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Original article

The effects of florfenicol on lymphocyte subsets and humoral immune response in mice

M. Lis, M. Szczypka, A. Suszko, M. Świtała, B. Obmińska-Mrukowicz

Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Norwida 31, 50-375 Wrocław, Poland

Abstract

Florfenicol is a broad-spectrum bacteriostatic antibiotic used in domestic animals. The aim of the study was to determine the effect of florfenicol on the total number of lymphocytes in the thymus, spleen and mesenteric lymph nodes and the percentage and the absolute number of T cell subsets ($CD4^+CD8^+$, $CD4^-CD8^-$, $CD4^+$, $CD8^+$) in the thymus and T ($CD3^+$, $CD4^+$, $CD8^+$) and B ($CD19^+$) lymphocytes in the peripheral lymphatic organs in non-immunized mice and humoral immune response in sheep red blood cells (SRBC)-immunized mice. Florfenicol was administered orally at a dose of 30 mg/kg six times at 24 h intervals to non-immunized mice and four or seven times at 24 h intervals to SRBC-immunized mice. SRBC was injected 2 hours prior to the first dose of the drug. Florfenicol increased the percentage of $CD4^-CD8^-$ thymocytes and the absolute number of $CD4^+$ and $CD8^+$ thymocytes on day 7. The increased percentage and absolute number of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes in mesenteric lymph nodes and decreased percentage of lymphocytes B were also observed 24 hours from the last administration of florfenicol. Florfenicol administered after SRBC immunization reduced the number of plaque forming cells (PFC) and the production of anti-SRBC antibodies on days 4 and 7 after priming.

Key words: florfenicol, B and T lymphocyte subsets, humoral immune response, mice

Introduction

Florfenicol is a bacteriostatic antibiotic, which similarly to chloramphenicol and thiamphenicol, belongs to the group of fenicols. Florfenicol is active against most aerobic and anaerobic Gram-positive and Gram-negative bacteria and may even have a bactericidal effect on *Histophilus somni* and *Pasteurella spp.*. It is also active against a wide range of fish pathogens, such as *Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum*, *Yersinia rucker* in salmon and trout and *Edwardsiella ictaluri* in catfish (Dawling 2006). The drug is a derivative of thiamphenicol in which the *p*-nitro group has been replaced by a sulfomethyxyl group and in addition has a fluorine atom instead of the hydroxyl group at 3'carbon position. This fluorine molecule makes the antibiotic more resistant to bacterial inactivation. The mechanism of action of florfenicol is connected with inhibition of peptidyl transferase activity and subsequent microbial protein synthesis (Cannon et al. 1990). Pharmacokinetic properties of the drug have been

Correspondence to: B. Obminska-Mrukowicz, e-mail: b.mrukowicz@gmail.com, tel.: +48 71 320 54 03

described in veal calves (Varma et al. 1986, Adams et al. 1987), lactating cows (Soback et al. 1995), horses (McKeller and Varma 1996), pigs (Liu et al. 2003), ducks (El-Banna 1998), broiler chickens (Afifi et al. 1997, Shen et al. 2003), turkeys (Świtała et al. 2007), pigeon (Ismail and El-Kattan 2009), rabbits (Park et al. 2007), dogs (Park et al. 2008), goats and sheep (Ali et al. 2003, Lane et al. 2004).

The presence of *p*-nitro group in chloramphenicol, the main representative of fenicols, is likely to induce idiosyncratic aplastic anemia in humans. For this reason, chloramphenicol has been banned for use in human medicine. However, a similar effect of florfenicol has not been observed, although it can cause dose-dependent reversible bone marrow suppression.

Florfenicol is only used in veterinary medicine and has been approved by the Committee for Veterinary Medicinal Products (the European Agency for the Evaluation of Medical Products – EMEA). It is a potent agent used as an alternative to chloramphenicol. It was introduced for the treatment of susceptible bacterial diseases in cattle, fish, pigs and chickens in the mid-1990s.

It is well-known that some groups of antibiotics despite their antimicrobial action exert a modulating effect on the function of the host immune system, which can increase or decrease their antimicrobial efficacy. Antibiotics can affect the immune system of the host, particularly due to intracellular penetration. There is still limited information about the influence of fenicols on the immune response. For this reason, the aim of the study was to determine the effect of florfenicol on the subsets of lymphocytes T in the thymus, spleen and mesenteric lymph nodes and B lymphocytes in the spleen and mesenteric lymph nodes in non-immunized mice and on humoral immune response in SRBC-immunized mice.

Materials and Methods

Animals

The studies were conducted on male and female Balb/c mice (8-10 weeks of age), each weighing 20-22 g. The mice were kept under conventional conditions. The animals were fed a commercial, granulated food and water *ad libitum*. The experimental animals were obtained from a Breeding Center of Laboratory Animals at the Institute of Occupational Medicine, Łódź, Poland. The studies were performed on non-immunized and sheep red blood cells (SRBC)-immunized mice. The mice were immunized intraperitoneally (i.p.) with 0.2 ml of 10% SRBC suspension (4 x 10^8 cells per mouse). The sheep blood was collected into Alsever's solution in sterile manner and kept there at 4°C for at least 3 days. The SRBC suspension was prepared *ex tempore* in phosphate buffered saline (PBS, Institute of Immunology and Experimental Therapy, Wrocław, Poland).

Principles of laboratory animal care (NIH publication No 86-23, revised 1985), as well as the specific national laws on the protection of animals were followed. The study protocol was approved by the II Local Ethics Commission in Wrocław, Poland (No 23/2008)

Drug and treatment

Florfenicol in powder form (Vetos-Pharma, Bielawa, Poland) at a dose of 30 mg/kg was administered orally (by stomach-tube) with a 1% starch jelly six times at 24 h intervals in non-immunized mice. SRBC-immunized mice were administered the drug orally, four or seven times at 24 h intervals. The first dose of florfenicol was administered 2 h after antigen injection. The trials in the control group were conducted in parallel. Animals in the control group received an equivalent amount of pure starch jelly. The volume of the drug was 0.2 ml/ mouse. Each control and experimental group consisted of eight mice.

Measurements

The following measurements were taken: (i) the total number of thymocytes, splenocytes and lymphocytes of mesenteric lymph nodes; (ii) the weight ratio of the thymus, spleen and mesenteric lymph nodes calculated according to the following formula: weight of organ (mg) /body weight of mouse (mg) x 100; (iii) the percentage and count of lymphocyte subpopulations in lymphatic organs; (iv) the number of leucocytes and the picture of leucocytes in blood; (v) the number of plaque forming cells (PFC) in spleen; (vi) anti-SRBC haemagglutinin titre in the serum.

The total number of thymocytes, splenocytes, lymphocytes of mesenteric lymph nodes, the weight ratio of the thymus, spleen and mesenteric lymph nodes, the percentage and count of CD subsets ($CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, $CD8^+$ in the thymus, $CD19^+$, $CD3^+$, $CD4^+$ and $CD8^+$ in spleen and mesenteric lymph nodes) as well as the number of leucocytes and the picture of leucocytes in the blood were determined on days 1, 3 and 7 after the last dose in non-immunized mice treated six times with florfenicol. The number of PFC and anti-SRBC haemagglutinin titres in SRBC-immunized mice treated four or seven times with florfenicol were determined on days 4 and 7 after priming.

Assays of thymocyte, splenocyte and lymphocyte of mesenteric lymph node subsets

The mice were anaesthetized with halothane (Narcotan, Zentiva, Prague, Czech Republic) 1, 3 and 7 days after the final dose of florfenicol administration. Thymuses, spleens and mesenteric lymph nodes were removed and placed in disposable Petri dishes containing a sterile, ice-cold PBS. The suspended cells were released from the lymphatic organs by passage through a nylon mesh and then centrifuged (2250 x g, 15 min, 4°C) on a layer of Ficoll 400 (Pharmacia, Fine Chemicals AB, Uppsala, Sweden)/ Urografin 76% (diatrizoate sodium and meglumine diatrizoate, Bayer Schering Pharma, Poland) in a 1 : 3 ratio at a density of 1.076. After centrifugation, the cells were collected from the interphase and washed twice (375 x g, 8 min, 4°C) with a sterile, ice-cold PBS supplemented with 1% bovine serum albumin (BSA, Sigma). After the second wash, the cells were suspended in PBS with 1% BSA at 1 x 10^7 cells/ml. The viability of each cell suspension was 90-98% according to a trypan blue dye-exclusion assay. The cells were resuspended in 100 µl PBS solution containing 1% BSA. The thymocytes, splenocytes and lymphocytes of the mesenteric lymph nodes were stained with Rat Anti-Mouse CD4: FITC / CD8: RPE dual color reagent (Serotec, Kidlington, UK) at the dilution recommended by the manufacturer. The splenocytes and lymphocytes of the mesenteric lymph nodes were also stained with Rat Anti-Mouse CD19:FITC / CD3:RPE dual color reagent (Serotec, Kidlington, UK) at a dilution recommended by the producer.

The cells were incubated at 4°C for 30 min. and washed (375 x g, 8 min, 4°C) three times with an ice-cold PBS buffer. The fluorescence was analyzed using a flow cytometer (FACS Calibur; Becton Dickinson Biosciences). The distribution of the thymocyte, splenocyte and lymphocyte of mesenteric lymph node markers was analyzed using the CellQuest 3.1f software.

Determination of plaque forming cells (PFC)

The mice were anaesthetized with halothane and then killed by cervical dislocation 24 h after the final dose of florfenicol administration. The spleens were removed and placed into sterile, ice-cold Hank's saline (Institute of Immunology and Experimental Therapy, Wrocław, Poland). The lymphocytes from the spleens were isolated as described above. After centrifugation at 4°C, the splenocytes were collected from the interface and washed twice (375 x g, 8 min, 4°C) in ice-cold Hank's saline. After the second wash, the cells were suspended in the Hank's saline at 1 x 10^6 cells/ml. The viability of the splenocyte suspension was 96-100% according to a trypan blue dye-exclusion assay. The number of splenocytes producing haemolytic anti-SRBC antibodies (plaque forming cells, PFC) was determined by a local hemolysis technique in agar gel as described by Mishell and Dutton (1967).

Determination of anti-SRBC antibodies in the serum

The blood samples were taken from retro-ocular arteria of halothane anaesthetized mice. The sera were obtained by blood centrifugation and inactived at 56°C for 30 min. The total and 2-mercaptoethanol-(2-ME) resistant serum haemagglutinin titres were defined by active haemagglutination test carried out on microplates (Hudson and Hay 1980). The titre of 2-mercaptoethanol resistant antibody is roughly equivalent to that of the IgG in the serum, so the greater titre obtained without 2-mercaptoethanol is due to the IgM. The results were expressed as a value of $\log_2$. It was found that the serum of non-immunized mice did not contain spontaneous anti-SRBC antibodies.

Statistical analysis

The data obtained in the study were analyzed statistically using a t-Student test. The differences were considered significant at $p<0.05$.

Results

The effects of florfenicol on the total number of lymphocytes in the thymus, spleen and mesenteric lymph nodes and the weight ratio of lymphatic organs in non-immunized mice

As can be seen in Table 1, administration of florfenicol six times at a dose of 30 mg/kg did not change the total number of lymphocytes in the thymus, spleen and mesenteric lymph nodes of non-immunized mice. However, six exposures to florfenicol temporarily decreased the weight ratio of the spleen on day 1 after the last dose of drug. The weight ratio of the mesenteric lymph nodes also decreased on day 7 after six florfenicol doses.

Table 1. The effects of florfenicol on the total number of thymocytes, splenocytes and mesenteric lymph nodes cells and weight ratio of thymus, spleen and mesenteric lymph nodes. The mean values (n=8) and standard deviations are presented.

Index	24 hours		72 hours		7 days	
	control	florfenicol	control	florfenicol	control	florfenicol
The total number of thymocytes (x10^7)	32.1 ± 7.7	28.1 ± 9.5	33.9 ± 5.1	33.2 ± 8.5	34.6 ± 4.6	36.0 ± 4.5
Weight ratio of thymus	0.188 ± 0.044	0.194 ± 0.05	0.165 ± 0.037	0.193 ± 0.028	0.177 ± 0.03	0.199 ± 0.033
The total number of splenocytes (x10^7)	59.8 ± 7.6	54.0 ± 9.5	56.6 ± 5.7	58.8 ± 11.2	65.3 ± 8.0	56.9 ± 9.6
Weight ratio of spleen	0.604 ± 0.062	0.426 ± 0.187*	0.578 ± 0.096	0.55 ± 0.114	0.625 ± 0.186	0.571 ± 0.07
The total number of mesenteric lymph nodes cells (x10^7)	28.9 ± 9.7	38.2 ± 8.2	31.2 ± 11.3	37.7 ± 5.8	36.7 ± 8.5	37.6 ± 7.4
Weight ratio of mesenteric lymph nodes	0.39 ± 0.113	0.324 ± 0.098	0.412 ± 0.141	0.372 ± 0.051	0.497 ± 0.138	0.361 ± 0.062*

* $p<0.05$ as compared to the control group

Table 2. Percentage of thymocytes, splenocytes and lymphocytes from mesenteric lymph node subpopulations in mice treated with florfenicol. The mean values (n=8) and standard deviations are presented.

Index	24 hours		72 hours		7 days	
	control	florfenicol	control	florfenicol	control	florfenicol
Thymocytes %						
$CD4^-CD8^-$	77.8 ± 3.3	74.3 ± 3.9	77.7 ± 4.3	75.2 ± 3.7	82.2 ± 4.0	79.4 ± 3.4
$CD4^+CD8^+$	2.2 ± 0.5	2.7 ± 0.7	2.0 ± 0.5	2.3 ± 0.3	1.5 ± 0.7	3.6 ± 2.2*
$CD4^+$	17.5 ± 2.6	20.0 ± 3.2	17.0 ± 2.0	19.4 ± 2.9	12.8 ± 0.9	14.9 ± 1.5*
$CD8^+$	2.5 ± 0.4	3.0 ± 0.6	2.5 ± 0.6	2.7 ± 0.7	1.4 ± 0.4	2.0 ± 0.3*
Splenocytes %						
$CD3^+$	31.8 ± 6.4	32.9 ± 3.8	31.1 ± 5.7	31.2 ± 4.6	25.4 ± 6.6	21.7 ± 4.7
$CD4^+$	26.5 ± 5.2	26.1 ± 4.7	25.7 ± 4.7	27.1 ± 4.5	19.7 ± 6.1	17.7 ± 3.1
$CD8^+$	5.6 ± 1.5	6.4 ± 1.8	5.4 ± 1.5	5.5 ± 1.7	2.9 ± 1.3	2.6 ± 0.5
$CD19^+$	55.2 ± 8.6	55.5 ± 5.4	57.3 ± 8.4	58.3 ± 6.3	69.1 ± 7.2	71.3 ± 5.0
Mesenteric lymph node cells %						
$CD3^+$	36.5 ± 6.0	45.8 ± 5.3*	39.3 ± 8.2	45.3 ± 8.3	32.6 ± 9.6	37.6 ± 8.4
$CD4^+$	35.9 ± 4.0	41.5 ± 4.7*	37.1 ± 4.7	38.1 ± 6.5	32.6 ± 5.3	32.7 ± 6.7
$CD8^+$	4.5 ± 2.2	7.6 ± 1.9*	5.8 ± 3.2	6.4 ± 2.5	4.6 ± 0.9	6.3 ± 2.1
$CD19^+$	55.6 ± 7.1	47.4 ± 5.7*	54.2 ± 8.6	50.3 ± 8.5	54.3 ± 11.8	56.9 ± 9.3

* $p<0.05$ as compared to the control group

The effects of florfenicol on the percentage and absolute number of lymphocyte subpopulations in the thymus, spleen and mesenteric lymph nodes in non-immunized mice

It has been found that six exposures to florfenicol at a dose of 30 mg/kg altered the percentage and absolute count of T cell subsets in the thymus and T and B lymphocytes in the mesenteric lymph nodes. No effect of multiple administration of florfenicol on the percentage and absolute count of T and B in the spleen were found (except a decrease in the absolute count of $CD3^+$ lymphocytes in the spleen on day 7 after the last dose of the drug). As can be seen in Tables 2 and 3, florfenicol administered orally at a dose of 30 mg/kg six times at 24 h intervals increased the percentage and absolute count of immature $CD4^-CD8^-$ thymic cells (double-negative cells) and mature, single-positive $CD4^+$ and $CD8^+$ thymocytes

Table 3. Absolute number of thymocytes, splenocytes and lymphocytes from mesenteric lymph node subpopulations in mice treated with florfenicol. The mean values (n=8) and standard deviations are presented.

Index	24 hours		72 hours		7 days	
	control	florfenicol	control	florfenicol	control	florfenicol
Thymocytes (x10^7)						
$CD4^-CD8^-$	7.1 ± 2.5	7.6 ± 3.6	0.7 ± 0.2	0.7 ± 0.2	0.5 ± 0.3	1.3 ± 0.8
$CD4^+CD8^+$	25.0 ± 6.0	21.1 ± 7.7	26.3 ± 4.0	25.0 ± 6.6	28.8 ± 4.4	28.1 ± 3.9
$CD4^+$	5.6 ± 1.5	5.4 ± 1.5	5.8 ± 1.3	6.4 ± 1.7	4.4 ± 0.7	5.2 ± 0.7*
$CD8^+$	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.4	0.5 ± 0.1	0.7 ± 0.1*
Splenocytes (x10^7)						
$CD3^+$	18.9 ± 4.4	17.6 ± 3.3	17.7 ± 4.4	18.3 ± 4.1	16.5 ± 4.2	12.3 ± 3.2*
$CD4^+$	15.7 ± 3.1	14.0 ± 3.0	14.6 ± 3.7	15.6 ± 3.9	12.8 ± 3.9	10.0 ± 2.1
$CD8^+$	3.4 ± 1.0	3.4 ± 1.1	3.1 ± 1.1	3.1 ± 1.1	1.9 ± 0.8	1.5 ± 0.3
$CD19^+$	33.1 ± 7.5	30.2 ± 7.5	32.2 ± 3.7	34.4 ± 8.0	45.2 ± 8.0	40.6 ± 7.9
Mesenteric lymph node cells (x10^7)						
$CD3^+$	10.7 ± 4.2	17.7 ± 5.2*	12.7 ± 6.2	17.1 ± 4.6	12.4 ± 6.0	14.0 ± 3.8
$CD4^+$	10.4 ± 3.8	16.0 ± 4.5*	11.7 ± 4.9	14.4 ± 3.4	12.1 ± 4.3	12.2 ± 3.1
$CD8^+$	1.3 ± 0.7	2.9 ± 1.2*	1.9 ± 1.5	2.4 ± 1.1	1.7 ± 0.6	2.3 ± 0.7
$CD19^+$	16.0 ± 5.7	17.9 ± 3.7	16.5 ± 5.7	18.9 ± 4.0	20.2 ± 6.8	21.6 ± 6.6

* $p<0.05$ as compared to the control group

Table 4. The number of leucocytes, lymphocytes, neutrophils, basophils, eosinophils, monocytes in blood of mice treated with florfenicol. The mean values (n=8) and standard deviations are presented.

Index	24 hours		72 hours		7 days	
	control	florfenicol	control	florfenicol	control	florfenicol
The number of leucocytes (10^3/µl)	9.1 ± 1.7	8.7 ± 1.9	9.3 ± 1.4	7.8 ± 0.7*	7.1 ± 2.3	6.5 ± 1.6
The number of lymphocytes (10^3/µl)	6.9 ± 1.4	7.3 ± 1.4	7.3 ± 1.0	6.4 ± 0.6	5.8 ± 1.8	5.2 ± 1.3
The number of baciliform neutrophils (10^3/µl)	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
The number of segmented neutrophils (10^3/µl)	1.5 ± 0.5	1.3 ± 0.6	1.5 ± 0.5	0.9 ± 0.2*	0.8 ± 0.3	0.9 ± 0.2
The number of basophils (10^3/µl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.05 ± 0.05	0.1 ± 0.1	0.03 ± 0.03
The number of eosinophils (10^3/µl)	0.03 ± 0.04	0.05 ± 0.05	0.04 ± 0.05	0.01 ± 0.03	0.02 ± 0.03	0.05 ± 0.05
The number of monocytes (10^3/µl)	0.2 ± 0.1	0.1 ± 0.05*	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

* $p<0.05$ as compared to the control group

on day 7 after the last dose of the drug. No changes in the percentage and absolute count of T cell subsets in the thymus as early as 1 and 3 days following the exposure to six florfenicol doses were found. However, administration of florfenicol six times at a dose of 30 mg/kg increased the percentage and absolute count of $CD3^+$ (Pan-T-cells), which corresponded with an increased percentage and absolute count of $CD4^+$ and $CD8^+$ lymphocytes and a decreased percentage, but not the absolute number of $CD19^+$ lymphocytes in the mesenteric lymph nodes. This effect was short-lasting. The effect of florfenicol on T and B lymphocytes in the mesenteric lymph nodes was observed only on day 1 after the last dose of florfenicol.

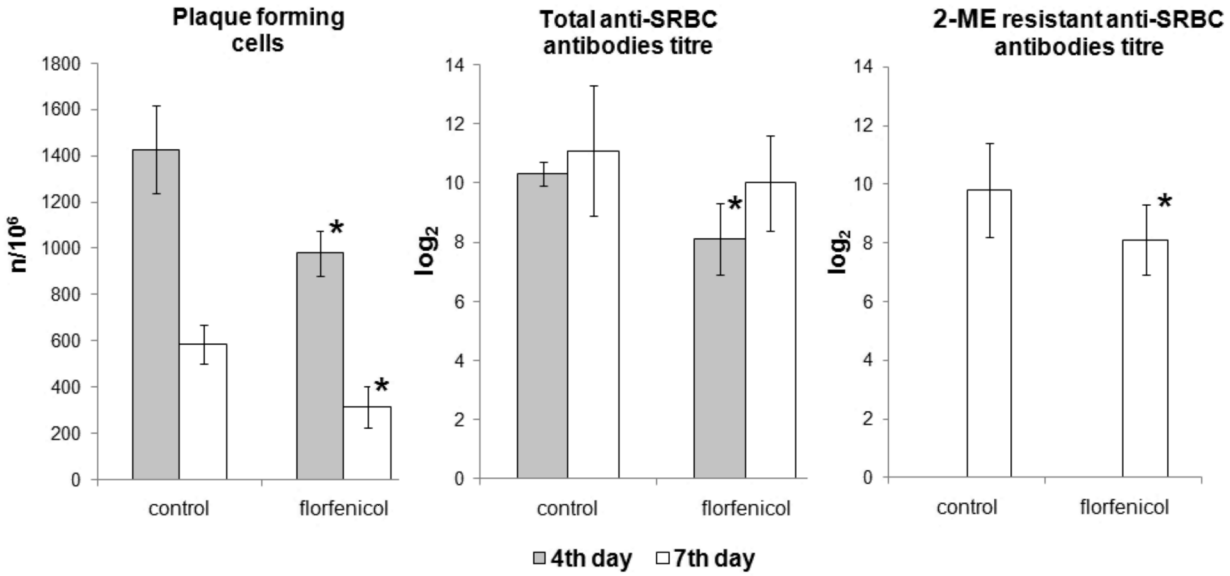

Fig. 1. The number of plaque forming cells and anti-SRBC haemagglutinin titres in SRBC-immunized mice treated with florfenicol four or seven times at 24 hours intervals after priming. The mean values (n=8) and standard deviations are presented. * $p<0.05$ as compared to the control group.

The effects of florfenicol on blood picture in non-immunized mice

It has been found that florfenicol did not change the count of white blood cells as early as 24 hours following the exposure to six florfenicol doses of 30 mg/kg, except monocytes the number of which decreased. However, 72 h after the last dose of florfenicol, a reduction in the count of leucocytes was observed and it corresponded with the diminished count of segmented neutrophiles. This effect of florfenicol was short-lasting. No changes in the count of white blood cells were observed (Table 4).

The effects of florfenicol on the primary humoral response in SRBC-immunized mice

As reported in Fig. 1, multiple administration of florfenicol at a dose of 30 mg/kg reduced the primary humoral immune response in SRBC-immunized mice resulting in a decreased number of splenocytes producing hemolytic anti-SRBC antibodies (PFC) and anti-SRBC antibody titers, both total and 2-mercaptoethanol-resistant. The suppressive effect of florfenicol on the humoral immune response did not depend on the number of subsequent doses applied.

Discussion

The results obtained in the present study conducted on non-immunized mice confirm a modulating effect of florfenicol on the percentage and absolute number of T cell subsets in the thymus and T and B lymphocytes in the mesenteric lymph nodes. It has been found that multiple administration of florfenicol at a dose of 30 mg/kg significantly increased the percentage and absolute count of immature, double-negative $CD4^-CD8^-$ thymic cells and mature T $CD4^+$ and $CD8^+$ cells in the thymus as well as the percentage and absolute count of $CD3^+$ mesenteric lymph node cells. This corresponded with the increases in the percentage and absolute count of $CD4^+$ and $CD8^+$ cells and decreases in the percentage and absolute count of $CD19^+$ cells (B lymphocytes) in the mesenteric lymph nodes. Although the results of our earlier studies conducted on Arian broiler chickens weighing 0.5-0.6 kg, 1.2-1.4 kg and 2.4-2.6 kg showed that florfenicol administered orally at a therapeutic dose of 30 mg/kg, three times at 24 h intervals decreased the percentage of T ($CD3^+$, $CD4^+$, $CD8^+$ and TCRγδ) and B (Bu-1) lymphocytes in the blood. The suppressive effect of florfenicol depended on the body weight of birds. The strongest suppressive effect of florfenicol on the percentage of T and B blood lymphocytes was observed in broiler chickens weighing 0.5-0.6 kg.

The results obtained in the present study also show that multiple exposures to florfenicol temporarily decreased the count of leucocytes, segmented neutrophiles and monocytes in the blood of non-immunized mice. Bretzlaff et al. (1987) reported a suppressive effect of florfenicol on the activity of neutrophiles. They observed that florfenicol at concentrations within the ranges of 5 to1000 µg/ml of cell

culture inhibited phagocytosis of ^{32}phosphorus-labelled *Staphylococcus aureus* by bovine blood neutrophils. The trials conducted *in vitro* on bovine neutrophils isolated from milk showed that chloramphenicol (the parent compound) at concentrations of 4000 and 2000 μg/ml reduced phagocytosis of these cells. However, no effects of florfenicol and thiamfenicol on the neutrophil function were observed, but the drugs altered neutrophil morphology (Paape et al.1990). The data reported by Lundèn et al. (1999) show that florfenicol did not change the antibody production and circulating leucocyte levels but caused a suppression in chemiluminescence response/phagocytic cell of rainbow trout immunized with a commercial oil-based divalent (furunculosis/vibrosis) vaccine.

The results of the present study also prove that florfenicol exerts a suppressive effect on humoral immune response in SRBC-immunized mice. Florfenicol decreased the number of splenocytes producing haemolytic anti-SRBC antibodies (PFC) and anti-SRBC haemagglutinin titers (total and 2-mercaptoethanol-resistant) when the drug was administered after priming. Couderc et al. (1983) also reported a suppressive effect of fenicols on humoral immune response. They observed that specific *in vitro* PFC responses to trinitrophenyl conjugated to SRBC are inhibited by chloramphenicol and thiamphenicol, which may impair the effects of B lymphocytes. The decreased number of PFC by florfenicol both on day 4 and 7 after SRBC immunization suggests that this antibiotic has a negative impact on the production of murine B lymphocytes capable of producing anti-SRBC antibodies. SRBC is a thymus dependent antigen which stimulates humoral immune response in cooperation with helper-inducer T lymphocytes and antigen presenting cells (APCs). T helper-inducer lymphocytes and cytokines, such as IL-4, IL-5, IL-6 significantly influence growth and differentiation of B lymphocytes. Recent data (Zhang et al. 2008) show that florfenicol *in vitro* reduced tumor necrosis factor (TNF) and interleukin-6 (IL-6) production by lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. At the same time, the drug had a little effect on IL-1β and IL-10 secretion. Further studies conducted *in vivo* on mice challenged with LPS also showed that florfenicol significantly attenuated TNF and IL-6 production in the murine serum (Zhang et al. 2009). The studies carried out *in vitro* showed that florfenicol modulated early cytokine response by blocking the NF-kappaB pathway in RAW 264.7 macrophages (Zhang et al. 2008). IL-6 plays a major role in the development of antigen-specific humoral response and is the essential factor responsible for ultimate differentiation of B lymphocytes, activated to the cells producing immunoglobulines by the antigen (Van Snick 1990). The results obtained in earlier studies suggest that the suppressive effect of florfenicol on humoral immune response in SRBC-immunized mice observed in our study is likely to be associated with inhibition of IL-6 synthesis and release by florfenicol.

In conclusion, it can be stated that florfenicol has a modulating effect on lymphocyte subsets, as it increased and/or decreased the number of specific CD antigens on the surface of thymocytes and lymph node cells. The most significant effect observed in the present study was an increase in the percentage and absolute count of T lymphocytes and a decrease in the percentage and absolute count of B cells in mesenteric lymph nodes. Florfenicol also exerts a suppressive effect on humoral immune response in SRBC-immunized mice, which shall not be neglected when choosing this antibiotic for animal treatment.

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