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 µl/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 iPPOV (Zylexis®) is used in veterinary field to stimulate natural immunity against infections [14]. It has been reported that iPPOV 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 iPPOV 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]. iPPOV 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 iPPOV 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 iPPOV in rats [2]. It has been also reported that iPPOV plays a role in inflammation and in the regulation of immune response in the canine phagocytic cells by activating canine phagocytic cells [19]. iPPOV is used for the prophylaxis and treatment of upper respiratory tract infections caused by equine herpesvirus (EHV) types 1 and 4. Strong antiviral efficacy of iPPOV 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 iPPOV in rats [2]. 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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 ml/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 measured in high concentrations at the last sampling times after iPPVO 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 iPPVO inoculation in mice. Moreover, experimental models of infection (Lipopolysaccharide, LPS in rat) report that it reaches to higher concentrations in shorter times [26]. In the present study, iPPVO administration stimulated TNF later and in lower concentrations. In addition, iPPVO administration caused elevation in IL-6 concentration at the last sampling times, similar to its effect on TNF-α concentration. Serum IL-6 level was measured to be at detectable concentrations at the 16th and 24th hr following iPPVO, whereas IL-6 level was at detectable concentration at the 12th, 16th and 24th hr. 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 iPPVO administration stimulated TNF later and in lower concentrations. Serum TNF-α level was measured to be at detectable concentrations at the 16th and 24th hr following iPPVO, whereas IL-6 level was at detectable concentration at the 12th, 16th and 24th hr. 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 iPPVO administration stimulated TNF later and in lower concentrations. In addition, iPPVO administration caused elevation in IL-6 concentration at the last sampling times, similar to its effect on TNF-α concentration.

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].
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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