NOTE *Virology*

Effects of *inactive parapoxvirus ovis* on cytokine levels in rats

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ABSTRACT. The aim of this study is to determine the effects of iPPOV on pro-inflammatory and anti-inflammatory cytokine levels in rats. iPPOV (1 ml/rat) was administered intraperitoneal route to 49 rats, except for 7 rats (Control, 0 group). Serum samples were collected from 7 rats at 1st, 2nd, 4th, 8th, 12th, 16th and 24th hr after treatments. Levels of TNF-α, IL-6, IL-12 and IL-10 were determined using ELISA. Administration of iPPOV stimulated TNF-α (16th and 24th hr) and IL-6 (12th, 16th and 24th hr) synthesis and caused fluctuations in IL-10 and IL-12 concentrations. In conclusion, increased cytokine levels could be attributed to immunomodulatory activity of iPPOV, however, detailed studies are required to fully understand effects of iPPOV on immune system.

KEY WORDS: cytokine, *inactive parapoxvirus ovis*, rat

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Parapoxvirus ovis (Orf virus; PPVO), which is a member of Poxviridae genus, causes pustular skin lesions in sheep, goat and human [9]. The commercial preparation that contains iPPVO (Zylexis®) is used in veterinary field to stimulate natural immunity against infections [14]. It has been reported that iPPVO reduces sensitivity against hepatitis B virus and herpes simplex virus in rats by inducing cytokine (IFN, IL-12p40, IL-18 and TNF-α) production [24]. *In vitro* studies have revealed that iPPVO enhances rate of phagocytosis in monocytes and neutrophils against *Listeria monocytogenes* and production of reactive oxygen species in the canine phagocytic cells by activating canine phagocytic cells [19]. iPPVO is used in horses for the prophylaxis and treatment of upper respiratory tract infections caused by equine herpesvirus (EHV) types 1 and 4. Strong antiviral efficacy of iPPVO against human hepatitis B virus (HBV) has been demonstrated in transgenic mice [15]. In addition, it has been reported that interferon (IFN)-γ and interleukin-10 (IL-10) expressions were induced after administration of iPPVO in rats [2]. It has been also reported that iPPVO plays a role in inflammation and in the regulation of immune response in addition to its immune-stimulating efficacy [7].

Natural immune response (nonspecific, innate immunity) against infections plays an important role in struggling with diseases. Substances that have regulatory characteristics due to effects on immune system are called as 'Immunomodulators' [3]. Cytokines are mainly synthesized by phagocytes, and syntheses of cytokines are stimulated by bacterial or viral factors [25]. They play an initial role in immune response [8, 17]. It has been stated that, after infectious stimulation, proinflammatory cytokines, which are among the cytokines that play a role as acute phase proteins, reach to peak level within the first 1–2 hr, and anti-inflammatory cytokines that increase thereafter reach to peak level in 6–8 hr and decrease to very low levels within 24 hr [18]. TNF is an early pro-inflammatory cytokine produced by different cells [macrophages (TNF-α) and T lymphocytes (TNF-β)] and stimulates IL-6 synthesis. The amount of TNF produced is the main factor for the control of its role in the disease process [2]. IL-6 is primarily synthesized by T and B lymphocytes and by some other cells [1]. IL-6 leads to synthesis of acute phase reactants (CRP, haptoglobulin, etc.), stimulation of IgG synthesis, differentiation of B lymphocyte, activation of T cell and stimulation of IL-2 production [5]. IL-10 is an anti-inflammatory cytokine synthesized and released particularly by immune system cells (T cell, B cell and monocyte/macrophage) under various circumstances [6, 18, 21]. IL-10, which is defined as Th2-type cytokine, plays an important role in balancing immune response [16] in addition to its role in the suppression of inflammatory response [12, 13, 22, 23]. IL-12 is a proinflammatory cytokine and is synthesized by activated mononuclear phagocytes and dendritic cells. It is effective on the immune response against intracellular pathogens and enhances cytolytic effect of NK [4].

iPPVO is a lyophilized solution for injection, which helps prevention of infection and stress-induced diseases by stimulating nonspecific immune system in cat, dog, horse, cattle and pigs [7, 24]. However, literature review demonstrated no study about the effects of iPPVO, which is recommended in the treatment of many infections in many kinds of animals, on cytokine synthesis, which are the first response mediators of immune system. The present study is based on the hypothesis that iPPVO might cause changes in the cytokine concentrations and aimed to determine the changes in proinflammatory and anti-inflammatory cytokine concentrations in serum samples collected at different times following administration of commercially product of iPPVO (Zylexis® flk), a viral immune stimulator in rats.

In the present study, 56 Wistar male rats (6–8 months, 225–250 g, Animal Laboratory Unit, Necmettin Erbakan

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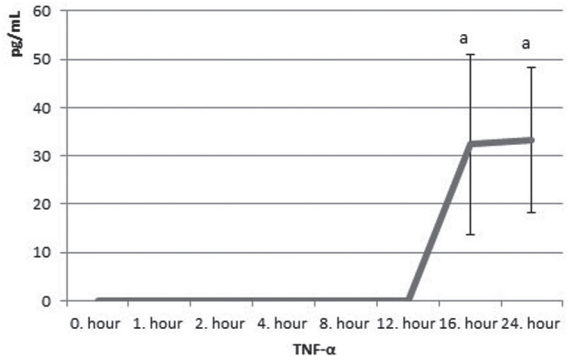

Fig. 1. Serum TNF-α (*p*g/m*l*) concentration after inoculation.

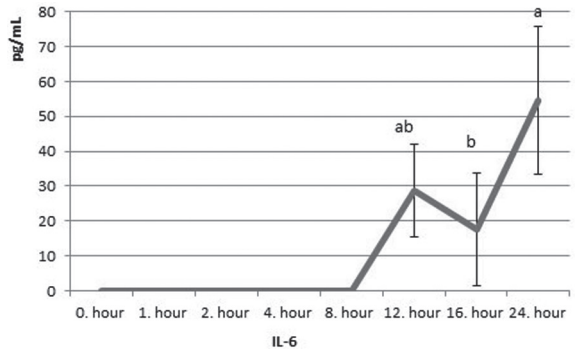

Fig. 2. Serum IL-6 (*p*g/m*l*) concentration after inoculation.

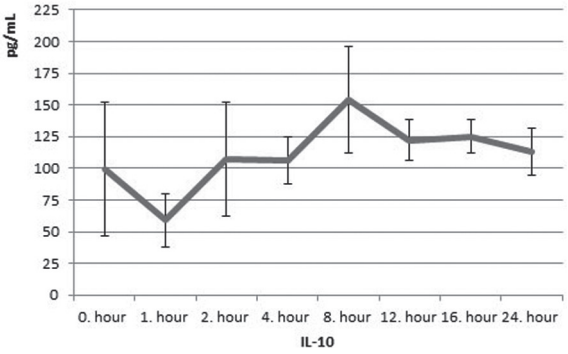

Fig. 3. Serum IL-10 (*p*g/m*l*) concentration after inoculation ($P \geq 0.05$).

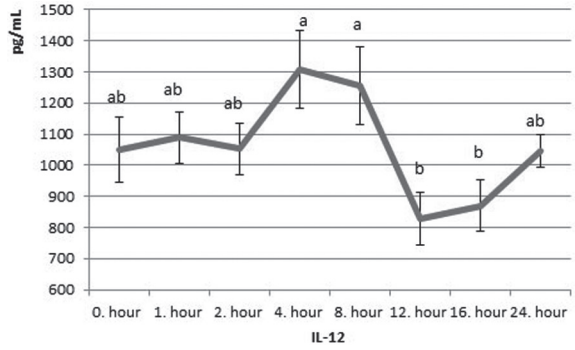

Fig. 4. Serum IL-12 (*p*g/m*l*) concentration after inoculation.

University, Konya, Turkey) were used. The study protocol was approved by the Ethics Committee of Necmettin Erbakan University (No: 2014-034). After 7 rats were left as the 0-hr sampling time group, iPPVO (1 m*l*/rat, Zylexis® flk, Zoetis, Rue Laid Burniat 1, Belgium) was administered via intraperitoneal route in all of the remaining 49 rats. Blood samples were collected by cardiac puncture under anesthesia (thiopental sodium, 70 mg/kg, intraperitoneal) both before (0 hr, Control) and after administration at the 1st, 2nd, 4th, 8th, 12th, 16th and 24th hr. Thereafter, all rats were immediately sacrificed by cervical dislocation. Serum TNFα (Catalog no: BMS622, eBioscience, Vienna, Austria), IL-6 (Catalog no: BMS625, eBioscience), IL-10 (Catalog no: BMS329, eBioscience) and IL-12 (Invitrogen, Nivelles, Belgium) levels were measured by ELISA reader (Rayto RT-2100C, Shenzhen, China). The test was performed in accordance with manufacturer's instructions.

Cytokine levels obtained at different sampling times were evaluated by ANOVA and post-hoc Duncan's tests (SPSS 10.0 for Windows/SPSS® Inc., Chicago, IL, U.S.A.). The results are presented as mean ± SE. $P<0.05$ was considered as the level of statistical significance.

Serum TNFα, IL-6, IL-10 and IL-12 levels are presented in Figs 1, 2, 3 and 4, respectively. Peak levels of TNF-α, IL-6, IL-10 and IL-12 were determined at 16th (Fig. 1), 12th (Fig. 2), 8th (Fig. 3) and 4th (Fig. 4) hr, respectively. Whilst serum TNF-α level was measured to be at detectable concentrations at the 16th and 24th hr following iPPOV, serum IL-6 level was at detectable concentration at the 12th, 16th and 24th hr. iPPOV caused fluctuations in serum IL-10 and IL-12 concentrations.

Zylexis® flacon is a commercially available nonspecific immunomodulator agent. It is a viral immune stimulator containing iPPOV D1701 strain and is used for protection against infectious diseases owing to its stimulating the immune system [20].

Based on the effects on organism, TNF-α, IL-6 and IL-12 are called as pro-inflammatory cytokines, whereas IL-10 is called as anti-inflammatory cytokine [10, 11]. In the present study, TNF-α was measured in high concentrations at the last sampling times after iPPOV administration (fig. 1). It has been reported by Anziliero *et al.* [1] that pro-inflammatory cytokines (TNF-α) reach to the peak level 24 hr after iPPOV inoculation in mice. Moreover, experimental models of infection (Lipopolysaccharide, LPS in rat) report that it reaches to higher concentrations in shorter times [26]. However, the present study determined that iPPOV administration stimulated TNF later and in lower concentrations. In addition, iPPOV administration caused elevation in IL-6 concentration at the last sampling times, similar to its effect on TNF-α concentration (Fig. 2). Studies conducted in rats using LPS revealed that it is elevated to higher concentrations at earlier sampling times [26]. In the present study, iPPOV administra-

tion had no remarkable effect on IL-10 and IL-12 concentrations (Figs. 3 and 4). A study conducted in healthy rats has been reported 1.5 times increase in IL-10 concentration after 72 hr of iPPOV administration [1], whereas there is no study on the effect of iPPOV on IL-10 and IL-12 concentrations in viral infections. However, it is reported that IL-12 increases IFN-γ level and regulates natural killer cells in viral infection [14]. In the present study, iPPOV may have triggered immune response on acute phase (at 4th and 8th hr) for stimulating natural killer cells.

In conclusion, although changes have been observed in the concentrations of some cytokines in rats that received parenteral (intra peritoneal) Zylexis® flacon (1 m*l*),experimental studies that comprise different methods of administration at different doses in the target species are needed for Zylexis® flacon to be used for protection against viral infections. Moreover, it can be stated that it might cause more remarkable effects at recurrent doses.

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