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The effect of polychlorinated biphenyls on some parameters of non-specific immunity in *Ovis musimon* and postpartum changes of enzyme activity in the glandular cells of the uterine endometrium

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ABSTRACT

The study was focused on the observation of alkaline and acidic phosphatase activity in the glandular cells of the uterine endometrium in puerperal moufflon ewes (*Ovis musimon*) after exposure to polychlorinated biphenyls. Fifteen animals divided into 2 groups (experimental group – n=8; control group – n=7) were included in the experiment. The immunotoxic effect was evaluated by the test of the ingestion ability of phagocytes (phagocytic activity and index of phagocytic activity) and blastogenic response of lymphocytes after mitogen stimulation. For thirty days moufflons in the experimental group were given per orally capsules of domestically made Delor 105 chemical preparation (100 μg/kg) containing polychlorinated biphenyls (PCB). The blood samples were taken on day 17 postpartum, i. e. 5 days from the termination of a 30-day PCB application; on day 25 postpartum, i.e. 17 days from the last application of PCB and on day 34 postpartum which was equivalent to day 28 from the termination of the application. The samples of endometrium were collected from the uterine horn by Palmer´s laparoscope on day 17, day 25 and on day 34 postpartum under general anaesthesia (Diprivan 1%, propofol) given i.v. When evaluating alkaline phosphatase (ALP) activity in the glandular cells of the endometrium in the control group, a statistically significant increase ($P<0.01$) was observed on day 25 ($P<0.001$) and on day 34 compared to day 17 postpartum. Acidic phosphatase activity in the glandular cells of the ewes' endometrium showed a statistically significant increase between day 17 and day 25 as well as day 17 and day 34 postpartum ($P<0.001$). No statistically significant differences in alkaline phosphatase (ALP) activity during the post-partum period were observed ($P>0.05$) in the experimental group. Acidic phosphatase density in the experimental group of ewes also showed no statistically marked change ($P>0.05$) at the observed intervals postpartum. Compared to control groups, all investigated functional activities of phagocytes (phagocytic activity

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and phagocytic index) and lymphocytes (blastogenic response to mitogens) were significantly suppressed in moufflons treated with polychlorinated biphenyls.

Key words: moufflons, non-specific immunity, alkaline phosphatase, acidic phosphatase, polychlorinated biphenyls

Introduction

Polychlorinated biphenyls commonly known as PCBs are man made chemicals originally widely used in industrial processes but later found to be dangerous environmental pollutants. They make up a group of 209 individual chlorinated biphenyl rings known as congeners. In the concentrated form, PCBs are either liquid or solids with no discernable taste or odour. Also, PCBs with large number of chlorines are more stable and thus resistant to biodegradation (BARBALACE, 2003). These congeners present the greatest risk for the environment because of their high thermal and chemical resistance that means they do not readily break down and they remain in the environment for very long periods of time. They can easily cycle between air, water and soil. The PCBs have been demonstrated to cause a variety of adverse health effects. PCBs have been shown to cause cancer in animals and a number of serious non-cancer health effects in animals, including effects on the immune system, reproductive system, nervous system, endocrine system and other health effects (FONNUM et al., 2006). The different health effects of PCBs may be interrelated, as alterations in one system may have significant implications for the other systems of the body.

Moufflons are wild animals that live in natural conditions and by that are possibly exposed to the effects of various chemicals present in environment. Environmental contamination and its effect on wild animal health are considered an urgent issue of veterinary and agricultural sciences, as it has serious consequences including reproduction problems and decreased immunocompetence.

The aim of this study was to observe the activity of enzymes of alkaline and acidic phosphatase in the glandular cells of the endometrium in puerperal ewes (moufflons – *Ovis musimon*) after exposure to polychlorinated biphenyls (applied in Delor 105 preparation) and to detect the possible effect of PCB on enzyme activity during regeneration and regressive processes in the above stated period. Simultaneously, the immunotoxic effect of this preparation on the functional activity of lymphocytes and phagocytes was evaluated.

Materials and methods

Animals. The experiment was approved by a permit based on accreditation of the

State Veterinary and Food Administration of the Slovak Republic (accreditation number 10115/02-220). 15 moufflons divided into 2 groups (the experimental group, n=8 and control group, n=7) were included in the experiment. The experimental group of ewes were given perorally capsules of domestically produced PCB preparation perorally (trade name Delor 105; Chemko, Slovakia), which is an equivalent to the foreign preparation Aroclor 1254 (CAS 11097691). The ewes were weighed individually before exposure to the chemical and Delor was then administered at the rate of 100 μg/kg of the body weight. The animals were kept in an experimental centre during the experiment. Feed ration per animal/day consisted of meadow hay, fodder concentrate, root crops. A mineral supplement and water were available ad libitum.

Sampling. The samples of endometrium were collected from the uterine horn by Palmer´s laparoscope on day 17, day 25 and on day 34 postpartum under general anaesthesia (Diprivan 1%, propofol) given i.v. Samples of the endometrium from the uterine body and uterine horns were frozen in liquid nitrogen vapour (-196 ^{o}C) and stored in a freezer at -20 ^{o}C until processed. The blood samples for immunological analysis were taken on day 17 postpartum, i. e. 5 days from the termination of a 30-day application; on day 25 postpartum, i.e. 17 days from the last application of PCB; on day 34 postpartum which was equivalent to day 28 from the termination of the application. The schedule of PCB administration and blood and endometrium sample collection is shown in Tab. 1

Table. 1. The schedule of PCB administration (30 days) and sample collection

PCB administration			Sample collection		
1st application	delivery	last application	I.	II.	III.
18 days PRP	P	12 days PP	17 days PP	25 days PP	34 days PP

Legend: PRP – praepartum; P – partus ; PP – postpartum

The determination of alkaline phosphatase and acidic phosphatase was performed by a modified method as described by LOJDA et al. (1979). Integrating absorbance was measured at a wavelength of 480 μm and 520 μm. Enzyme density was analysed cytophotometrically with a VICKERS 85 (UK) microdensitometer. The measurements were accomplished using a x40 objective in scanning area of 28.3 μm^3 and 0.5 μm scanning spot. A masking shield was placed minimally on 30 areas along uterine sections. The activity of the observed enzymes was calculated as absorbance values min/ μm^3 recorded by the instrument in the glandular uterine epithelial cells.

Blastogenic response of blood lymphocytes to mitogens. Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech Ab, Sweden). The cultivation (culture medium contained 10% of autologous serum), mitogen stimulation

and the measurement of blastogenic response of lymphocytes by the fluorescence method were performed according to NAGAHATA et al. (1986). Concanavalin A (Con A, Sigma Chemical co., USA) was used for stimulation in the concentration 25 µg.ml-1 (10). The level of the blastogenic response of the lymphocytes was expressed as the stimulation index (SI).

Phagocytic activity of blood neutrophils was examined as described by VĚTVIČKA et al. (1982) using 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 µm, Artim Prague, Czech Republic). The phagocytic activity (PA) of neutrophils (Ne) was expressed as the percentage of the neutrophils phagocytizing 3 and more MSHP, and as the index of phagocytic activity (IPA) representing the ingestion ability of neutrophils (the ratio of the number of phagocytized MSHP and the number of potentially phagocytizing Ne).

Statistic evaluation. Statistical evaluation was accomplished using one-way variance analysis (ANOVA). Variance significance between the groups and statistical significance among individual days of samplings at postpartum period was determined by the Tukey test.

Results

Alkaline phosphatase (AP) activity in the glandular cells of the endometrium (Tab. 1) had a rising tendency with statistical significance detected between day 17 and day 25 postpartum ($P<0.01$). In the control group the range of values of AP activity showed a significant difference ($P<0.001$) between day 17 and day 34 of the experimental period in the control group.

No statistically significant differences were detected ($P>0.05$) when alkaline phosphatase activity was studied in the glandular cells of the endometrium (Tab. 1) in the experimental group of ewes. In the control group, mean values of its density in the evaluated period were below the level of values of day 17.

The evaluation of acidic phosphatase activity in the glandular cells of the endometrium in the control group of ewes is presented in Tab. 2. Its activity showed a statistically significant increase between day 17 and day 25 as well as day 34 postpartum ($P<0.001$). It is evident that acidic phosphatase activity observed in the glandular cells of the endometrium of puerperal ewes in the control group had an increasing tendency. Acidic phosphatase activity in the glandular cells of the ewes' endometrium in the experimental group (Tab. 2) did not vary significantly at the observed intervals of the postpartum period ($P>0.05$). Its values were below the level of day 17 in the control group. The range of mean values of acidic phosphatase in the experimental group was significantly lower ($P<0.001$) on evaluated days of the puerperal period than values in the control group. We

concluded, that available data and our current results indicate that exposure of animals to polychlorinated biphenyls (Delor 105) given to the experimental group of ewes in this experiment, had an inhibitory effect on acidic and alkaline phosphatase activity in reparative and regressive processes occurring in the uterus during the puerperal period.

Table 2 The mean values of the activity of alkaline phosphatase in the endometrial glandular cells of the control and experimental groups of ewes after parturition

Days postpartum	Alkaline phosphatase	
	Control	Experimental
17	5.394 ± 0.243 a	5.088 ± 0.63 a
25	6.240 ± 0.497 b**	5.169 ± 0.61 b
34	6.664 ± 0.396 b***	5.228 ± 0.62 b

Statistical significance compared to day 17 and other days after parturition ** = P<0.01; *** = P<0.001

Significant differences between the control and experimental groups during the observed days after parturition a : a = P>0.05 ; b : b = P<0.001

Functional status of the immune system cells (Tab. 3) was already altered during the first sampling (day 17). A non-significant decrease was found in phagocytic activity and the index of phagocytic activity of neutrophils that was lower in experimental group and in the last sampling showed a significant decrease (P<0.001) in comparison to the control group. The ability of moufflon lymphocytes to react to mitogen by proliferation expressed as the stimulation index (SI), has significantly decreased after PCB application in the first sampling (P<0.001). When these altered values were compared to values of the control group, it persisted until the end of our observation.

Table 3 The mean values of the activity of acidic phosphatase in the endometrial glandular cells of the control and experimental groups of ewes after parturition

Days postpartum	Acidic phosphatase	
	Control	Experimental
17	7.860 ± 0.32 b	6.78 ± 0.48 b
25	9.017 ± 0.63 b***	6.86 ± 0.45 b
34	10.17 ± 0.35 b***	7.13 ± 0.28 b

Statistical significance compared between day 17th and other days after parturition***=P<0.001.

Significance of differences between the control and experimental groups of ewes during observation days post partum. b:b = P<0.001

Discussion

The proliferation process of endometrial structures of sheep and goats as observed by KRAJNIČÁKOVÁ et al. (2002) coincides with the presence of alkaline phosphatase activity which has a role in transformation mechanisms and metabolic processes occurring in the postpartum uterus. In our study the activity of alkaline phosphatase activity in the glandular cells of the endometrium had a rising tendency in the control group. Similar results were reported in goats by KRAJNIČÁKOVÁ et al. (2002) during regressive changes occurring on the uterus in the postpartum period. KHAN and THOMAS (2001) claimed that exposure to PCB was the cause of a decrease in the concentration of hypothalamic serotonin (5-hydroxytriptamine) and the inhibitory effect on the activity of hypothalamic tryptophan hydroxylase. If the above stated facts regarding the PCB inhibitory effect on the enzymatic system are taken into account, then the stagnation of alkaline phosphatase activity observed by the authors after exposure of ewes to PCB confirms the above mentioned facts. The results of acidic phosphatase activity found in our study are comparable with mean values of its activity in postpartum goat as reported by KRAJNIČÁKOVÁ et al. (2003). The authors presume that the increasing tendency of acidic phosphatase in the postpartum period is due to the onset of gradual postpartum restoration of the uterine endometrium, which plays an important role in physiological regressions. Experiments conducted on mice, rats (ANDRIC et al., 2000) and sheep (JAN et al., 1999) showed that exposure to polychlorinated biphenyls markedly affected: sperm motility, follicular growth and maturation and embryonal development with an inhibitory effect on the enzymatic system at circulating steroidal hormones conversion. Acidic phosphatase activity in the experimental group observed by the authors is probably linked with the fact mentioned previously.

Optimal functional status of the immune system is a decisive factor limiting the susceptibility of an organism to pathogenic agents. Any alteration of the particular immune components has serious consequences for the health of the affected individual. Polychlorinated biphenyls are substances that can interfere with the normal function of the immune cells, negatively influencing humoral and cellular immunity (BANERJEE and HUSSAIN, 1987) and the incidence of tumours (LEŠNÍK, 2004). Alteration of non-specific immunity results in higher incidence of infections, occurrence of the diseases caused by opportunistic pathogens and the presence of recurrent diseases resistant to therapy. The studies in humans and animals suggest that PCBs may have serious potential effects on the immune systems of exposed individuals. It was shown that PCBs induce formation of reactive oxygen species (ROS) in neutrophils. This takes place primarily through phosphorylation and subsequent activation of NADPH oxidase. The production of ROS may have an adverse effect on the immune system (FONNUM et al., 2006). An immunotoxic effect of PCBs on human leukocyte phagocytosis has been documented.

The study suggests an aryl hydrocarbon receptor (AhR) independent pathway through which non-coplanar PCBs modulate phagocytosis, possibly increasing the risk of developing infectious disease (LEVIN et al., 2005a). In free-living mammalian species PCBs are known to have detrimental effects on the innate immune system because of their bioaccumulation to high concentration. The immunotoxic effect on phagocytosis, respiratory burst and cytotoxic activity of the leukocytes of marine mammals following incubation with PCBs was reported in the study by HAMMOND et al. (2005). These authors suggest a direct relationship between the vulnerability of the immune cells to immunotoxic effect of such contaminants and disease susceptibility of the animal species. Our findings are in agreement with previously published results and also confirm the significant immunosuppressive effect of PCB on the functional status of blood phagocytes regarding their activity and ingestion capacity.

Lymphocyte proliferation is an important part of adaptive immunity and any alteration of this function presents the possibility of increased susceptibility to diseases. The negative effect of PCBs on lymphocyte function was shown in the study by LYCHE et al. (2004). Goat kids, whose mothers were dosed with low doses of PCBs during pregnancy, exhibited significantly lower mean lymphocyte response to phytohaemagglutinin and to concanavalin A. The finding supports the hypothesis that PCBs mediate immunotoxic effects through both AhR – dependent and – independent mechanisms. Similar data are reported in the studies of the biological effect of PCBs on lipopolysacharide (LPS) – induced splenocyte proliferation and LPS induced antibody secretion that was probably inhibited through AhR –independent pathway (SMITHWICK et al., 2003). The relationships between mitogen-induced peripheral blood lymphocyte proliferation and PCB in harbour seal pups that could result in increased susceptibility to infections was revealed in a study by LEVIN et al. (2005b). Similarly, significant modulation of lymphocyte proliferation by organochlorines was reported by MORI et al (2006) in marine mammals and mice. The alteration of the proliferative activity of lymphocytes after stimulation with mitogen ConA that was proved in our study is in agreement with the results mentioned above.

In conclusion we can confirm the suppressive effect of PCB application on the functional status of lymphocytes and phagocytes in moufflons in vivo conditions that may affect their susceptibility to infection and reproduction ability. The manufacture of PCBs has gradually diminished; but PCBs are still persistent environmental contaminants and a potential health hazard for free-living animals. The results found in this study may have important implication for risk assessment and management strategies in moufflon breeding and their protection.

Acknowledgements cv

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MOJŽIŠOVÁ, J., I. VALOCKY, M. GOLDOVÁ, V. LETKOVÁ, V. BAJOVÁ: Učinak polikloriranih bifenila na pokazatelje nespecifične imunosti u endometriju muflona. Vet. arhiv 76, S151-S159, 2006.

SAŽETAK

Prikazana je aktivnost enzima kisele i alkalne fosfataze u žljezdanim stanicama endometrija muflona u tijeku puerperija, a nakon izlaganja polikloriranim bifenilima (PB). Ukupno 15 životinja podijeljeno je u pokusnu (n=8) i kontrolnu skupinu (n=7). Imunotoksičnost je provjeravana testom fagocitoze (indeks i aktivnost fagocita) i blastogenim odgovorom limfocita nakon podražaja. U tijeku 30 dana, mufloni su u pokusnoj skupini per/oralno primili kapsule preparata Delor 105 (100 μg/kg) s PB. 17 dana nakon janjenja (5 dana nakon zadnje aplikacije PB-a) uzeti su prvi uzorci krvi, drugi uzorci 25 dana poslije porođaja (17 dana nakon zadnje aplikacije PB-a) i treći uzorci 34 dana poslije porođaja (28 dana nakon zadnje aplikacije PB-a). Uzorci endometrija prikupljeni su Palmerovim laparoskopom 17., 25. i 34. dana nakon janjenja u uvjetima opće anestezije (Diprivan 1%, propofol, izravno u venu). Statistički značajan porast ($p<0,01$) aktivnosti enzima alkalne fosfataze u žljezdanim stanicama endometrija zabilježen je 25. i 34. dana nakon janjenja, a u usporedbi sa 17. danom. Aktivnost enzima kisele fosfataze značajno je porasla od 17. do 25. dana, kao i od 17. do 34. dana poslije janjenja ($p<0,001$). U usporedbi s kontrolnom skupinom aktivnost fagocita i limfocita značajno je umanjena u muflona obrađenih PB-ima.

Ključne riječi: muflon, nespecifična imunost, kisela fosfataza, alkalna fosfataza, poliklorirani bifenili