Effects of orally administered β – 1,3/1,6 – glucan on vaccination responses and immunological parameters in dogs

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ABSTRACT

The aim of this study was to evaluate immunomodulatory effects of orally administered β-1,3/1,6-glucan on leukocyte functions and vaccination responses in dogs. Thirty puppies were divided into two groups (G and C) of 15 individuals each and vaccinated against rabies and canine parvovirus-2. Dogs from the group G were orally administered 4 mg/kg soluble glucans once daily for 98 days. The phagocytic and metabolic activity of leukocytes and proliferation activity of lymphocytes were evaluated on days 0, 14, 28, 42 and 56. The antibody response to canine parvovirus type 2 and rabies virus were measured weekly. Comparing both groups, we noticed a significant increase in phagocytic activity of leukocytes (p < .001). Protective levels of antibodies against rabies virus, as well as against CPV-2 were reached earlier in the glucan treated group G. Our results suggest positive effects of glucan on some nonspecific immunity parameters, as well as on evaluated vaccination response.

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Introduction

β-Glucans are linear or branched glucose polymers found in the cell walls of fungi, yeasts, bacteria, algae, mushrooms and as callose in higher plants (Otabasi et al., 2006; Stone, 2004; Thompson, Oyston, & Williamson, 2010), useful in treating and/or preventing various diseases (Barsanti, Passarelli, Evangelista, Frassanito, & Gualtieri, 2011; Sakalauskiene et al., 2012; Samuelsen, Schrenzenmeir, & Knutsen, 2014). They have the capacity to activate innate immunity, thereby enhancing defense barriers (Goodridge, Wolf, & Underhill, 2009). A recent study by Shen, Wang, Dong, Xiang, and Liu (2016) has also revealed that glucans exert favorable effects in preventing colon cancer in mice. β-Glucans can vary in solubility, molecular mass, tertiary structure, polymer charge and solution conformation. All of these characteristics may influence their immunomodulating effects (Kulicke, Lettau, & Thielking, 1997; Sonck, Stuyven, Goddeeris, & Cox, 2010). In mammals, myeloid cells express several receptors capable of recognizing β-glucans; with the C-type lectin receptor dectin-1, in conjunction with Toll-like receptor 2 (TLR2), considered the key receptors for recognition of β-glucans (Goodridge et al., 2009; Goodridge et al., 2011;
Taylor et al., 2007). Adams et al. (2008) reported that the presence of other than β – (1,3) linked glucose residues in the backbone could limit interaction with Dectin-1, as the beta-glucan specific receptor. β-Glucans have been shown to stimulate both the specific and non-specific immune response (LeBlanc, Albina, & Reichner, 2006; Mucksova, Babicek, & Pospisil, 2001). Glucans can have an adjuvant effect on systematically co-administered vaccines trough increasing antigen specific IgG and IgA levels in the serum (Stuyven et al., 2010). They exert most of these effects by binding to specific receptors on macrophages, neutrophils, monocytes, dendritic cells and natural killer cells (Brown et al., 2003). As such, glucans stimulate phagocytosis and the production of inflammatory cytokines by macrophages (Brown et al., 2007), and furthermore, they have the ability to stimulate neutrophils and monocytes by the production of reactive oxygen species (Rubin-Bejerano, Abeijon, Magnelli, Grisafi, & Fink, 2007). The reports on the immune responses to glucans can be quite confusing, as what is observed for one preparation, is often inappropriately extrapolated to all glucans. In addition, the immunological potency of beta-glucans is not only dependent on molecular mass, solution conformation and backbone structure, but also the degree of branching is an important factor. As reported by Stuyven et al. (2010), a lot is already known about the effects of beta-glucans on the innate immune system, however, a great deal still needs to be learned about their effects on the adaptive immune system in mammals. In the present study, we analyzed the effect of oral supplementation of glucans on serum antibody response in healthy dogs after vaccination against rabies virus and canine parvovirus-2, as well as on some nonspecific immunity parameters.

**Materials and methods**

**Animals and sample collection**

For this study, 30 healthy 6 weeks old puppies were selected and divided into groups G and C. Group G was represented by 15 healthy puppies who orally received glucan for a period of 3 months. Group C (the control) was represented by 15 healthy puppies of the same age received a placebo instead of the glucan over the same period of time. Puppies of both groups were vaccinated against canine parvovirus-2, canine adenovirus-2, canine parainfluenza-2, canine distemper, leptospirosis and rabies using the Eurican vaccines DHPPi2, DHPPi2-L and DHPPi2-LR (Merial, France). The design of the trial (sampling and vaccination) is shown in Table 1. No clinical signs indicating illnesses were observed during the trial. All dogs were fed the same commercially available, nutritionally balanced complete dog diet. No dietary supplements or medications, other than routine antihelmintic drugs, were administered. The study was performed in compliance with the institutional guidelines for animal welfare issued by the ethical committee of the University of Veterinary Medicine and Pharmacy in Košice. Written informed consent was obtained from all of the dog owners.

**Immunomodulation**

Dogs from group G were treated orally with β-(1,3/1,6)-glucan (VET-P-IM, Pleuran, Slovakia) in the manufacturer’s recommended dose of 4 mg/kg once a day. The concentration of glucan (obtained from Pleurotus ostreatus) was 10 mg in 1 ml of syrup.
Determination of specific antibodies

Specific antibodies against canine parvovirus type 2 were detected using the hemagglutination inhibition test (HIT) according to Carmichael, Joubert, and Pollock (1980). Samples with an end point HI titer ≥ 1:80 were considered as protective. Antibodies against rabies were determined by an ELISA test developed in our laboratory by the Benisek, Suliova, Svrcek, and Zavadova (1989) and Suliova, Benisek, Svrcek, Durove, and Zavadova (1994). The results were expressed as equivalent units (EU/ml). All samples with antibody concentrations higher than 1 EU/ml were considered as protective (Clignet, Sagné, Schereffer, & Aubert, 2000; Meslin, Kaplan, & Koprowski, 1996).

Lymphocyte proliferation test

The lymphocyte activity was determined using the Cell Proliferation ELISA Kit, BrdU colorimetric (Roche, Germany). Briefly, the density of the cell suspension was adjusted to 10^6 cells/ml of RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% heat inactivated fetal bovine serum, 100,000 U/ml penicillin and 0.2 ml streptomycin. The cells were transferred into a 96 well microtiter plate and incubated at 37°C in a 5% CO₂ atmosphere for 3 days. For stimulation of lymphocytes, concanavalin A (ConA, Sigma) at a concentration of 10 µg/ml was used. The incorporation of BrdU was performed according to the manufacturer’s protocol. The stimulation index (SI) of lymphocytes was calculated as the ratio of the optical density (OD) values of stimulated cells and nonstimulated controls.

Phagocytosis assays

For the determination of the phagocytic activity (PA) of neutrophils and monocytes, the phagocytosis of methacrylate particles (MSHP, Artim, Prague) was used according to Vetvicka et al. (1982). After one hour of incubation, the percentage of cells in which at least three particles were engulfed was assessed from blood smears.
The metabolic activity (MA) test was performed according to Prochazkova and John (1986). For stimulation of isolated neutrophils and monocytes we used 10 µl of Zymosan A solution (Sigma-Aldrich, USA). INT (2-(4-jodfenyl-3-(4-nitrofenyl)-5-fenyl-tetrazolum chloride) (Sigma-Aldrich, USA) was used as an indicator of the respiratory burst. The index of metabolic activity (IMA) was calculated as the ratio of OD values of stimulated cells and nonstimulated controls.

**Statistical analysis**

The data were expressed as the mean (±) standard deviation (SD). Statistical evaluation was performed using the Mann–Whitney test. The analyses were performed using the software Prism 5.0 (GraphPad).

**Results**

**Phagocytosis assays**

As seen in Figure 1, we noticed a significant increase ($p < .001$) in the PA of leukocytes in group G on days 14, 28, 42 and 56 in comparison to group C. The highest value of PA (55.9 ± 8.5) was reached in group G on day 56. Values of the IMA did not significantly differ between the two groups (Figure 2).

**Lymphocyte proliferation test**

No significant changes in the SI of lymphocytes were observed between the two groups.

![Figure 1. Changes in PA of leukocytes (%) in blood samples from the two groups. Data are expressed as mean (SD). ***p < .001.](image-url)
Determination of specific antibodies

The effect of glucan on serum antibody response against CPV-2 is shown in Figure 3. Protective HI titers (1:80) were reached earlier (week 9 in the Figure 3) in group G and rose to levels higher than 1:1000. In group C, the protective levels of anti CPV-2 antibodies were

![Figure 2](image2.png)

**Figure 2.** Comparison of the IMA of leukocytes in group G and C.

**Determination of specific antibodies**

The effect of glucan on serum antibody response against CPV-2 is shown in Figure 3. Protective HI titers (1:80) were reached earlier (week 9 in the Figure 3) in group G and rose to levels higher than 1:1000. In group C, the protective levels of anti CPV-2 antibodies were

![Figure 3](image3.png)

**Figure 3.** Changes in concentrations of neutralizing antibody titer against canine parvovirus type 2 after vaccination in the two groups. Primovaccination (V1), first revaccination (V2), second revaccination (V3). Data are expressed as mean (SD). *p < .05, **p < .01, ***p < .001.
reached at week 11 in the Figure 3. Serum antibody response against rabies virus in groups G and C is shown in Figure 4. The protective levels (≥1EU/ml) of antibodies against rabies in group G were reached at week 16 in the Figure 4 (1.068 ± 0.202) and in group C at week 19 in the Figure 4.

Discussion

In recent years, a numbers of clinical trials have been conducted on the effects following oral administration of beta-glucan preparations (Samuelsen et al., 2014; Sonck et al., 2010). Glucans have been proven to be beneficial in various animal species, such as fish and shellfish (Bagni et al., 2005; Jorgensen & Robertsen, 1995; Supamattaya, Pongmaneerat, & Klowklieng, 2000), mice (Hetland & Sandven, 2002; Instanes, Ormstad, Rzdzjord, Wik, & Hetland, 2004; Yun, Estrada, Van Kessel, Park, & Laarveld, 2003), pigs (Stuyven et al., 2009) and horses (Krakowski, Krzyzanowski, Wrona, & Siwicki, 1999). Furthermore, authors Chau, Collier, Welsh, Carroll, and Laurenz (2009) investigated the effect of β-glucan on the production of specific Actinobacillus pleuropneumoniae antibodies in piglets passively immunized during neonatal life. Nevertheless, although they did not confirm an increase of immunity in piglets, the authors do not exclude the positive effects of β-glucan as an oral adjuvant to enhance immunoglobulin production in response to vaccinations. In the present study, we evaluated the effects of orally administered beta-glucan on the vaccination responses of dogs to CPV-2 and rabies virus, as well as on some immunological parameters, such as proliferation activity of lymphocytes, phagocytic and MA of leukocytes in six weeks old healthy puppies. In our previous study by Haladová et al. (2011), the favorable effects of β-(1,3/1,6)-D-glucan were observed in 4-months-old immunosuppressed shelter dogs with unknown vaccination histories.
Since glucans are found in microorganisms, they are classified as pathogen associated molecular patterns and can be recognized by pattern recognition receptors on host immune cells and hence trigger immune response and are, therefore considered immunomodulating compounds (Samuelsen et al., 2014). The best known effect of beta-glucans consist of the augmentation of phagocytosis of professional phagocytes (Abdullah, Abdulghani, Ismail, & Abidin, 2017; Vetvicka, 2011). This also supports our findings in dogs, where the values of PA were significantly higher in dogs receiving glucan. We also noticed that the MA of leukocytes did not change during the trial with soluble glucan. This is in line with Sonck et al. (2010), where soluble beta-glucans did not influence the MA, while particulate glucans had a stimulating effect. Therefore, the authors suggested a strong dependence of the MA of leukocytes on the concentration and solubility of beta-glucans. Upon evaluating the humoral immunity response after vaccination against canine parvovirus type 2 and rabies virus, we noticed an earlier onset of protective immunity in glucan treated dogs, as well as higher levels of serum antibodies. There are only few reports on the effect of glucans on the humoral immunity response in dogs. Stuyven et al. (2010) reported that oral administration of glucans increased the serum IgM levels, decreased serum and mucosal IgA concentrations, whereas no effect on the total IgG and specific anti Bordetella bronchiseptica IgG concentrations was seen. Khalkhane, Abbasi, Zadeh, and Arian (2013) reported that oral supplementation of beta-glucan resulted in increased serum IgG concentrations in lambs. Other studies also showed that serum IgG levels could be affected by immunomodulatory properties of beta-glucans (Krakowski, Krzyzanowski, Wrona, Kostro, & Siwicki, 2002; Zhang, Guo, & Wang, 2008).

**Conclusion**

Our results indicate that dietary supplementation of β-(1,3/1,6)-glucans could enhance the humoral immune response after vaccination, as well as some nonspecific immunity parameters. Therefore, we suggest that oral long-term administration of glucan has a beneficial effect on the vaccination response against canine parvovirus and rabies virus in dogs. Although further studies are necessary, we recommend glucans as suitable dietary supplement in an attempt to increase the canine immunocompetence.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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