subsets determination in the peripheral blood of pygmy goats. Lymphocyte subpopulations were enumerated by flow cytometry using monoclonal antibodies against surface antigens of bovine or ovine leukocytes, but not all of these were found to cross-react. Therefore, only CD2, 5, 8, 11a/18, 18, 14 and B-cells were calculated (Tab. III). Our results were in accordance with those of Davis and Ellis (1991). They used the same mAbs for CD2, CD4, CD5, and CD8 identification in the peripheral blood of goats and they received similar percentages of labeled lymphocytes. Navarro et al. (1996), who used different mAbs, in their experiment of four goats seven months

I. Monoclonal antibodies to leukocyte antigens used for the experiment

mAb	Specificity	Source	Ig isotype	Dilution	Cross-reactivity
CC42	CD2	Serotec	IgG1	1 : 200	Yes
MM1A	CD3	VMRD	IgG1	1 : 500	No
CC30	CD4	Serotec	IgG1	1 : 100	No
CC17	CD5	Serotec	IgG1	1 : 200	Yes
CC63	CD8	Serotec	IgG2a	1 : 100	Yes
BAT75A	CD11a/18	VMRD	IgG1	1 : 50	Yes
MM10A	CD11b	VMRD	IgG2a	1 : 100	No
BAQ153A	CD11c	VMRD	IgM	1 : 100	No
BAQ30A	CD18	VMRD	IgG1	1 : 100	Yes
CC1	CD45	Serotec	IgG1	1 : 200	No
VPM65	CD14	Serotec	IgG1	1 : 20	Yes
VPM30	B-cells	Serotec	IgM	1 : 20	Yes

II. Total and differential leukocyte counts in the peripheral blood of pygmy goats with different ages

Parameter	Young goats (n = 3)		Adult goats (n = 8)	
	%	10^9/l	%	10^9/l
Leukocytes		16.6 ± 3.0		14.5 ± 3.5
Lymphocytes	80.5 ± 3.2	13.4 ± 2.9	60.8 ± 14.0**	9.2 ± 3.9
Neutrophils	13.5 ± 3.7	2.2 ± 0.3	35.4 ± 13.4**	4.8 ± 1.2**
Monocytes	4.4 ± 1.3	0.7 ± 0.2	2.0 ± 1.0	0.3 ± 0.2
Eosinophils	1.6 ± 0.6	0.3 ± 0.1	2.0 ± 1.2	0.3 ± 0.2

** $p < 0.01$

III. Lymphocyte subpopulations in the peripheral blood of pygmy goats with different ages

Subpopulation	Young goats (n = 3)		Adult goats (n = 8)	
	%	10^9/l	%	10^9/l
CD2	46.8 ± 4.7	6.4 ± 2.1	67.9 ± 7.6**	6.3 ± 2.8
CD5	55.7 ± 3.7	7.4 ± 2.0	68.8 ± 7.1**	6.4 ± 2.9
CD8	17.0 ± 3.1	2.3 ± 0.9	27.1 ± 8.1*	2.5 ± 1.3
B-cells	35.0 ± 4.0	4.7 ± 0.9	15.3 ± 7.5**	1.3 ± 0.8**

** $p < 0.01$
* $p < 0.05$

old, obtained results which were analogous with the results of the adult goats in our study. Moreover, we detected higher number of B-cells. It could be caused by different mAbs for B-cells identification. Unlike Navarro et al. (1996), who used antibody against surface IgM, we used mAb VPM30. This antibody was described to be recognize antigen on B-cell surface (Naessens and Howard,1991), more recently was determined to be bind to antigen on the surface of activated T-cells as well (Campbell et al., 1998).

The mAbs against CD11a/18 and CD18 reacted with 98.9 ± 0.9 and 99.0 ± 0.5 percent of lymphocytes, respectively. Monocytes and granulocytes were also labelled by these antibodies. The mAb against CD14 reacted with 28.9 ± 5.0 percent of monocytes in the young goats and with 56.1 ± 12.3 in the adult goats.

Both, leukocyte counts and lymphocyte subsets of the two goats, which were identified to be shedder of *M. a. paratuberculosis* of the most common DNA type B-C1 were comparable with the parameters of the remaining goats (data not shown). These results were in accordance with our previous results, in which we observed changes during infection of cattle with *M. a. paratuberculosis* (Toman et al., 1999). Our observation showed that changes in both leukocyte counts and lymphocyte subsets did not occurr before the onset of clinical signs of the disease. Accordingly, we decided to include these two goats in our research.

The effect of age on lymphocyte subsets distribution was documented. Adult goats had statistically highly significant ($p < 0.01$) more T-lymphocytes and less B-lymphocytes than the young ones. The age-related changes in lymphocyte subsets are also known in other animal species (Joling et al., 1994; Sellon et al., 1996; Wilson et al., 1996; Greeley et al., 1996; Faldyna and Toman, 1998).

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REFERENCES

Campbell J. D. M., Hopkins J., Howie S. E. M., Oliver R. A., Nichani A. K., Goel P., Spooner R. L., Glass E. J. (1998): A novel cell surface proliferation-associated marker expressed on T-cells and up-regulated on germinal center B cells. J. Leukoc. Biol., *63*, 567–574.

Davis W. C., Ellis J. A. (1991): Individual antigens of goats. Vet. Immunol. Immunopathol., *27*, 121–131.

Faldyna M., Toman M. (1998): The effect of age on the distribution of lymphocyte and neutrophil granulocyte subsets in the peripheral blood of dog. Vet. Med. – Czech, *43*, 193–199.

Greeley E. H., Kealy R. D., Ballam J. M., Lawler D. F., Segre M. (1996): The influence of age on the canine immune system. Vet. Immunol. Immunopathol., *55*, 1–10.

Jain N. C. (1993): Comparative hematology of common domestic animals. In: Jain N. C. (ed.): Essentials of Veterinary Hematology. Philadelphia, USA, Lea & Febiger.

Joling P., Bianchi A. T. J., Kappe A. L., Zwart R. T. (1994): Distribution of lymphocyte subpopulations in thymus,

spleen, and peripheral blood of specific pathogen free pigs from 1 to 40 weeks of age. Vet. Immunol. Immunopathol., *40*, 105–117.

Kunze Z. M., Portales S. F., McFadden J. J. (1992): Biologically distinct subtypes of *Mycobacterium avium* differ in possession in insertion sequence *IS901*. J. Clin. Microbiol., *30*, 2366–2372.

Merkal R. S., McCullough W. G. (1982): A new mycobactin, mycobactin J, from *Mycobacterium paratuberculosis*. Curr. Microbiol., *7*, 333–335.

Naessens J., Olubayo R. O., Davis W. C., Hopkins J. (1993): Cross-reactivity of workshop antibodies with cells from domestic and wild ruminants. Vet. Immunol. Immunopathol., *39*, 283–290.

Naessens J., Howard C. J. (1991): Monoclonal antibodies reacting with bovine B cells (BoWC3, BoWC4 and BoWC5). Vet. Immunol. Immunopathol., *27*, 77–85.

Navarro J. A., Caro M. R., Seva J., Rosillo M. C., Gomez M. A., Gallego M. C. (1996): Study of lymphocyte subpopulations in peripheral blood and secondary lymphoid organs in the goat using monoclonal antibodies to surface markers of bovine lymphocytes. Vet. Immunol. Immunopathol., *51*, 147–156.

Pavlík I., Horváthová A., Dvorská L., Bartl J., Švastová P., du Maine R., Rychlík I. (1999): Standardization of restriction fragment length polymorphism analysis for *Mycobacterium avium* subsp. *paratuberculosis*. J. Microbiol., Meth., in press.

Sellon R. K., Levy J. K., Jordan H. L., Gebhard D. H., Tompkins M. B., Tompkins W. A. (1996): Changes in lymphocyte subsets with age in perinatal cats: late gestation through eight weeks. Vet. Immunol. Immunopathol., *53*, 105–113.

Somvanshi R., Biswas J. C., Sharma B., Koul G. L. (1987): Haematological studies on Indian pashmina goats. Res. Vet. Sci., *42*, 124–126.

Sopp P., Howard C. J. (1997): Cross-reactivity of monoclonal antibodies to defined human leucocyte differentiation antigens with bovine cells. Vet. Immunol. Immunopathol., *56*, 11–25.

Toman M., Pavlík I., Faldyna M., Mátlová L., Hořín P. (1999): Immunological characteristics of cattle with various forms of *Mycobacterium avium* subsp. *paratuberculosis* infection. Vet. Microbiol., submitted.

Whipple D. L., Callihan D. R., Jarnagin J. L. (1991): Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardised procedure. J. Vet. Diagn. Invest., *3*, 368–373.

Wilson R. A., Zolnai A., Rudas P., Frenyo L. V. (1996): T-cell subsets in blood and lymphoid tissues obtained from fetal calves, maturing calves, and adult bovine. Vet. Immunol. Immunopathol., *53*, 49–60.

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RHYTHMIC CHANGES OF MELATONIN IN THE CIRCULATION AND TISSUES OF BROILER CHICKENS*

RYTMICKÉ ZMENY MELATONÍNU V CIRKULÁCII A NIEKTORÝCH ORGÁNOCH BROJLEROVÝCH KURČIAT

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ABSTRACT: Besides the well known role of melatonin in regulation of daily rhythmicity in birds and seasonal cycles in photoperiodic mammals, pleiotropic effects of melatonin mediated by interactions with endocrine and immune systems or through specific receptors in peripheral tissues, were suggested. In the present study we measured melatonin levels in the pineal gland, plasma and tissues of 5–7-day-old broiler chicks in the middle of the light : dark cycle 12 : 12 and assessed whether melatonin concentrations exert a daily rhythm. Melatonin levels were measured by radioimmunoassay, directly in plasma and after solvent extraction with methanol in pineal glands and chloroform in the duodenum, jejunum, pancreas, spleen, kidneys and bursa of Fabricius. As expected, melatonin concentrations in the pineal gland and plasma exerted the distinct daily rhythm with high level during the dark period. Similarly, the clear daily melatonin rhythm was observed in the duodenum, jejunum, pancreas, spleen and bursa of Fabricius ($P < 0.001$, t-test). On the contrary, melatonin levels in the kidneys were low and did not exert rhythmic changes. Daytime melatonin levels in the spleen, kidneys and bursa of Fabricius were lower than melatonin levels in the gastrointestinal tract and pancreas (34.0–63.0 pg/g of wet tissue versus 116.3–179.3 pg/g of wet tissue, respectively). Our results demonstrated that rhythmic melatonin synthesis in the pineal gland results in rhythmic changes of melatonin concentrations in peripheral tissues. Since bright light suppresses melatonin biosynthesis, high light intensities should be avoided in broiler rearing because they can negatively influence physiological processes and efficiency of production.

pineal gland; duodenum; jejunum; pancreas; spleen; kidneys; bursa of Fabricius; rhythm

ABSTRAKT: Pleiotropným účinkom melatonínu sa venuje v posledných rokoch veľká pozornosť. Bolo dokázané, že melatonín môže zasahovať do fyziológie organizmu cez interakcie s endokrinným a imunitným systémom, ale pravdepodobne pôsobí aj priamo, prostredníctvom špecifických receptorov lokalizovaných v periférnych tkanivách. Preto sme sa zamerali na spôsob regulácie hladín melatonínu v niektorých tkanivách brojlerových kurčiat vo veku päť až sedem dní chovaných na svetelnom režime svetlo : tma 12L : 12D a zisťovali sme, či obsah melatonínu vykazuje rytmické zmeny. Hladiny melatonínu v epifýze, plazme, dvanástniku, lačníku, pankrease, slezine, obličkách a Fabriciovej burze sme merali rádioimunoanalyticky, v plazme priamo, v epifýze po extrakcii metanolom a v ostatných tkanivách po extrakcii chloroformom. Obsah melatonínu v epifýze a plazme vykazoval v súlade s očakávaním výrazný denný rytmus. Podobne sme zaznamenali signifikantné rozdiely aj v dvanástniku, lačníku, pankrease, slezine a Fabriciovej burze, zatiaľ čo hladiny v obličkách boli nízke a nevykazovali rytmické zmeny. Uvedené výsledky naznačujú, že v podmienkach svetelného režimu, ktorý nepotláča endogénnu produkciu melatonínu, môže tento indolamín v periférnych tkanivách prispievať k synchronizácii fyziologických dejov, alebo modulovať činnosť uvedených tkanív.

epifýza; dvanástnik; tenké črevo; pankreas; slezina; obličky; Fabriciova burza; rytmus

ÚVOD

Melatonín je dominantný epifyzárny hormón, ktorého syntéza prebieha rytmicky a odráža sa na rytme hladín melatonínu v plazme. Rytmus melatonínu participuje na regulácii denných rytmov u vtákov a sezónnych rytmov u fotoperiodických cicavcov, pričom tieto účinky sú sprostredkované melatonínovými receptormi v hypotalame a hypofýze (Vaněček a i., 1987; Rivkees a i., 1989). Na druhej strane je málo známe o účinkoch

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melatonínu na úrovni periférnych tkanív, hoci pleiotropným účinkom melatonínu sa venuje v posledných rokoch veľká pozornosť.

Melatonín ako lipofilná molekula má vysokú penetračnú schopnosť a keďže jeho hladiny v plazme vykazujú denný rytmus, je pravdepodobné, že sa tieto zmeny prenášajú aj na hladiny melatonínu v tkanivách, kde rytmus hladín melatonínu môže prispievať k synchronizácii fyziologických procesov. Navyše bolo dokázané, že melatonín môže zasahovať do fyziológie organizmu cez interakcie s endokrinným (Binkley, 1988; Zeman a i., 1993) a imunitným (Maestroni, 1993) systémom a aj keď úroveň interakcií nie je známa, je možné, že participuje aj na činnosti iných systémov, resp. tkanív.

Keďže primárnym predpokladom pre fyziologické účinky je prítomnosť aktívnej látky, zamerali sme sa najmä na spôsob regulácie hladín melatonínu v niektorých tkanivách a zisťovali sme, či obsah melatonínu vykazuje rytmické zmeny.

MATERIÁL A METÓDY

V našej práci sme rádioimunologicky stanovovali melatonín v plazme, epifýze a tkanivách päť- až sedemdňových kurčiat brojlerovej línie hybrida Hybro. Kurčatá, ktoré boli dodané ako jedno- až dvojdňové (Unigalex Bratislava, liaheň Častá) sa odchovávali vo zverinci so svetelným režimom 12L : 12D (svetlo od 24.00 do 12.00) v skupinových klietkach 650 x 500 x 250 mm, po 15 až 20 zvierat s voľným prístupom k vode a potrave (kompletná kŕmna zmes pre brojlerové kurčatá BR 1). Svetlo malo intenzitu 100 až 200 luxov. Teplota vo zverinci bola počas prvých piatich dní udržiavaná na úrovni 35 °C a do konca prvého týždňa na 33 °C. Vzorky krvi, proximálnej časti dvanástnika, lačníka, pankreasu, obličiek, sleziny a Fabriciovej burzy, ako aj epifýzy sme odoberali v strede svetlej a tmavej fázy svetelného režimu.

Krv bola odobraná hneď po dekapitácii do heparinizovaných skúmaviek a po scentrifugovaní (1 500 G, 10 min) boli vzorky plazmy uložené pri teplote –18 °C až do stanovenia koncentrácie melatonínu.

Hneď po dekapitácii sme extirpovali epifýzy, ktoré sme skladovali v mikroskúmavkách s uzáverom pri teplote –18 °C až do ďalšieho spracovania. Epifýzy sme zhomogenizovali v mikrohomogenizátoroch v 3 x 0,1 ml metanolu. Po odparení metanolu sme vzorky rekonštituovali v 0,3 ml tricínového pufru s 0,1% azidom sodným a želatínou a uskladnili do stanovenia pri teplote –18 °C.

Vzorky gastrointestinálneho traktu (asi 400 mg) sme odoberali čo najrýchlejšie po dekapitácii, pričom odber trval v priemere tri až osem minút. Vzorky čriev sme po rozstrihnutí očistili od tráveniny, uložili do mikroskúmaviek s uzáverom a skladovali pri teplote –18 °C.

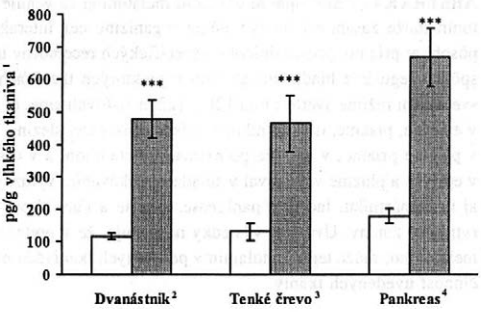
Extrakcia melatonínu z tkanív bola modifikovaná na podmienky nášho laboratória (Herichová a i., 1998). Množstvo melatonínu v plazme, epifýze a tkanivách gastrointestinálneho traktu sme merali rádioimunologickou metódou (RIA) (Fraser a i., 1983) modifikovanou na podmienky nášho laboratória (Zeman a i., 1992). Použili sme rádioindikátor ^{3}H-melatonin (acetyl-5-methoxy-tryptamine-N-[2-aminoethyl-2-3H]; výrobca: Du Pont, NEN, USA; špecifická aktivita: 0,925 až 1,85 TBq/mmol) a melatonínové antisérum pripravené proti melatonínu konjugovanému na hovädzí tyreoglobulín (výrobca: Stockgrand Ltd., University of Surrey, Veľká Británia) riedenej v pomere 1 : 2 000. Celkovú koncentráciu melatonínu vo vzorkách gastrointestinálneho traktu sme vypočítali pre každú skúmavku osobitne berúc do úvahy individuálnu účinnosť extrakcie. Účinnosť bola meraná pomocou interného štandardu (približne 2 500 dpm ^{3}H-melatonínu na skúmavku) a predstavovala v priemere 80 %.

Významnosť zistených rozdielov sa posudzovala nepárovým *t*-testom.

VÝSLEDKY

Obsah melatonínu zistený v epifýze bol v noci (7 720,9 ± 994,7 pg na epifýzu) viac ako 20-násobne vyššií v porovnaní s hodnotami zistenými cez deň (374,6 ± 69,2 pg na epifýzu). Podobne aj koncentrácie melatonínu zistené v plazme vykazovali zreteľný denný rytmus, t.j. 347,9 ± 36,3 pg/ml v noci a 29,0 ± 3,77 pg/ml cez deň.

Ako vyplýva z výsledkov znázornených na obr. 1 a 2, aj vo väčšine študovaných tkanív sme zaznamenali rozdiely medzi koncentráciami melatonínu zistenými vo svetlej a tmavej časti fotoperiódy. V tmavej fáze fotoperiódy sme zaznamenali signifikantne vyššie hladiny melatonínu ako vo svetlej fáze ($P < 0,001$) vo



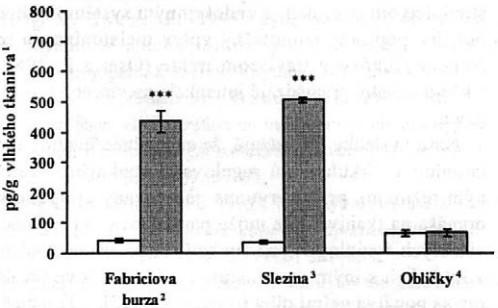
1. Hladiny melatonínu v gastrontestinálnom trakte a pankrease brojlerových kurčiat. Každý stĺpec predstavuje aritmetický priemer ± SEM. Biele stĺpce predstavujú hodnoty zistené v strede svetlej fázy fotoperiódy, tmavé stĺpce indikujú odbery v strede tmavej fázy fotoperiódy. *t*-test: *** $P < 0,001$ – Melatonin levels in gastrointestinal tract and pancreas of broiler chickens. Each column represents arithmetic mean ± SEM. White columns are the values found in the middle of the light phase of photoperiod, dark columns indicate samplings in the middle of dark phase of photoperiod. *t*-test: *** $P < 0.001$

[1]pg/g of wet tissue, [2]duodenum, [3]jejunum, [4]pancreas

všetkých sledovaných častiach tráviaceho traktu a pankrease (obr. 1). Podobne aj koncentrácie melatonínu v slezine a Fabriciovej burze boli preukazne vyššie počas tmavej fázy fotoperiódy ($P < 0,001$; obr. 2). Na rozdiel od týchto tkanív, hladiny v obličkách boli nízke a zistené hladiny melatonínu rytmické zmeny nevykazovali (obr. 2).

Okrem rozdielov medzi koncentráciami melatonínu, zistenými v strede svetlej a tmavej fázy fotoperiódy, zaznamenali sme aj rozdiely v rámci jednotlivých svetelných fáz, ktoré udáva tab. I. Zatiaľ čo sa hladiny melatonínu v tráviacom trakte a pankrease vo svetlej časti fotoperiódy pohybovali v rozmedzí od 116,3 do 179,3 pg/g tkaniva, koncentrácie melatonínu vo Fabriciovej burze, slezine a obličkách boli podstatne nižšie (od 34,0 do 63,0 pg/g tkaniva). Tieto rozdiely boli potvrdené aj analýzou variancie (tab. I), a to aj napriek malému počtu vzoriek v prípade sleziny, keďže kvôli malej hmotnosti tkaniva jedna vzorka pozostávala z troch slezín.

V tmavej časti fotoperiódy boli hladiny melatonínu vo všetkých tkanivách podobné, s výnimkou koncentrácie zistenej v obličkách, resp. analýza variancie neodhalila signifikantné rozdiely. Numericky najvyššie koncentrácie počas svetlej a tmavej fázy dňa boli zistené v pankrease.



2. Hladiny melatonínu vo Fabriciovej burze, slezine a obličkách brojlerových kurčiat. Každý stĺpec predstavuje aritmetický priemer ± SEM. Biele stĺpce predstavujú hodnoty zistené v strede svetlej fázy fotoperiódy, tmavé stĺpce indikujú odbery v strede tmavej fázy fotoperiódy. *t*-test: *** $P < 0,001$ – Melatonin levels in bursa of Fabricius, spleen and kidneys of broiler chickens. Each column represents arithmetic mean ± SEM. White columns are the values found in the middle of the light phase of photoperiod, dark columns indicate samplings in the middle of dark phase of photoperiod. *t*-test: *** $P < 0.001$

[1]pg/g of wet tissue, [2]bursa of Fabricius, [3]spleen, [4]kidneys

DISKUSIA

Z našich výsledkov vyplýva, že melatonín je prítomný tak v tkanivách tráviceho traktu, ako aj v pankrease, obličkách, slezine a Fabriciovej burze, pričom s výnimkou obličiek jeho hladiny vykazujú zreteľné zmeny počas dňa. Denný rytmus hladín melatonínu bol dokázaný aj v dvanástniku holuba (Vakkuri a i., 1985), tráviacom trakte zebričky (Vant Hof a Gwinner, 1996) a brojlerových kurčiat (Herichová a i., 1998), pričom rozsah zistených koncentrácií publikovaný inými autormi je zhodný s našim. Naznačuje to, že melatonín môže v periférnych tkanivách prispievať k synchronizácii fyziologických funkcií, čomu nasvedčuje aj prítomnosť väzobných miest 2-[^{125}I]jódomelatonínu v tráviacom trakte kurčiat (Pontoire a i., 1993), kačíc (Lee a i., 1995), holubov a prepelíc japonských (Poon a i., 1997) ako aj v obličkách (Song a i., 1993), slezine (Yu a i., 1991) a Fabriciovej burze (Poon a Pang, 1994).

Je dobre známe, že galiformné vtáky chované na svetelnom režime prijímajú potravu takmer výlučne počas svetlej fázy dňa (fotofáza) (Savory, 1980). Napriek tomu dokážu energeticky pokryť aj obdobie tmavej fázy svetelného režimu (skotofáza), kedy potravu nekonzumujú. Bolo dokázané, že príjem potravy u brojlerových kurčiat chovaných na svetelnom režime vykazuje počas 24 h cyklu dva vrcholy, pričom jeden je spojený s koncom a druhý so začiatkom fotofázy (Buyse a i., 1993).

I. Hladiny melatonínu v gastrointestinálnom trakte, pankrease, Fabriciovej burze, slezine a obličkách. Výsledky sú udávané ako aritmetický priemer ± SEM (pg/g vlhkého tkaniva) – Melatonin levels in gastrointestinal tract, bursa of Fabricius, spleen and kidneys of broiler chickens. Each column represents arithmetic mean ± SEM (pg/g of wet tissue)

	Dvanástnik[1]	Tenké črevo[2]	Pankreas[3]	Fabriciova burza[4]	Slezina[5]	Obličky[6]
L	$116,31^{ac}$ ± 8,84 ($n = 6$)	$131,12^{ad}$ ± 26,70 ($n = 6$)	$179,31^{a}$ ± 23,04 ($n = 6$)	$40,55^{b}$ ± 5,32 ($n = 9$)	$34,03^{bc}$ ± 5,09 ($n = 3$)*	$63,06^{bcd}$ ± 10,05 ($n = 6$)
D	$479,10^{a}$ ± 58,42 ($n = 6$)	$464,75^{a}$ ± 87,60 ($n = 6$)	$667,26^{a}$ ± 89,32 ($n = 6$)	$433,77^{a}$ ± 37,04 ($n = 5$)	$505,66^{a}$ ± 8,02 ($n = 2$)*	$66,31^{b}$ ± 11,37 ($n = 6$)

Hodnoty s nerovnakým abecedným označením sú signifikantne odlišné (ANOVA; $P < 0,05$) – Levels with different alphabetic denotation are significantly different (ANOVA; $P < 0.05$)

* kvôli malej hmotnosti tkaniva, jedna vzorka obsahovala tri sleziny – due to low weight of tissue, one sample contained three spleens

L = odber počas svetlej fázy fotoperiódy – sampling during light phase of photoperiod

D = odber počas tmavej fázy fotoperiódy – sampling during dark phase of photoperiod

[1]duodenum, [2]jejunum, [3]pancreas, [4]bursa of Fabricius, [5]spleen, [6]kidneys

Prvý vrchol v konzumácii slúži na vytvorenie si zásoby krmiva, ktoré sa potom pomaly uvoľňuje a trávi po celú noc. Počas skotofázy dochádza k predĺženiu času potrebnému na prechod tráveniny tráviacim traktom. Raňajší vrchol príjmu potravy je pravdepodobne dôsledkom postabsorbčného stavu na konci noci. Tento cyklus v príjme potravy sa odráža aj na objeme tráveniny v jednotlivých častiach tráviaceho traktu. Najvyšší objem krmiva v hrvoli a proventrikule sa nachádza na začiatku tmavej fázy fotoperiódy. V tomto období je najvyšší objem krmiva aj v hrubom čreve, pričom v uvedenom segmente GIT sa nachádza trávenina najmä z ranného vrcholu príjmu potravy. Najnižší objem krmiva bol vo všetkých študovaných častiach tráviaceho traktu na konci tmavej fázy svetelného režimu (Buyse a i., 1993).

Úloha svetelného režimu pri regulácii príjmu potravy sa potvrdila aj v pokuse, v ktorom sa sledovali viaceré fyziologické parametre prepelice japonskej pri troch odlišných svetelných režimoch (Wilson a McFarland, 1969). Autori zistili, že hmotnosť pečene je najvyššia na konci fotofázy pri svetelnom režime 6L : 6D, 12L : 12D ako aj 16L : 8D. Zmeny v obsahu lipidov v pečeni boli paralelné so zmenami hmotnosti pečene a objem tráveniny v jednotlivých častiach tráviaceho traktu tiež úzko koreloval s daným svetelným režimom.

Je teda zrejmé, že svetelný režim môže u galiformných vtákov ovplyvniť príjem potravy, pričom predĺženie svetlej fázy svetelného režimu má za následok zvýšenie príjmu a skrátenie zase pokles príjmu krmiva. Je tiež dobre známe, že svetelný režim reguluje aj výdaj melatonínu epifýzou. Melatonín sa podieľa na synchronizácii denných rytmov pohybovej aktivity vtákov (Gwinner a Benzinger, 1978; Chabot a Menaker, 1992; Heigl a Gwinner, 1995) a je možné, že participuje aj na synchronizácii prijímania potravy s určitým svetelným režimom.

Z doteraz známych účinkov melatonínu na aktivitu GIT možno spomenúť vplyv melatonínu na črevnú peristaltiku, aj keď v týchto štúdiách boli ako experimentálny model použité cicavce. Bolo dokázané, že melatonín má vplyv na spontánnu aj serotonínom indukovanú peristaltiku črevného aparátu potkana (Harlow a Weekley, 1986; Bubenik, 1986) a rýchlosť prechodu tráveniny tráviacim traktom laboratórnych myší (Bubenik a Dhanvantari, 1989). Hoci uvedené štúdie popisujú predovšetkým účinky exogénne aplikovaného melatonínu, predpokladá sa, že aj endogénny melatonín môže pôsobiť podobne, a to predovšetkým formou parakrinnej regulácie. Zdroj melatonínu v GIT síce nebol definitívne identifikovaný, ale viacerí autori predpokladajú, že melatonín v tráviacom trakte je tvorený *in situ* (Raiklin a Kvetnoy, 1976; Bubenik a i., 1977; Huether, 1994), konkrétne v enterochromafinných bunkách mukózy GIT, v ktorých prebieha syntéza melatonínového prekurzoru serotonínu (Espamer a Asero, 1952). V tráviacom trakte sa taktiež nachádza N-acetyltransferáza (Hong a Pang, 1995), ktorá konvertuje serotonín na N-acetylserotonín a hydroxyindol-O-methyltransferáza (Quay a Ma, 1976), ktorá metyluje N-acetylserotonín na melatonín, čo dokazuje, že v GIT sa nachádza substrát aj enzýmová výbava na produkciu melatonínu.

Okrem priamych účinkov melatonínu na funkcie GIT je popísaný aj vplyv melatonínu na energetický metabolizmus. Bolo dokázané, že u kurčiat epifyzektómia (t.j. odstránenie hlavného zdroja melatonínu v organizme) alebo vystavenie zvierat ostrému svetlu, ktoré potláča syntézu melatonínu v epifýze, má za následok pomalší rast a pokles hladiny T_3 oproti kurčatám chovaným v tme (Osol a i., 1980), zatiaľ čo aplikácia melatonínu takéto účinky epifyzektómie alebo ostrého svetla eliminovala (Osei a i., 1989). Aplikácia melatonínu prepeliciam japonským v pitnej vode mala za následok vzostup hladiny T_3, pokles plazmatickej koncentrácie kortikosterónu a zníženie obsahu abdominálneho tuku oproti kontrolným zvieratám chovaným v podmienkach konštantného svetla (Zeman a i., 1993). Potvrdzuje to úlohu melatonínu pri konverzii krmiva pravdepodobne prostredníctvom interakcií s endokrinným systémom, hoci bol tiež popísaný stimulačný vplyv melatonínu na resorpciu glukózy v tráviacom trakte (Osei a i., 1989), takže do úvahy prichádzajú interakcie na viacerých úrovniach.

Naše výsledky potvrdzujú, že endogénne hladiny melatonínu v cirkulácii sú regulované vonkajším svetelným režimom, pričom rytmus generovaný epifýzou sa prenáša na tkanivá, kde môže participovať na regulácii viacerých fyziologických funkcií, či už priamo, alebo v interakcii s inými systémami. Keďže v chove brojlerov sa používa veľmi dlhá fotoperióda (23L : 1D) alebo konštantné svetlo, je pravdepodobné, že pri použití vyššej intenzity osvetlenia dochádza k potlačeniu syntézy melatonínu, čo môže negatívne ovplyvňovať viaceré fyziologické procesy. Je zaujímavé, že najnižšie hladiny za svetla sme zistili v orgánoch spojených s imunitným systémom (Fabriciova burza, slezina), čo naznačuje možnú inhibíciu imunitného systému intenzívnym osvetlením. Je potrebné získať ďalšie údaje o vzťahu medzi intenzitou osvetlenia a produkciou melatonínu u brojlerových kurčiat a regulovať intenzitu osvetlenia tak, aby negatívne neovplyvňovala efektívnosť produkcie.

LITERATÚRA

Binkley S. (1988): The Pineal: Endocrine and Neuroendocrine Function. New York, Prentice Hall, Engewood Cls. 1–288.

Bubenik G. A. (1986): The effect of serotonin, N-acetylserotonin and melatonin on spontaneous contractions of isolated rat intestine. J. Pineal Res., *3*, 41–54.

Bubenik G. A., Dhanvantari S. (1989): Influence of serotonin and melatonin on some parameters of gastrointestinal activity. J. Pineal Res., *7*, 333–344.

Bubenik G. A., Brown G. M., Grota L. J. (1977): Immunohistological localization of melatonin in the rat digestive system. Experientia., *33*, 662–663.

Buyse J., Adelson D. S., Decuypere E., Scanes C. G. (1993): Diurnal-nocturnal changes in food intake, gut storage of

ingesta, food transit time and metabolism in growing broiler chickens: a model for temporal control of energy balance. Br. Poultry Sci., *34*, 699–709.

Chabot C. C., Menaker M. (1992): Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons. J. Comp. Physiol. A, *170*, 615–622.

Espamer V., Asero B. (1952): Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature, *169*, 800–801.

Fraser S., Cowen P., Franklin M., Franey C., Arendt J. (1983): Direct radioimmunoassay for melatonin in plasma. Clin. Chem., *29*, 396–397.

Gwinner E., Benzinger I. (1978): Synchronization of a circadian rhythm in pinealectomized European starlings by daily injections of melatonin. J. Comp. Physiol., *127*, 209–213.

Harlow H. J., Weekley B. L. (1986): Effect of melatonin on the force of spontaneous contractions of in vitro rat small and large intestine. J. Pineal Res., *3*, 277–284.

Heigl S., Gwinner E. (1995): Synchronization of circadian rhythms of house sparrows by oral melatonin: effects of changing period. J. Biol. Rhythm., *10*, 225–233.

Herichová I., Zeman M., Veselovský J. (1998): Effect of tryptophan administration on melatonin levels in the pineal gland, plasma and gastrointestinal tract of broiler chicks. Acta Vet. (Brno), *67*, 89–95.

Hong G. X., Pang S. F. (1995): N-acetyltransferase activity in the quail (*Coturnix coturnix japonica*) duodenum. Comp. Biochem. Physiol., *112B*, 251–255.

Huether G. (1994): Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin. Ann. NY Acad. Sci., *719*, 146–158.

Lee P. N., Shiu S. Y. W., Chow P. H., Pang S. F. (1995): Regional and diurnal studies of melatonin and melatonin binding sites in the duck gastrointestinal tract. Biol. Signals., *4*, 212–224.

Maestroni G. J. M. (1993): The immunoneuroendocrine role of melatonin (Mini-review). J. Pineal Res., *14*, 1–10.

Osei P., Robbins K. R., Shirley V. (1989): Effects of exogenous melatonin on growth and energy metabolism of chickens. Nutr. Res., *9*, 69–81.

Osol J. G., Foss D. C., Carew Jr. L. B. (1980): Effects of light environment and pinealectomy on growth and thyroid function in the broiler cockerel. Poultry Sci., *59*, 647–653.

Pontoire C., Bernard M., Silvian C., Collin J. P., Voisin P. (1993): Characterization of melatonin binding sites in chicken and human intestines. Eur. J. Pharmacol., *247*, 111–118.

Poon A. M., Pang S. F. (1994): Different effects of quanosine 5-O-(3-thiotriphosphate) (GTP gamma S) on 2-[^{125}I]iodomelatonin binding sites in the chicken bursa of Fabricius and spleen. Neurosci Lett., *173*, 167–171.

Poon A. M. S., Chow P. H., Mak A. S. Y., Pang S. F. (1997): Autoradiographic localization of 2[^{125}I]iodomelatonin binding sites in the gastrointestinal tract of mammals including humans and birds. J. Pineal Res., *23*, 5–14.

Quay W. B., Ma Y. H. (1976): Demonstration of gastrointestinal hydroxyindole-O-methyltransferase. Int. Rep. Clin. Sci., Med. Sci. Lib. Compend., *4*, 563.

Raiklin N. T., Kvetnoy I. M. (1976): Melatonin and enterochromaffin cells. Acta Histochem., *55*, 19–24.

Rivkees S. A., Cassone V. M., Weaver D. R., Reppert S. M. (1989): Melatonin receptors in chick brain: characterization and localization. Endocrinology, *125*, 363–368.

Savory C. J. (1980): Diurnal feeding patterns in domestic fowl: a review. Appl. Anim. Ethol., *6*, 71–82.

Song Y., Ayre E. A., Pang S. F. (1993): [^{125}I]iodomelatonin binding sites in mammalian and avian kidneys. Biol. Signals, *2*, 207–220.

Vakkuri O., Rintamaki H., Leppaluoto J. (1985): Presence of immunoreactive melatonin in different tissues of the pigeon (*Columbia livia*). Gen. Comp. Endocrinol., *58*, 69–75.

Vant Hof T. J., Gwinner E. (1996): Cicadian variation in Zebra finch (*Poephila guttata*) gastrointestinal tract melatonin. Ital. J. Anat. Embryol., *101*, 78.

Vaněček J., Pavlík A., Illnerová H. (1987): Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res., *435*, 359–362.

Wilson W. O., McFarland L. Z. (1969): Diurnal changes in livers and digestive systems of Coturnix as related to three photoperiodic regimes. Poultry Sci., *48*, 447–482.

Yu Z. H., Yuan H., Lu Y., Pang S. F. (1991): [^{125}I]iodomelatonin binding sites in spleens of birds and mammals. Neurosci. Lett., *125*, 175–179.

Zeman M., Gwinner E., Somogyiová E. (1992): Development of melatonin rhythm in the pineal gland and eyes of chick embryo. Experientia, *48*, 765–768.

Zeman M., Výboh P., Juráni M., Lamošová D., Košťál Ľ., Bilčík B., Blažíček P., Jurániová E. (1993): Effects of exogenous melatonin on some endocrine, behavioural and metabolic parameters in Japanese quail (*Coturnix coturnix japonica*). Comp. Biochem. Physiol., *105A*, 323–328.

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ÚSTŘEDNÍ ZEMĚDĚLSKÁ A LESNICKÁ KNIHOVNA, PRAHA 2, SLEZSKÁ 7

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METHODS OF LAPAROSCOPIC STERILIZATION OF BITCHES

METÓDY LAPAROSKOPICKEJ STERILIZÁCIE U SÚK

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ABSTRACT: At present the question of sterilisation of bitches with the aim of inducing permanent sterility is paid much attention. So far preferred techniques have required surgical intervention *per laparoscopiam*. In this submitted study we focused on using laparoscopy at sterilisation of bitches in three ways: by creating mechanical occlusion of oviducts using endo-stapler technique (n = 3), by forming oviduct occlusion by electrocoagulation with the aid of endo-coagulator (n = 3), by performing ovariectomy using modified endo-suture technique (n = 2). At the interventions effectiveness, complications and difficulty were observed. The effect of laparoscopic intervention on reproductive apparatus and condition of the patient was observed as well. Patients (n = 8) of German shepherd breed aged 2–7 years were included in the evaluation. Interventions were performed with the aid of two MLW Germany laparoscopic units with 180 degree angle and 0.5–1 cm working diameter. Electrocoagulation was carried out with Eltom, Chirana Slovakia, equipment. Occlusions of oviducts were performed with endo-stapler titanium clip applicator. Ovariectomy was carried out with the aid of extracorporal Orsilon technique with non-traumatic units. We have found that it is possible to make a permanent occlusion of oviducts by placing one or two clips on the left or right oviduct concerned. The intervention lasted 20–30 minutes. By using electro-coagulation of oviduct 1–2 occlusions were made on each oviduct. The occlusion of oviducts by endo-coagulation took 10 minutes. At ovariectomy two ligatures were applied cranially to the ovary and a single ligature caudally from the same ovary. The ovary was removed with endoscissors. The whole endoprocedure in both ovaries took 70 minutes. In comparison with similar laparoscopic interventions the above described techniques have certain advantages. The result of abdominal cavity perforation is just small wounds which require 2 deep and 2 superficial sutures at the most. As the interventions are relatively simple, they can be performed on older patients as well. Small laparoscopic incisions contribute to a considerable decrease of postoperative pain in surgical wound.

female dog; laparoscopy; sterilisation; endoscopic surgical technique; ovary; oviduct

ABSTRAKT: V súčasnosti sa dostáva do popredia otázka sterilizovania súk s cieľom navodenia trvalej sterility. Doposiaľ uprednostňované techniky si vyžadovali chirurgickú intervenciu *per laparoscomiam*. V predloženej práci sme sa zamerali na použitie laparoskopie pri sterilizácii súk tromi spôsobmi: vytvorením oklúzie vajcovodov mechanicky použitím endo-stapler techniky (n = 3), vytvorením oklúzie vajcovodov elektrokoaguláciou použitím endo-koagulátora (n = 3), vykonaním ovariektómie použitím modifikovanej endo-suture techniky (n = 2). Počas zákrokov sme sledovali účinnosť, komplikácie a náročnosť laparoskopických výkonov. Tiež sme sledovali vplyv laparoskopickej intervencie na reprodukčný aparát a zdravotný stav pacienta. Do vyhodnotenia boli zaradení pacienti (n = 8) plemena nemecký ovčiak vo veku dva až sedem rokov. Zákroky sme vykonávali pomocou dvoch laparoskopických jednotiek MLW Germany so 180° uhlom s pracovným priemerom od 0,5 do 1 cm s príslušenstvom. Elektrokoagulácia bola vykonávaná pomocou prístroja Eltom (Chirana, Slovakia). Oklúzie vajcovodov boli uskutočnené endostaplerom, aplikátorom titánových klipov. Ovariektómia bola vykonaná extrakorporálnou technikou použitím Orsilonových atraumatických návlekov. Zistili sme, že je možné vytvoriť trvalú oklúziu vajcovodov naložením jedného alebo dvoch klipov na predmetný vajcovod. Trvanie samotného výkonu predstavovalo 20 až 30 minút. Pri použití elektrokoagulácie vajcovodov boli vykonané jedna až dve oklúzie na každom vajcovode. V priemere oklúzie vajcovodov endokoaguláciou trvali 10 minút. Pri ovariektómii boli naložené dve ligatúry kraniálne a jedna kaudálne od vaječníka. Ováriá boli odňaté endo-nožnicami. Celkovo zákrok trval 70 minút. V porovnaní s obdobnými laparotomickými zákrokmi majú uvedené metódy určité výhody. Výsledkom perforácie dutiny brušnej sú iba malé rany vyžadujúce maximálne dva hlbkové a dva povrchové stehy. Keďže zákroky sú relatívne jednoduché, laparoskopické incízie vedú k významnému zníženiu postoperačnej bolesti v operačnej rane.

suka; laparoskopia; sterilizácia; endoskopia; vaječník; vajcovod

INTRODUCTION

Laparoscopy is one of the few techniques tested first on humans and later adjusted to the needs of veterinary medicine. One of the most interesting applications of laparoscopy in the management of reproduction is doubtless sterilisation. In human medicine, the uterine tube sterilisation is routinely used (Gomel, 1977). Daniel and Herbert (1984) described the salpingostomy technique using CO_2 laser through a laparoscope.

Only in 1985 did Wildt and Fawler describe the laparoscopic sterilisation of bitches by bipolar highfrequency coagulation of a salpinx. Sterilisation of bitches by the salpinx interruption, however, does not eliminate accompanying signs of oestrus (attractiveness of female to males, sanguineous vaginal discharge). For this reason, some owners prefer ovariectomy or ovariohysterectomy. Ovariectomy in female dogs by a laparoscope was first described by Thiele et al. (1993). Videocamera with a monitor was used for simplification and better making. The extracorporal Roeder's knots and the system ethi-endo-ligature (Ethicon) were used for ligatures. It was not possible to perform a surgical intervention successfully only in one of 62 female dogs due to synechias in the umbilical area.

Development of a laparoscopic surgery in gynecology of women has enabled operations with a laparoscope on adnexa (Semm, 1979; Semm and Mettler, 1980) as well as treatment of extrauterine pregnancy (Shapiro and Adler, 1973; Wilke and Kuhn, 1992). These interventions were carried out by direct visualisation using a laparoscope, which limited possibilities of a surgeon and his team. The use of videotechnique has widened a diapason of possibilities of laparoscopic techniques because also other members of the operation team are involved in the operation process. This has made it possible to perform also complicated surgical interventions with a laparoscope such as the first laparoscopic per vaginam assisted hysterectomy (Reich et al., 1989). Semm (1991) performed the first pelviscopic hysterectomy. Within a short time, these techniques, causing relatively only little trauma, have gained a great recognition among the professional personnel and popularity among the patients (Padial and Stolongo, 1992).

The first laparoscopic ovariohysterectomy in a bitch was carried out by von Siegel et al. (1994). The operation did not last more than 60 minutes.

The aim of this research is to discuss three different methods of spaying:

- mechanical occlusion of oviducts with endostaples,
- forcing occlusion of oviducts with electrocoagulation,
- ovariectomy by using endosuture technique.

During those procedures that were mentioned above, we followed the effect, complications and difficulties which accompanied the surgical manipulations. We also observed the influence upon the reproductive apparatus, and the health condition of the patients after treating them with laparoscope.

MATERIAL AND METHODS

Animals

For mechanical occlusion of oviducts we operated 3 bitches, for occlusion of oviducts by electrocoagulation 3 bitches were operated, and for ovariectomy procedure we operated 2 bitches. The age of the patients was between 2–7 years, bitches were German Shepherd breed. The animals were kept in clinical kennels and received on commercial food and were given water *ad libitum*. The weight of bitches ranged from 35 to 40 kg. All the bitches were in the stage of anestrus.

Optic equipment and accessories

Two laparoscope units consisting of laparoscope MLW Germany, 180 degrees, 1 cm in diameter, source of light, manual insufflator with regulator and T shaped pressure gauge connected to CO_2 tank container. For endocoagulation we used electrocautering unit, Eltom Chirana Slovakia.

Invasive and surgical instruments

For pneumoperitoneum we used Veress needle. For insertion of laparoscope we used a set of trocars and cannulas in the range of 0.5–1.5 cm with safety protection for the sharp tip. For manipulation with internal organs we used rigid metal probe. For grasping and fixation of internal organs and different parts or various instruments, we used endo pean forceps. For cutting and/or coagulation of soft tissue, we used endo scissors. For occlusion of oviducts we used titanium clip and its applicator (endo-stapler). For internal suturing we used the extra corporal Orsilon technique with non-traumatic units and adapter for tightening the internal surgical knot, plus specially devised internal needle holder.

Anesthesia and preoperational preparation of the patients

Within all the animals, the same material and substances were used. Food was withheld for 24 hours and water for 6 hrs. before operation. We used tranquiliser: 15 mg diazepam *i.m.* (Apaurin, Léčiva Praha) pro toto, 15 minutes before introducing anesthesia. For sedation we used xylazin 2 mg/kg b.w. *i.m.* (Rometar, Spofa Praha) and after 10 min. neuroleptic analgetic ketamin 10 mg/kg b.w. *i.m.* (Narkamon, Spofa Praha) was injected. The animals were fixed in a recumbent position with the head down and total body plane laid between 30–40 degrees in relation to the ground. The surface between the pubic bone and 4 cm cranially to the umbilicus was shaved and scrubbed including 10 cm to each side.

Formation of pneumoperitoneum

Puncture of abdominal cavity with Veress needle located 2 cm cranially to pelvic bone and 2 cm laterally on each side (according to the convenience of surgeon) of *linea alba*. To confirm an intact bladder we aspirate with syringe through the Veress needle. If urine is present, the Veress needle is reinserted. The Veress needle must penetrate through all the abdominal wall layers to control subcutis, muscle or subperitoneal dislocation. The volume of insufflated CO_2 ranged from 20 to 30 mm Hg, which represented in dependence on the tightness of the system initial consumption as large as 2.0 l.

Mechanical occlusion of oviducts

Laparoscopic trocar and cannula were penetrated 1 cm behind the umbilicus, and a second trocar was inserted for titanium clips applicator Endo clip II ML Auto Suture, USA (endo stapler) 0.5 cm in diameter, 6–10 cm behind the umbilicus and 2–3 cm laterally from specific udder unit. A third trocar and cannula for endo pean were introduced contralaterally. After confirmation of accurate location, and fixation with endo pean, the site of occlusion was grasped with the jaws of applicator, and the endo stapler released onto the oviduct, and the procedure was repeated 0.5 cm away from the former, on the same oviduct.

Occlusion of oviducts by coagulation

Laparoscope and cannula were inserted using the same procedure described in the former chapter „mechanical occlusion of oviducts". After identification of oviduct, it is fixed by the jaws of endo pean. Electrocoagulation was carried out with unipolar electrocautering unit (Chiratom 170, Chirana, Slovak Republic) and coagulation level was set to grade 2. After controlling the location of endo pean, coagulation is performed for a few seconds (2–3) until complete occlusion is achieved. This procedure requires a very delicate manipulation with care in order to prevent touching other organs with the endo pean, which may cause permanent damage to them.

Ovariectomy by laparoscopic technique

Trocar for laparoscopic cannula is located 1 cm behind the umbilicus. Two additional trocars with adjacent cannulas in diameter of 10 mm are located 5 cm behind the umbilicus 6–8 cm laterally to each side of *linea alba*. *Ligamentum proprium* is held with endo pean, and using manipulative movements of pushing and pulling proximally, tension appears on the *ligamentum latum uteri*, causing its flattening. The *lig. latum uteri* is then sutured by using endo needle and atraumatic Orsilon 1 A unit (Spofa, Czech Republic). The suture is made craniolaterally (in relation to ovary) around ligamentum suspensorium ovarii. The endo needle is then inserted through the cannula and circling the lig. suspenpensorium, creating a loop around it, and is led out through the same cannula where an outer surgical knot is made. The knot is led inside along the cannula, under a specially designed endo ligature adapter, and tightened on the desired location. Using this technique we suture the ovarian artery and vein. Coagulation of those vessels was also performed successfully. With the second suture we used the same technique, but the location was caudally to the ovary, through and around lig. proprium. Using endo scissors and endo coagulation simultaneously, we separate between ligamentum suspensorium ovarii caudally to the suture, and ligamentum ovarii proprium cranially to the knot. The tissue remnants which are composed of bursa ovarica, part of ligamentum suspensorium ovarii and part of ligamentum ovarii proprium, are cleared through the working cannula. The procedures are repeated contralaterally. Before drawing back the laparoscope and its accessories, it is necessary to check for blood leaks from the stumps.

End of laparoscopic surgery and postoperative care

After observation of the place of surgery and its surroundings, the instruments are collected, and through the working channel of laparoscope the CO_2 which created the pneumoperitoneum is released, the cannules are removed and internal plus external sutures are performed. Incisions with 1.5 cm in diameter were sutured with two deep and two superficial ligatures. We used a single wide spectrum long acting parenteral antibiotic injection (Antipen, Werfft-Chemie, Austria or Clamoxyl, Smith Kline Beecham, UK).

RESULTS

In spaying by using laparoscopy, we used two different methods for occlusion of oviducts (Tab. I, II) and a single ovariectomy technique (Tab. III).

During the procedure of mechanical occlusion using endostapler technique, 1–2 staples were applied to each oviduct in each of the three bitches. The procedure had only one single complication; the clip slipped from the site of application. The released clip was drawn out, and replaced. The whole procedure lasted 30 min (Tab. I). In one case, bleeding, which appeared after the cannula was removed from the abdominal wall, was treated with suture. In each of the 3 bitches clips were placed on both oviducts and their fixity was checked mechanically with endo pean.

By using coagulation of oviducts in three bitches, 1–2 occlusions were performed on each oviduct. During the procedure, bleeding appeared on abdominal wall in the peritoneal cavity, during the insertion of cannula for adjacent endo pean. This complication does not put at risk either the patient or the surgical progress/prognosis. The bleeding was treated at the end of the sur-

I. Mechanical occlusion of oviducts: endo-stapler technique

No. of bitches	No. of clips used on oviducts		Complication during surgery	Duration of occlusion (min)	Duration of surgery (min)	Complication after surgery
	left	right				
1	+	++	replacing one clip	25	45	refusing food 24 h
2	+	+	none	20	40	none
3	++	++	none	30	40	bleeding abdominal wall

II. Occlusion of oviducts: endo-coagulation

No. of bitches	No. of coagulations in oviduct		Complication during surgery	Duration of occlusion (min)	Duration of surgery (min)	Complication after surgery
	left	right				
4	++	++	none	15	25	none
5	+	+	bleeding abdominal wall	10	25	refusing food 42 h
6	++	++	none	15	25	none

III. Ovarioectomy by using modified endo-suture technique

No. of bitches	Number of ligatures on oviduct				Complication during surgery	Duration of occlussion (min)		Duration of surgery (min)	Complication after surgery
	left		right						
	caudal	cranial	caudal	cranial		left oviduct	right oviduct		
7	+	++	+	++	slight bleeding from incision	25	30	65	none
8	+	+	+	+	slight bleeding from incision	30	30	70	none

gery, after emptying the gas from the abdominal cavity, which produced pneumoperitoneum, necessary for easy access to the internal organs. The occlusion of oviducts by endo coagulation took 10 minutes, and the whole surgery 25 min (Tab. II). In each of the three bitches electrocoagulation was performed on each oviduct and damage to the tissue was visually checked.

Ovariectomy by using modified endosuture technique was carried out successfully on two bitches. Two ligatures were applied cranially to the ovary, and a single ligature caudally from the same ovary. The ovary was removed with endoscissors. This procedure is repeated on the other ovary as well. The most important critical step in this stage, which has a direct effect on the patient's life, is making sure the ovarian artery was either ligated with the cranial ligature to the ovary or a separate ligature specific to this artery should be done. However, bleeding appeared at the site of right incision in both cases. It did not require any treatment. The whole endoprocedure in both ovaries took 70 min. (Tab. III).

DISCUSSION

At present, the question of bitch sterilisation to induce permanent sterility is getting in the limelight. Up to now preferred methods have required a surgical intervention *per laparoscopiam*. The methods of sterilisation were elaborated and they have been widely used in the human gynecology (Gomel, 1977; Daniell and Herbert, 1984). The endo-stapler technique used to form permanent occlusions of the biliary duct and corresponding blood canal was adapted for formation of salpinx occlusions. It has been found that the permanent occlusion of salpinx can be formed by putting one or two clips on the salpinx concerned. The intervention itself lasted from 20 to 30 minutes. However, it is possible to shorten this time by gaining skill. The great advantage appears to be the fact that disposable applicator is available, with a clip cartridge that can last for several operations. Working canals of the 0.5 cm diameter are necessary at salpinx occlusion by coagulation, however, an electrocauter is also necessary. Demand for extreme caution at coagulation alone not to cause burning of other organs is a disadvantage. The present electrocauters are protected by several circuits and so the work with them, under observing the conditions given by the producer, is comfortable and relatively fast, 10–15 minutes. The presented methods have certain advantages in comparison with similar laparotomy intervention. They require a shorter time interval. Only small wounds requiring at maximum two deep and two superficial sutures are the result of the abdominal cavity perforation. As the intervention is relatively simple, it may be carried out also in dogs of older age categories. The advantages of minimally invasive surgery performed laparoscopically are apparent. Therefore, there is still greater interest in them. They can be applied in veterinary gynecology of bitches at ovariectomy, even at ovariohysterectomy. Small laparoscopic incisions lead to a significant decrease in postoperative

pain. Direct measurements of the pressure in operated wounds have confirmed this fact (Emmermann, 1992). So it is true that the smaller the incision, the less the pain. The laparoscopic procedure may lead in female dogs to fast postoperative normalisation of their health status. This is an advantage, above all, in obese animals and in those with bad wound healing. The owner's postoperative care of the patient is much easier then (Thiele et al., 1993). Not only ovariectomy but also ovariohysterectomy may be performed safely with adequate equipment of the laparoscopic unit (von Siegl, 1994). This possibility is given by relativelly good accessibility of all the tissues in question by endo-instruments *per laparoscopiam*. At present, the stapler technique endo GIA is very topical. Its only disadvantage, however, is its high price, but its using ensures for a surgeon maximum comfort at the intervention.

The use of laparoscopic techniques has its contraindications such as advanced endometritis, progressive stages of cysts or tumours and suspicion of extended intra-abdominal adhesions. Using the laparoscopic methods at ovariectomy and especially ovariohysterectomy remains a domain of specialised clinics due to high prices of endoscopic unit, special endoinstruments and videotechnique. As these interventions are relatively long, there is a probability of the failure of cardiovascular circulation, or lung collapse, especially due to Trendelenburg's position and necessity of pneumoperitoneum (Windberg et al., 1993). The inhalation anesthesia with assisted breathing through Elvent-unit with ensuring internal homeostasis by solutions appears to be optimal. Further development in veterinary gynecology will show the importance of ovariohysterectomy *per laparoscopiam*.

CONCLUSION

Technical parameters of the endoscopic optic systems have underwent considerable quantitative development. Therefore today, laparoscopes provide an authentic panoramic picture of the cavity observed. In addition, there is a possibility of some enlargement, which makes visualisation more precise and the picture clearer. At using the laparoscopic techniques the tissue impairment does not occur. Since the surgeon can see details of the organ surfaces (structures about 1.0 mm and less), he can avoid the blood vessels and so prevent unnecessary bleeding. A great advantage of laparoscopy is an immediate possibility of recording on videosystems, compact disk or photographic film. That is why laparoscopic techniques have been adopted not only in human medicine but also in veterinary medicine.

REFERENCES

Daniell J. F., Herbert C. M. (1984): Laparoscopic salpingostomy utilizing the CO_2 laser. Fertil. Steril., *41*, 558–563.

Emmermann B. (1992): Vortrag: Bauchdeckenspannung nach MIC. In: 1. MIC-Symposium Hamburg, 6–7. 11. 1992.

Gomel V. (1977): Salpingostomy by laparoscopy. J. Reprod. Med., *18*, 265–268.

Padial J. G., Stolongo J. (1992): Laparoscopy assisted vaginal hysterectomy: report of seventy-five consecutive cases. J. Gynecol. Surg., *8*, 81–84.

Reich H., De Caprio J., McGlynn F. (1989): Laparoscopic hysterectomy. J. Gynecol. Surg., *5*, 213–216.

Semm K. (1979): New methods of pelviscopy (gynecologic laparoscopy) for myectomy, ovarectomy, tubectomy and adnectomy. Endoscopy, *2*, 85–93.

Semm K., Mettler L. (1980): Technical progress in pelvic surgery via operative laparoscopy. Am. J. Obstet. Gynecol., *138*, 121–127.

Semm K. (1991): Hysterectomie per laparotomiam oder per pelviscopiam: Ein neuer Weg ohne Kolpotomie durch CASH. Geburtsh. Frauenheilkd, *51*, 996–1003.

Shapiro H. I., Adler D. H. (1973): Excision of an ectopic pregnancy through the laparoscope. Am. J. Obstet. Gynecol., *117*, 290–291.

Thiele S., Kelch G., Gerlach K. (1993): Kastration der Hündin durch laparoskopische Ovarektomie. Kleintierpraxis, *38*, 463–466.

Von Siegl H., Bohm R., Ferguson J., Friedrich M., Losert U. M. (1994): Laparoskopische Ovariohysterektomie bei einem Hund. Wien. Tierarztl. Mschr., *81*, 149–152.

Wildt D. E., Fawler D. F. (1985): Laparoscopic sterilisation of the bitch and queen by uterine horn occlusion. Am. J. Vet. Res., *46*, 864–869.

Wilke G., Khun W. (1992): Pelviskopische Salpingektomie bei rupturierter Extrauteringravidität mit Hilfe eines automatischen Klammer-Schneidegerates. Geburtsh. Frauenheilkd., *52*, 347–350.

Windberger U., Siegl H., Woisetschläger R., Schrenk P., Bodesser B., Losert U. M. (1993): Hemodynamic changes during prolonged laparoscopic surgery. Eur. Surg. Res., *195*, 1–8.

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IDENTIFICATION OF TOXIGENIC STRAINS OF *PASTEURELLA MULTOCIDA* BY PCR AND BIOLOGICAL METHODS

DETEKCE TOXINOGENNÍCH KMENŮ *PASTEURELLA MULTOCIDA* METODOU PCR A BIOLOGICKÝMI METODAMI

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ABSTRACT: Sensitivity and specificity of methods for the identification of toxigenic strains of *Pasteurella multocida* were compared. The production of dermonecrotoxin was determined by a skin test in guinea pigs, a cell culture test using bovine embryonic lung cells, and PCR. The highest sensitivity and specificity were found for PCR that allows the identification of strains producing only small amounts of the toxin *in vitro* that are not detectable by biological methods. However, strains producing structurally different toxins with the same biological effect are not identifiable by this technique owing to its high specificity. Therefore, biological and particularly cell culture tests are necessary for a comprehensive assessment of toxigenicity of individual strains.

Pasteurella multocida; dermonecrotoxin; PCR; toxA gene; atrophic rhinitis

ABSTRAKT: Byla porovnána citlivost a specifita metod ke stanovení toxinogenních kmenů *Pasteurella multocida*. Produkce dermonekrotoxinu byla stanovována pomocí kožního testu na morčatech, testem na linii embryonálních bovinních plicních buněk a PCR. Nejvyšší citlivost a specifita pro určení schopnosti produkce dermonekrotoxinu byla zjištěna u vyšetření PCR. S jeho pomocí je možné diagnostikovat kmeny v *in vitro* podmínkách, produkující nízké množství toxinu nedetekovatelné biologickými metodami. Z důvodů vysoké specifity PCR není možné odhalit kmeny, které produkují strukturálně odlišné toxiny se stejným biologickým účinkem. Z tohoto důvodu zůstávají biologické metody, především pak test na buněčné linii, důležité pro komplexní posouzení toxigenity jednotlivých kmenů *Pasteurella multocida*.

Pasteurella multocida; dermonekrotoxin; PCR; toxA gen; sípavka

INTRODUCTION

Pasteurella multocida is a common inhabitant of the upper respiratory tract of farm animals. Dermonecrotoxin (DNT), produced by some strains, is the causative agent of atrophic rhinitis of swine (Diemen et al., 1994) and causes also specific skin lesions and damage to bovine embryonic pulmonary cells (EBL) already at very low concentrations (Kamp et al., 1987).

Toxigenic strains of *P. multocida* (TPM) present on the nasal mucosa and in tonsillar crypts release DNT which is absorbed into blood and induces generalized toxaemia leading to a specific dysbalance of osteoblast and osteoclast differentiation affecting particularly the ossification of the nasal septum and turbinates in swine (Gwaltney et al., 1997). The result of the DNT effect is not only rhinitis, deviation of the nasal septum and atrophy of turbinates, but also a considerable decrease of performance in the affected herds (Ackermann et al., 1996).

Direct diagnostic methods for atrophic rhinitis are based on the demonstration of TPM and, in particular, production of DNT. The earliest diagnostic methods included the lethality test in mice and the skin test in guinea pigs (De Jong et al., 1980), as well as several tests of DNT demonstration in cell cultures (Pennings and Storm, 1984). Most suitable for DNT detection are EBL cells that have been tested in various modifications (Rutter and Luther, 1984; Chanter et al., 1986). The recent techniques for DNT detection include ELISA using monoclonal antibody to DNT, and demonstration by PCR of the toxA gene coding the production of DNT (Foged et al., 1988; Bocowersock et al., 1992; Nagai et al., 1994). Currently, several modifications of PCR, each detecting a specific fragment of the toxA gene of TPM isolates, are used (Kamp et al., 1996; Hotzel et al., 1997).

The high sensitivity and specificity of PCR allows a direct demonstration of DNT in nasal and tonsillar swabs avoiding the necessity to isolate the causative agent (Lichtensteiger et al., 1996). The objective of our experiments was to test the identification of TPM by PCR and to assess the correlation of results of PCR and of the two biological methods, i.e. skin test in guinea pigs and test in an EBL cell culture.

MATERIAL AND METHODS

Strains

Field strains collected with swabs (Copan-Bovezo) from the caudal part of nasal cavity of swine were

tested. Solid culture media were used for bacteriological examinations. The material from the swabs was inoculated on selective blood agar containing 2.5 mg neomycin and 0.2 I.U. bacitracin per 1 ml and the plates were incubated at 37 °C overnight. On the subsequent day, the bacterial colonies were re-inoculated onto antibiotic-free blood agar. The isolated strains were maintained on the slant Dorset egg medium.

Skin test

The isolated strains of *P. multocida* were incubated in stationary cultures in the liquid medium BHI at 37 °C for 24 h, centrifuged at 10 000 g for 10 min and tested for the presence of DNT by intradermal inoculation of 0.2 ml of the supernatant in the shaved dorsal region of albinotic guinea pigs of 300 to 400 g live weight. Four to six samples were tested in each animal.

The guinea pigs were sacrificed 72 h after the inoculation and inflammatory reactions were assessed. The reaction was classified as positive if local inflammation with or without central necrosis developed at the inoculation site. The severity of the reaction was classified as + to ++++.

Cell culture test

The tests in a culture of embryonic bovine lung cells were done using the method described by Rutter and Luther (1984) in two modifications differing in the preparation of the tested cultures. Either stationary cultures grown in the liquid medium BHI at 37 °C for 24 h or a suspension in PBS (pH 7.4) of colonies grown on blood agar were used.

An EBL cell culture was grown in MEM supplemented with 8% bovine foetal serum and 100 µg streptomycin, 50 µg gentamycin, and 100 IU penicillin per 100 ml.

DNT was detected in microplates (Gama comp.). The supernatants of the cultures were diluted 1 : 10 with MEM and 50 µl were pipetted into each well. Then all the wells were completed with 75 µl of the EBL cell suspension with a density of approx. 3×10^5 cells per 1 ml. Positive and negative controls were run with each set of the tested samples. The microplates were incubated at 37 °C and 3% CO_2 for four days. After rinsing, the cells were fixed with 8% formaldehyde and stained with 1% crystal violet, and CPE was assessed macroscopically or microscopically.

PCR

PCR was run in 20 µl of the reaction mixture containing 2 µl tenfold concentrated reaction buffer, 200 µM $sNTP_s$, 10 pmol each primer, 0.5 U Taq DNA polymerase (Promega), and 2 µl DNA (tested sample), and the volume was completed with deionized water (Millipore). Positive and negative controls were run with each set of samples.

The following primers were used:

Gene accession Z28388, FEBS Lett. 277(1–2), 59–64 (1990) was selected for proposal of the primers.

PM1: 5'AGA ACA GAA GAA GAT ATT CC 3'

PM2: 5'ACT TCA CTT ACT AAA GAA CC 3'

These oligonucleotide primers amplify a 266 bp fragment.

PCR sensitivity

Sensitivity of PCR was determined by a diluting series of toxicogenic *P. multocida* culture propagated for 16 h in BHI and subsequent inoculation of 100 µl of the diluted culture from BHI onto blood agar.

Specificity of the primers

Specificity of the primers was tested by examinations of 15 toxigenic and 15 nontoxigenic strains. Possibility of cross reaction with the following bacterial species: *M. intracelullare, Str. intestinalis, M. avium, A. pyogenes, Str. suis, P. aeruginosa, E. coli, H. parasuis, K. pneumonie, A. suis, S. choleraesuis, E. rhuseopathiae, Y. pseudotuberculosis, Str. pneumoniae, B. bronchiseptica, A. pleuropneumoniae* was also assessed.

DNA isolation

A colony of *P. multocida* was resuspended in 50 µl deionized water (Millipore) and boiled for 10 min. Cell debris was removed by centrifugation at 10 000 g for 2 min and 2 µl of the supernatant was collected as a DNA sample.

Amplification

The initial temperature of 95 °C for 3 min was followed by 30 cycles of 45-s periods of 92 °C, 55 °C and 72 °C, and the amplification was completed at 72 °C for 3 min (Nagai et al., 1994). The amplification product of 266 bp was detected by electrophoresis in 2% agarose gel containing 0.5 µg ethidium bromide per 1 ml at 5 V per 1 cm and visualized in UV light using a transilluminator.

RESULTS

The sensitivity of PCR was determined to be $2.6.10^4$ CFU/ml, which is necessary for obtaining 266 bp of the amplification product. Examination of 15 reference toxicogenic strains yielded positive results and examination of 15 negative reference strains yielded negative results. Negative results were also obtained in other tested bacterial species.

PCR and skin tests were used to test a set of 286 strains of *P. multocida* for the production of dermonecrotoxin (Tab. I). Identical results by both methods were obtained in 217 strains (75.9%), of which 155 (54.2%), were non-toxigenic. Positive skin test and negative PCR were found in 55 strains (19.2%), of which 80% elicited skin reactions with scores + or ++. Positive by

PCR and negative by skin test were 14 strains (4.9%). Positive by both methods were 62 strains (21.7%).

The results of toxigenicity tests using PCR and the EBL cell culture method were compared in 550 strains of *P. multocida* (Tab. II). The cell culture tests were performed in 281 strains grown in the BHI medium and 269 strains were processed by resuspension in PBS. Identical results by both methods were obtained in 517 strains (94%) of which 341 (62%) were non-toxigenic. Negative results of the cell culture test and positive results of PCR were obtained in 19 strains (3.5%). Positive results of the cell culture test with BHI medium and negative results of PCR were obtained in 9 strains (3.2%). Resuspension of the strains in PBS showed 5 strains (1.9%) positive in cell culture and negative in PCR. Processing of the isolated strains of *P. multocida* for toxigenicity examination showed besides the mentioned lower correlation also a less pronounce cytopathic effect. Four dubious findings were recorded, which gave negative results at repeated examinations.

Results of cell culture tests, PCR and skin tests were compared in 261 strains of *P. multocida* (Tab. III). A certain type of discrepancy was found in 72 strains (27.6%). 37 strains of the total of 261(14.2%) were positive only by skin test and in 33 strains (12.6%), the scores of the skin reactions were + or ++. Five strains (1.9%) were positive by PCR only, and five strains (1.9%) were PCR negative but positive in skin test and cell culture.

DISCUSSION

PCR is a rapid, accurate and highly sensitive method for the identification of TPM strains (Kamp et al., 1996; Hotzel et al., 1997). Simultaneous use of PCR and skin test for toxigenicity of *P. multocida* isolates revealed discrepant results in 69 strains (24.1%). In most of the samples negative by PCR and positive by skin test, the scores of the skin reactions were + or ++. Skin tests yielded a high number of dubious results and the overall accordance between the results of the two methods was lower than that reported by Nagai et al. (1994).

Simultaneous use of PCR and skin and cell culture tests revealed 55 strains negative by PCR and positive by skin test, and 14 strains negative by PCR and positive by cell culture test. Negative PCR and positive results of skin and cell culture tests were obtained only in 5 strains.

I. Comparison of results of skin tests in guinea pigs (ST) and PCR for toxigenicity of the strains of *Pasteurella multocida*

	ST negative	ST positive	Assessment of the ST positive*			
			+	++	+++	++++
PCR negative	155	55	28	16	8	3
PCR positive	14	62	9	16	24	13

* + to ++++ = classification of inflammatory reactions after intradermal administration of supernatant of the tested culture *P. multocida*
number of the strains tested = 286

II. Comparison of results of PCR and test in EBL cells (TBL) for toxigenicity of *P. multocida* strains

	Total TBL negative	Total TBL positive	TBL-A negative	TBL-A positive	TBL-B negative	TBL-B positive
PCR negative	341	14	198	9	143	5
PCR positive	19	176	13	61	6	115

TBL-A – samples prepared of cultures grown in the liquid BHI medium
TBL-B – samples prepared of colonies grown on blood agar plates and resuspended in PBS
number of the strains tested = 550

III. Comparison of results of PCR, test in EBL cells (TBL) and skin tests (ST) in guinea pigs for toxigenicity of *P. multocida* strains

	Total	Percentage of total number	Percentage of discrepancies	Assessment of the ST positive*			
				+	++	+++	++++
TBL– PCR+ KT+	9	3.5	12.5	3	4	1	1
TBL– PCR– KT+	37	14.2	51.4	21	12	2	2
TBL+ PCR– KT+	5	1.9	6.9	1	1	2	1
TBL– PCR+ KT–	5	1.9	6.9	–	–	–	–
TBL+ PCR+ KT–	10	3.8	13.9	–	–	–	–
TBL+ PCR– KT–	6	2.3	8.4	–	–	–	–
TBL+ PCR+ KT+	47	18.0	–	2	10	24	11
TBL– PCR– KT–	142	54.4	–	–	–	–	–

* + to ++++ = classification of inflammatory reactions after intradermal administration of supernatant of the tested culture *P. multocida*
number of the strains tested = 261

Due to high sensitivity and specificity of PCR, direct detection of DNT is an important complementary method. Cases of negative PCR and positive skin and/or cell culture tests can be interpreted as a consequence of too high specificity of PCR. A change in the structure of the toxA gene can make the production of DNT undetectable by PCR although no qualitative alterations in its biological activity are found. This assumption is supported by the finding of 3 strains that were negative by PCR but induced CPE in cell culture tests and their scores in skin tests were +++ to ++++. The same scores were obtained for four strains that were negative by PCR and cell culture tests. A similar hypothesis was suggested by Alexa et al. (1997) who investigated strains of *Escherichia coli*. It must be noted, however, that no TPM strains with an altered structure of the toxA gene have been isolated so far (Hoskins and Lax, 1996).

Correlation between serotype and production of DNT was not investigated because the structures of toxA genes of the serotypes A and D are almost identical (Nagai et al., 1994). Nonspecific positive reactions in skin and cell culture tests might be due to a subjective assessment of the results. With regard to the factors like preparation of the application field, way of application, animal's individuality, housing etc, the reactions with the scores + to ++ are difficult to assess objectively whether the changes are caused by DNT or a nonspecific skin reaction following application. Of the total of 51 discrepacies in the skin test, 42 (82.3%) were assessed as + to ++. For these reasons the skin test does not appear very suitable for TPM detection.

Negative results of skin and/or cell culture tests in strains positive by PCR can be interpreted rather as weak than as missing production of DNT during *in vitro* examinations because, as stated by Lichtensteiger et al. (1996), all toxA-positive isolates express toxin.

As a conclusion we can state, that examinations of *P. multocida* strains by PCR offer a rapid and accurate assessment of toxigenicity of the strains isolated from nasal swabs, which can be used for the diagnosis and prevention of TPM transmission. Nagai et al. (1994) suggest that PCR can be used as a rapid and feasible examination method for the detection of TPM even from the rinsed cultures of nasal and tonsillar swabs.

REFERENCES

Ackermann M. R., Register K. B., Stabel J. R., Gwaltney S. M., Howe T. S., Rimler R. B. (1996): Effect of *Pasteurella multocida* toxin on physeal growth in young pigs. Am. J. Vet. Res., *57*, 848–852.

Alexa P., Rychlík I., Nejezchleb A., Hamřík J. (1997): Identification of enterotoxin-producing strains of *Escherichia coli* by PCR and biological methods. Vet. Med. – Czech, *42*, 97–100.

Bowersock T. L., Hooper T., Pottenger R. (1992): Use of ELISA to detection toxigenic *Pasteurella multocida* in atrophic rhinitis of swine. J. Vet. Diagn. Invest., *4*, 419–422.

Chanter N., Rutter J. M., Luther P. D. (1986): Rapid detection of toxigenic *Pasteurella multocida* by an agar overlay methods. Vet. Rec., *119*, 629–630.

De Jong M. F., Oei M. L., Tetenburg G. J. (1980): AR – pathogenicity – test for *Pasteurella multocida* isolates. In: Proc. 6th IPVS Congr., Copenhagen. 211.

Diemen P. M., De Jong M. F., Reilingh G. V., Hel P., Schrama J. W. (1994): Intranasal administration of *Pasteurella multocida* toxin in a challenge-exposure model used to induce subclinical signs of atrophic rhinitis in pigs. Am. J. Vet. Res., *55*, 49–54.

Foged N. T., Nielsen J. P., Pedersen K. B. (1988): Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by enzyme-linked immunosorbent assay. J. Clin. Microbiol., *26*, 1419–1420.

Gwaltney S. M., Galvin R. J. S., Register K. B., Rimler R. B., Ackermann M. R. (1997): Effects of *Pasteurella multocida* toxin on porcine bone marrow cell differentiation into osteoclasts and osteoblasts. Vet. Pathol., *34*, 421–430.

Hoskins I. C., Lax A. J. (1996): Constitutive expression of *Pasteurella multocida* toxin. FEMS Microbiol. Lett., 141, 189–193.

Hotzel H., Erler W., Schimmel D. (1997): Nachweis des Dermonekrotoxin-Gens in *Pasteurella multocida* Stämmen mettels Polymerase-Kettenreaktion (PCR). Dtsch. Tierärztl. Wschr., *110*, 139–142.

Kamp E. M., Heijden P. J., Tetenburg B. J. (1987): Purification of a heat labile dermonecrotic toxin from culture fluid of *Pasteurella multocida*. Vet. Microbiol., *13*, 235–248.

Kamp E. M., Bokken G. C. A., Vermeulen T. M. M., Jong M. F., Buys H. E. C. M., Reek F. H., Smits M. A. (1996): A specific and sensitive PCR assay suitable for large-scale detection of toxigenic *Pasteurella multocida* in nasal and tonsillar swabs specimens of pigs. J. Vet. Diagn. Invest., *8*, 304–309.

Lichtensteiger C. A., Steenbergen S. M., Lee R. M., Polson D. D., Vimr E. R. (1996): Direct PCR analysis for toxigenic *Pasteurella multocida*. J. Clin. Microbiol., *34*, 3035–3039.

Nagai S., Someno S., Yagihashi T. (1994): Differentiation of toxigenic from nontoxigenic isolates of *Pasteurella multocida* by PCR. J. Clin. Microbiol., *32*, 1004–1010.

Pennings A. M. M. A., Storm P. K. (1984): A test in Vero cell monolayers for toxin production by strains of *Pasteurella multocida* isolated from pigs suspected of having atrophic rhinitis. Vet. Microbiol., *9*, 503–508.

Rutter J. M., Luther P. D. (1984): Cell culture assay for toxigenic *Pasteurella multocida* from atrophic rhinitis of pigs. Vet. Rec., *114*, 393–396.

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RADIOPROTECTIVE EFFECT OF BRONCHO-VAXOM ON THE DEVELOPMENT OF LATENT INJURY IN RAT LIVER

RÁDIOPROTEKTÍVNY ÚČINOK BRONCHO-VAXOMU NA ROZVOJ LATENTNÉHO POŠKODENIA PEČENE POTKANOV

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ABSTRACT: The influence of immunomodulator Broncho-Vaxom on the rise and restoration of latent radiation injury in rat liver was studied. Broncho-Vaxom was administered intraperitoneally at the dose of 1.5 mg/rat or 3 mg/rat 24 h before whole body irradiation with the doses of 3, 6 and 9 Gy of gamma radiation. Animals in all groups were partially hepatectomized (2/3 of liver mass removed) within 30 min or on the 6th and 13th day after irradiation and examined on the 30th h after the operation, i.e. on the 1st, 7th and 14th day after irradiation. We have found that i.p. administration of 1.5 mg/rat resp. 3 mg/rat of Broncho-Vaxom 24 h before whole body irradiation with the doses of 3, 6 and 9 Gy of gamma radiation resulted in an alleviation of latent injury. The alleviation has been manifested by smaller range of radiation-induced changes during regeneration of liver 30 h after partial hepatectomy. It is indicated by increasing of mitotic index (Fig. 1, Tab. I) and decreasing of chromosome aberrations (Fig. 2, Tab. II) in comparison with irradiated nonprotected animals. The range of radioprotective effect of investigated immunomodulatory agent has partially depended on the dose of Broncho-Vaxom and radiation. It follows from our results that the Broncho-Vaxom has not only radioprotective effect but favourable influence the processes of compensational regeneration as well. Administration of 1.5 mg/rat and particulary 3 mg/rat of Broncho-Vaxom 24 h before partial hepatectomy markedly stimulated proliferation also in regenerative liver of non-irradiated animals (Fig. 1, Tab. I). We suggest that stimulation of regenerative process of liver after administration of immunomodulators Broncho-Vaxom including is not propably realised directly but via mediators released from stimulated liver macrophages – Kuppfer cells (Šimek et al., 1986; Beyer et al., 1990; Nagata et al., 1994).

Broncho-Vaxom; gamma irradiation; mitotic index; chromosomal aberrations

ABSTRAKT: Vyšetrovali sme vplyv imunomodulačného preparátu Broncho-Vaxomu na vznik a úpravu latentného radiačného poškodenia pečene potkanov. Broncho-Vaxom sme aplikovali intraperitoneálne vo forme suspenzie vo fyziologickom roztoku (PBS; pH 7,4) v dávke 1,5 mg na potkana a 24 h pred celotelovým ožiarením jednorazovými dávkami 3, 6 a 9 Gy gama žiarenia (0,348 Gy/min), alebo v dávke 3 mg potkana 24 h pred celotelovým ožiarením jednorazovou dávkou 6 Gy. Všetky skupiny zvierat boli podrobené 2/3 parciálnej hepatektómii (PHE) do 30 min po ukončení žiarenia a na 6. a 13. deň po ožiarení a vyšetrené na 30. h po operácii. Podanie Broncho-Vaxomu zmiernilo vznik latentného radiačného poškodenia v pečeni, o čom svedčí vzrast mitotického indexu v porovnaní s ožiarenými, nechránenými zvieratami. Rádioprotektívny účinok vyšetrovaného preparátu sa prejavil aj v znížení frekvencie chromozómových aberácií indukovaných žiarením.

Broncho-Vaxom; gama žiarenie; mitotický index; chromozómové aberácie

ÚVOD

Väčšina parenchymatických buniek pečene dospelých zvierat sa nachádza reverzibilne v G_0 štádiu bunkového cyklu a udržuje si schopnosť syntetizovať DNA a deliť sa, ak sú k deleniu stimulované. V pokusoch sú bunky pečene najčastejšie stimulované parciálnou hepatektómiou (2/3 PHE), po ktorej je zvyšok pečeňového tkaniva vystavený značnej funkčnej a metabolickej záťaži. Predpokladá sa, že po resekcii sa v reziduálnej pečeni náhle zvýši prítok krvi *v. portae*, ktorou sú privádzané látky rôzneho pôvodu, ktoré stimulujú regeneračný proces. Na aktivácii regeneračného procesu pečene v prvých hodinách po PHE sa významne podieľajú mnohé hormóny a rastové faktory, najmä inzulín, glukagon, somatotropný hormón, prostaglandíny, HGF (hepatocyte growth factor), EGF (epidermal growth factor), TGF (transforming growth factor) a hepatopoetín (Chamuleau a Bosman, 1988; Šimek a i., 1989; Michalopoulos, 1990; Bucher, 1991; Zarnegar, 1992; Andus a Holstege, 1994).

Určitú úlohu majú aj komitogény pečeňových rastových faktorov, ku ktorým patria pravdepodobne aj niektoré cytokíny: tumor necrosis factor (TNF), interleukin-1 (IL_1), produkované pečeňovými makrofágmi – Kuppferovými bunkami (Beyer a i., 1990; Nagata a i., 1994).

Po 2/3 PHE sa vo zvyšku pečene začína veľmi intenzívne rozvíjať regeneračný proces už v priebehu prvého pooperačného dňa. V prvých hodinách sú aktivované protoonkogény, ktoré sa tiež zúčastňujú na regulácii rastu buniek, ktorý vrcholí delením (napr. produkt protoonkogénu c-met je receptorom pre HGF) (Chamuleau a Bosman, 1988; Zarnegar, 1992). Na regeneračnom procese sa podieľajú všetky bunky pečene, ale nie rovnakou rýchlosťou a rozsahom. Nástup a rozvoj regenerácie, ktorá sa uskutočňuje kompenzačnou hypertrofiou a hyperpláziou prejavujúcou sa zvýšenou mitotickou aktivitou buniek, ktorú pozorujeme najskôr v hepatocytoch (Michalopoulos, 1990). Na 4. hodinu po PHE vstupuje veľká časť hepatocytov (až 90 %) synchrónne z G_0 štádia do presyntetickej G_1 fázy (Chamuleau a Bosman, 1988) nasledovanej ďalšími fázami interkinézy a mitózy. V regenerujúcom zvyšku pečene prudko vzrastá syntéza DNA a mitotická aktivita hepatocytov s maximom na 20. až 24. hodinu, resp. 28. až 30. hodinu po operácii (Šimek a i., 1986; Keppler, 1987; Michalopoulos, 1990; Bucher, 1991). Syntetická a proliferatívna aktivita neparenchymatických buniek, ako sú Kuppferove bunky, endoteliálne bunky a Itoove bunky vystielajúce pečeňové sinusoidy, nastupuje spravidla neskôr, asi s jednodňovým oneskorením (Michalopoulos, 1990; Bucher, 1991).

Ionizujúce žiarenia a niektoré chemické látky, (napríklad cyklofosfamid) vyvolávajú v máloproliferujúcom tkanive intaktnej pečene latentné poškodenie, ktoré zasahuje najmä genetický aparát bunky. Takéto poškodenie sa prejaví po stimulácii buniek k deleniu rôznymi biochemickými zmenami, vrátane inhibície syntézy DNA a mitózy a cytogenetickými alteráciami v regenerujúcom zvyšku pečene (Barbason a i., 1983; Kropáčová a Mišúrová, 1981, 1988). Rozsah inhibície mitózy a tvorby chromozómových aberácií po jednorazovom ožiarení závisia od veľkosti dávky (Kropáčová a Mišúrová, 1981). Pri kontinuálnom ožarovaní zvierat dochádza ku kumulácii latentného poškodenia (Kropáčová a Mišúrová, 1988).

Rozvoj latentného poškodenia vyvolaného ionizujúcim žiarením možno zmierniť podaním rádioprotektívnych látok, napríklad cysteamínu, adeturonu alebo gamafosu v určitú dobu pred ožiarením (Kropáčová a Mišúrová, 1990). Niektoré hepatoprotektívne preparáty, napríklad Flavobion, Thioctacid a Essentiale priaznivo ovplyvňujú metabolizmus pečeňových buniek a tým stimulujú reparačné procesy pečene (Kropáčová a Mišúrová, 1992, 1995).

Doteraz je málo preskúmaná úloha lymfatických tkanív a imunomodulátorov na proces regenerácie. Lymfoidné bunky získané po PHE zo sleziny stimulovali u neoperovaných recipientov mitotickú aktivitu hepatocytov a retikuloendoteliálnych buniek (Babajeva a i., 1969). Aj niektoré bakteriálne lipopolysacharidy (endotoxín *Escherichia coli*, prodigiosan) urýchľujú nástup a intenzitu syntézy DNA a mitózy v regenerujúcej pečeni (Babajeva a i., 1969; Majanskij, 1981; Šimek a i., 1986; Beyer a i., 1990).

Bakteriálne komponenty pôsobia imunostimulačne predovšetkým prostredníctvom aktivácie makrofágov.

Broncho-Vaxom je lyofilizovaný bakteriálny lyzát používaný ako polyvalentný imunobioterapeutický preparát najmä v liečbe infekcií respiračného traktu (Mauel a i., 1989). Stimuluje produkciu sérových imunoglobulínov (Puigdollers a i., 1980) a sekréciu cytokínov – IL_1, TNF a prostaglandínov z makrofágov (Neta a i., 1986; Walden, 1987; Bottex a i., 1988), ktoré majú aj rádioprotektívny účinok. Rádioprotektívny účinok Broncho-Vaxomu podaného myšiam 24 hodín pred jednorazovým ožiarením dávkou 9,5 Gy sa prejavil výrazným zvýšením prežívania zvierat (Fedoročko a i., 1992). Broncho-Vaxom akceleroval úpravu počtu kmeňových krvotvorných buniek v kostnej dreni (CFU-S, GM-CFC) aj úpravu počtu buniek v periférnej krvi. U neožiarených zvierat 24 hodín po podaní Broncho-Vaxomu vzrástlo v kostnej dreni percentuálne zastúpenie CFU-S a GM-CFC v S fáze (Fedoročko, 1994).

Podanie Broncho-Vaxomu v dávke 1,5 mg na potkana pred celotelovým ožiarením dávkami 3, 6 a 9 Gy zmiernilo postradiačné zmeny koncentrácie a obsahu histónov (Kožurková a Fedoročko, 1998) a hlavne DNA v regenerujúcej pečeni a slezine (Haková a i., 1997).

V tejto práci sme vyšetrovali vplyv imunomodulačnej látky Broncho-Vaxomu na vznik a úpravu latentného radiačneho poškodenia pečene potkanov. Účinok Broncho-Vaxomu sme hodnotili na základe zmien mitotickej aktivity a chromozómových aberácií v regenerujúcej pečeni (po parciálnej hepatektómii) chránených a nechránených potkanov ožiarených rôznymi dávkami gama žiarenia.

MATERIÁL A METÓDY

Vyšetrenia sme robili na dospelých samcoch bielych potkanov kmeňa Wistar (SPF) z veľkochovu Velaz (Praha, Česká republika) s priemernou telesnou hmotnosťou 290 ± 20 g na začiatku pokusu. Zvieratá boli chované v štandardných podmienkach, kŕmené laboratórnou potravou a napájané pitnou vodou *ad libitum*.

Podľa kombinácie dávky Broncho-Vaxomu a žiarenia boli potkany rozdelené do ôsmich skupín po piatich až šiestich zvierat. Spolu s pokusnými zvieratami boli vyšetrené aj kontroly. Vyšetrenia sme robili v troch časových intervaloch, na prvý, siedmy a 14. deň po ožiarení.

Aplikácia Broncho-Vaxomu

Broncho-Vaxom (Biogal Pharmaceutical Works, Debrecen, Hungary, vyrobený v licencii OM Laboratories, Geneva, Switzerland) je lyofilizovaný bakteriálny lyzát z ôsmich bakteriálnych kmeňov: *Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Staphylococcus pyogenes, Streptococcus viridans, Neisseria ca-*

tarrhalis. Preparát sme podávali intraperitoneálne vo forme suspenzie vo fyziologickom roztoku (PBS; pH 7,4) v dávke 1,5 mg na potkana v objeme 1,2 ml 24 hodín pred celotelovým ožiarením jednorazovými dávkami 3, 6 a 9 Gy, alebo v dávke 3 mg na potkana 24 hodín pred celotelovým ožiarením jednorazovou dávkou 6 Gy. Kontrolným zvieratám sme podali v rovnakom čase pred PHE fyziologický roztok v objeme 1,2 ml.

Podmienky ožarovania

Zvieratá boli celotelove ožiarené jednorazovými dávkami 3, 6 a 9 Gy gama žiarenia (0,348 Gy/min) 24 hodín po aplikácii Broncho-Vaxomu. Ako zdroj žiarenia bol použitý ^{60}Co (Chisostat, Česká republika).

Parciálna hepatektómia (PHE)

Všetky skupiny potkanov boli podrobené 2/3 parciálnej hepatektómii (odstránený bol stredný lalok – *lobus medialis* a ľavý laterálny lalok – *lobus sinister lateralis*) podľa štandardného postupu do 30 minút po ukončení ožiarenia, resp. na 6. a 13. deň po ožiarení. Zvieratá sme operovali v celkovej éterovej narkóze vždy v ranných hodinách (8.00 až 10.00) a vyšetrili na 30. hodinu po operácii, t.j. v čase maxima proliferatívnej aktivity indukovanej parciálnou hepatektómiou.

Metódy

Z tkaniva regenerujúceho zvyšku pečene sme zhotovili roztlakové preparáty farbené Feulgenovou metódou. Pri vyhodnotení približne 50 000 buniek v každej vyšetrovanej skupine sme zaznamenali všetky mitotické figúry a chromozómové aberácie (mostíky a acentrické fragmenty) v postmetafáze. Na základe týchto údajov sme vypočítali mitotický index (MI), ktorý udáva počet mitotických figúr pripadajúcich na registrovaných 1 000 buniek a percento chromozómových aberácií z celkového počtu postmetafázových figúr nájdených medzi uvedeným počtom vyhodnotených buniek.

Štatistickú významnosť sme hodnotili Peritzovým *F*-testom (Harper, 1984).

VÝSLEDKY

Mitotický index (MI) v regenerujúcej pečeni kontrolných zvierat (K) v čase maxima, t.j. na 30. hodinu po PHE, dosiahol hodnotu 27,14 ± 0,51 ‰ (obr. 1, tab. I). Podanie Broncho-Vaxomu (BV) výrazne stimulovalo proces regenerácie pečene neožiarených zvierat. Hodnoty MI v regenerujúcej pečeni neožiarených zvierat, ktorým bol 24 hodín pred operáciou podaný Broncho-Vaxom, boli v porovnaní s príslušnými kontrolami (K) štatisticky významne zvýšené v prvý deň po dávke 1,5 mg (BV – 1,5 mg) a najmä po dávke 3 mg (BV – 3 mg) a udržiavali sa približne na rovnakej úrovni počas 14dňového vyšetrovaného obdobia (tab. IA, B, C, resp. D).

Jednorazové ožiarenie dávkami 3, 6 a 9 Gy spôsobilo výraznú inhibíciu mitotickej aktivity, ktorá sa prejavila redukciou počtu mitotických figúr v porovnaní s príslušnými kontrolami v priebehu celého vyšetrovaného obdobia. Rozsah inhibície mitotickej aktivity závisel od veľkosti dávky. Po jednorazovom ožiarení dávkou 3 Gy (O – 3 Gy) počet mitotických figúr klesol na hodnotu 15,11 ± 0,32 ‰, t.j. bol takmer dvojnásobne nižší ako v regenerujúcej pečeni kontrolných zvierat (K) (obr. 1A, tab. IA). Ožiarenie dávkami 6 Gy (O – 6 Gy) a 9 Gy (O – 9 Gy) spôsobilo inhibíciu mitotickej aktivity, ktorá sa prejavila približne štvornásobným, resp. deväťnásobným znížením počtu mitóz v porovnaní s príslušnými neožiarenými kontrolnými zvieratami (obr. 1B, C, tab. IB, C).

Inhibícia mitotickej aktivity indukovaná žiarením bola zmiernená podaním Broncho-Vaxomu. Aplikácia tohto imunomodulátora (1,5 mg) zvieratám ožiareným dávkou 3 Gy (BV – 1,5 mg + O – 3 Gy) sa prejavila signifikantným zvýšením hodnôt MI na prvý a siedmy deň vyšetrenia v porovnaní s nechránenými ožiarenými zvieratami (O – 3 Gy) (obr. 1A, tab. IA). Mierny protektívny účinok Broncho-Vaxomu na zmeny MI spôsobené ožiarením dávkami 6 Gy (BV – 1,5 mg + O – 6 Gy) a 9 Gy (BV – 1,5 mg + O – 9 Gy) sa prejavil počas celého vyšetrovaného obdobia (obr. 1B, C, tab. IB, C). Účinok imunomodulačnej látky aplikovanej v dávke 3 mg na potkana sa prejavil u zvierat ožiarených dávkou 6 Gy štatisticky signifikantným zmiernením zmien MI v približne rovnakom rozsahu ako po dávke 1,5 mg (obr. 1D, tab. ID).

Aberantné postmetafázy v regenerujúcej pečeni kontrolných zvierat (K) a zvierat, ktorým bol 24 hodín pred operáciou podaný Broncho-Vaxom (BV – 1,5 mg) a (BV – 3 mg), sme zaznamenali v 1,5 až 2,5 % postmetafázových buniek v priebehu celého vyšetrovaného obdobia (obr. 2, tab. II).

Celotelové ožiarenie zvierat vyvolalo prudký vzrast počtu chromozómových aberácií v regenerujúcej pečeni v závislosti od veľkosti dávky do výšky 6 Gy. Po jednorazovej dávke 3 Gy (O – 3 Gy) sme na prvý deň vyšetrenia zaznamenali 52,44 ± 1,72 % chromozómových aberácií (obr. 2A, tab. IIA). Po dávke 6 Gy (O – 6 Gy) vzrástlo percento aberácií na 98,15 ± 1,75 % a po dávke 9 Gy (O – 9 Gy) až na 100 % (obr. 2B, resp. 2C, tab. IIB, resp. IIC).. Počas ďalších vyšetrovaných intervalov, t.j. na siedmy a 14. deň po ožiarení dávkami 3 a 6 Gy sme zaznamenali mierny pokles percenta chromozómových aberácií.

Podanie Broncho-Vaxomu v dávke 1,5 mg 24 hodín pred ožiarením 3 a 6 Gy (BV – 1,5 mg + O – 3 Gy a BV 1,5 mg + O – 6 Gy) zmiernilo vzrast frekvencie chromozómových aberácií v porovnaní s ožiarenými nechránenými zvieratami počas celého vyšetrovaného obdobia (obr. 2A, B, tab. IIA, B).

I. Mitotický index (‰) v regenerujúcej pečeni potkanov po ožiarení rôznymi jednorázovými dávkami gama žiarenia (O) a aplikácii Broncho-Vaxomu (BV); priemerné hodnoty S.E.M. – Mitotic index in regeneration rat liver after irradiation by different single doses of gamma radiation (O) and administration of Broncho-Vaxom (BV); means ± S.E.M.

Skupina[1]		Dni po ožiarení[2]		
		1. deň[3]	7. deň[4]	14. deň[5]
A	K	27,11 ± 0,51	27,68 ± 0,43	26,84 ± 0,56
	BV – 1,5 mg	31,06 ± 0,75^{x}	30,23 ± 0,76^{x}	32,64 ± 0,48^{xx}
	O – 3 Gy	14,80 ± 0,31^{xx}	14,16 ± 0,52^{xx}	15,74 ± 0,28^{xx}
	BV – 1,5 mg + O – 3 Gy	17,24 ± 0,27$^{xx\ o}$	16,48 ± 0,38$^{xx\ o}$	15,24 ± 0,32^{xx}
B	K	27,11 ± 0,51	27,68 ± 0,43	26,84 ± 0,56
	BV – 1,5 mg	31,06 ± 0,75^{x}	30,23 ± 0,76^{x}	32,64 ± 0,48^{xx}
	O – 6 Gy	7,08 ± 0,31^{xx}	6,88 ± 0,24^{xx}	7,24 ± 0,64^{xx}
	BV – 1,5 mg + O – 6 Gy	7,78 ± 0,32^{xx}	8,05 ± 0,74$^{xx\ o}$	8,55 ± 0,82^{xx}
C	K	28,64 ± 0,58	27,01 ± 0,91	27,92 ± 0,85
	BV – 1,5 mg	32,84 ± 0,24^{xx}	31,05 ± 0,50^{xx}	32,64 ± 0,32^{xx}
	O – 9 Gy	3,28 ± 0,27^{xx}	3,02 ± 0,27^{xx}	3,98 ± 0,43^{xx}
	BV – 1,5 mg + O – 3 Gy	4,58 ± 0,33^{xx}	4,98 ± 0,36$^{xx\ o}$	4,88 ± 0,44^{xx}
D	K	28,64 ± 0,56	27,01 ± 0,91	27,92 ± 0,85
	BV – 3 mg	34,24 ± 0,98^{xx}	34,27 ± 0,81^{xx}	32,98 ± 0,44^{xx}
	O – 6 Gy	6,72 ± 0,24^{xx}	7,44 ± 0,84^{xx}	6,88 ± 0,32^{xx}
	BV – 3 mg + O – 6 Gy	8,84 ± 0,72$^{xx\ o}$	9,24 ± 0,34$^{xx\ o}$	8,29 ± 0,64$^{xx\ o}$

x = $P \leq 0,05$; xx = $P \leq 0,001$ v porovnaní s kontrolami – x = $P \leq 0.05$; xx = $P \leq 0.001$ in comparison with controls
o = $P \leq 0,05$ v porovnaní s ožiarenými nechránenými zvieratami – o = $P \leq 0.05$ in comparison with irradiated nonprotected animals
K = kontrola – control

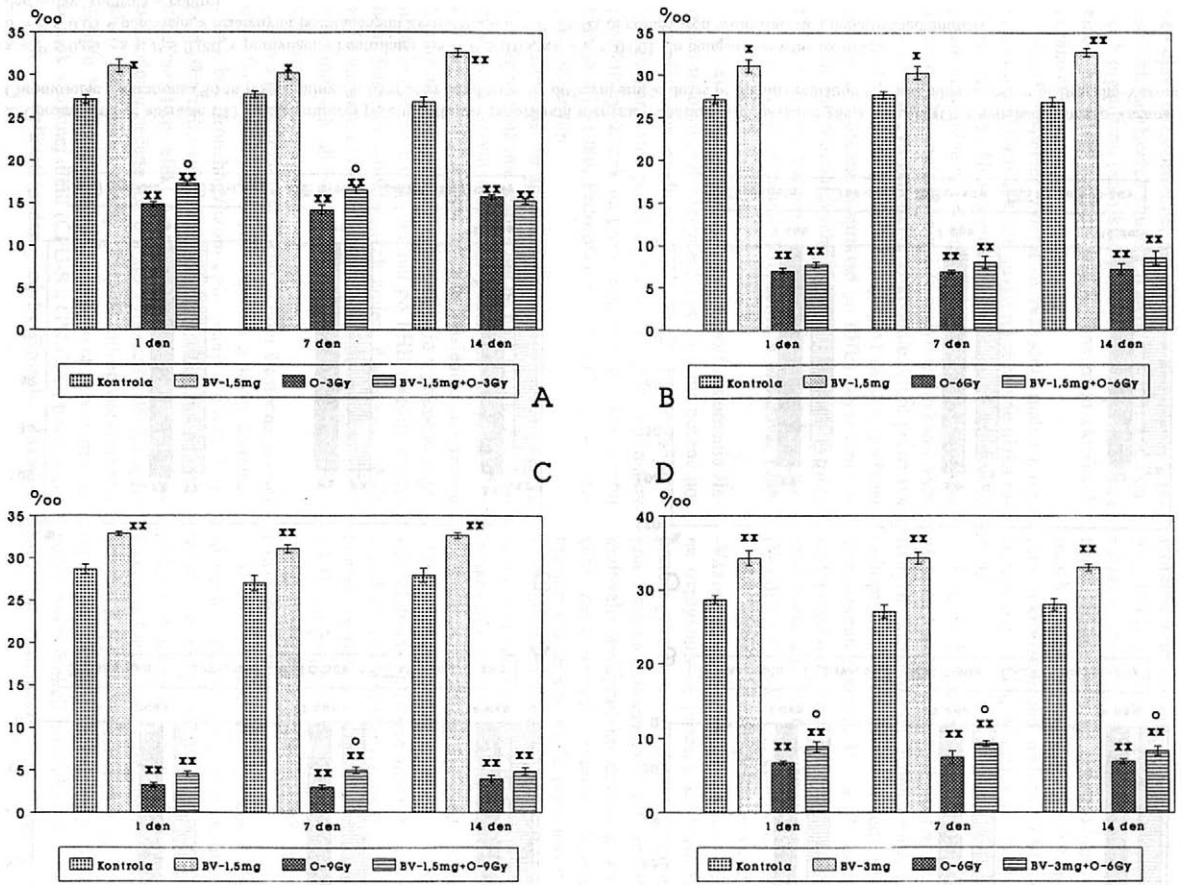
[1]group, [2]days after irradiation, [3]day 1, [4]day 7, [5]day 14

II. Chromozómové aberácie (%) v regenerujúcej pečeni potkanov po ožiarení rôznymi jednorázovými dávkami gama žiarenia (O) a aplikácii Broncho-Vaxomu (BV); priemerné hodnoty S.E.M. – Chromosome aberrations (%) in regenerating rat liver after irradiation by different single doses of gamma radiation (O) and administration of Broncho-Vaxom (BV); means ± S.E.M.

Skupina[1]		Dni po ožiarení[2]		
		1. deň[3]	7. deň[4]	14. deň[5]
A	K	1,33 ± 1,08	2,27 ± 1,05	1,78 ± 1,24
	BV – 1,5 mg	1,85 ± 1,04	1,55 ± 0,89	2,04 ± 1,54
	O – 3 Gy	52,44 ± 1,72^{xx}	53,78 ± 2,83^{xx}	50,56 ± 2,40^{xx}
	BV – 1,5 mg + O – 3 Gy	48,52 ± 1,56^{xx}	49,72 ± 2,84^{xx}	47,05 ± 2,50^{xx}
B	K	1,33 ± 1,08	2,27 ± 1,05	1,78 ± 1,24
	BV – 1,5 mg	1,85 ± 1,04	1,55 ± 0,89	2,04 ± 1,54
	O – 6 Gy	98,15 ± 1,85^{xx}	94,56 ± 0,89^{xx}	92,68 ± 1,85^{xx}
	BV – 1,5 mg + O – 6 Gy	94,28 ± 2,49^{xx}	88,94 ± 1,94^{xx}	85,75 ± 2,85$^{xx\ o}$
C	K	1,84 ± 1,02	2,33 ± 2,04	2,05 ± 1,84
	BV – 1,5 mg	1,04 ± 0,95	1,94 ± 0,98	1,75 ± 1,06
	O – 9 Gy	100,00 ± 0,00^{xx}	97,22 ± 2,08^{xx}	98,98 ± 2,09^{xx}
	BV – 1,5 mg + O – 3 Gy	98,70 ± 2,06^{xx}	97,02 ± 2,82^{xx}	96,54 ± 2,36^{xx}
D	K	1,084 ± 1,02	2,33 ± 2,04	2,05 ± 1,84
	BV – 3 mg	0,94 ± 0,45	1,05 ± 0,52	1,52 ± 0,82
	O – 6 Gy	94,44 ± 2,09^{xx}	96,85 ± 1,82^{xx}	92,34 ± 1,65^{xx}
	BV – 3 mg + O – 6 Gy	86,54 ± 2,15$^{xx\ o}$	90,45 ± 2,85$^{xx\ o}$	88,44 ± 2,28^{xx}

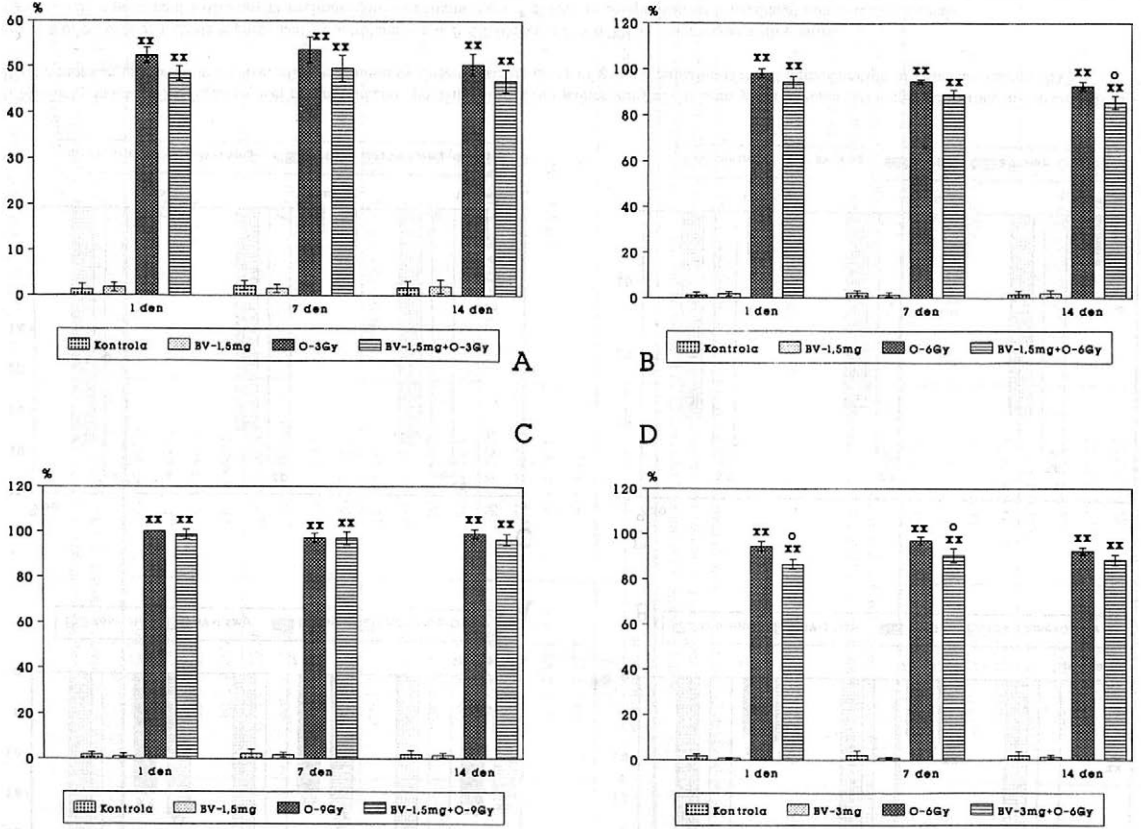
x = $P \leq 0,05$; xx = $P \leq 0,001$ v porovnaní s kontrolami – x = $P \leq 0.05$; xx = $P \leq 0.001$ in comparison with controls
o = $P \leq 0,05$ v porovnaní s ožiarenými nechránenými zvieratami – o = $P \leq 0.05$ in comparison with irradiated nonprotected animals
K = kontrola – control

[1]group, [2]days after irradiation, [3]day 1, [4]day 7, [5]day 14



1. Mitotický index (‰) v regenerujúcej pečeni potkanov po ožiarení rôznymi jednorazovými dávkami gama žiarenia (O) a aplikácii Broncho-Vaxomu (BV) – Mitotic index in regeneration rat liver after irradiation by different single doses of gamma radiation (O) and administration of Broncho-Vaxom (BV)

x = $P \leq 0{,}05$; xx = $P \leq 0{,}001$ v porovnaní s kontrolami – x = $P \leq 0.05$; xx = $P \leq 0.001$ in comparison with controls
o = $P \leq 0{,}05$ v porovnaní s ožiarenými nechránenými zvieratami – o = $P \leq 0.05$ in comparison with irradiated nonprotected animals
den – day, kontrola – control



2. Chromozómové aberácie (%) v regenerujúcej pečeni potkanov po ožiarení rôznymi jednorazovými dávkami gama žiarenia (O) a aplikácii Broncho-Vaxomu (BV) – Chromosome aberrations (%) in regenerating rat liver after irradiation by different single doses of gamma radiation (O) and administration of Broncho-Vaxom (BV)

x = $P \leq 0{,}05$; xx = $P \leq 0{,}001$ v porovnaní s kontrolami – x = $P \leq 0.05$; xx = $P \leq 0.001$ in comparison with controls
o = $P \leq 0{,}05$ v porovnaní s ožiarenými nechránenými zvieratami – o = $P \leq 0.05$ in comparison with irradiated nonprotected animals
den – day, kontrola – control

Účinok imunomodulačnej látky aplikovanej v dávke 3 mg bol podobný ako po dávke 1,5 mg (obr. 2D, tab. IID).

DISKUSIA

Skúmanie rádioprotektívnych účinkov niektorých fyzikálnych zásahov, chemických alebo imunostimulačných preparátov je založené väčšinou na sledovaní životne dôležitých ukazovateľov poškodenia ako je mortalita zvierat alebo stav krvotvorby (Fedoročko a i., 1992, 1994; Nagata a i., 1994). Z teoretického i praktického hľadiska pokladáme za potrebné poznať možnosti ochrany aj pred latentným poškodením, ktoré sa neprejaví bezprostredne, ale môže sa manifestovať pri určitých záťažových situáciách alebo patologických stavoch organizmu (napríklad po rozsiahlych poraneniach alebo parciálnej resekcii proliferačne málo aktívnych orgánov).

Pečeň dospelých zvierat sa vyznačuje malou proliferatívnou aktivitou, s čím súvisí jej relatívne nízka rádiosenzitivita. Histologické a cytologické zmeny sú priamo zistiteľné až pri letálnych dávkach ionizujúceho žiarenia. Ožiarenie rôznymi (i malými) dávkami však vyvoláva aj cytogenetické poškodenie, ktoré pretrváva v latentnej forme dlhú dobu a prejaví sa až v priebehu regenerácie pečene (napríklad po PHE) zvýšeným výskytom chromozómových aberácií, inhibíciou syntézy DNA a mitózy, zmenou kinetiky bunkového cyklu a niektorými inými zmenami (Kropáčová a Mišúrová, 1988, 1995).

V našej práci sme sledovali možnosť zmiernenia žiarením indukovaného latentného poškodenia pečene podávaním imunomodulačného preparátu Broncho-Vaxomu. Preparát sme podávali v čase, kedy má optimálny protektívny účinok na prežívanie zvierat a krvotvorbu (Šimek a i., 1986; Fedoročko a i., 1994), t.j. 24 hodín pred ožiarením.

Zistili sme, že aplikácia Broncho-Vaxomu v dávke 1,5 mg na potkana, resp. 3 mg na potkana *i.p.* 24 hodín pred celotelovým ožiarením použitými dávkami 3, 6 a 9 Gy gama žiarenia, mala za následok zmiernenie latentného poškodenia, čo sa prejavilo v priebehu regenerácie pečene na 30. hodinu po PHE menším rozsahom žiarením indukovaných zmien, čomu nasvedčuje zvýšenie mitotickej aktivity a zníženie výskytu chromozómových aberácií v porovnaní s ožiarenými nechránenými zvieratami.

Rozsah rádioprotektívneho účinku čiastočne závisel od veľkosti dávky Broncho-Vaxomu a dávky žiarenia.

Ak bol Broncho-Vaxom aplikovaný v dávke 3 mg pred ožiarením subletálnou dávkou 6 Gy, bol jeho rádioprotektívny efekt na rozvoj latentného radiačného poškodenia pečene iba o málo výraznejší ako po aplikácii polovičnej dávky. Stupeň rádioprotektívneho účinku hodnotený počtom kmeňových krvotvorných buniek v kostnej dreni (CFU-S a GM-CFC) bol závislý nielen od veľkosti podanej dávky Broncho-Vaxomu, ale aj od jeho aplikácie pred, resp. po ožiarení subletálnou dávkou (Fedoročko a i., 1992).

Naše nálezy potvrdzujú údaje autorov Haková a i. (1997), svedčiace o beneficiálnom účinku Broncho-Vaxomu (1,5 mg na potkana *i.p.* 24 hodín pred ožiarením dávkami 3, 6 a 9 Gy), ktorý sa prejavil počas celého vyšetrovaného obdobia zmiernením postiradiačných zmien RNA a DNA v regenerujúcej pečeni i slezine potkanov. Podanie Broncho-Vaxomu zmiernilo aj zmeny koncentrácie a obsahu histónov v regenerujúcej pečeni potkanov (Kožurková a i., 1998).

Mechanizmus rádioprotektívneho účinku Broncho-Vaxomu nie je presne známy. Experimentálne štúdie naznačujú, že Broncho-Vaxom zvyšuje celulárnu a humorálnu imunitnú odpoveď (Bosch a i., 1983; Emerich a i., 1990). Zvyšuje počet a fagocytárnu aktivitu makrofágov, stimuluje sekréciu cytokínov a mediátorov, napríklad interferónov, interleukínov, faktorov stimulujúcich kolónie hematopoetických buniek (CSF) a faktora nekrotizujúceho nádory (TNF) (Podleski, 1985; Bottex a i., 1988; Mauel a i., 1989; Fedoročko a i., 1992).

Viacero biologických účinkov tohto bakteriálneho extraktu umožňuje predpokladať aj viacero rádioprotektívnych mechanizmov. Jeden z možných mechanizmov rádioprotektívneho účinku je teda Broncho-Vaxomom stimulovaná sekrécia IL_1 a prostaglandínov, ktorých rádioprotektívny efekt bol sledovaný v podmienkach *in vivo* (Neta a i., 1986; Schwartz a i., 1989) aj *in vitro* (Walden a i., 1987; Galicchio a i., 1989).

Z výsledkov našich pokusov vyplýva, že Broncho-Vaxom nemá len rádioprotektívny účinok, ale pozitívne ovplyvňuje aj procesy kompenzačnej regenerácie, pretože jeho podanie v dávke 1,5 mg na potkana, a najmä 3 mg na potkana 24 hodín pred PHE, výrazne stimulovalo proliferatívnu aktivitu aj v regenerujúcej pečeni neožiarených zvierat. Domnievame sa, že regeneráciu pečene môže podporovať sekrécia prostaglandínov z aktivovaných makrofágov tým, že stimulujú prechod hepatocytov z interkinetickej G_0 do G_1 fázy bunkového cyklu (Kanzaki, 1979).

Imunomodulačné látky, medzi ktoré patrí aj Broncho-Vaxom, pôsobia stimulačne predovšetkým prostredníctvom aktivácie makrofágov, ktoré sú známe v pečeni ako Kuppferove bunky. Z celkového počtu buniek vystielajúcich pečeňové sinusoidy pripadá až 40 % na Kuppferove bunky. Zabezpečujú spojenie medzi parenchýmom pečene a kostnou dreňou, kde sa tvoria monocyty.

Tkanivové makrofágy pečene – Kuppferove bunky sú najdôležitejšou zložkou mononukleárneho fagocytárneho systému. Ak je tento systém poškodený, napríklad tetrachlórmetánom, môžu sa rozvinúť rôzne infekčné ochorenia alebo orgánové zmeny. Od funkčnej aktivity Kuppferových buniek závisí teda nielen proces reparačnej regenerácie pečene po PHE, ale aj obnova pečeňových funkcií poškodených rôznymi patogénnymi noxami (Majanskij, 1981; Nagata a i., 1994).

Kuppferove bunky sú zásobárňou lyzozómových enzýmov. Po stimulácii Kuppferových buniek bakteriálnym polysacharidom vzrastá fagocytárna aktivita týchto tkanivových makrofágov a sekrécia a aktivita lyzozómových enzýmov. Lyzozómové enzýmy môžu byť uvoľňované z aktivovaných makrofágov do prostredia a môžu sa podieľať na regulácii syntézy DNA a proliferatívnej aktivity hepatocytov po parciálnej hepatektómii. Mitogénne vlastnosti majú napríklad lyzozómové proteázy (Majanskij, 1981). Aktivácia pečeňových makrofágov môže mať vplyv aj na migráciu lymfocytov do regenerujúcej pečene, čo prispieva k stimulácii proliferatívnej aktivity hepatocytov (Babajeva a i., 1969). V aktivovaných makrofágoch vzrastá aj sekrécia cytokínov, ktoré ovplyvňujú bunkový rast, diferenciáciu a aktiváciu imunokompetentných buniek organizmu (Andus a Holstege, 1994). Medzi cytokíny produkované aktivovanými makrofágmi patrí aj faktor nekrotizujúci nádory (TNF), ktorý stimuluje syntézu DNA a mitotickú aktivitu hepatocytov v intaktnej i regenerujúcej pečeni (Beyer a i., 1990).

Niektoré baktérie črevnej mikroflóry tiež produkujú lipopolysacharid, ktorý patrí k najúčinnejším aktivátorom makrofágov stimulujúcich regeneráciu pečene aj u ožiarených zvierat (Majanskij, 1981).

Ak boli Kuppferove bunky stimulované endotoxínom *Escherichia coli* alebo prodigiosanom (polysacharid zo *Serratia marcenses*) 24 hodín pred PHE, nástup a rozvoj regeneračného procesu bol intenzívnejší v porovnaní s nestimulovanými potkanmi. Keď bol endotoxín injikovaný tri hodiny po PHE, syntéza DNA bola čiastočne inhibovaná (Šimek a i., 1986). Ak bola aktivita Kuppferových buniek potlačená aj proces regenerácie pečene po PHE bol zabrzdený približne o 10 hodín (Majanskij, 1981).

Z uvedených informácií vidno, že zmeny aktivity Kuppferových buniek majú vplyv na proliferatívnu aktivitu hepatocytov. Stimulácia procesu regenerácie pečene po podaní imunomodulátorov vrátane Broncho-Vaxomu nie je pravdepodobne spôsobená priamo, ale prostredníctvom mediátorov uvoľnených zo stimulovaných makrofágov.

LITERATÚRA

Andus T., Holstege A. (1994): Cytokines and the liver in health and disease. Effects on liver metabolism and fibrogenesis. Acta Gastroenterol. Belg., *57*, 236–244.

Babajeva A. G., Kraskina N. A., Liozner L. D. (1969): Stimulacija proliferativnoj aktivnosti kletok pečeni neoperirovanych myšej limfoidnimi kletkami častično gepatektomirovannych donorov. Byull. Exp. Biol. Med., *34*, 91–94.

Barbason H., Rassenfosse C., Betz E. H. (1983): Promotion mechanism of phenobarbital and partial hepatectomy in DENA hepatocarcinogenesis cell kinetics effect. Brit. J. Cancer., *47*, 517–525.

Beyer H. S., Stanley M., Theologides A. (1990): Tumor necrosis factor-α increases hepatic DNA and RNA and hepatocyte mitosis. Biochem. Int., *22*, 405–410.

Bosch A., Lucena F., Pares R., Jofre J. (1983): Bacterial immunostimulant (Broncho-Vaxom) versus levamisole on the humoral immune response in mice. Int. J. Immunopharmacol., *5*, 107–116.

Bottex C., Cristan B., Corazza J. L., Mougin B., Fontanges R. (1988): Effect of two bacterial extracts, OM-89 and Broncho-Vaxom, on IL-l release and metabolic activity of murine macrophage cell-line. Int. J. Immunother., *4*, 203–212.

Bucher U. L. R. (1991): Liver regeneration: An overview. J. Gastroenterol. Hepatol., 615–624.

Chamuleau, R. A. F. M., Bosman, D. K. (1988): Liver regeneration. Hepato-gastroenterol., *35*, 300–312.

Emmerich B., Emslander H. P., Pachman K., Hallek M., Milatovic D., Busch R. (1990): Local immunity in patients with chronic bronchitis and the effects of a bacterial extract, Broncho-Vaxom, on T lymphocytes, macrophages, gamma interferon and secretory immunoglobulin A in bronchoalveolar lavage fluid and other variables. Respiratorion, *57*, 90–97.

Fedoročko P., Brezáni P., Macková N. O. (1992): Radioprotection of mice by the bacterial extract Broncho-Vaxom: haemopoietic stem cells and survival enhancement. Int. J. Radiat. Biol., *61*, 511–518.

Fedoročko P., Brezáni P., Macková N. O. (1994): Radioprotective effects of WR-2721, Broncho-Vaxom and their combinations: survival, myelopoietic restoration and induction of colony-stimulating activity in mice. Int. J. Immunopharmac., *16*, 177–184.

Galicchio V. S., Hulette B. C., Messino M. J., Gass C., Bieschke M. W., Doukas M. A. (1989): Effect of various interleukins (IL-l, IL-2 and IL-3) on the *in vitro* radioprotection of bone marrow progenitors (CFU-GM and CFU--MEG). J. Biol. Respon. Modif., *8*, 479–487.

Haková H., Mišúrová E., Kropáčová K. (1997): Modification of postradiative changes of nucleic acids by the bacterial extract Broncho-Vaxom in rat tissues. Folia Biol. (Praha), *4*, 231–237.

Harper J. (1984): Basic program of a robust multiple comparison test for statistical analysis of all differences among group means. Comput. Biol. Med., *14*, 437–445.

Kanzaki Y., Mahmud I., Miura Y. (1979): Tromboxane as possible trigger of liver regeneration. Cell. Mol. Biol., *25*, 147–152.

Kožurková M., Fedoročko P. (1998): Vplyv Broncho-Vaxomu a indometacínu na históny v pečeni ožiarených myší. In: Fedoročko P., Brezáni P., Toropila (eds.): Aktuálne problémy súčasnej biológie a rádiobiológie. Košice, 97–101.

Kropáčová K., Mišúrová E. (1981): Mitotic activity and chromosomal aberrations in the rat regenerating liver after x-rays exposure (in Russian). Byull. Exp. Biol. Med., *91*, 359–361.

Kropáčová K., Mišúrová E. (1988): Duration of latent injury to liver after the cessation of continuous gamma-irradiation (in Russian). Radiobiologiya, *28*, 44–47.

Kropáčová K., Mišúrová E. (1990): Radiation-induced latent injury in liver and its influencing by radioprotectants. Curr. Trends Cosmic Biol. Med., *1*, 143–148.

Kropáčová K., Mišúrová E. (1992): Influence of age and gamma irradiation on the proliferative activity in regenerating rat liver. Physiol. Res., *41*, 135–140.

Kropáčová K., Mišúrová E. (1995): The influence of essential phospholipids (ESSENTIALE) on liver regeneration in gamma irradiated rats. Physiol. Res., *44*, 241–247.

Majanskij, D. N. (1981): Kletka Kuppferai i sistema mononuklearnych fagocitov. Nauka, 172.

Mauel J., Pham T. V., Kreis B., Bauer J. (1989): Stimulation by a bacterial extract (B-V) of the metabolic and functional activities of murine macrophages. Int. J. Immunopharmacol., *11*, 637–645.

Michalopoulos G. K. (1990): Liver regeneration: molecular mechanisms of growth control. Faseb J., *4*, 176–187.

Nagata, Y., Tanaka N., Orita K. (1994): Endotoxin-induced liver injury after extended hepatectomy and the role of Kuppfer cells in the rat. Surg. Today, *24*, 441–448.

Neta R., Douches S., Oppenheim J. J. (1986): Interleukin 1 is a radioprotector. J. Immunol, *136*, 2483–2485.

Podleski W. K. (1985): Immunomodulation of allergic autocytotoxicity in bronchial asthma by a bacterial lysate – Broncho-Vaxom. Int. J. Immunopharmacol., *7*, 713–718.

Puigdollers J. M., Rodis Serna G., Hernandez del Rey L., Tillo Barruffet M. T., Jofre Torroella J. (1980): Stimulation de la production d'immunoglobulines chez l'homme par l'homme par l'administration orale d'un lysat bacterien. Respiration, *40*, 142–149.

Schwartz G. N., Pathechn M. L., Neta R., Mac Vittie T. J. (1989): Radioprotection of mice with interleukin-1: Relationship to the number of spleen colony-forming units. Radiat. Res., *119*, 101–112.

Šimek J., Shcherbakov V. I., Mayansky D. N., Vošvrdová H., Červinková Z., Holeček M. (1986): Effect of the activation of macrophages on the course of regeneration of rat liver following partial hepatectomy. Physiol. Bohemoslov,. *35*, 473–480.

Šimek J., Červinková Z., Holeček M. (1989): Úloha funkční zátěže v mechanismu jaterní regenerace. Československ. Fysiol., *38*, 219–240.

Walden T. L., Patchen M. L., Snyder S. L. (1987): Dimethyl prostaglandin E_2 increases survival in mice following irradiation. Radiat. Res., *109*, 440–448.

Zarnegar R. (1992): Regulatory signals in liver regeneration. Cell. Mol. Asp. Cirrhosis., *216*, 245–254.

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Key words should make it possible to retrieve the paper on the basis of the animal species investigated, characteristics of their health, husbandry conditions, applied substances, etc. The terms used in the paper title should not be used as keywords.

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The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telephone and fax number, or e-mail.

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