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INDUCTION OF FIRST POSTPARTUM OVULATION BY GnRH ANALOGUE IN SUCKLED DAIRY COWS

INDUKCIA PRVEJ POSTPARTÁLNEJ OVULÁCIE POMOCOU GnRH U DOJNÍC S ODCHOVOM TELIAT CICANÍM

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ABSTRACT: The aim of the experiment was to assess the ovarian response to synthetic Gonadotropin-releasing hormone (GnRH) treatment in suckled dairy cows. Experimental cows ($n = 10$) were treated i.m. with 25 and 50 μg GnRH analogue (Ferring – Léciva, Praha, Czech Republic) on days 12 and 13 post partum. Five control cows were left untreated. The ovarian response to hormonal treatment was evaluated daily by rectal palpation of the ovaries. From days 4 to 8 (second treatment = day 0) serum progesterone concentrations (P4; RIA-test PROGS kit, HUMA-LAB, Košice, Slovak Republic) were quantified. Animals in the control group were checked in the same way as described above. The calves from parturition throughout the experimental period were reared near their mother by suckling *ad libitum*. In the experimental group dominant follicles ovulated in 4/10 cows (Tab. 1) with elevated P4 concentrations in blood serum. In another four cows (4/10) P4 elevated without ovulation which was probably due to luteinization of the large follicle. The elevation of P4 in these cows continued 6 days after treatment and then gradually decreased as compared to the continual elevation until the end of P4 determination in the ovulated group (Fig. 1). In non-treated control animals four cows ovulated on day 27.3 ± 2.9 . The first postpartum dominant follicle in one control cow become cystic. It can be concluded that the treatment with by GnRH in early puerperal period may induce ovulation and/or luteinization of large follicles in suckled dairy cows.

dairy cattle; suckling; GnRH; induction of ovulation

ABSTRACT: Zámerom práce bolo zistiť ovariálnu odpoveď u dojníc s odchovom teliat pri matkách cicaním na podanie syntetického gonadotropín-uvolňujúceho hormónu (GnRH) v skorom popôrodnom období. Dojniciam v pokusnej skupine ($n = 10$) na 12. a 13. deň po pôrode bolo intramuskulárne podané 25 a 50 μg superanalógu GnRH (Ferring – Léciva, Praha, ČR). Ovariálna odpoveď na podaný preparát sa sledovala denným rektálnym vyšetrením vaječníkov a od 4. do 8. dňa (deň 2. aplikácie = deň 0) sa súčasne vyhodnocovala koncentrácia progesterónu (P4; RIA-test PROGS, HUMA-LAB, Košice, SR) v krvnom sére, z odberov periférnej krvi (v. *jugularis*). V neošetrenej kontrolnej skupine ($n = 5$) sa ovariálna aktivita posudzovala obdobne. Teliatá od pôrodu, ako aj počas celého obdobia sledovania, boli odchovávané pri kravách s možnosťou cicania matiek. V pokusnej skupine sme zaznamenali v 40 % prípadov (4/10) indukovanú ovuláciu a tvorbu žltého telieska. U ďalších 40 % dojníc (4/10) sa zistilo zvyšovanie koncentrácie P4 v jugulárnej krvi bez potvrdenej ovulácie dominantného folikulu. V tejto skupine kráv od 6. dňa od ošetrovania sme zaznamenali pokles priemerných koncentrácií P4 oproti kontinuálnemu zvyšovaniu P4 s potvrdenou ovuláciou až do konca sledovania. Ošetrovanie dojníc v skorom postpartálnom období s odchovom teliat cicaním vo väčšine prípadov môže indukovať ovuláciu, resp. luteinizáciu folikulov.

dojnice; dojenie teliat; GnRH; indukovaná ovulácia

ÚVOD

Pôrodom končí jedna dôležitá etapa reprodukčného cyklu kráv a po rôzne dlho trvajúcom „oddychu“ je organizmus matky znova pripravený k novej koncepcii. Skôr než je maternica schopná poskytnúť vhodné prostredie pre spermie a oplodnené vajíčko, na pohlavnom aparáte prebiehajú involučné pochody a vaječníky sú v tzv. období kľudu. Tento stav označujeme ako popôrodná anestria. Dĺžka anovulačného obdobia je ovplyvňovaná výživou, telesnou kondíciou, cicaním te-

liat, laktáciou, dystókiami, plemennou príslušnosťou, vekom, mesiacom otelenia, patológiou maternice a chronickými oslabujúcimi ochoreniami (Zemjanis, 1961; Kaltenbach, 1980; Lamming a i., 1981; Tucker, 1982 a iní).

Postpartálne obdobie kráv je charakterizované veľmi skorým začiatkom rastu folikulov na vaječníkoch s odchovom teliat napájaním mimo matiek (Savio a i., 1990; Roche a i., 1992), ale aj s odchovom teliat cicaním (Dimmick a i., 1991). Na druhej strane prvá postpartálna ovulácia u kráv s odchovom teliat cica-

ním je zisťovaná signifikantne neskoršie v porovnaní s dojnicami pri odchove teliat napájaním.

Pominutie inhibičného účinku gravidity a opätovná citlivosť hypofýzy na podanie GnRH, ako aj folikulárny rast vytvárajú predpoklady pre prvú popôrodnú ovuláciu (Britt a i., 1974; Kesler a i., 1977). Ošetrenie kráv v skorom popôrodnom období bolo vyvolávané z dôvodov experimentálnych, ako aj klinických. Odchov teliat cicaním pri kravách oddaluje prvú postpartálnu ovuláciu, avšak podanie syntetického GnRH u mäsových plemien môže indukovať ovuláciu (Crowe a i., 1993; Twagiramungu a i., 1994). Indukcia prvej popôrodnej ovulácie u mliekového typu kráv s odchovom teliat cicaním nebola popísaná.

Zámerom nášho príspevku bolo sledovať ovariálnu odpoveď na podanie syntetického GnRH u dojníc v skorom popôrodnom období s odchovom teliat pri kravách a s možnosťou voľného cicania.

MATERIÁL A METÓDA

Do sledovania v terénnych podmienkach sme zaradili 15 dojníc držaných v klasickej maštali s vážnym ustajnením. Kravy sa telili začiatkom marca. Dve kravy, ktoré mali puerperálnu endometritídu sa rozdelili do pokusnej a kontrolnej skupiny. Ostatné kravy mali nekomplikovaný priebeh puerperia. Po pôrode teliat boli priviazané ku kŕmnyň žliabom k svojim matkám. Ich odchov sa praktizoval cicaním vlastných matiek. Na 12. a 13. deň po pôrode desiatim kravám (pokusná skupina) bolo vnútrošvalovo podané 25 a 50 µg superanalógu GnRH – Lecirelinum (Ferring – Lčičiva, Praha, ČR). V deň podania prvej dávky sa vykonalo klinické vyšetrenie každej dojnice. Gynekologické vyšetrenie sa zameralo najmä na priebeh involúcie pohlavných orgánov a popôrodný rast folikulov na vaječníkoch. Rektálne vyšetrenie sme robili denne až do 9. dňa od podania prvej dávky prípravku. Od 4. dňa po druhom ošetrení sme spolu s rektálnym vyšetrením odobrali krv z v. jugularis, ktorá sa bezprostredne po odbere spracovala a krvné sérum po transporte v prenosnom termoboxe s ľadovou drťou sa až do stanovenia uskladnilo pri teplote -18 °C. Rovnako sme postupovali aj v kontrolnej skupine. Vo vzorkách krvného séra komerčnou súpravou RIA-test PROGS, (HUMA-LAB, Košice, SR) sa radioimunologicky stanovil progesterón. Deklarovaná citlivosť testu vyjadrená ako priemerné množstvo progesterónu v skúmavke odlišiteľné s 95% pravdepodobnosťou od nulového štandardu bola 0,5 nml/l, a koeficient variácie pre intra-assay a inter-assay bol 5,8 %, resp. 8,4 %. Ovulácia sa v pokusnej skupine stanovila na základe rektálneho vyšetrenia a hodnôt progesterónu v krvnom sére. Kritériom pre indukovanú ovuláciu podaním GnRH bolo zvýšenie koncentrácie progesterónu nad 3 nmol/l po treťom dni od podania. Prvá ovulácia v kontrolnej skupine sa stanovila na základe nálezov rektálneho vyšetrenia a hodnôt progesterónu vo vzorkách krvného séra.

VÝSLEDKY A DISKUSIA

Odchovávanie teliat pri matkách je väčšinou praktizované u mäsových plemien kráv s voľným spôsobom chovu. Dojčenie u mliekových plemien kráv pri väčšom počte kráv s modernou technológiou maštali je zriedkavosťou, avšak v niektorých starších typoch maštali sa klasickou technológiou je praktizovaný aj odchov teliat pri kravách.

Prvá postpartálna ovulácia u väčšiny kráv sa nevykytuje skôr ako do 10. až 15. dňa po pôrode, ale anestrické obdobie v mnohých prípadoch pretrváva oveľa dlhšie. Príčina postpartálneho anestrického obdobia je polyfaktorálna, ale vo všeobecnosti je to zlyhanie a predĺžovanie obnovenia normálnej funkcie hypotalamo-hypofyzárno-ovariálnej osi. Jednou z príčin, ktorá predlžuje postpartálne anestrické obdobie, je cicanie teliat (Short a i., 1990; Williams, 1990).

Cicanie teliat vyvolá atenuáciu gonadotropnej sekrécie (Williams a i., 1996) ale nebráni skorému rastu folikulov u mäsových (Dimmick a i., 1991), ani u mliekových plemien kráv (Savio a i., 1990).

Z metód navodenia prvej postpartálnej ovulácie je uvádzané podanie GnRH. V našom sledovaní 4/10 kravy ovulovali po podaní superanalógu GnRH oproti 0/5 kravam v kontrolnej skupine (tab. I). Z praktických dôvodov väčšia pozornosť je venovaná ovplyvňovaniu anestrického obdobia u mäsových plemien kráv. V literárnych prameňoch chýbajú sledovania s indukciou ovulácie u mliečnych plemien kráv s odchovom teliat cicaním.

V predchádzajúcich prácach na mäsových plemenách kráv po ošetrení s GnRH po jednom, opakovaných alebo pulzatívnych podaniach, sa zaznamenala variabilná odpoveď a ovulácia od 10–80 % (Fonseca a i., 1979; Riley a i., 1981; Walters a i., 1982; Edwards a i., 1983; Crowe a i., 1993).

Opakované ošetrenie superanalógom GnRH v našom sledovaní kráv (4/10) vyvolalo následne zvyšovanie koncentrácie progesterónu (tab. II), ale u hodnotených kráv sme na vaječníkoch nezaznamenali ovulačnú jamku ani ovulačnú papulu. Pravdepodobne išlo o luteinizáciu folikulu. Macmillan a Thatcher (1991) po ošetrení kráv s GnRH a následnom zabíí zistili pri nulovej indukcii ovulácie jeden zaujímavý fakt. Ošetrenie v skupine stredných a veľkých folikulov vyvolalo luteinizáciu. Obdobne Crowe a i. (1993)

I. Ovariálna odpoveď kráv po podaní 25 a 50 µg superanalógu GnRH (Spofa) na 12. a 13. deň po pôrode – Ovarian response of cows after administration of 25 and 50 µg of superanalogue GnRH (Spofa) on days 12 and 13 after parturition

Skupina ¹	Počet ⁴	Ovulovalo ⁵		Neovulovalo ⁶	
		n	%	n	%
Pokusná ²	10	4	40	6	60
Kontrolná ³	5	0	0	5	100

¹group, ²experimental, ³control, ⁴number, ⁵ovulated, ⁶non-ovulated

II. Koncentrácie progesterónu v krvnom sére kráv (nmol/l) po podaní 25 a 50 µg supernalógu GnRH na 12. a 13. deň po pôrode (13. deň = 0. deň) – Concentration of progesterone in blood serum of cows (nmol/l) after administration of 25 and 50 µg of supernalogue GnRH on days 12 and 13 after parturition (day 13 = day 0)

Deň ¹	Skupina ²		
	pokusná s ovuláciou ³ (n = 4)	pokusná bez ovulácie ⁴ (n = 4)	kontrolná ⁵ (n = 4)
0	0,9 ± 0,6	0,9 ± 0,2	0,9 ± 0,9
4	3,6 ± 2,5	4,7 ± 5,1	0,8 ± 0,6
5 *	8,9 ± 7,2	6,8 ± 6,6	0,9 ± 0,4
6	7,4 ± 2,4	9,4 ± 9,5	0,4 ± 0,3
7	10,8 ± 3,8a	6,0 ± 2,9	0,9 ± 0,3a
8	9,7 ± 3,3a	3,4 ± 2,8	0,5 ± 0,3a

t-test; a-a P < 0,05

¹day, ²group, ³experimental with ovulation, ⁴experimental without ovulation, ⁵control

popisujú pri zlyhaní indukcie ovulácie luteinizáciu dominantného folikulu.

Hoci kvalita výšky progesterónovej produkcie v oboch skupinách (so zistenou ovuláciou a bez nej) je v našom sledovaní do 6. dňa takmer rovnaká (tab. II), v skupine bez ovulácie je tendencia k skoršiemu ukončeniu luteálnej fázy a od 6. dňa sme zaznamenali graduálny pokles. Macmillan a Thatcher (1991) uvádzajú štyri- až šesťdňové trvanie účinku zakalených a luteinizovaných folikulov, rovnako ako Wettemann a i. (1982) po jednej dávke GnRH u anestrických kráv pozorovali zvýšenie koncentrácie progesterónu v trvaní tri až šesť dní.

V kontrolnej skupine v sledovanom čase sme nezistili ovuláciu (0/5; tab. I). Prvú postpartálnu ovuláciu v tejto skupine sme zaznamenali v priemere za 27,3 ± 2,9 dňa. Koncentrácie progesterónu v tab. II sú uvedené od štyroch kráv. Jedna od začiatku sledovania mala folikulárnu cystu, ktorá sa neskôr transformovala na luteálnu s produkciou progesterónu nad limitnú hodnotu. Uvedenú dojniciu sme nezahrnuli do hodnotenia. Lopez Diaz a Bosu (1992) uvádzajú, že cystická degenerácia vaječnikov nie je statický proces, ale môže nastať luteinizácia folikulárnej cysty s produkciou progesterónu v luteinizovaných štruktúrach.

U dvoch kráv, ktoré nereagovali na podaný GnRH v pokusnej skupine, v našom sledovaní sme zaznamenali ovuláciu na 28. a 30. deň po pôrode.

U másových plemien kráv napriek prítomnosti a cicanie teliat u dojnic nebráni skorému rastu stredných a veľkých folikulov a že ošetrenia s GnRH na 12. a 13. deň po pôrode môže navodiť ovuláciu dominantného folikulu alebo jeho luteinizáciu, prípadne aj luteinizáciu ďalších folikulov.

Z uvedeného vyplýva, že predlžovanie anestrického obdobia u kráv s prítomnosťou teliat je skôr v dôsledku zlyhania dozretia folikulu a ovulácie, ako v predlžovaní začiatku rastu folikulov, respektíve dominantného folikulu.

Záverom z nášho sledovania môžeme uviesť, že cicanie teliat u dojnic nebráni skorému rastu stredných a veľkých folikulov a že ošetrenia s GnRH na 12. a 13. deň po pôrode môže navodiť ovuláciu dominantného folikulu alebo jeho luteinizáciu, prípadne aj luteinizáciu ďalších folikulov.

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IODINE IN MILK BY SUPPLEMENTING FEED: AN ADDITIONAL STRATEGY TO ERASE IODINE DEFICIENCY*

HLADINA JODU V MLÉČE DOJNIC PŘI OBOHACENÉ KRMNÉ DÁVCE: CESTA K ELIMINACI JODOVÉHO DEFICITU

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ABSTRACT: Central Europe including Germany and the Czech Republic is an endemic goitrogenic area with persisting iodine deficiency which is the cause of the occurrence of goitre and other disorders. Farm animals are also affected with iodine deficiency, therefore new ways of prophylaxis and possibilities to increase iodine concentration in cow's milk are being sought. In this study a metabolic efficiency of continuously increasing iodine supplementation in the form of potassium iodide with a defined iodine uptake in the ration, productional supplement, and a balance supplement was investigated on 25 dairy-cows. A 17-week experiment was divided into 5 cycles (four 3-week cycles and one 5-week cycle) so that the basic ration was supplemented with 0, 20, 60, and 150 mg of iodine per day. Iodine content calculated per 1 kg of dry matter of the whole ration was gradually increased from 0.1 mg to 7.6 mg of iodine per 1 kg of dry matter. The results indicate that a gradual iodine supplementation had a significant effect on increased iodine excretion in milk. Iodine level in milk reached 128.1 µg per 1 l prior the experiment on a standard ration, however after a iodine supplementation at the end of the experiment the level rose to 470.0 µg per 1 l. In the first week of a 3-week cycle iodine level of 819.0 µg per 1 l was found. The experiment further included quality evaluation of milk, functional evaluation of thyroid gland based on the contents of triiodothyronine (T₃), thyroxine (T₄), and thyrotropic hormone of hypophysis (TSH). No effect of the described model of iodine intake on quality parameters of milk (sensual characters, contents of urea, lipid, proteins, lactose, and cell elements count), and the mean concentrations of T₃ (2.87 ± 0.24 nmol/l) and T₄ (65.29 ± 7.19 nmol/l) in blood was found. Non-significant variation of TSH was due to other factors than iodine supplementation.

dairy cows; thyroid gland; urine; thyroxine; triiodothyronine; milk; iodine supplementation; urea; thyrotropic hormone of hypophysis

ABSTRAKT: Střední Evropa, včetně SRN a ČR, je endemickou strumigenní oblastí s přetrvávajícím deficitem jodu jako příčinou strumy a dalších poruch. Nedostatečné zásobení jodem postihuje rovněž hospodářská zvířata, proto jsou hledány způsoby profylaxe a možnosti, jak zvýšit koncentraci jodu v kravském mléce. Na 25 dojnících byla studována metabolická účinnost kontinuálně zvyšovaného přídávku jodu ve formě jodidu draselného s definovaným příjmem jodu v krmné dávce, v produkčním přídávku a ve vyrovnávacím doplňkovém přídávku. Pokus trval 17 týdnů a byl rozdělen do pěti cyklů – čtyřikrát tři týdny a jednou pět týdnů tak, že základní krmná dávka byla obohacena o 0, 20, 60 a 150 mg jodu denně. Obsah jodu přepočtený na 1 kg sušiny celkové krmné dávky se postupně zvyšoval z 0,1 mg na 7,6 mg I na kg sušiny. Z výsledků je zřejmé, že toto stupňovité obohacení krmné dávky jodem mělo signifikantní vliv na zvyšující se vylučování jodu mlékem. Zatímco před pokusem na standardní krmné dávce dosáhla hladina jodu v mléce 128,1 µg/l, po aditivním příjmu jodu na konci pokusu se jeho obsah v mléce zvýšil na 470,0 µg/l. V prvním týdnu třetího cyklu byla dokonce zjištěna hodnota 819,0 µg/l. Experiment dále zahrnoval hodnocení jakostních znaků mléka, funkce štítné žlázy podle stavu trijodotyroninu (T₃), tyroxinu (T₄) a tyreotropního hormonu hypofýzy (TSH). Výše popsaný vývoj příjmu jodu neovlivnil parametry jakosti mléka (senzorické vlastnosti, obsah močoviny, tuku, bílkovin, laktózy a počtu buněčných elementů) a průměrnou koncentraci T₃ (2,87 ± 0,24 nmol/l) a T₄ (65,29 ± 7,19 nmol/l) v krvi. Na statisticky nevýznamném kolísání TSH se podílely jiné faktory než suplementace jodu.

dojnice; štítná žláza; moč; tyroxin; trijodotyronin; mléko; přídavek jodu; močovina; tyreotropní hormon hypofýzy

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INTRODUCTION

Iodine deficiency and its consequences (goiter, physical/mental retardation, cretinism) are known in more than 100 countries throughout the world (De Lange, 1996). Germany and the Czech Republic as well as other European countries have not erased iodine deficiency yet. In the Czech Republic insufficient iodine supply in humans causes symptoms of moderate iodine deficiency in 6–15% of children and 25% of adults (Hníková, 1995). Zamrazil et al. (1996) found renal iodine excretion rates below 50 µg/l in 70.2% of boys, 10.4% of girls, 12.2% of men and 21.1% of women. These results indicate a serious iodine deficiency problem. High struma prevalences were found in calves in the Czech Republic resulting not only from low iodine intake but also from high consumption of feed with goitrogenic substances (Čada, 1988; Kurša et al., 1992, 1998). Two studies published in 1996 show that iodine supply in Germany is still insufficient. Hampel et al. (1995b) collected urine samples from 5932 persons from 32 regions in Germany. They found a median value of iodine excretion of 72.4 µg/g creatinine. (Urinary iodine excretions above 100 µg/g creatinine are regarded as sufficient.) There were no differences between Northern and Southern regions of Germany. Only 9% of the observed population showed sufficient iodine status. Metges et al. (1996) determined urinary iodine excretion in 1 040 students and employees of five universities in Southern Germany. They found the total mean urinary iodine excretion of 70.7 µg/g creatinine, only 16% of all observed persons had an adequate iodine excretion. Herzig et al. (1996) determined urinary iodine excretion to evaluate iodine intake. Regular assessments of renal iodine excretion in dairy cows in different regions of the Czech Republic in the years 1993–1997 showed that 70% of the animals have moderate iodine deficiency, only 30% have a normal iodine intake. As a result of the insufficient iodine supply thyroid changes typical of iodine deficiency are widespread. This is confirmed in both studies. Recently Hampel et al. (1995a) observed an enlarged thyroid in 50% of the adult persons, while Metges et al. (1996) showed in the study at universities in Southern Germany that 21% of all students had struma.

The use of iodized salt is regarded as the most promising strategy for the effective elimination of iodine deficiency. In Germany, the iodized salt used in households does not contribute to the iodine supply to a large extent. Both Hampel et al. (1995b) and Metges et al. (1996) showed that persons using iodized salt have an only slightly increased iodine excretion of 7 µg/g creatinine. To be effective iodized salt has to be used not only in households but also in food manufacturing and in restaurants. Since the use of iodized salt is voluntary in Germany there is not enough food produced with iodized salt. Therefore an additional strategy in the fight against iodine deficiency is necessary

(Rambeck et al., 1997). We know from the countries like Great Britain (1995) and Finland (Lamberger, 1993) that milk can be a good source of iodine if the animals get feed enriched with iodine. For this reason we wanted to determine whether human's basal iodine supply can be improved by optimizing the iodine content in feed for dairy cows and consequently in milk. Special attention was focused on animal performance (including thyroid status) and milk quality.

MATERIAL AND METHODS

Feeding experiment

25 dairy cows (Holstein HF; day of lactation 5–189) were used for this iodine feeding experiment. 14 days before the start of the experiment udder disinfection with iodine containing detergents was stopped to avoid additional iodine contamination.

The animals received normal feed during the whole run of the experiment except for different amounts of iodine supplemented. The basic feed that was fed *ad libitum* consisted of 50% grass silage, 40% corn silage and 10% hay. Concentrate A – of which the animals received up to 12 kg depending on their milk yield – consisted mainly of soya, wheat and barley. It also contained an iodized mineral mix that led to an overall iodine concentration in concentrate A of 3 mg I/kg feed.

Each cow received 1 kg of concentrate B – independent on their milk yield. The composition was the same as in concentrate A with the exception that it contained non-iodized mineral feed. For these reasons we used concentrate B as a carrier for controlled iodine supplementation to dairy cows.

We conducted 4 different successive feeding periods (3 weeks each) with iodine concentrations in concentrate B of 0, 20, 60 and 150 mg I/kg (since the cows received 1 kg concentrate B per day these concentrations resulted in additional iodine intakes of 0, 20, 60 and 150 mg/d) – Tab. I.

Feed intake was controlled and registered automatically. Milk and serum samples were collected at the end of each feeding period.

Quality parameters in milk

The sensorial test was carried out in accordance with the rules of the DLG (Deutsche Landwirtschaftsgesellschaft). Three trained testers examined every milk sample approximately 6 hours after milking. They gave a maximum of 5 points in the categories "appearance", "smell" and "taste" for perfect milk. Discrepancies led to a devaluation in the corresponding category.

Fat, protein and lactose were determined by measuring infrared absorption (fat: 3.50 resp. 5.73 µm, protein: 6.50 µm, lactose: 9.50 µm) that is proportional to the concentration in milk.

I. Iodine supplementation in concentrate B (mg/kg as potassium iodate), measured iodine concentration (mg/kg dry matter) in concentrate B and calculated iodine supplementation (mg/kg total dry matter) in different feeding periods of the feeding experiment with dairy cows

Feeding period	Iodine addition to concentrate B (mg/kg)	Iodine concentration (measured) (mg/kg dry matter)	Iodine supplementation (calculated) (mg/kg total dry matter ¹)	Duration (weeks)
0	0	2.21	0.1	3
I	20	22.76	1.1	3
II	60	65.78	3.1	3
III	150	158.94	7.6	3

¹ assuming dry matter intake of 20.9 kg/d (= estimated mean dry matter intake)

The cell counts were determined by counting particles with colored cell nucleus-DNA with the help of fluorescence microscopy.

Urea was determined in an autoanalyzer based on the following principle: Urea reacts with diacetylmonoxime in acid medium to a triazine-derivate: its yellow color is intensified by thiosemicarbacid. The yellow color, which is proportional to the urea concentration, is measured at 8.83 μ m.

Biochemical parameters of the thyroid status

T₃ and T₄ were determined in the serum of the cows with ELISAs of Boehringer, Mannheim.

Thyroid stimulating hormone (TSH) was analyzed with a RIA of DPC Biermann GmbH, Bad Nauheim.

Iodine determination in milk

Iodine concentration in milk was determined by gas chromatography. The analysis was based on the following principle: Iodine from milk reacts with 2-butanone resulting in 1-iodo-2-butanone that is quantified by gas chromatography with electron-capture detector (Baker, 1977). The advantage of this method is the possibility to do it without an ashing step since all interfering substances are removed by phase changes. The detailed instruction is published by Prei et al. (1997).

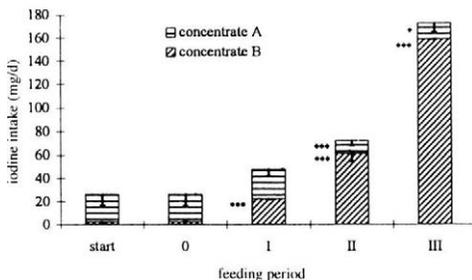
Statistical evaluation

Statistical analyses were performed with SPSS 6.1 for Windows. Differences were tested for statistical significances with the MANOVA-test for repeated measurements. Significant differences compared to the preceding period are labelled with * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

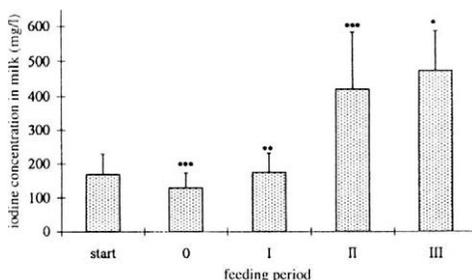
RESULTS AND DISCUSSION

Iodine intake from concentrate

Corresponding to the experimental design the mean iodine intake from concentrate B increased significantly in feeding periods I, II and III (Fig. 1). Mean iodine intake from concentrate A decreased in period



1. Mean daily iodine intake (mg/d) from concentrate before experiment start and at the end of each feeding period with iodine supplementations of 20 (I), 60 (II) and 150 (III) mg/d ($n = 25$)



2. Mean iodine concentration in milk (μ g/l) before experiment start and at the end of each feeding period with iodine supplementations of 20 (I), 60 (II) and 150 (III) mg/d ($n = 17$)

II because of reduced feed intake and remained at this level.

Milk yield

Before the experiment started, the mean milk yield was 26.1 ± 6.7 l/d. It decreased until experiment end constantly to 19.9 ± 5.0 l/d. This corresponds to 23.8% in 17 weeks and is therefore normal during lactation.

Iodine in milk

Before the experiment started, mean iodine concentration in milk was 168.6 ± 60.0 μ g/l (Fig. 2). Iodine concentration in milk shows seasonal changes since it

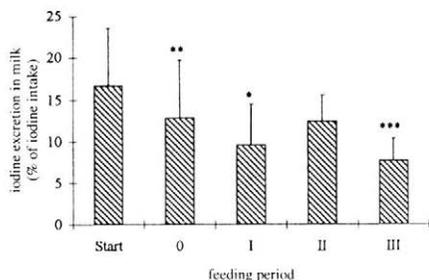
depends on the concentrate intake (which is mostly iodized) very much and concentrate is reduced while grazing. Therefore iodine concentration in milk is usually higher in winter than in summer. The dairy cows used for this feeding experiment did not have any chance to graze. Therefore values for iodine concentrations in their milk can be compared best with values published for winter milk. This is in very good accordance with values published by Prei et al. (1997), who found mean iodine concentration in Bavarian dairy milk of 160.8 µg/l in December and 180.4 µg/l in January.

Milk iodine concentration declined significantly in feeding period 0 (without iodine supplementation) to 128.7 ± 43.4 µg/l. This decline in milk iodine concentration despite constant iodine intake seems to occur because the udder disinfection with iodine containing detergents was probably stopped too late. We stopped it 14 days before the experiment started because Swanson et al. (1990) found this period to be sufficient to remove iodine contamination completely. The decrease in milk iodine that we found 5 weeks after stopping the disinfection indicates that iodine contamination was not completed. One part of the iodine content in milk seemed to originate from the disinfectant – independent of the alimentary iodine intake.

In the following feeding periods I (+ 20 mg I/d), II (+ 60 mg I/d) and III (+ 150 mg I/d) iodine concentration in milk increased significantly corresponding to the increased iodine intake. A comparison of these dose-response data with published values (for example by Hillman and Curtis, 1980; Berg et al., 1988; Anke et al., 1989) is very difficult, since parameters that influence milk iodine (besides iodine intake) vary very much, but are not described exactly (like iodine compounds, goitrogens, udder treatment and milk yield).

Carry over of iodine from feed to milk

Iodine carry over rate from feed to milk was calculated as follows:



3. Mean iodine carry over from feed to milk (%) before experiment start and at the end of each feeding period with iodine supplementations of 20 (I), 60 (II) and 150 (III) mg/d ($n = 17$)

$$\text{iodine carry over (\%)} = \frac{\text{iodine excretion in milk (mg/d)}}{\text{iodine intake (mg/d)}} \cdot 100$$

Before the experiment started, 16.6 ± 6.9% of the iodine taken from feed was excreted in milk. This result is in the range of the values published by Kirchgessner (1959) (7.0–27.0%) and Binnerts (1989) (7–10%). During the run of the experiment higher iodine intakes resulted in lower carry over rates for iodine in milk (except period II) – Fig. 3. Negative correlations between iodine intake and the carry over rate into milk were also observed by Berg et al. (1988) and Swanson et al. (1990).

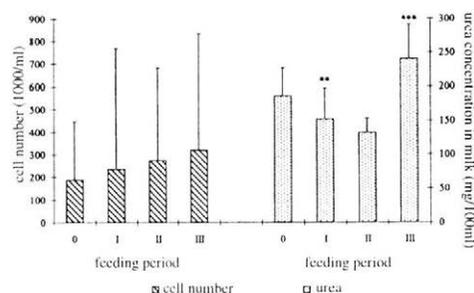
Milk quality

The sensorial quality of all milk samples was without any defect. There were not any significant differences between the different feeding periods.

The mean concentration of fat, protein and lactose was within the normal range during the whole run of the experiment. Iodine supplementation did not show any influence on the concentration of these milk components.

The mean cell counts increased from 188 × 10³/ml to 319 × 10³/ml during the run of the experiment (Fig. 4). Differences between two successive periods were not statistically significant because of high standard deviations. Even the highest value is still below the maximum legal limit for German consumer milk (400 × 10³/ml). One reason for the constant increase is the dispense with iodized udder disinfectants. Koskinen et al. (1996) observed the same effect after withdrawing these detergents. Another reason may be seasonal changes since cell counts can increase during hot periods (Koskinen et al., 1996).

The mean urea concentration in milk decreased constantly from experiment start until end of period II (+ 60 mg I/d) but remained in the normal range. In period III urea concentration in milk increased significantly compared to the preceding period because basic feed was renewed (Fig. 4).

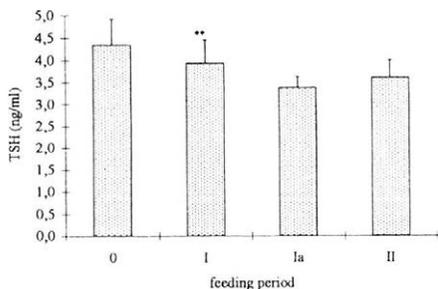


4. Mean cell number (1000/ml) and urea concentration (mmol/l) in milk at the end of each feeding period with iodine supplementations of 20 (I), 60 (II) and 150 (III) mg/d ($n = 25$)

Thyroid status of dairy cows

The mean T_3 concentration amounts to 2.87 ± 0.24 nmol/l, mean T_4 concentration was 65.29 ± 7.19 nmol/l. T_3 and T_4 concentrations in serum were not influenced by iodine supplementation rates up to 150 mg/d. This is in good accordance with results reported by Hillman and Curtis (1980), who did not find any thyroidal changes after the supplementation of 164 mg/d. T_3 and T_4 remain very stable even under iodine deficiency conditions (Kroupová et al., 1996).

TSH concentration in serum declined constantly from period 0 (without iodine supplementation) to period II (+ 60 mg I/d) – Fig. 5. It increased slightly in period III (+ 60 mg I/d). Differences between two successive feeding periods were not significant. Since other influences on TSH in serum (like lactation phase and climate) changed during the run of our experiment we can not conclude whether iodine intake influenced TSH secretion.



5. Mean TSH concentration (ng/ml) in serum at the end of each feeding period with iodine supplementations of 20 (I), 60 (II) and 150 (III) mg/d ($n = 6$)

Benefit of iodine enriched milk for an iodine deficient society

The results of our feeding experiment prove that iodine concentrations in milk can be increased without any losses in quality or milk yield. To get information about the benefit of milk with 250 μg I/l we recalculated the iodine intake according to the German National Consumption Study. The results show that an increase of milk iodine from 75 μg /l to 250 μg /l gives an increased daily iodine intake of 30 μg /d in children and 15 μg /d in adults. Though this additional iodine intake does not erase iodine deficiency in Germany completely, it contributes markedly to the iodine supply (especially for the high risk group "children").

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CLINICAL AND ULTRASONOGRAPHIC FINDING ON THYROID GLAND OF RAMS DURING ADMINISTRATION OF KALIUM IODATUM

KLINICKÝ A ULTRASONOGRAFICKÝ NÁLEZ NA ŠTÍTNEJ ŽLÁZE BARANOV V PRIEBEHU APLIKÁCIE KALIUM JODATUM

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ABSTRACT: The dynamics of triad values, general health condition, palpation and ultrasonographic findings on thyroid glands of rams with various form of clinical manifestation of goitre during therapy with kalium iodatum. An experiment included 9 breeding Merino rams at the age of 2.5 year with clinically developed goitre form. Experimental group (6 rams) was administered kalium iodatum in the feed mixture at a dose of 0.5 g/animal/day during 42 days. The control group without treatment consisted of three rams. Dynamics of body temperature had a similar trend in both the groups and differences at this parameter found between treated and untreated animals ranged within physiological boundaries. Breath and heart rates reached during the whole experiment statistically insignificantly higher values in experimental rams than in control rams. Increase in the weight of experimental group started to be manifested compared with initial values from 15th day and the highest weight was in these animals at the end of experiment (88.5 ± 3.85 kg). The difference in weight between experimental and control rams was on day 42 of the experiment on the level of significance $p < 0.05$. A slight degree of apathy and inappetence was observed in all animals at the beginning of experiment. Improved intake of the feed in treated animals started to be manifested from day 8 but the improvement of the general health condition and reactions to external stimuli were found on day 21 day from the beginning of therapy. The changed palpation finding of thyroid gland (hypertrophy, solid-elastic consistency) during application of kalium iodatum was gradually modified and the size of thyroid gland was 2–4 x 1.5–2 cm on day 42 in different experimental animals. An original solid-elastic consistency of thyroid gland changed in treated rams to softer and soft. Symmetry, temperature, sensitivity and mobility of thyroid gland was not affected by therapeutic procedure. Heteroechoic character of ultrasonographic examination of thyroid gland in experimental and control rams was characteristic at the beginning of experiment. Sonogramme showing almost homogenous echogeneity was typical for thyroid gland in treated rams on day 42 of the trial. This finding corresponded to modified thyroid gland with relatively high representation of colloid and lower representation of cells.

goitre; sheep; kalium iodatum; clinical finding; ultrasonography

ABSTRAKT: V práci sa hodnotila dynamika triasových hodnôt, všeobecný zdravotný stav, palpačný a ultrasonografický nále z na štítnej žláze baranov s rôznou formou klinickej manifestácie strumy v priebehu terapie s kalium jodatum. Do experimentu v klinických podmienkach sa zaradilo deväť plemenných baranov plemena merino vo veku 2,5 roka s klinicky rozvinutou formou strumy. Experimentálna skupina (šesť baranov) dostávala cestou kŕmnej zmesi kalium jodatum v dávke 0,5 g/kus/deň počas 42 dní. Kontrolnú skupinu bez ošetrovania tvorili tri barany. Dynamika telesnej teploty mala v oboch skupinách podobný priebeh a rozdiely, ktoré sa pri tomto parametri zistili medzi liečenými a neliečenými zvieratmi, sa pohybovali v rámci fyziologických hraníc. Frekvencia dychu a pulzu dosahovala v priebehu celého experimentu štatisticky nevýznamne vyššie hodnoty u experimentálnych baranov ako u kontrolných. Zvýšenie hmotnosti u experimentálnej skupiny sa začalo oproti východiskovým hodnotám prejavovať od 15. dňa a najvyššia hmotnosť u týchto zvierat bola na konci experimentu ($88,5 \pm 3,85$ kg). Rozdiel hmotnosti medzi experimentálnymi a kontrolnými baranmi ležal na 42. deň experimentu na hladine významnosti $p < 0,05$. U všetkých zvierat na začiatku experimentu sa pozoroval mierny stupeň apatie a inapetencie. Zlepšený príjem krmiva sa u ošetrovaných zvierat začal prejavovať od 8. dňa, ale zlepšenie všeobecného zdravotného stavu a reakcií na vonkajšie podnety sa zistili na 21. deň po začatí terapie. Zmenený palpačný nále z štítnej žlázy (hypertrofia, tuhoelastická konzistencia) sa počas aplikácie kalium jodatum postupne upravoval a na 42. deň bola veľkosť štítnej žlázy u jednotlivých experimentálnych zvierat 2 až 4 x 1,5 až 2 cm. Pôvodná tuhoelastická konzistencia štítnej žlázy sa zmenila u liečených baranov na mäkkšiu až mäkkú. Symetrickosť, teplota, citlivosť a pohyblivosť štítnej žlázy nebola terapeutickým postupom ovplyvnená. Heterochoégnny charakter ultrasonografického vyšetrenia štítnej žlázy u experimentálnych a kontrolných baranov bol charakteristický na začiatku experimentu. Sonogram vykazujúci takmer homogénnu echogenitu bol typický pre štítnu žlázu u ošetrovaných baranov na 42. deň pokusu. Tento nále z zodpovedal upravenej štítnej žláze s pomerne vysokým zastúpením koloidu a nižším zastúpením buniek.

struma; ovce; kalium jodatum; klinický nále z; ultrasonografia

Podľa údajov World Health Organization až 20 % ľudskej populácie žijúcej v 95 krajinách sveta trpí rôznou formou porúch metabolizmu jódu (WHO, 1992). Rovnaká situácia sa v náraste subklinických a klinických foriem primárnych a sekundárnych karencií jódu pozoruje aj u zvierat v štátoch strednej Európy (Bíreš a i., 1996; Herzig a Suchý, 1996; Kursa a i., 1996).

Aplikácia najnovších poznatkov v diagnostike, prevencii a terapii metabolických porúch jódu u zvierat je predpokladom úspešného zvládnutia tohto problému taktiež vo veterinárnej medicíne. Z tohto pohľadu štítnej žľazy na základe priameho vzťahu k metabolizmu jódu má v diagnostike ochorení u zvierat dominujúce postavenie. Štítina žľaza ako párový orgán uložený vo ventrálnej časti hornej tretiny krku dosahuje u malých prežívavcov za fyziologických podmienok hmotnosť okolo 2 g (Blod a kol., 1989). Prístupná ku klinickému vyšetreniu (posúdenie veľkosti, tvaru, symetrie, teploty, konzistencie, povrchu, citlivosti a pohyblivosti) je u zvierat hyperplastická štítina žľaza na rôznom etiologickom základe (Reddi a Rajan, 1986; Bíreš a i., 1993). Kvantitatívna a kvalitatívna scintigrafia je zobrazenie štítnej žľazy pomocou rádionuklidu ^{123}I alebo $^{99\text{m}}\text{Tc}$. Aj napriek tomu, že táto metóda poskytuje dôležité informácie o funkcii palpovateľného tkaniva štítnej žľazy, vo veterinárnej medicíne sa zatiaľ nevyužíva. Perspektívne, rovnako ako v humánnej medicíne (Tajtáková a i., 1987) sa v ostatnom čase ukazuje zavedenie ultrasonografického vyšetrenia štítnej žľazy aj u zvierat. Jej prednosťou je jednoduchosť, objektívnosť pri vyšetrení veľkosti (makromorfologie) štítnej žľazy a možnosť opakovateľnosti vyšetrenia bez obmedzenia (Tajtáková a i., 1994). Princíp vyšetrenia je založený na odraze ultrazvukových vln o frekvencii 3 až 10 MHz od rozhraní medzi tkaninovými štruktúrami štítnej žľazy o rozličnej hustote, prípadne ostatným prostredím. Ak je hustota prostredia za rozhraním blízka hustote pred rozhraním, je odrazená frakcia nepatrná. V prípade, že je hustota oveľa väčšia, môže dôjsť k úplnému odrazu (Tajtáková a i., 1987, 1994).

Cieľom práce bolo posúdiť klinický a ultrasonografický nález na štítnej žľaze baranov s rôznou formou klinickej manifestácie strumy v priebehu aplikácie kalium jodatum.

MATERIÁL A METÓDY

Do pokusu v klinických podmienkach sa zaradilo deväť plemenných baranov plemena merino vo veku 2,5 roka. U všetkých baranov sa diagnostikovala v rôznej forme klinická struma. Zvieratá zaradené do experimentu pochádzali z endemickej oblasti s výskytom primárnej karencie jódu (Bíreš a i., 1993).

Barany boli umiestnené v troch kotercoch po tri kusy na hlbokú podstielku. Krmná dávka pozostávala

z lúčneho sena (2 kg/kus/deň) a kompletnej krmnej zmesi u všetkých baranov – BAK (0,50 kg/kus/deň). Vodu prijímali *ad libitum*. Koncentrácia jódu v pôvodnej hmote bola u skrmovaného sena 0,201 mg/kg, v jadrovej zmesi 0,235 mg/kg a vo vode 1,94 µg/l. Príjem jódu z krmiva (2 kg lúčneho sena; 0,5 kg kompletnej krmnej zmesi; 5 l vody) predstavoval u všetkých baranov na kus a deň 0,529 mg. Experimentálna skupina (šesť baranov) dostávala v krmnej zmesi kalium jodatum v dávke 0,5 g/kus/deň po dobu 42 dní, čo bolo 382,2 mg jódu. Celkový príjem jódu bol u experimentálnych baranov 382,72 mg/kus/deň a u kontrolných 0,529 mg/kus/deň. Analýza jódu vo vyšetrovanom krmive a vode bola robená fotometrickým stanovením podľa Tušla (1983).

Triasové hodnoty (frekvencia dychu, pulzu a telesná teplota) sa zisťovali individuálne u všetkých zvierat ráno a večer. Vo výsledkoch sú uvedené priemerné hodnoty (\bar{x}) s udaním smerodajnej odchýlky (\pm SD) za experimentálnu a kontrolnú skupinu pred začatím experimentu, 8., 15., 21., 29., 35. a 42. deň. Individuálne vázenie baranov sa robilo na začiatku experimentu, 8., 15., 21., 29., 35. a 42. deň. Komparácia výsledkov triasových hodnôt a živjej hmotností medzi experimentálnymi a kontrolnými baranmi bola robená Studentovým *t*-testom.

Klinické vyšetrenie zvierat pozostávajúce z posúdenia celkového správania zvierat, príjmu krmiva, výskytu ostatných ochorení, vyšetrenia štítnej žľazy (tvar, veľkosť, konzistencia, symetrickosť, pohyblivosť, teplota voči okoliu a citlivosť) sa robilo individuálne u všetkých zvierat pred začatím experimentu, 8., 21. a 42. deň.

Ultrasonografické vyšetrenie štítnej žľazy sa robilo u jedného kontrolného a dvoch pokusných baranov na začiatku experimentu, 8., 21. a 42. deň. Príprava zvierat pre sonografické zobrazenie štítnej žľazy spočívala vo vystrihaní a vyholení kože v oblasti štítnej žľazy. Zvieratá boli fixované pomocníkom v stojacej polohe. Ultrasonografické vyšetrenie sa robilo prístrojom ALOCA Co., Ltd fy Japan, kamera SSD-500 V za použitia ultrazvukového vlnenia v rozsahu 5 MHz lineárnou sondou v transverzálnom reze.

VÝSLEDKY

Triasové hodnoty

Dynamika triasových hodnôt u ošetrovaných a neošetrovaných baranov je uvedená v tab. I. Telesná teplota mala u obidvoch skupín podobný priebeh. Štatisticky nevyznamné rozdiely, ktoré sme u tohto ukazovateľa zistili počas sledovania medzi liečenými a neliečenými zvieratmi, sa pohybovali v rámci fyziologických hraníc. Frekvencia dychu a pulzu dosahovala v priebehu celého experimentu vyššie hodnoty u experimentálnych baranov ako u kontrolných. Namerané rozdiely vo frekvencii dychu a pulzu medzi experimentálnou

Ukazovateľ ¹		Experimentálna skupina ⁹ (n = 6)			Kontrolná skupina ¹⁰ (n = 3)		
		telesná teplota ¹¹	frekvencia pulzu ¹²	frekvencia dychu ¹³	telesná teplota	frekvencia pulzu	frekvencia dychu
Pred započatím experimentu ²	\bar{x}	39,66	77,33	95,0	39,57	67,67	62,33
	SD	0,28	7,86	30,37	0,21	7,50	28,36
8. deň ³	\bar{x}	38,85	82,66	55,33	38,87	67,33	33,33
	SD	0,22	13,12	16,52	0,15	23,09	3,06
15. deň ⁴	\bar{x}	38,96	77,00	68,17	39,27	73,33	42,00
	SD	0,15	10,02	17,02	0,45	4,16	15,87
21. deň ⁵	\bar{x}	39,07	77,80	54,83	39,03	74,00	33,33
	SD	0,08	10,55	22,81	0,23	6,00	6,11
29. deň ⁶	\bar{x}	39,23	82,0	62,50	38,63	69,00	55,33
	SD	0,38	9,38	10,87	2,19	7,55	4,62
35. deň ⁷	\bar{x}	38,93	76,17	70,33	38,80	76,00	67,33
	SD	0,14	4,99	7,12	0,17	2,0	12,22
42. deň ⁸	\bar{x}	39,10	74,67	49,33	39,10	68,67	45,33
	SD	0,18	8,64	17,28	0,10	8,08	19,73

¹indicator, ²before beginning of experiment, ³day 8, ⁴day 15, ⁵day 21, ⁶day 29, ⁷day 35, ⁸day 42, ⁹experimental group, ¹⁰control group, ¹¹body temperature, ¹²heart rate, ¹³breath rate

a kontrolnou skupinou neboli v priebehu experimentu signifikantne významné. Najvyššie rozdiely vo frekvencii dychu sa medzi experimentálnymi a kontrolnými zvieratmi pozorovali od začiatku experimentu do 29. dňa pozorovania.

Živá hmotnosť

Živá hmotnosť u experimentálnych a kontrolných baranov v priebehu aplikácie kalium jodatum je zaznamenaná v tab. II. Živá hmotnosť u pokusných zvierat bola na začiatku experimentu $85,0 \pm 2,86$ kg a u kontrolných $83,5 \pm 0,82$ kg. Zvýšenie hmotnosti u experimentálnej skupiny sa začalo oproti východiskovým hodnotám prejavovať od 15. dňa a najvyššia hmotnosť u týchto zvierat bola na konci experimentu ($88,5 \pm 3,85$ kg). Rozdiel hmotnosti medzi experimentálnymi a kontrolnými baranmi ležal na 42. deň experimentu na hladine významnosti $p < 0,05$. Pokles hmotnosti u kontrolných baranov sa začal pozorovať v porovnaní s hmotnosťou na začiatku experimentu od 15. dňa a na konci sledovania hmotnosť dosiahla $82,17 \pm 0,23$ kg.

Všeobecný klinický nález

Všetky zvieratá prejavovali na začiatku experimentu mierny stupeň apatie. Väčšinou ležali a bol pozorovaný ľahký stupeň inapetencie. Trus bol nezmenený. Zlepšený príjem krmiva sa u liečených zvierat začal v porovnaní s neošetrenými prejavovať od 8. dňa experimentu. Rovnako experimentálne barany v tomto období živšie reagovali na vonkajšie okolie. K úplnej úprave apetítu a správania došlo u liečených baranov od 21. dňa sledovania. Znížený príjem krmiva a celková apatia sa

II. Živá hmotnosť baranov (kg) – Live weight of rams (kg)

Ukazovateľ ¹		Experimentálna skupina ⁹ (n = 6)	Kontrolná skupina ¹⁰ (n = 3)
Pred začatím experimentu ²	\bar{x}	85,0	83,5
	SD	2,86	0,82
8. deň ³	\bar{x}	85,92	82,83
	SD	4,07	0,85
15. deň ⁴	\bar{x}	86,67	83,0
	SD	3,60	1,08
21. deň ⁵	\bar{x}	86,75	83,0
	SD	4,05	0,82
29. deň ⁶	\bar{x}	87,5	82,83
	SD	3,87	0,62
35. deň ⁷	\bar{x}	87,83	82,33
	SD	3,94	0,47
42. deň ⁸	\bar{x}	88,5 ⁺	82,17
	SD	3,85	0,23

+ $p < 0,05$

For 1–10 see Tab. I

s rôznymi výkyvmi u neliečených baranov počas sledovania prehľbovali. V priebehu experimentu sa vyvinula u dvoch experimentálnych a jedného kontrolného barana katarálna forma bronchopneumónie, pre terapiu ktorej sa použil prípravok Clamoxyl inj. LA (Beecham, Anglicko).

Klinický nález na štítnej žľaze

Klinické zmeny na štítnej žľaze (veľkosť, tvar, konzistencia) u experimentálnych a kontrolných baranov

sú zaznamenané v tab. III. a IV. Veľkosť štítnej žľazy bola u liečených baranov pred zahájením experimentu 5 až 12 x 3,5 až 8 cm a u kontrolných zvierat 6 až 9 x 4 až 6 cm. Tvar štítnej žľazy bol v tomto období u jednotlivých zvierat rozdielny od fazule cez slepačie alebo holubacie vajce až po plochý gaštan. Na začiatku experimentu bola štítna žľaza u všetkých vyšetrovaných baranov väčšinou symetrická, pohyblivá voči podložke a pokožke, teplota voči okoliu nebola zmenená a rovnako sa nemenila ani citlivosť. Konzistencia štítnej žľazy bola u jednotlivých zvierat pred začatím terapie tuhoelastická a v jednom prípade slabo elastická.

Veľkostné pomery sa začali upravovať u liečených baranov už od 8. dňa sledovania. Najvýraznejšie zmenšenie štítnej žľazy v porovnaní s východiskovou veľkosťou sme pozorovali na 42. deň, kedy parametre veľ-

kosti sa u jednotlivých experimentálnych zvierat pohybovali 2 až 4 x 1,5 až 2 cm. Veľkosť štítnej žľazy u neliečených baranov mala u dvoch zvierat v priebehu experimentu tendenciu narastať a u jedného sa významne nezmenila. Na konci experimentu veľkosť štítnej žľazy u kontrolných baranov dosahovala 5,5 až 10 x 4 až 6 cm.

Tvarové vlastnosti štítnej žľazy nevykazovali výraznú závislosť od aplikácie kalium jodatum a s minimálnymi odchýlkami sa zachovali u experimentálnych aj kontrolných zvierat do konca sledovania. Pôvodná tuhoelastická konzistencia štítnej žľazy sa menila u liečených baranov na mäkkšiu až mäkkú od 8. dňa začatia terapie. Tuhoelastická konzistencia štítnej žľazy u kontrolných baranov na začiatku experimentu väčšinou v priebehu pozorovania prechádzala na tuhú. Ostatný

III. Veľkosť, tvar a konzistencia štítnej žľazy u kontrolných baranov – Size, shape and consistency of thyroid gland in control rams

Dátum vyšetrenia ¹	Baran č. 7 ⁶			Baran č. 8 ⁷			Baran č. 9 ⁸		
	V	T	K	V	T	K	V	T	K
Pred začatím experimentu ²	8 x 5	holubacie vajce ⁹	TE	9 x 6	holubacie vajce	TE	6 x 4	gaštan ¹⁰	TE
8. deň	9 x 5	holubacie vajce	T	10 x 6	holubacie vajce	TE	5 x 4	gaštan	TŠ
21. deň	10 x 5,5	holubacie vajce	T	11 x 7	holubacie vajce	T	6 x 4	gaštan	TŠ
42. deň	10 x 5	holubacie vajce	T	10 x 6	holubacie vajce	T	5,5 x 4	gaštan	TŠ

V = veľkosť (cm) – size (cm)

T = tvar – shape

K = konzistencia – consistency

TE = tuhoelastická – solid-elastic

T = tuhá – solid

TŠ = tuhšia – more solid

¹date of examination, ²before beginning of experiment, ³day 8, ⁴day 21, ⁵day 42, ⁶ram No. 7, ⁷ram No. 8, ⁸ram No. 9, ⁹dove's egg, ¹⁰chestnut

IV. Veľkosť, tvar a konzistencia štítnej žľazy u experimentálnych baranov – Size, shape and consistency of thyroid gland in experimental rams

Dátum vyšetrenia ¹	Baran č. 1 ⁶			Baran č. 2 ⁷			Baran č. 3 ⁸		
	V	T	K	V	T	K	V	T	K
Pred začatím experimentu ²	5 x 3,5	fazuľa ¹²	TE	12 x 8	slepačie vajce ¹³	TE	7 x 5	gaštan ¹⁴	TE
8. deň ³	5 x 2	fazuľa	M	8 x 2,5	slepačie vajce	MŠ	2 x 1,5	gaštan	MŠ
21. deň ⁴	4 x 3	fazuľa	M	7 x 5	slepačie vajce	MŠ	2 x 1	gaštan	MŠ
42. deň ⁵	2,5 x 1,5	fazuľa	M	4 x 2	slepačie vajce	M	2 x 1,5	gaštan	M
Dátum vyšetrenia	Baran č. 4 ⁹			Baran č. 5 ¹⁰			Baran č. 6 ¹¹		
	V	T	K	V	T	K	V	T	K
Pred začatím experimentu	11 x 6	slepačie vajce	TE	10 x 7	slepačie vajce	TE	6 x 4	holubacie vajce ¹⁵	SE
8. deň	6 x 3	slepačie vajce	MŠ	6 x 4	slepačie vajce	MŠ	4 x 2	holubacie vajce ¹⁵	MŠ
21. deň	5 x 2	slepačie vajce	MŠ	4 x 3	slepačie vajce	MŠ	3 x 2	holubacie vajce	MŠ
42. deň	3 x 1,5	slepačie vajce	MŠ	2,5 x 1,5	slepačie vajce	MŠ	2 x 1,5	holubacie vajce	MŠ

V = veľkosť (cm) – size (cm)

T = tvar – shape

K = konzistencia – consistency

TE = tuhoelastická – solid elastic

SE = slabaelastická – weak-elastic

MŠ = mäkkšia – softer

M = mäkká – soft

¹date of examination, ²before beginning of experiment, ³day 8, ⁴day 21, ⁵day 42, ⁶ram No. 1, ⁷ram No. 2, ⁸ram No. 3, ⁹ram No. 4, ¹⁰ram No. 5, ¹¹ram No. 6, ¹²bean, ¹³hen egg, ¹⁴chestnut, ¹⁵dove's egg

klinický nález na štítnej žľaze (symetrickosť, pohyblivosť, teplota, citlivosť) sa u experimentálnych a kontrolných baranov v priebehu experimentu výrazne nemenili a zachovali si aj napriek predchádzajúcim zmenám vlastností, ktoré sa pozorovali na začiatku experimentu.

Ultrasonografické vyšetrenie

Ultrasonografické vyšetrenie štítnej žľazy dvoch experimentálnych a jedného kontrolného barana pred začiatkom pozorovania poukázalo na heteroechogenitu

(obr. 1). Heteroechogénny charakter ultrasonografického vyšetrenia u analyzovaných baranov bol spôsobený v štítnej žľaze prítomnosťou difúzne nepravidelne usporiadaných útvarov rôznej veľkosti a tvaru, ktoré boli miestami anechogénne, hypoechogénne a hyperechogénne. Postupná úprava ultrasonografického nálezu sa u liečených baranov začala prejavovať od 21. dňa, ale najmä na 42. deň. V tomto období sonogram vykazoval takmer homogénnu echogenitu, ktorá bola vyššia ako v okolí (obr. 2). Takto ultrasonograficky formovaná štítina žľaza u experimentálnych baranov na konci experimentu bola charakterizovaná pomerne vysokým zastúpením koloidu a pomerne nižším zastúpením bu-



1. Ultrasonografický obraz hypertrofovej štítnej žľazy (echogenita difúzne zmenená); experimentálny baran č. 1 na začiatku experimentu – Ultrasonographic picture of hypertrophic thyroid gland (echogeneity diffusely changed); experimental ram No. 1 at the beginning of experiment



2. Ultrasonografický obraz štítnej žľazy po terapeutickom ovplyvnení s kalium jodatum (echogenita homogénna, primeraná); experimentálny baran č. 1 na 42. deň experimentu – Ultrasonographic picture of thyroid gland after therapeutic influence of kalium iodatum (homogenous echogeneity, adequate); experimental ram No. 1 on day 42 of experiment

niek. Obráz hyp- až aneochogenity štítnej žľazy pretrvával u kontrolného barana aj na konci experimentu.

DISKUSIA

Klinická diagnostika ochorení štítnej žľazy je u zvierat často problematická. Subklinické formy unikajú pozornosti a preto klinické vyšetrenia sa využívajú pri rozvinutej hyperplazii štítnej žľazy.

Dynamika triasových hodnôt ako súčasť klinického vyšetrenia u baranov s vyvinutou strumou v priebehu aplikácie kalium jodatum nevykazovala významnú závislosť od terapeutického postupu a zistené rozdiely v telesnej teplote a frekvencii pulzu medzi ošetrovanou a neošetrovanou skupinou boli v referenčnom rozpätí (Slanina, Dvořák a kol., 1993). Zvýšená frekvencia dychu nad fyziologickú hranicu predovšetkým u experimentálnych baranov bola v priebehu sledovania pravdepodobne ovplyvnená aj výskytom bronchopneumónií a zoohygienickými podmienkami (barany s dlhým rúnom boli držané v miestnosti s relatívne vysokou vlhkosťou a teplotou). V prípade, že berieme do úvahy zistenia autorov Jones a i. (1986), Bate a i. (1993), Bíreš a i. (1996), ktorí zmeny vo frekvencii dychu, pulzu a telesnej teploty považujú pri jódopéniách ako výsledok nízkej syntézy tyroxínu, poruchy termoregulácie a vývoja pľúcneho parenchýmu skôr u mláďat ako u dospelých zvierat, potom vplyv suplementácie kalium jodatum u 2,5-ročných experimentálnych baranov bol minimálny.

Striedavá inapetencia, apatia a zhoršený výživný stav sa považujú za všeobecné príznaky karence jódu a pri poruche syntézy tyroïdných hormónov sú prejavom narušenia celkového metabolizmu (Hetzel a Dunn, 1989; Kaptein a i., 1994). V zhode s uvedenými zisteniami dochádzalo u liečených baranov, v porovnaní s kontrolnými, k postupnej úprave príjmu krmiva, zlepšil sa výživný stav a zvieratá čulejšie reagovali na vonkajšie podnety.

Vývoju vo všeobecnom klinickom obraze u experimentálnych a kontrolných baranov zodpovedal v priebehu terapeutického postupu aj palpačný nález na štítnej žľaze. Tuhoelastická konzistencia štítnej žľazy u liečených baranov na začiatku experimentu prechádzala na mäkkšiu a rovnako sa od 8. dňa experimentu upravovali, na rozdiel od kontrolných baranov, veľkostné pomery. Tvar, pohyblivosť, citlivosť, symetrickosť a teplota štítnej žľazy sa v závislosti od terapeutického postupu významne nemenili. Tuhoelastická konzistencia hypertrofovanej štítnej žľazy, aká sa diagnostikovala na začiatku experimentu u všetkých baranov, súvisí pri deficiencii jódu s histologicky nahromadením drobných folikulov vyslaných kubických alebo plošných epitelom, ktoré sú umiestnené v edematyckom rozšírenom spojive infiltrovanom okrúhlo-jadernými bunkami (Bíreš et al., 1993). Prechod tuhoelastickej alebo tuhej konzistencie na mäkkšiu súvisí počas aplikácie kalium jodatum u experimentálnych baranov s normalizáciou anatomickej štruktúry štítnej žľazy, kedy

v parenchýme je vysoko zastúpený koloid s pomerne nižším obsahom buniek (Tajtáková et al., 1994). Pretrvávanie tuhoelastickej až tuhej konzistencie so zväčšovaním veľkosti štítnej žľazy je u neliečených baranov v priebehu experimentu dôkazom pomnoženia spojivového tkaniva a postupnou fibrotizáciou parenchýmu.

V korelácii s palpačným nálezom na štítnej žľaze bol počas terapeutického postupu u liečených baranov ultrasonografický obraz. Heterochochogenita diagnostikovaná v dôsledku nehomogénnej štruktúry štítnej žľazy na začiatku experimentu u všetkých baranov prechádzala vplyvom realizovanej terapie u experimentálnych zvierat v homochochogenitu, ktorá je charakteristická pre fyziologicky makrofolikulárne formovanú štítnu žľazu (Tajtáková a i., 1987; Itoh a i., 1995).

Výsledky klinických vyšetrení, ktoré sa realizovali v priebehu terapie strumy u baranov potvrdili diagnostický význam ich využitia pri ochoreniach štítnej žľazy u zvierat. Rovnako na základe ultrasonografických zmien počas terapeutického postupu pri ultrasonografickom vyšetrení štítnej žľazy malo zaujať pevné miesto v diagnostike tyreopatií aj u zvierat, tak ako o tom referujú u ľudí Zamrazil a i. (1986). Napriek dosiahnutým výsledkom objektívna diagnostika ochorenia štítnej žľazy aj na základe porúch metabolizmu jódu vyžaduje u zvierat súčasnú aplikáciu klinických, biochemických, prípadne ostatných špeciálnych vyšetrení (Bíreš a i., 1993; Ramakrishna a i., 1994; Herzig a i., 1996).

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Deutsche Veterinärmedizinische Gesellschaft e.V.



Fachgruppe: "Epidemiologie und Dokumentation" der DVG

Wusterhausen, den 27. Februar 1998

Tagung der Fachgruppe „Epidemiologie und Dokumentation“ vom 02.-04. 09. 1998 in Wusterhausen

Sehr geehrte Frau Kollegin, sehr geehrter Herr Kollege!

die o.g. Internationale Fachtagung wird als Gemeinschaftsveranstaltung der „Fachgruppe Epidemiologie und Dokumentation“ der Deutschen Veterinärmedizinischen Gesellschaft, des „Forums für Epidemiologie und Tiergesundheit“, Schweiz und der „ARGE Quama“, Österreich

unter dem Thema „Informationsgewinnung und -verarbeitung“ stattfinden.

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Für die Referate werden voraussichtlich 30 Minuten, incl. Diskussion, zur Verfügung stehen. Die Beiträge werden in der DVG Schriftenreihe veröffentlicht.

Falls Sie sich zu einem Beitrag entschließen, senden Sie bitte das beiliegende Antwortformular und ein kurzes Abstract (bevorzugt über Diskette (Word) oder e-mail: matthias.kramer@wus.bfav.de)

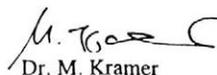
spätestens bis zum 17.04.1998

zurück.

Herzlichen Dank für Ihre Mitarbeit und Unterstützung schon im Voraus.

Mit freundlichen Grüßen


Dr. H. Schlüter


Dr. M. Kramer

THE EFFECT OF TWO β_2 ADRENERGIC AGONISTS ON THE ULTRASTRUCTURE OF THE AIRWAY EPITHELIUM IN RABBITS*

VLIV DVOU β_2 -SYMPATOMIMETIK NA ULTRASTRUKTURU EPITELU DÝCHACÍCH CEST KRÁLÍKŮ

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ABSTRACT: The ultrastructure of the rabbit tracheal epithelium was studied 30 min after intratracheal administration of Ventolin (0.2 mg of salbutamol) and Berotec (0.4 mg of fenoterolhydrobromide), respectively. The injury to the tracheal epithelium due to the treatment with two puffs of Ventolin was considered moderate to severe and morphological signs of impaired self-cleaning ability were encountered. After administration of a similar dose of Berotec, only a mild damage to this epithelium was revealed. Marked differences exist in the degree of damage to the airway epithelium due to the administration of two drugs that belong to the same family of the selective β_2 adrenergic agonists.

airways; epithelium; β_2 adrenergic agonists; ultrastructure

ABSTRAKT: Studovali jsme ultrastrukturu tracheálního epitelu králíků 30 minut po intratracheálním podání Ventolinu (0.2 mg salbutamolu) a Berotecu (0.4 mg fenoterolhydrobromidu). Poškození tracheálního epitelu po podání dvou vdechů Ventolinu bylo hodnoceno jako střední až těžké s výskytem morfoložických známek narušení samočistící schopnosti. Po podání obdobné dávky Berotecu bylo zjištěno jen lehké poškození epitelu. Dvě léčiva, která patří do stejné skupiny selektivních β_2 -sympatomimetik, způsobují výrazně rozdílný stupeň poškození epitelu dýchacích cest.

dýchací cesty; epitel; β_2 -sympatomimetika; ultrastruktura

INTRODUCTION

In our previous study, the effects of an adrenergic agonist salbutamol (Ventolin) and a cholinergic antagonist ipratropium bromide (Atrovent) on the ultrastructure of the tracheal epithelium were studied (Konrádová et al., 1997). Only slight differences in the injury to the airway epithelium due to the administration of a single therapeutic dose of both drugs were revealed. Two puffs of both bronchospasmolytics caused moderate to severe damage to the tracheal epithelium. To test the hypothesis that all the bronchospasmolytic drugs induced similar injury to the airway epithelium, it was decided to compare the effect of a therapeutic dose of salbutamol with that of another β_2 adrenergic agonist – fenoterolhydrobromide (Berotec) – on the ultrastructure of the tracheal epithelium.

MATERIAL AND METHODS

The same group of three healthy rabbits used in our previous study (Konrádová et al., 1997) served as

untreated controls. Under general anesthesia induced by i.m. administration of a mixture of ketamine and xylazine (Konrádová et al., 1997), two groups of three healthy rabbits (body weight of 1,500–3,000 g) were treated with two puffs Ventolin (Glaxo, Aranda de Duero, Spain) containing 0.2 mg of salbutamol and two puffs of Berotec (Boehringer, Ingelheim am Rhein, FRG) with 0.4 mg of fenoterolhydrobromide, respectively. For administration of both bronchospasmolytic aerosols, the method described in our previous paper was used (Konrádová et al., 1997). The material for the electron microscopic examination was collected 30 min post exposure. Tiny fragments of the tracheal mucous membrane were processed using the same methods as described in our previous paper (Konrádová a Šrajer, 1987; Konrádová et al., 1997). For quantitative estimation, in controls and after administration of two puffs of Ventolin and Berotec, 1,058 μm^2 , 2,145.25 μm^2 and 1,614.25 μm^2 of ciliary border with 10,252, 11,092 and 11,334 kinocilia were evaluated, respectively. In those experimental groups also a total of 186, 384 and 609 goblet cells were

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studied, respectively. The goblet cells were classified into three categories: mucus-filled, mucus-discharging and degenerated ones. Kinocilia were classified into four categories: intact 9 + 2 cilia, slightly damaged pathological cilia with local swellings of the ciliary membrane or with tiny vacuoles situated in their shafts, degenerating cilia, represented by axonemes incorporated into the cytoplasmic blebs or by isolated axonemes, and malformed cilia with either abnormal arrangement or number of microtubules in their axonemes.

For statistical evaluation, relative values of the 3 categories of goblet cells and 4 categories of cilia were evaluated by the chi-square test of homogeneity in frequency tables. To specify categories causing deflections from the hypothesis of homogeneity, adjusted standardized deviations were used. Means of cilia/ μm^2 were compared by the one-way analysis of variance (ANOVA) and by a non-parametric analogy of the ANOVA – Kruskal-Wallis test. The differences between groups were assessed by the Tukey's test for multiple comparison. The Leven's test of homogeneity of variance was also performed.

RESULTS

Control rabbits

In the tracheal ciliated pseudostratified columnar epithelium of control rabbits, ciliated cells of standard ultrastructure and isolated mucus-filled goblet cells were found. $3 \pm 1\%$ of secretory elements discharged their secretion by means of gradual evacuation of the individual apical mucous granules (Tab. I). 9.7 ± 0.3 cilia/ μm^2 were found in the regular ciliary border. $98.8 \pm 0.1\%$ of cilia were intact. The proportions of individual types of cilia were given in Tab. I.

30 minutes after administration of two puffs of Ventolin (0.2 mg of Salbutamol)

30 min post exposure, rabbits' tracheae were lined with an altered pseudostratified ciliated epithelium with narrow intercellular spaces and intact apical junctional complexes.

On the apical portions of the ciliated cells, rather numerous small to medium sized cytoplasmic protrusions were developed (Fig. 1). Inside those cytoplasmic blebs, usually only a few intact or disintegrating axonemes of kinocilia were revealed, but in some voluminous protrusions, a great number of axonemes of degenerating cilia were encountered. In the deeper portions of ciliated cells' cytoplasm, a slight increase in the number of small vacuoles and tiny lysosomes, dilatation of the cisternae of granular endoplasmic reticulum and Golgi complex were observed (Fig. 2).

The goblet cells were found as isolated elements among the ciliated ones. Only $9 \pm 1\%$ of them were arranged in tiny groups. $91 \pm 1\%$ of the goblet cells were stimulated to discharge mucus (Tab. I). The stimulated goblet cells communicated widely with the lumen of the respiratory passages. Mucus was evacuated simultaneously from numerous apical mucous granules and packets of mucous granules were also detached from the goblet cells. In some stimulated goblet cells, chain fusion of the adjacent mucous granules' membranes was noticed. The completely exhausted degenerated secretory cells amounted to $79 \pm 3\%$ (Fig. 3). After losing their contacts with the basal lamina, the exhausted elements were mostly encountered in the apical portion of the epithelium and after sloughing off, remnants of their electron-dense degenerated cytoplasm were observed in the area of the ciliary border.

Isolated cells equipped with short irregular microvilli containing a few small rather electron dense secretory granules in their undifferentiated cytoplasm, or secretory elements filled with small mucous granules separated by voluminous cytoplasmic septa were encountered in the epithelium.

The regular arrangement of the ciliary border was slightly impaired. The mean number of cilia was $7.4 \pm 0.2/\mu\text{m}^2$. The altered elements represented $8.6 \pm 2.9\%$. The proportions of the individual types of altered cilia were given in Tab. I. In the area among the kinocilia, not very numerous bacteria and clumps or layers of inspissated mucus were observed (Figs. 4, 5).

30 minutes after administration of two puffs of Berotec (0.4 mg of fenoterolhydrobromide)

30 min after administration of Berotec aerosol, slightly altered pseudostratified ciliated epithelium with narrow intercellular spaces sealed with intact apical junctional complexes was observed in the tracheae of treated rabbits.

Only small isolated apical cytoplasmic blebs containing a few axonemes of degenerating kinocilia appeared on the apical portions of the ciliated cells. In their cytoplasm, a slight increase in number of tiny vacuoles and lysosomes was recorded. Only $7 \pm 4\%$ of goblet cells formed small groups. In $18 \pm 3\%$ of the mucus discharging elements, the gradual evacuation of individual apical mucous granules was noticed. The detachment of groups of mucous granules was revealed only exceptionally. $4 \pm 1\%$ of goblet cells were completely exhausted. The remnants of their degenerated cytoplasm were found only exceptionally among free cilia.

On average, 7.0 ± 0.3 kinocilia/ μm^2 were revealed in the slightly altered ciliary border where small remnants of membranes and isolated bacteria were observed among mostly intact cilia. The altered kinocilia amounted only to $1.3 \pm 0.3\%$ (Tab. I).

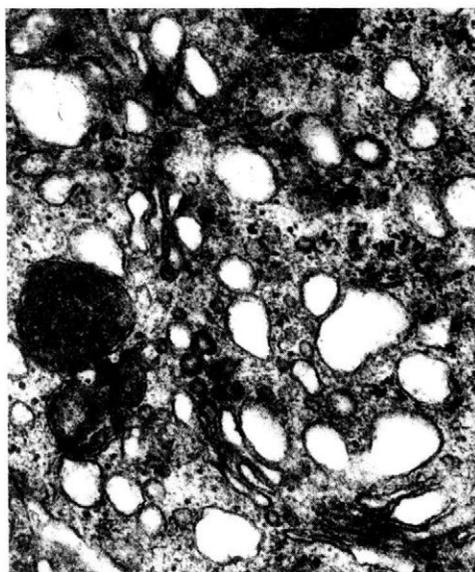
1. Quantitative evaluation of the goblet cells (GC) and ciliary border in the trachea of rabbits 30 minutes after inhalation of 2 puffs of two β_2 adrenergic agonists

	Controls	Salbutamol	Fenoterol hydrobromide
Nonstimulated GC	97 ± 1%	*9 ± 1%	*78 ± 3%
Mucus-discharging GC	3 ± 1%	*12 ± 3%	*18 ± 3%
Degenerated GC	0	*79 ± 3%	*4 ± 1%
Stimulated GC total	3 ± 1%	*91 ± 1%	*22 ± 3%
GC arranged in groups	6 ± 3%	9 ± 1%	7 ± 4%
Number of cilia per 1 μm^2 of ciliary border	9.7 ± 0.3	*5.2 ± 0.2	*7.0 ± 0.5
Intact cilia	98.8 ± 0.1%	*91.4 ± 2.9%	*98.7 ± 0.3%
Pathological cilia	0.5 ± 0.2%	0.8 ± 0.4%	0.6 ± 0.2%
Degenerating cilia	0.3 ± 0.1%	*7.4 ± 2.1%	*0.1 ± 0%
Malformed cilia	0.4 ± 0.2%	0.4 ± 0.5%	0.6 ± 0.2%
Altered cilia total	1.2 ± 0.1%	*8.6 ± 2.9%	1.3 ± 0.3%

$n = 3$, mean \pm SD, * values differ significantly ($p < 0.01$) from controls, — values differ significantly ($p < 0.01$) from each other



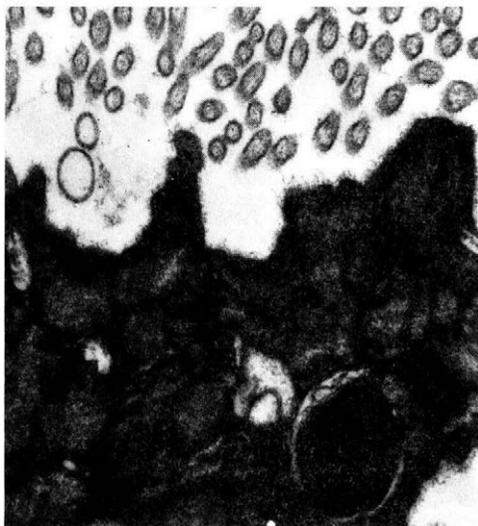
1. Cytoplasmic bleb containing axonemes of degenerating kinocilia on the apical portion of a ciliated cell (rabbit tracheal epithelium 30 min after administration of two puffs of Ventolin); 25,000x



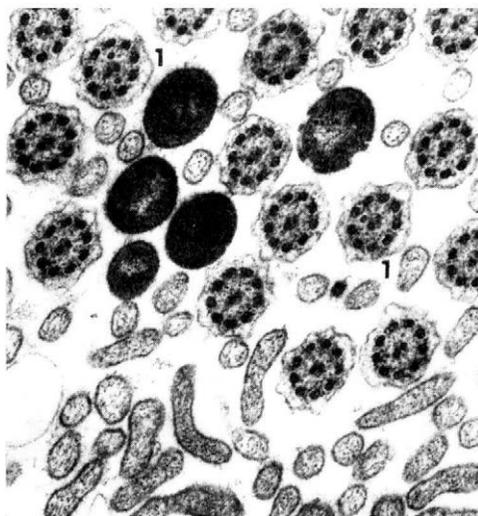
2. Dilated cisternae of the Golgi complex and tiny vacuoles in the cytoplasm of a ciliated cell (rabbit tracheal epithelium 30 min after administration of two puffs of Ventolin); 50,000x

DISCUSSION

On the basis of our previous experiments, a classification of the degree of injury to the airway epithelium was proposed (Konrádová, 1991) – Tab. II. According to this classification, the injury to the tracheal epithelium due to the treatment with two puffs of Ventolin (0.2 mg of salbutamol) was considered moderate to severe. On the other hand, after administration of a similar dose of Berotec (0.4 mg of fenoterolhydro-



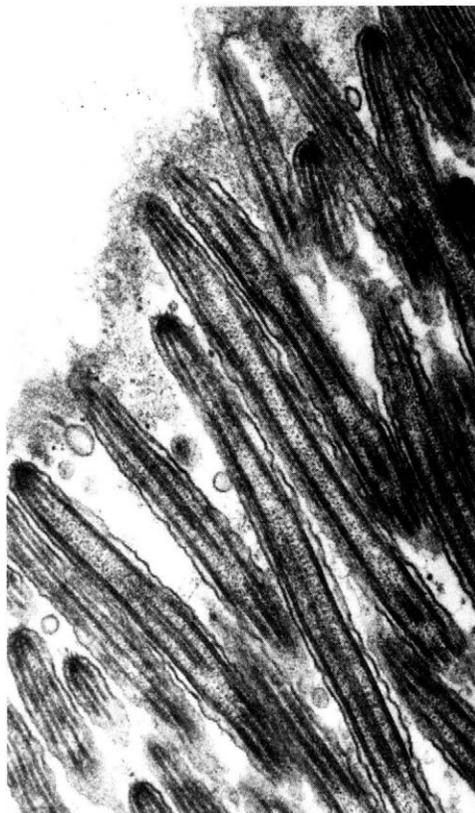
3. Apical portion of an exhausted, degenerated goblet cell (rabbit tracheal epithelium 30 min after administration of two puffs of Ventolin); 37,500x



5. Bacteria among kinocilia two of which are malformed (1) (rabbit tracheal epithelium 30 min after administration of two puffs of Ventolin); 50,000x

bromide), only a mild damage of this epithelium was revealed.

As we described in our previous paper (Konrádová et al., 1997), our results describing the degree of damage to the tracheal epithelium due to the inhalation of Ventolin aerosol were in accordance with those obtained by Spahr-Schopfer and Shorten with



4. Inspissated secretion in the area of the ciliary border (rabbit tracheal epithelium 30 min after administration of two puffs of Ventolin); 37,500x

their fellow workers using the light microscope (Spahr-Schopfer et al., 1994; Shorten et al., 1995). Significantly less pronounced damage was noticed in the airway epithelium after administration of another β_2 adrenergic agonist – Berotec.

Due to the treatment with Ventolin, the secretory elements were highly affected. More than 90% of goblet cells were stimulated to discharge their mucus, while Berotec caused mucus evacuation only in about one fifth of all secretory elements. Inhalation of Ventolin aerosol significantly accelerated mucus release and influenced the mechanism of secretion. Signs of an apocrine type of secretion were frequently encountered and also compound exocytosis (Neutra and Schaffer, 1977; Specian and Neutra, 1980; Roumagnac and Laboisse, 1987; Specian and Oliver, 1991; Konrádová et al., 1996; Newman et al., 1996) representing the most rapid way of mucus discharge was noticed in numerous cells. After Berotec administration, the merocrine type of secretion still prevailed.

II. Evaluation of the degree of damage to the airway epithelium of rabbits 30 minutes after inhalation of 2 puffs of two β_2 adrenergic agonists

	Control rabbits	Degree of damage			Salbutamol	Fenoterol hydrobromide
		mild	moderate	severe		
Stimulated GC	<3%	3-50%	50-90%	>90%	91%	22%
Ratio discharging GC degenerated GC	degenerated GC not found	>1	0.1-1	<0.1	0.15	4.50
Number of cilia/ μm^2	>9	7-9	3-7	<3	5.2	7.0
Altered cilia	<1.2%	1.2-2.0%	2.0-10.0%	>10.0	8.6%	1.3%
Signs of impairment of the self cleaning ability	0	±	+	++	+	±

GC – goblet cells

Two puffs of Ventolin not only overstimulated, but also damaged the secretory elements. After rapid mucus discharge, 79% of the exhausted goblet cells degenerated and gradually sloughed off. Due to the Berotec administration, only a few degenerated goblet cells were noticed in the epithelium.

On the basis of our previous experiments (K o n r á d o v á et al., 1990), we expected massive differentiation of new secretory elements resulting in the appearance of intraepithelial mucous glands to occur in the tracheal epithelium after contact with Ventolin aerosol, but 30 min post exposure rather numerous differentiating secretory cells without any changes in their distribution were noticed in the epithelium. The proportion of goblet cells arranged in groups did not differ significantly compared with that of healthy control rabbits. The administration of Berotec did not even stimulate an increase in number of differentiating goblet cells.

Due to the administration of both bronchospasmodic aerosols, the ciliated cells were less injured compared to the goblet ones. Nevertheless, the inhalation of Ventolin caused more serious pathological alteration of these cells. Apical blebbing associated with destruction of rather numerous kinocilia and an increase in number and volume of vacuoles and secondary lysosomes were revealed in their cytoplasm. Due to the contact with Berotec aerosol, only a few tiny apical cytoplasmic blebs and a small increase in the number of vacuoles were revealed in the ciliated cells' cytoplasm.

In the area of the ciliary border, significant decrease in the mean number of kinocilia to $5.2/\mu\text{m}^2$ and a significant increase in percentage of the degenerating cilia were revealed 30 min post exposure of Ventolin. Due to the Berotec inhalation, only a slight decrease of the average number of kinocilia was noticed. The proportion of altered kinocilia did not significantly increase compared with the findings in the healthy control rabbits. Both bronchospasmodic drugs did not cause an increase in the proportion of malformed cilia showing thus that they did not affect the ciliogenesis.

In accordance with other authors who studied the relation of the cilia to the layer of secretion in the respiratory passages (Y o n e d a, 1976; S t u r g e s s, 1977; S t r a t m a n n et al., 1991; G e i s e r et al.,

1997), we consider the appearance of inspissated mucus and bacteria in the area of the ciliary border as morphological signs of impaired mucus flow in the airways. After Ventolin inhalation, mark impairment of the self-cleaning ability of the tracheal epithelium was revealed. Due to the inhalation of two puffs of Berotec, a few isolated bacteria revealed among the kinocilia represented the first sign of local mucus flow disturbance.

Our results demonstrate, that from the morphological point of view, marked differences exist in the effect on the airway epithelium due to the administration of individual related drugs that belong to the same family of the selective β_2 adrenergic agonists. These differences were more pronounced compared with those of an adrenergic agonist and a cholinergic antagonist described in our previous paper (K o n r á d o v á et al., 1997).

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THE EFFECT OF AGE ON THE DISTRIBUTION OF LYMPHOCYTE AND NEUTROPHIL GRANULOCYTE SUBSETS IN THE PERIPHERAL BLOOD OF DOG*

VLIV VĚKU PSŮ NA ZASTOUPENÍ SUBPOPULACÍ LYMFOCYTŮ A NEUTROFILNÍCH GRANULOCYTŮ V PERIFERNÍ KRVI

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ABSTRACT: Samples of peripheral blood obtained from Beagle dogs of different age were examined for total and differential leukocyte counts and distribution of lymphocyte subpopulations. Subsets of leukocytes were counted using the cell counter and light microscope of blood films. Monoclonal antibodies against canine homologue of CD3, CD5, CD4, CD8, and CD21 were used for the detection of lymphocyte subsets by flow cytometry. First day after the parturition the neutrophil to leukocyte ratio was 2.22. Over the first week a decrease of this ratio occurred till the values about 0.63. Since the second week of age an increase of neutrophil count was recorded connected with extended neutrophil to leukocyte ratio till the value 1.87 at one month of age. The ratio had been extending till the value 1.97 at the age 3–5 years. The ratio between T and B lymphocytes was 1.2 and 1.3 first day after parturition and at the age of one month, respectively. The ratio had been extending till the value 6.3 at the age of 1–2 years. The extension was caused essentially by a decrease of the absolute B lymphocyte count. On day 1 after parturition almost all T-lymphocytes were CD4 positive and their count even increased over the first week. Since the second week of age no changes in CD4 positive lymphocyte count have been recorded. CD8 positive lymphocyte count increased over the age of the examined dogs. Thus the ratio of CD4 and CD8 positive lymphocytes was reduced from 13.7 on day 1 after parturition to 2.0 in age category 3–5 years.

dog; age; lymphocytes; neutrophils; subpopulations; flow cytometry

ABSTRAKT: Ve vzorcích periferní krve psů plemene beagle různého věku byl pomocí počítače krvinek a světelnou mikroskopii krevních nátěrů zjištěn celkový počet a diferenciální rozpočet leukocytů. Zastoupení subpopulací lymfocytů bylo detekováno metodou průtokové cytometrie za použití monoklonálních protilátek proti psím homologům antigenů CD3, CD5, CD4, CD8 a CD21. První den po porodu byl zjištěn poměr neutrofilů k lymfocytům 2,22. V průběhu prvního týdne došlo k poklesu tohoto poměru až na hodnoty okolo 0,63. Od druhého týdne věku bylo zjištěno zvýšení počtu neutrofilů a tím i rozšiřování poměru neutrofilů k lymfocytům až na hodnoty okolo 1,87 ve věku jednoho měsíce. Poměr neutrofilů k lymfocytům se s věkem rozšiřoval až na hodnotu 1,97 ve věku tří až pěti let. První den po porodu byl poměr mezi T a B lymfocyty 1,2, ve věku jednoho měsíce 1,3. Poměr se s věkem rozšiřoval až na hodnotu 6,3 ve věku jednoho až dvou let. Toto rozšíření bylo způsobeno hlavně poklesem absolutního počtu B lymfocytů. První den po narození byly téměř všechny T lymfocyty CD4 pozitivní. Jejich počet se v průběhu prvního týdne ještě zvýšil. Od druhého týdne života nebyly zaznamenány výraznější změny v absolutních počtech CD4 pozitivních lymfocytů. Počet CD8 pozitivních lymfocytů se zvyšoval s věkem vyšetřovaných psů. Tím se poměr CD4 a CD8 pozitivních lymfocytů zužoval z 13,7 první den po porodu na 2,0 u kategorie tří až pět let.

pes; věk; lymfocyty; neutrofilů; subpopulace; průtoková cytometrie

INTRODUCTION

Hematological parameters and the influence of age on different leukocytes are summarized in the book of veterinary hematology by Jain (1993).

Since the 90's, monoclonal antibodies against surface antigens on dogs' leukocytes have been prepared by several authors (Moore et al., 1990, 1992; Gebhard and Carter, 1992; Danilenko et al., 1992;

Voss et al., 1993). The determination and comparison of the specificity of monoclonal antibodies was presented during the First International Canine Leukocyte Antigen Workshop (CLAW), which was held in July 1993 in Cambridge (Cobbold and Metcalfe, 1994). A panel of 127 monoclonal antibodies from laboratories of USA, France, UK and FRG was presented and as well as antibodies against canine equivalents of human CD5, CD4, CD8 and Thy1 antigens on

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T-lymphocytes were defined. In addition, other antibodies against the canine homologues of the CD11/18 group, CD44 and the CD45/CD45R antigen family, antigens on the surface of B-lymphocytes (including CD21), granulocytes (possibly CD15) and platelets (probably CD41) were defined too. Antibodies against canine MHC-II and immunoglobulins were also presented (Cobbold and Metcalfe, 1994).

The effect of age on the lymphocyte subsets distribution in different animals was described in various papers. Joling et al. (1994) described distribution of lymphocyte subpopulations in the thymus, spleen, and peripheral blood of specific pathogen-free pigs from 1 to 40 weeks of age. Sellon et al. (1996) described changes in lymphocyte subsets with age in perinatal cats, and Wilson et al. (1996) referred about T-cell subsets in blood and lymphoid tissues obtained from fetal calves, maturing calves, and adult cattle. Grealey et al. (1996), in their study of 47 dogs (Labrador Retrievers), described changes in the distribution of lymphocyte subpopulations, activity of natural killer cells, antibody production, and the mitogen-induced lymphoproliferative responses. Dogs were divided into three groups according to their age: young (mean 2.4 years), middle-aged (mean 5.8 years) and old (mean 9.1 years). There is no information about distribution of leukocyte subsets in the peripheral blood of neonatal puppies.

In our research, we studied changes in total and differential counts and lymphocyte subpopulations in the peripheral blood of Beagle dogs, in both sexes and different ages, including neonatal puppies.

MATERIAL AND METHODS

Animals

Thirty Beagle dogs (examined at the department of immunology of the Veterinary Research Institute, Brno) were divided into different groups according to their age. Eighteen puppies were observed till the age of 6 months. Twelve adult dogs were divided, based on their age, into two groups. All dogs were kept in experimental premises. Their health condition was good during our investigation.

Blood collection

In neonatal puppies, blood was collected from the jugular vein, blood from older dogs was collected by venipuncture of *vena cephalica antebrachii*. Heparin (15 i.u./ml) was used as an anticoagulant. Blood was processed on the same day of collection.

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.

Flow cytometry

Enumeration of lymphocyte subsets was performed using indirect whole blood lysis technique. Fifty μ l of blood was incubated with monoclonal antibodies at laboratory temperature for 15 min. Hemolytic solution (8.26 g NH_4Cl , 1 g KHCO_3 and 0.037 g Na_4EDTA in 1 litre of distilled water) was used for erythrocyte hemolysis. After centrifugation and removal of the supernatant, secondary antibodies were added to the suspension and tubes were incubated at 4 °C for 20 min. Three ml of washing solution (1 g sodium-azid and 1.84 Na_4EDTA in 1 litre of PBS) was added into all tubes, then centrifuged, supernatant was removed, and resuspended cells were kept in washing solution with paraformaldehyde. Analysis was performed immediately after the process or the samples were stored at 4 °C until the next day.

Murine monoclonal antibodies anticanine CD3 (CA17.2A12), CD4 (CA13.1E4), CD8 α (CA9.JD3), and CD21 (CA2.1D6) kindly provided by P. Moore (Davis, USA) and murine anti-CD8 (DOG10.8E) and rat antibody anti-CD5 (DOG17.4.8) kindly provided by E. Kremmer (Munich, Germany) were used as primary antibodies. Fluorescein isothiocyanate-labelled swine anti-mouse immunoglobulin (SwAM-FITC, Sevac, Prague) and fluorescein isothiocyanate-labelled mouse anti-rat immunoglobulin (MAR-FITC, Jackson ImmunoResearch, West Grove, PA) were used as secondary antibodies. Our analysis was performed by flow cytometer FACSCalibur (Becton Dickinson, Mountain View, CA).

Statistical analysis

Statistical analysis was performed by the program STAT plus version 1.10 (Matoušková et al., 1992). Mean values, standard deviations and statistical significance using Student's *T*-test were calculated.

RESULTS

Changes in neutrophil to lymphocyte ratio in blood of six puppies of one litter in the period since parturition up to the age of one month are shown in Tab. I. On day 1 after parturition the neutrophil to lymphocyte ratio ranged between 1.34 and 3.1 (mean 2.22). Over the first week of age decrease of neutrophil count and increase of lymphocyte count was recorded. Thus the Ne to Ly ratio changed to 0.39–0.77 (mean 0.63). Since the second week of age increase of neutrophil count and consequently extension of the neutrophil to lymphocyte ratio up to 1.49–2.29 (mean 1.87) at the age of one month were recorded. Statistical significance of the changes is shown in Tab. V.

I. The effect of age on neutrophil to lymphocyte ratio in the peripheral blood of dog in early postnatal phase of the development (mean \pm SD)

Age		1st day	1st week	2nd week	3rd week	1st month
No. of animals		6	6	6	6	6
Leukocytes	($10^9/l$)	10.0 \pm 1.1	11.9 \pm 4.7	10.0 \pm 0.9	10.7 \pm 2.1	12.7 \pm 3.2
Lymphocytes	($10^9/l$)	2.8 \pm 1.1	5.9 \pm 3.0	4.4 \pm 0.7	3.9 \pm 1.1	4.2 \pm 1.0
Neutrophils	($10^9/l$)	5.4 \pm 0.9	3.5 \pm 1.3	4.5 \pm 0.4	5.8 \pm 1.7	7.7 \pm 2.8
Ne : Ly ratio		2.2 \pm 0.9	0.6 \pm 0.2	1.0 \pm 0.2	1.6 \pm 0.7	1.9 \pm 0.6

II. The effect of age on the distribution of lymphocyte subsets in the peripheral blood of dog in early postnatal phase of the development (mean \pm SD)

Age		1st day	1st week	2nd week	3rd week	1st month
No. of animals		3	6	6	6	6
CD3	%	47.9 \pm 6.1	65.6 \pm 7.5	57.1 \pm 10.3	55.2 \pm 7.6	55.0 \pm 5.2
	abs.c.($\times 10^9/l$)	1.2 \pm 0.4	3.8 \pm 0.8	2.5 \pm 0.8	2.2 \pm 0.9	2.0 \pm 0.7
CD5 total	%	49.9 \pm 7.3	65.4 \pm 11.4	56.5 \pm 11.0	52.9 \pm 8.3	54.2 \pm 7.0
	abs.c.($\times 10^9/l$)	1.2 \pm 0.5	3.8 \pm 1.9	2.5 \pm 0.8	2.1 \pm 0.9	2.0 \pm 0.6
CD5 high	%	*	*	*	39.1 \pm 10.1	43.8 \pm 9.1
	abs.c.($\times 10^9/l$)				1.6 \pm 0.8	1.6 \pm 0.6
CD5 low	%	*	*	*	13.8 \pm 4.2	10.4 \pm 4.5
	abs.c.($\times 10^9/l$)				0.5 \pm 0.2	0.4 \pm 0.2
CD4	%	36.9 \pm 5.2	50.2 \pm 6.1	43.8 \pm 7.8	35.6 \pm 9.5	39.9 \pm 5.1
	abs.c.($\times 10^9/l$)	0.9 \pm 0.3	2.9 \pm 1.2	1.9 \pm 0.6	1.4 \pm 0.7	1.4 \pm 0.4
CD8	%	2.7 \pm 0.4	3.3 \pm 1.7	10.9 \pm 1.5	13.7 \pm 2.5	11.7 \pm 2.0
	abs.c.($\times 10^9/l$)	0.1 \pm 0.1	0.2 \pm 0.2	0.5 \pm 0.1	0.5 \pm 0.2	0.4 \pm 0.2
CD21	%	41.9 \pm 1.3	35.6 \pm 7.0	36.1 \pm 10.6	41.2 \pm 9.8	41.1 \pm 7.2
	abs.c.($\times 10^9/l$)	1.0 \pm 0.3	2.1 \pm 1.0	1.5 \pm 0.4	1.6 \pm 0.3	1.5 \pm 0.5
CD5/CD21		1.2 \pm 0.1	2.1 \pm 0.6	1.7 \pm 0.8	1.4 \pm 0.5	1.3 \pm 0.4
CD4/CD8		13.7 \pm 3.1	15.3 \pm 6.3	4.0 \pm 0.6	2.7 \pm 0.9	3.2 \pm 0.9

* these subpopulations could not be distinguished

Development of changes of lymphocyte subpopulations in the same group of puppies is shown in Tab. II. On day 1 after parturition nearly half of all the lymphocytes were CD21 positive. Almost all CD5 positive lymphocytes present in peripheral blood were CD4 positive. CD8 positive lymphocytes were minimum. Over the first week of age an increase of all lymphocyte types occurred, especially CD4 positive (and therefore also CD3 and CD5 positive) and to a lesser extent also CD21 positive. Since the second week of age CD3, CD5, CD4 and CD21 positive lymphocyte counts remained relatively invariable. Number of CD8 positive lymphocytes increased with age over the whole experimental period. In CD5 positive lymphocytes, two subpopulations could be distinguished since the age of three weeks based on the amount of expressed molecules of the antigen. Statistical significance of these changes is shown in Tab. V.

Tab. III shows the effect of age on neutrophil to lymphocyte ratio in dogs older than 1 month. Generally, a relatively invariable number of neutrophils and drop of lymphocyte count based on age was observed. This determined the extension of the neutrophil to lymphocyte ratio from 1.29 \pm 0.51 in 1 month to 1.97 \pm 0.96 in 3–5 years of age. Statistical significance of these changes is shown in Tab. VI.

Proportion of lymphocyte subpopulations in the peripheral blood of dogs of different age categories is shown in Tab. IV. In a lymphocyte population expressing antigens CD3 and CD5 the same dynamics was observed. Percentual proportion of these populations rose up to the age category 1–2 years. Increased numbers of cells with high expression of this antigen were found in CD5 positive lymphocytes. Proportion of CD4 positive lymphocytes appeared as relatively invariable. However, the proportion of CD8 positive lymphocytes increased with age of the examined animals. The greatest changes in their proportion were determined in CD21 positive lymphocytes. A marked decrease of percentual proportion as well as final counts of those lymphocytes were recorded between the age categories 3 months and 1–2 years. Statistical significance of these changes is shown in Tab. VI.

Fig. 1 shows the charts of CD4 antigen expression on neutrophils in neonatal puppies (above) and adult dogs (below).

III. The effect of age on neutrophil to lymphocyte ratio in the peripheral blood of dog (mean \pm SD)

Age		1 month	3 months	6 months	1-2 years	3-5 years
No. of animals		18	9	5	7	5
Leukocytes	($10^9/l$)	11.5 \pm 2.1	13.5 \pm 1.6	13.9 \pm 3.3	11.3 \pm 3.5	10.6 \pm 2.4
Lymphocytes	($10^9/l$)	4.8 \pm 1.3	5.3 \pm 0.9	4.2 \pm 0.5	4.0 \pm 0.3	3.4 \pm 0.8
Neutrophils	($10^9/l$)	5.8 \pm 1.4	6.7 \pm 1.3	7.7 \pm 3.2	5.9 \pm 2.8	6.2 \pm 2.4
Ne : Ly ratio		1.3 \pm 0.5	1.3 \pm 0.5	1.9 \pm 0.9	1.5 \pm 0.7	2.0 \pm 0.9

IV. The effect of age on the distribution of lymphocyte subsets in the peripheral blood of dog (mean \pm SD)

Age		1 month	3 months	6 months	1-2 years	3-5 years
No. of animals		18	9	5	7	5
CD3	%	54.3 \pm 11.5	61.2 \pm 5.7	76.4 \pm 3.6	78.8 \pm 5.7	75.6 \pm 11.1
abs.c.	($\times 10^6/l$)	2.5 \pm 0.7	3.1 \pm 0.6	2.6 \pm 0.7	3.1 \pm 0.3	2.5 \pm 0.8
CD5 total	%	52.5 \pm 10.6	62.5 \pm 9.9	not tested	78.9 \pm 7.2	70.8 \pm 9.4
abs.c.	($\times 10^6/l$)	2.4 \pm 0.7	3.2 \pm 0.7	not tested	3.0 \pm 0.3	2.3 \pm 0.8
CD5 high	%	41.8 \pm 10.5	47.5 \pm 9.3	not tested	66.1 \pm 6.1	58.3 \pm 8.1
abs.c.	($\times 10^6/l$)	1.9 \pm 0.6	2.6 \pm 0.4	not tested	2.5 \pm 0.3	2.0 \pm 0.6
CD5 low	%	10.5 \pm 3.7	14.9 \pm 2.6	not tested	13.3 \pm 1.4	12.3 \pm 2.3
abs.c.	($\times 10^6/l$)	0.5 \pm 0.2	0.8 \pm 0.2	not tested	0.5 \pm 0.1	0.4 \pm 0.2
CD4	%	36.5 \pm 7.8	37.8 \pm 6.2	44.6 \pm 8.3	43.4 \pm 8.3	38.5 \pm 8.9
abs.c.	($\times 10^6/l$)	1.7 \pm 0.5	1.9 \pm 0.4	1.7 \pm 0.6	1.7 \pm 0.3	1.3 \pm 0.5
CD8 α	%	10.5 \pm 3.8	15.1 \pm 3.3	12.8 \pm 3.1	18.7 \pm 3.4	20.2 \pm 3.3
abs.c.	($\times 10^6/l$)	0.5 \pm 0.2	0.7 \pm 0.2	0.5 \pm 0.2	0.8 \pm 0.1	0.7 \pm 0.2
CD21	%	40.5 \pm 10.2	40.8 \pm 11.0	not tested	15.2 \pm 4.3	18.6 \pm 6.1
abs.c.	($\times 10^6/l$)	2.0 \pm 0.9	2.2 \pm 0.7	not tested	0.6 \pm 0.2	0.7 \pm 0.3
CD5/CD21		1.3 \pm 0.4	1.5 \pm 0.5	not tested	6.3 \pm 2.0	4.7 \pm 2.4
CD4/CD8		3.5 \pm 0.8	2.5 \pm 0.7	3.5 \pm 1.2	2.5 \pm 0.5	2.0 \pm 0.9

V. Statistical significance of differences between age categories in parameters shown in Tabs. I and II

	a/b	a/c	a/d	a/e	b/c	b/d	b/e	c/d	c/e	c/d
Leukocytes										
Lymphocytes		*		*						
Neutrophils	*	*				*	**		*	
Ne : Ly ratio	**	*			**	*	**		*	
CD3	%	**				*	*			
abs.c.		**	*		*	**	**			
CD5 total	%									
abs.c.			*							
CD4	%	*				*	**			
abs.c.		*	*			*	*			
CD8 α	%		**	**	**	**	**	*		
abs.c.			**	*	*	**	*			
CD21	%									
abs.c.			*							
CD5/21		*					*			
CD4/8			*	*	*	**	**	**	*	

Sign of category:

a - 1st day

d - 3rd week

b - 1st week

e - 1st month

c - 2nd week

Statistical significance:

* $P < 0.05$

** $P < 0.01$

VI. Statistical significance of differences between age categories in parameters shown in Tabs. III and IV

	a/b	a/c	a/d	a/e	b/c	b/d	b/e	c/d	c/e	e/d
Leukocytes	*						*			
Lymphocytes			*	*	*	**	**			
Neutrophils										
Ne : Ly ratio				*						
CD3		**	**	**	**	**	**			
%	*									
abs.c	*		*							
CD5 total		NT	**	**	NT	**		NT	NT	
%	*	NT	*		NT			NT	NT	
abs.c.	**	NT	*		NT		*	NT	NT	
CD5 high		NT	**	*	NT	**		NT	NT	
%		NT	*		NT		*	NT	NT	
abs.c.	**	NT	*		NT	**	**	NT	NT	
CD5 low		NT	*		NT			NT	NT	
%	**	NT	*		NT			NT	NT	
abs.c.	**	NT	*		NT	**	**	NT	NT	
CD4										
%										
abs.c.										
CD8			**	**			*	**	**	
%	**		**	**			*	**	**	
abs.c.	*		**					*		
CD21		NT	**	**	NT	**	**	NT	NT	
%		NT	**	**	NT	**	**	NT	NT	
abs.c.		NT	**	**	NT	**	**	NT	NT	
CD5/21		NT	**	*	NT	**	*	NT	NT	
%		NT	**	*	NT	**	*	NT	NT	
abs.c.		NT	**	*	NT	**	*	NT	NT	
CD4/8	**		**	**						

Sign of category: Statistical significance:

- a - 1st month * $P < 0.05$
- b - 3rd month ** $P < 0.01$
- c - 6th month
- d - from 1 to 2 years
- e - from 3 to 5 years

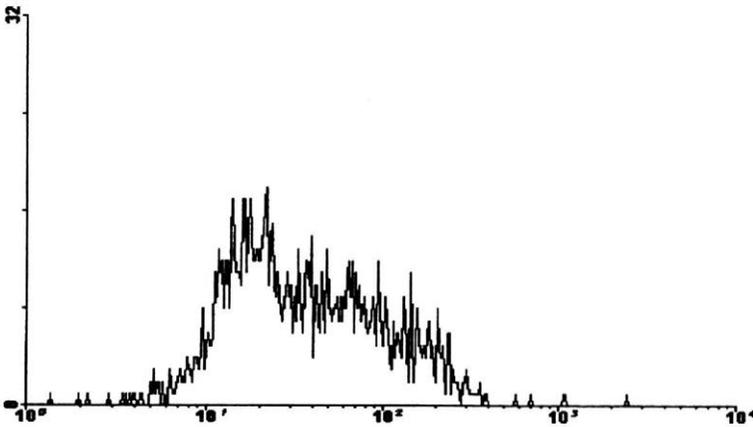
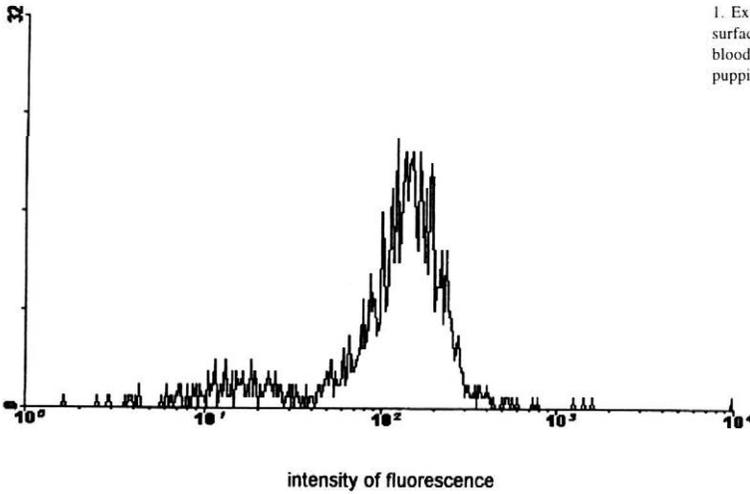
DISCUSSION

It is known that young dogs, cats and horses have higher lymphocyte counts compared with adult ones. Jain (1993) reports in Beagle dogs, in the age group 0-3 days, the values $3.7 \pm 2.3 \times 10^9/l$ lymphocytes and $9.8 \pm 7.1 \times 10^9/l$ neutrophils. Further he describes a reduction of the ratio between neutrophils and lymphocytes till the age of 40 to 45 days. In this age he reports the values $7.6 \pm 2.6 \times 10^9/l$ neutrophils and $6.1 \pm 1.9 \times 10^9/l$ lymphocytes. In our investigations, neonatal puppies had at first higher neutrophil counts and within the first week there was a shift towards the prevalence of lymphocytes. Higher neutrophil count was probably due to cortisol concentration which increases in fetal blood up to 10 times 1 to 2 days prior to parturition. Application of corticoids results in the decrease of lymphocytes and increase of neutrophils (our unpublished data). Our results (Fig. 1) suggest that a part of neutrophils of peripheral blood in neonatal puppies is less matured. Our suggestion is based on the observation by Moore et al. (1992). The authors describe a phenomenon known so far only from the peripheral blood of dog - expression of CD4 antigen on nonstimulated neutrophils. Importance of this phenomenon has not been explained yet. The authors divided bone mar-

row neutrophils by high- and low-density gradients of ficoll (1.077 g/cm^3 and 1.119 g/cm^3) according to the stage of granulation into more and less matured. Analysis of expression of CD4 antigen revealed that expression of CD4 antigen on neutrophils is a sign of maturation. We investigated the expression shown in Fig. 1 till the age of one month. Since that age the expression of CD4 antigen on neutrophils has been of a character as shown in Fig. 1 (below). Phagocytary and metabolic activity of neutrophils in the peripheral blood of neonatal puppies requires further research.

Antigen CD5 in dog, unlike in rodents and men, has not been detected on any B-lymphocytes, B-cell lines nor B-cell areas by immunohistology (Cobbold and Metcalfe, 1994). Therefore in our investigations we considered the antigen CD5 as a marker of T-lymphocytes. The expression of CD5 on canine T cells could be resolved into major high expressing, and minor low expressing subpopulations. The subpopulation with low expressing remains to be seen $\gamma\delta$ T cell or NK-cell populations (Cobbold and Metcalfe, 1994). We have detected this resolution only since the 3rd week of the age of a puppies (Tab. II). Antibodies against CD3 antigen were not present on I.C.LAW. In spite that such antibodies already exist, detailed relations bet-

1. Expression of CD4 antigen on the surface of granulocytes in peripheral blood of adult dog (up) and neonatal puppies (below)



between CD3 and CD5 positive lymphocyte populations are not known. Antigen CD4 is in dog expressed on subpopulation of T-lymphocytes and neutrophils (Moore et al., 1992). While in the rat and men CD4 is expressed on T lymphocytes and also on monocytes. The antigen CD8 as a co-receptor for class I. MHC is expressed on cytotoxic T-lymphocytes in the form of $\alpha\beta$ heterodimers, although NK cells may express α chain homodimers. Cells CD4/CD8 double positive do not occur in dog's blood under normal conditions. The antibody against caCD21 (on 1.CLAW designed as CLAW#105) were used for detection of B-lymphocytes.

Some authors used in their studies the methods of detection of lymphocyte subpopulations in peripheral dog's blood. Dirscherl et al. (1995) used in their study 12 Beagle dogs (five females and seven males) aged 3.5–4.5 years. They compared the proportion of lymphocyte subpopulations in bronchoalveolar lavages

and peripheral blood. They found in blood $69.0 \pm 3.9\%$ CD5-positive lymphocytes, $44.5 \pm 3.2\%$ CD4 positive, and $25.7 \pm 4.8\%$ CD8 positive lymphocytes. Ratio between CD4 and CD8 positive lymphocytes is given as 1.81 ± 0.3 . Compared with our results, they detected higher proportion of all the examined populations. Proportions between CD4 and CD8 positive lymphocytes have been the same.

Greeley et al. (1996) described the effect of age on the immune system of dog. They also recorded the changes in proportion of subpopulations in peripheral blood. 47 Labrador Retrievers were divided according to the age into three groups: young (mean 2.4 years), middle-aged (mean 5.8 years) and old (mean 9.1 years). They described a statistically significant decline of percentual proportion of B lymphocytes and increase of percentual proportion of T-lymphocytes between the age categories 2.4 and 5.8 years. Increased T-lympho-

cyte count was a result of an increase of CD8 positive lymphocytes. General trends in the increase of CD8 positive cells (and thus of all T-lymphocytes) and decrease of B-lymphocytes are in accordance with our results.

Chabanne et al. (1995) used the method of immunophenotyping for study of abnormalities of lymphocyte subpopulations at German Shepherd dogs with pyoderma. Authors described the distribution of lymphocyte subpopulations in the peripheral blood of 12 German Shepherds of good health (seven males and five females) aged 1–7 years. Those results are not comparable with ours, because dogs were not divided to groups according to their age. However, based on our results, we premise that exist differences in hematological parameters and proportion of lymphocyte subpopulations in German Shepherds compared with other breeds of dogs. This phenomenon may play an important role in pathogenesis of some diseases. We referred about the decreased total lymphocyte count and a different proportion of lymphocyte subpopulations in peripheral blood of Beagle dogs (Toman et al., 1997).

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A CONCISE CATALOGUE OF SELECTED PHARMACEUTICALS**V. Šutiak***University of Veterinary Medicine, Košice, Slovak Republic. 1997.*

At the beginning of December 1997 a new pharmacological publication appeared in the University of Veterinary Medicine Library, dealing with selected pharmaceuticals for students of various branches of medicine and science (e.g. veterinary medicine, pharmacy, zoology, zootechnique, natural history and others).

I am sure that this publication may help many students, especially English-speaking increase and deepen the scope of their pharmacological knowledge not only to improve their practice in field conditions and but also to help increase the prosperity of their country of origin. It may also serve however as a good guidebook to special terminology for those Slovak and Czech students and practitioners who are thinking of working abroad, especially in connection with the application of the Slovak and Czech Republics to become members

of the European Union, and also to be successful in veterinarian activities after accession.

The text about the pharmaceuticals focuses, above all, on the most important characteristics of the selected pharmaceuticals (their exact name, composition indications, packaging, method of dosage, etc.) and in some cases on other important characteristics (cautions, contraindications, ways of administration, etc.). In writing it, the author consulted various foreign sources, especially those in the most industrially-developed countries in addition to Slovak and Czech literature, concentrating on the last six years to bring the information up to date.

It was the author's intention to enable all those studying this catalogue to use it as an opportunity to acquire important data from the text.

Doc. MVDr. A. Jurčina, CSc.

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