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Research paper

Toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of florfenicol

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ABSTRACT

Florfenicol (FLO) is a broad-spectrum antibacterial agent for treatment of bacteriosis of piglets in veterinary practice. To study the toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of FLO, 20 healthy weaned piglets were selected and randomly divided into two groups. Piglets in the FLO group were fed with fodder supplemented with 30 mg/kg BW of FLO twice a day for 10 days. Blood samples were drawn at four time points: 1 day before FLO administration and 1, 7, and 14 days post-withdrawal. Three or four piglets were euthanized at each time point post-withdrawal and tissue samples (bone marrow, thymus and spleen) were collected for fixation and cryostorage. The levels of classical swine fever virus (CSFV) antibody against the vaccine, the concentrations of Hsp70 and IL-6 in serum and Hsp70 in tissues, and the mRNA expression levels of B-cell lymphoma 2 (bcl-2) and tumor suppressor p53 were detected, the hematology of the piglets were analyzed, and the histopathology and the status of apoptosis of the hematopoietic and lymphoid organs was examined. The results showed changes in several indicators in the FLO group 1 day post-withdrawal: the concentration of red blood cells (RBCs) was decreased, and that of platelets (PLTs) was significantly lower ($p < 0.05$); the volumes of RBC and PLT were increased; the sum of blood lymphocytes was statistically decreased ($p < 0.05$); the concentration of IL-6 was significantly increased ($p < 0.05$); the concentrations of Hsp70 in serum and tissues were increased; obvious atrophy of the hematopoietic cell lines and partial replacement by fat cells were observed in bone marrow; thymus and spleen tissues showed lower concentrations and sparser arrangement of lymphocytes in the thymic medulla and white pulp of the spleen respectively; and the mRNA expression levels of bcl-2 in the three tissues were up-regulated, while that of p53 was down-regulated. With time after cessation of FLO administration, the indicators of the FLO group gradually returned to close to that of the control group and the histological lesions of the tissues gradually recovered, and the differences in the densities of lymphocytes and cell arrangements in the tissues between two groups gradually decreased. In conclusion, a therapeutic dose of FLO induces temporary toxicity in the hematopoietic and lymphoid organs of piglets to some extent, and influences hemopoiesis and immune function. These effects gradually decrease after cessation of FLO administration.

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1. Introduction

Florfenicol (FLO) is a synthetic broad-spectrum antibacterial chloramphenicol (CLP) derivative with a fluoro substitution at C3 and replacement of a nitro group ($-NO_2$) with a sulfomethyl group ($-SO_2CH_3$) that is used in veterinary practice for treatment of most Gram-positive and -negative bacterial infections (Syriopoulou et al., 1981; Lis et al., 2011). Because of its specific molecular structure, FLO has considerable activity against a broad spectrum of CLP–thiamphenicol-resistant, Gram-negative bacteria (Varma et al., 1986). In addition, FLO is more effective than CLP for bacterial inactivation since the nitro group has been replaced and it does not convey the potential fatal side effects of CLP (Skolimowski et al., 1983). FLO has been widely used in the prevention and control of infectious diseases, such as porcine respiratory diseases caused by *Mycoplasma*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Pasteurella multocida*, as well as digestive disorders caused by *Escherichia coli* and *Salmonella* (Ueda et al., 1995; Priebe and Schwarz, 2003; Shin et al., 2005).

The antibacterial actions of FLO result from inhibition of peptidyl transferase activity and subsequent microbial protein synthesis by the binding of the drug to the ribosomes (Cannon et al., 1990), but it has also been reported that fenicols can combine with the ribosomes of mitochondria and inhibit the synthesis of mitochondrial proteins (Yunis et al., 1970; Pohl et al., 1978; Wiest et al., 2012). With the wide use of FLO in veterinary medicine, several forms of FLO-induced toxicity have been reported, especially damage to hematopoietic and immune function, such as inhibition of phagocytosis (Bretzlaff et al., 1987), lymphocyte proliferation (Sieroslawska et al., 1998), the immune response (Guan et al., 2011), and hematopoiesis (Hassanin et al., 2014).

FLO is widely used to treat or prevent bacterial infectious diseases, such as swine enzootic pneumonia and swine plague, in piglets at the end of the suckling period or during the early stages of nursing. The dosage of FLO and treatment duration are often increased in the period which is crucial to piglet growth and the maturation of the immune response to vaccinations. To determine whether the therapeutic dose of FLO can influence the development of hematopoietic and lymphoid organs or damage hematopoietic and immune function, and further to guide the scientific use of FLO in pig production, we evaluated FLO-induced toxicity and side effects to piglets, especially to the hematopoietic and lymphoid organs, in blood and tissue samples obtained from piglets treated or untreated with a therapeutic dose of FLO after being vaccinated against CSFV. Here, we monitored the dynamics of CSFV antibody response and the concentrations of Hsp70 and IL-6, as detected using ELISA, analyzed differences in hematological parameters, detected histological differences in tissues among groups by hematoxylin and eosin (H&E) staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis, and mRNA expression levels of the apoptosis-related genes bcl-2 and p53 by real-time PCR.

2. Materials and methods

2.1. Animals, facilities and management

Twenty healthy 28-day-old weaned piglets, weighing approximately 6.89 ± 0.17 kg, were randomly divided into two groups. All of them were weaned at the age of 22 days and were left to adapt to the experimental environment for 6 days. They were housed in standard conditions of temperature, humidity and light, and all animal experiments were performed in strict accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Florfenicol (98% white crystalline powder; Guobang Co., Ltd., Zhejiang, China) was mixed to part of the daily fodder prepared for the piglets of the FLO (30 mg/kg BW, twice a day), and the piglets were provided enough pure fodder when they finished eating all the mixed fodder for 10 days since the age of 29 days. All the piglets were vaccinated with live CSFV vaccine (Winsun Pharmaceutical Co., Ltd., Guangdong, China) after blood sampling at the age of 28 days. The experiment had been repeated once in order to verify the results of the first-time experiment.

2.2. Experimental design and samples

Blood samples of 3 or 4 piglets were drawn from anterior vena cava before gradual-fill CO_2 euthanasia at the age of 39 days (1 day post-withdrawal), 45 (7 days post-withdrawal) and 52 (14 days post-withdrawal) respectively to detect the level of CSFV antibodies against the vaccine, the concentrations of Hsp70 and IL-6 to identify the influences of FLO to the inhibition and damage of hematopoietic and lymphoid cells, and do hematology analysis. Bone marrow, thymus and spleen sample that collected from each of the selected CO_2-euthanized piglets were divided into two parts respectively, the cryopreserved part for the detections of the mRNA expression levels of bcl-2 gene and p53 gene and the concentrations of Hsp70 in the tissues to study the influence of FLO to apoptosis; and the other part were fixed in 10% buffered formalin for histopathology (H&E staining) and TUNEL reaction. Bone marrow was collected from sawn-off thighbones by using puncture needle.

2.3. Hematology analysis and ELISA

The whole blood samples were analyzed by Hematology Analyzer PE-6800 (Prokan, China) according to the instructions. Commercially available CSFV antibody test kit (IDEXX, 99-43220-A151, USA) was used for the detection of serum antibody to CSFV. Pig Hsp70 ELISA Kit (CSB-E08317p, CUSABIO, China) was used for the detection of Hsp70 in serum and tissues. Pig IL-6 ELISA Kit (CSB-E06786p, CUSABIO, China) was used for the detection of IL-6 in serum. The kits were used according to the manufacturer's instructions. Optical density values were read at 450 nm using ELISA Micro-plate reader (BioTek ELx808, USA).