IMMUNE RESPONSE AND IMMUNOMODULATORY EFFECT OF LEVAMISOLE IN IMMUNOSUPPRESSED DOGS VACCINATED AGAINST PARVOVIRUS

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Abstract

The specific and non-specific immune response and immunomodulatory effect of levamisole were studied in dogs with altered immune function due to giardiosis and vaccinated against canine parvovirus (CPV) infection. In immunosuppressed dogs combination of vaccine with levamisole treatment enhanced depressed phagocytic ability and stimulated proliferation activity of lymphocytes. CPV antibody production was one week delayed in dogs with immunosuppression. Marked higher titer of CPV antibodies was found after levamisole application.

Key words: dogs, immunomodulation, vaccination, levamisole.

Suppression of the immune system activity is often described as a phenomenon accompanying infectious and parasitic diseases (19). Immunosuppressive conditions can influence the effect of vaccination with the consequences on the protection of animals against infectious diseases. In small animal practice, canine parvovirus infection and canine distemper are infectious diseases that can be characterized by a high morbidity and mortality in puppies between the ages 6 weeks to 6 months (14). Therefore, it is a common practice to immunize the puppies with modified live or inactivated vaccine. Immunomodulatory effect of levamisole has been used to enhance immune system reactivity and specific response after vaccination (5, 16). The aim of present study was to evaluate phagocytic activity of leukocytes, proliferation activity of lymphocytes and production of specific antibodies after vaccination against CPV infection in immunosuppressed animals and possible immunostimulation with levamisole.

Material and Methods

Animals. Fifteen dogs of different breeds and sexes at the age of 4 months were used. The dogs were divided into 3 groups:

- group 1 - 5 dogs coming from home for stray dogs, suspected of immunosuppression due to giardiosis (Giardia intestinalis) confirmed by parasitological examination: these dogs were injected with levamisole (3.5 mg/kg) followed by vaccination against canine parvovirus (CPV) infection using commercially available modified live vaccine (Biocan P, Bioweta a.s., Ivanovice na Hané, Czech Republic, Parvovirus enteritidis canis min. 10^5.0 TCID₅₀ or 512 HAU); levamisole was injected two days before the vaccination, at day of vaccination and three days post vaccination;
- group 2 - 5 dogs coming from home for stray dogs, suspected of immunosuppression due to giardiosis and vaccinated against CPV infection;
- group C – control group - 5 healthy dogs coming from private owner, vaccinated against CPV infection.

Poor growth rate and intermittent diarrhea were observed in dogs suffering from giardiosis. Immunosuppression was suspected because of the origin and healthy status of 10 experimental dogs and it was later confirmed by immunological tests (sampling 0).

Blood collection. Peripheral blood samples were obtained by v. cephalica puncture and placed into plastic tubes containing heparin for immunological tests and into glass tube for evaluation of specific antibodies in serum. The scheme of blood sampling is presented in Table 1.

Blastogenic response of blood lymphocytes to mitogens. Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech AB, Sweden). The cultivation (at 37°C and 5% CO₂ in humidified air for 96 h), mitogen stimulation and measurement of the blastogenic response of lymphocytes were performed using the ethidium bromide fluorescence method (11). Concanavalin A (Con A, Sigma Chemical Co., USA) and phytohemagglutinin (PHA-P Sigma Chemical Co., USA) were used for the stimulation in the optimum concentration of 25 µg/ml and 20 µg/ml, respectively (17). The level of the blastogenic response of the lymphocytes was expressed as the stimulation index (SI). The SI was calculated according to formula SI = (A
The phagocytic ability of blood leukocytes was examined using 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 µm, ARTIM Prague, the Czech Republic) (22). The phagocytic activity of leukocytes was expressed as the percentage of the cells phagocytizing 3 and more MSHP, and as the phagocytic index representing the ingestion capacity of leukocytes (the ratio of the number of phagocytized MSHP and the number of all potentially phagocytizing leukocytes).

The level of specific antibodies against CPV was evaluated by hemagglutination inhibition test using 1% swine erythrocytes. Before testing the serum was inactivated by heating to 56°C. Serial twofold dilutions of the serum in buffer were added to microtiter plates. Then 4 HAUs (hemagglutination units) of the virus were added into each well and the plate was kept at room temperature for 1 h. After adding the suspension of swine erythrocytes and incubation at 4°C for 1 h the results were read.

Statistical analysis. The data were characterized by mean and standard deviation. The significance of difference was checked by Mann-Whitney U-test.

### Table 1

<table>
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<tr>
<th>Day of experiment</th>
<th>No. of sampling</th>
<th>Group 1 (n=5)</th>
<th>Group 2 (n=5)</th>
<th>Group C (n=5)</th>
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<tbody>
<tr>
<td>0</td>
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<td>Vaccine</td>
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<td>Levamisole</td>
<td>Vaccine</td>
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<td>3</td>
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<td>Levamisole</td>
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</tbody>
</table>

Results

Phagocytic activity of leukocytes was significantly lower in groups of dogs suffering from giardiosis at the beginning of the experiment comparing with the control (group C). One day after levamisole application (sampling 1) there was significantly higher phagocytic activity in group 1 comparing with group 2 (without levamisole) and no significantly higher comparing with control group. In all the next sampling there was a significant increase in this parameter in group 1 comparing to group 2 and control. At the last sampling the phagocytic activity of leukocytes in all three groups was comparable (Fig. 1).

Index of phagocytic activity of leukocytes at sampling 0 in groups 1 and 2 was significantly lower comparing with control. Then, at sampling 1 this parameter has significantly increased comparing with group 2 as well as control. At sampling 2, the index was significantly higher in groups 1 and 2 comparing to control. Later, there were no significant differences (Fig. 2).

Stimulation index (SI) of lymphocytes stimulated with ConA has increased at sampling 2 in group 2 comparing with control group. At sampling 3 there was a significant increase of SI in group 1 (vaccine + levamisole) comparing to group 2 (vaccine). At all other samplings there were no significant differences among experimental groups as is shown in Fig. 3.

SI of lymphocytes after stimulation with PHA-P (Fig. 4) was at the beginning of the experiment comparable in all groups of dogs. Vaccination and levamisole application resulted in a significant increase in lymphocyte proliferation in group 1 in comparison to group 2 and control group at the first sampling, with control group at the second sampling and with group 2 and control group at the third sampling. In group 2 (vaccinated without levamisole application) this parameter has increased only at the second sampling (after vaccination) in comparison with control group. Later, the SI was similar in all the dogs examined.

The development of the antibody titer after vaccination was delayed of one week in groups 1 and 2 comparing to controls. At sampling 4 the titer in group 1 treated with levamisole was significantly higher comparing to group 2. The antibody titer in all the groups decreased gradually until the end of observation and succeeded to stay at protective level (Fig. 5).
Fig. 1. Phagocytic activity of leukocytes.
Significant differences (P≤0.05) groups 1 and 2 versus control group at sampling 0, group 1 versus group 2 at sampling 1, group 1 versus group 2 and control group at samplings 2, 3, 4 and 5.

Fig. 2. Index of phagocytic activity of leukocytes.
Significant differences (P≤0.05) groups 1 and 2 versus control group at sampling 0, group 1 versus group 2 and control group at sampling 1, groups 1 and 2 versus control group at sampling 2.

Fig. 3. Stimulation index of lymphocytes to ConA.
Significant differences (P≤0.05) group 2 versus control group at sampling 2, group 1 versus group 2 at sampling 3.

Fig. 4. Stimulation index of lymphocytes to PHA.
Significant differences (P≤0.05) group 1 versus group 2 and control group at sampling 1, group 1 versus control group at sampling 2, group 1 versus group 2 and control group at sampling 3.
Fig. 5. Titre of antibodies against CPV.

Significant differences (P≤0.05) groups 1 and 2 versus control group at sampling 3, group 1 versus group 2 and control group at samplings 4 and 5.

Legend to the figures: 1 – group of dogs with giardiosis, inoculated with levamisole prior vaccination against parvovirois.
2 – group of dogs with giardiosis vaccinated against parvovirois,
C – control dogs vaccinated against parvovirois.

Discussion

Secondary immunodeficiency accompanying some infectious and parasitic diseases can negatively influence non-specific and specific immune response in animals. Giardiosis belongs to the parasitic diseases associated with immunosuppression (20). Clinical signs of the infection include abdominal pain, diarrhoea, nausea, malabsorption, wasting (2). Giardia infection is usually limited by the host’s immunocompetence that is presented by effective defense mechanisms against strict luminal parasites (2, 13). Marked immunosuppression in dogs with giardiosis was detected in the study where most frequent finding was a decrease in total immunoglobulin level, neutrophil counts, while depressed phagocytosis and lymphocyte activity were less common (20). In our study marked alteration of the phagocytic ability in all the dogs, in which giardia infection was confirmed by parasitological examination, was clearly demonstrated. The degree of proliferation activity of lymphocytes after their stimulation with mitogens remained unchanged. The most common host for the protozoa infection is immunosuppressed individual (6). Depression of some immune parameters in dogs under our study can present optimal conditions for the development of giardia infection. The real cause of this immunosuppression is unknown. The dogs examined came from the home for stray dogs, so we can presume that poor nutrition and stress contributed to the alteration of the immune system activity that predisposed them to opportunistic infection.

Vaccination response may be influenced by a variety of factors. The effects of environmental factors, stress, nutrition (21), chemotherapy (4), surgery (9), and long-term antibiotic treatment (10) on humoral and cellular immunity were monitored. Vaccination against canine distemper and canine parvovirus infection caused enhanced lymphocyte blastogenesis and decreased lymphocyte and leukocyte count in puppies (8, 9). A positive effect of inactivated CPV vaccine on lymphocytes in puppies was used in order to improve immunosuppression after surgery (18). Other authors described mild suppressive effect of vaccination on lymphocyte blastogenesis (7) that was of short duration. Phagocytosis and intracellular killing was not significantly modified by vaccination (15). Our results confirm a stimulatory effect of the vaccination against parvovirois on proliferative response of lymphocytes that was more significant and had persisted for longer time in dogs treated with levamisole. The enhancement of depressed phagocytic functions of neutrophils in dogs suffering from immunosuppression accompanied by giardiosis was probably caused by levamisole application, because in control dogs that were vaccinated only no significant changes were found. In contrast to some previous studies (4, 10, 21), where no significant differences in antibody response after vaccination between immunosuppressed and fully immunocompetent animals were reported, our results show marked difference in the development of CPV antibody response in dogs with alteration of some immunological parameters. CPV antibody titer measured by HIT was considered protective at 80 (4). This titer was detected one week later in immunosuppressed dogs in comparison to the control animals. Later, the level of antibodies was comparable and protective enough in all three groups of dogs. Significant changes were found in dogs treated with levamisole. Levamisole at the dose of 3.5 mg/kg b.w. caused, beside the marked improvement of phagocytic functions, also a significant increase in CPV antibody titer that persisted until the end of the experiment. Similarly, the proliferation activity of lymphocytes was higher for a longer time in the group of dogs receiving levamisole. In both veterinary and human medicine, levamisole has been used to reduce the immunosuppression (12). The effect of levamisole on
the immunity is variable and depends on the dose, scheme of application and immunocompetence of the host. Its immunostimulatory effect is more pronounced on altered immunological parameters (1) what was confirmed also in our work. According to the published data (3, 5) and our findings, levamisole is a suitable immunomodulator for the improvement of the immune reactivity in immunocompromised individuals and enhances the efficacy of protective vaccination.

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References