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Immune response in pigs treated with therapeutic doses of enrofloxacin at the time of vaccination against Aujeszky's disease

Małgorzata Pomorska-Mól [a,*], Ewelina Czyżewska-Dors [a], Krzysztof Kwit [a], Jarosław Rachubik [b], Andrzej Lipowski [a], Zygmunt Pejsak [a]

[a] Department of Swine Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland
[b] Department of Radiobiology, National Veterinary Research Institute, 24-100 Pulawy, Poland

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ABSTRACT

The effect of treatment with enrofloxacin was studied on the postvaccinal immune response in pigs.

Forty pigs were used (control not vaccinated (C), control vaccinated (CV), vaccinated, received enrofloxacin (ENRO)). From day −1 to day 3 pigs from ENRO group received enrofloxacin at the recommended dose. Pigs from ENRO and CV groups were vaccinated twice against Aujeszky's disease virus (ADV). There was a significant delay in the production of humoral response of enrofloxacin dosed pigs when compared with CV group. Moreover, in ENRO group the significant decrease in IFN-γ production and significantly lower values of stimulation index after ADV restimulation was noted, as compared with CV group. The secretion of IL-6, IL-10 and TNF-α by PBMC after recall stimulation was also affected in ENRO group.

The results indicate that enrofloxacin, in addition to its antimicrobial properties, possess significant immunomodulatory effects and may alter the immune response to vaccines.

1. Introduction

Enrofloxacin is an anti-bacterial agent which belongs to fluoroquinolones (Ziółkowski et al., 2014). The fluoroquinolones have become an increasingly popular class of antibiotics for use in a variety of infections in humans and animals. They are also known to have direct effects on the immune system (Dalhoff, 2005; Dalhoff and Shalit, 2003). The production and secretion of various cytokines and chemokines *in vitro* (*i.e.* IL-1, IL-3, IL-6, IL-8, TNF-α, and IFN-γ) was affected by various fluoroquinolones (Dalhoff, 2005; Khan et al., 1998; Riesbeck et al., 1994; Shalit et al., 2006). Similar results were confirmed in experiments on animals (Dalhoff, 2005; Khan et al., 1998; Purswani et al., 2002). The immunomodulatory effects of fluoroquinolones are probably due to their effects on intracellular cyclic AMP and phosphodiesterases, on transcription factors such as NF-kappa B and activator protein 1 (Dalhoff and Shalit, 2003).

Enrofloxacin is very popular in veterinary medicine because of its pharmacokinetic properties, low toxicity and a broad spectrum of activity (Brown, 1996; Vancutsem et al., 1990; Ziółkowski et al., 2014). In pigs it is used for treatment of gastrointestinal and respiratory tract infections.

It is common practice in pigs industry to give antibiotics at the time of vaccination. It cannot be excluded that this might affect the development and persistence of postvaccinal response in antibiotic-treated pigs, as it has been shown earlier in various animal species (Khalifeh et al., 2009; Pomorska-Mól et al., 2014; Tokarzewski, 2002). There are only few reports that investigated the effect of enrofloxacin treatments on the immune response (Khalifeh et al., 2009; Schoevers et al., 1999; Tokarzewski, 2002), however, the influence of enrofloxacin on the immune response in pigs has not been studied to date. The results obtained by Schoevers et al. (1999) revealed that enrofloxacin may accumulate in porcine phagocytes however had no effect on its chemotaxic action (Schoevers et al., 1999). Khalifeh et al. (2009) reported that enrofloxacin targeted the humoral response in chickens and resulted in a decrease in antibody production after vaccination. Tokarzewski (2002) revealed that enrofloxacin decreased level of specific IgY in hens stimulated with live Salmonella and lipopolysaccharide (LPS). Enrofloxacin is metabolized into pharmacologically active metabolite, ciprofloxacin, which is also known to have modulatory effect on the immune system (Dalhoff, 2005; Dalhoff and Shalit, 2003; Jiménez-Valera et al., 1998; Williams et al., 2005). Ciprofloxacin has been shown to modulate phagocytic and killing capacity of neutrophils and macrophages as well as affects the expression of toll-like receptors in monocytes (Cacchillo and Walters, 2002; Kaji et al., 2008; Katsuno et al., 2006). Moreover, ciprofloxacin decreased and/or delayed the synthesis of IL-1, IL-6, IL-12, TNF-α (Bailly et al., 1991; Khan et al., 1998; Purswanie et al., 2002).

* Corresponding author. Department of Swine Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland. Tel.: +48818893476; fax: +48 81 8893346.
E-mail address: mpomorska@piwet.pulawy.pl (M. Pomorska-Mól).

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These data suggest that enrofloxacin (and its pharmacologically active metabolite- ciprofloxacin) may affect cellular and humoral immunity by influence on the cytokine production and secretion.

In view of the potential immunomodulatory properties of enrofloxacin we evaluated the effect of therapeutic doses of enrofloxacin on selected immune parameters, with special emphasis on postvaccinal immune response.

2. Materials and methods

2.1. Animals

Forty, 10-week-old pigs, both sexes were used in this study. Animals were sourced from herd with high health status, seronegative to Aujeszky's disease virus (ADV). Only pigs that not received any of antibiotics were involved in the experiment.

Pigs were randomly divided into three groups: control not vaccinated (C, n = 10), control vaccinated (CV, n = 15), and vaccinated during enrofloxacin administration (ENRO, n = 15). During the experiment, pigs were housed in isolated units, one for each group. Food and water were offered *ad libitum*.

Animal use and handling protocols were approved by Local Ethical Commission (University of Life Sciences in Lublin, Poland).

2.2. Drug and vaccine

The commercially available product containing enrofloxacin was used (Enrobioflox 5% Injectio, 50 mg/ml, Vetoquinol Biowet).

For vaccination the live-attenuated gE^- deleted vaccine against ADV (Akipor 6.3, Merial, France) was used.

2.3. Experimental design

From day –1 to day 3 animals from ENRO group received enrofloxacin intramuscularly, at the recommended dose (1 ml/10 kg of body weight per day). Pigs from ENRO and CV groups were vaccinated intramuscularly at 10 and 12 weeks of age with 2 ml of vaccine (0 and 14 days of study). Piglets from C group were not vaccinated and did not receive any antibiotics. Detailed design of the experiment is presented in Table 1.

2.4. Laboratory examination

2.4.1. Immunoglobulin (Ig) G, M and A concentrations in serum

Total IgG, IgM and IgA concentrations in serum were determined by ELISA assays (ELISA Quantitation Kit, Bethyl Laboratories Inc, USA) according to the manufacturer instructions. Prior to analysis all samples were diluted as follows: 1:20,000 for IgA and IgM, 1:150,000 for IgG. The serial dilutions of standard samples of pig's reference serum were tested in order to receive calibration curve. Values of unknown Ig concentration samples were calculated with the use of FindGraph software. The absorbance was recorded at 450 nm using an ELISA plate reader (Multiskan RC, Labsystems, Finland).

2.4.2. Humoral response against ADV

Specific antibodies to the glycoprotein B (gB) and glycoprotein E (gE) antigen were determined using a blocking ELISA tests (HerdChek*Anti-PRVgB or HerdChek*Anti-PRVgp1, IDEXX Laboratories, USA), as directed by the manufacturer. Optical density (OD) was measured at 650 nm wavelength. The presence or absence of specific antibodies was determined by calculating the sample to negative ELISA (S/N) ratio (OD of test serum/mean OD of negative reference serum). Samples were considered to be positive for gB if ELISA S/N ratio will be ≤0.5, while for gE if ELISA S/N ratio will be ≤0.6.

2.4.3. Lymphocyte proliferation assay

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by centrifugation onto Histopaque 1.077 (Sigma, USA) and were seeded in vials at a density of 1×10^6 viable cells/ml medium (RPMI 1640 containing 10% fetal bovine serum, 2 mM L-glutamine and 1% of antibiotic–antimycotic solution).

PBMCs were restimulated with live ADV; strain NIA-3 (titer $10^{7.0}$ $TCID_{50}$). In control vials the PBMCs were cultivated without ADV (mock-control) or with 5 μg/ml of concanavalin A (ConA) (viability control). All samples were tested in triplicate.

After 72 hours of incubation at 37°C in 5% CO_2 atmosphere, the cultures were pulsed with 0.5μCi [^{3}H]-thymidine (PerkinElmer, USA) and were harvested after next 18 hours. The incorporated radioactivity was measured in liquid scintillation counter (Quantulus, PerkinElmer, USA). Proliferation was expressed as a stimulation index (SI) calculated as the number of counts per minute (cpm) of ADV stimulated PBMCs divided by the number of cpm of the mock-stimulated cells (in each cases taking mean of triplicate vials).

Based on the SI values (the mean value plus 3 × standard deviation) observed at day –1 (before vaccination) and in non-vaccinated animals, a SI ≥ 2.20 was considered positive for ADV-specific proliferation.

2.4.4. In vitro *secretion of interleukin (IL)-4, IL-6, IL-10, interferon (IFN)-γ, tumor necrosis factor (TNF)-α*

For analysis the ability of PBMC to secrete cytokines, the concentrations of IL-4, IL-6, IL-10, IFN-γ, and TNF-α after stimulation with live ADV ($10^{7.0}$ $TCID_{50}$) and ConA (5 μg/ml) were determined (ELISA kits specific for porcine IL-4, IL-6, IL-10, IFN-γ, and TNF-α; Invitrogen Corporation, Camarillo, USA; Abcam, Cambridge, UK). Untreated cells served as control (mock control).

PBMCs were isolated and incubated under the same conditions as described earlier. In each experiment, serial dilutions of each cytokine standards were tested to determine calibration curves. Concentrations of cytokines were calculated from calibration curve generated with the use of FindGraph software.

2.5. Statistical analysis

A nonparametric Kruskal–Wallis test with *post hoc* multiple comparisons for comparison of all pairs was used (STATISTICA 8.0; StatSoft). For all analyses $p \leq 0.05$ was considered significant.

Table 1
Characterization of groups, time point of vaccinations, enrofloxacin administration, bleeding schedule and laboratory examination.

Group (number of pigs)	Antibiotic administration (day of study)	Time of vaccination (day of study)	Bleeding schedule	Laboratory examination
Control (C) (n = 10)	Not administered	Not vaccinated	–1, 0, 2, 4, 6, 9, 14, 21, 63	Immunoglobulins
Control, vaccinated (CV) (n = 15)	Not administered	0 and 14	–1, 0, 4, 6, 9, 14, 21, 35, 49, 63	Lymphocyte proliferation assay
Received enrofloxacin and vaccinated (ENRO) (n = 15)	–1 to 3	0 and 14	–1, 4, 6, 9, 14, 21, 35, 49, 63 –1, 0, 4, 6, 9, 14, 21, 35, 49, 63	Cytokines Serology (antibodies specific to ADV)