POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, SI 2, pp. 79–84

EFFECTS OF MARBOFLOXACIN ON THE ACTIVITY OF MACROPHAGES AND T AND B CELLS IN NON-INFECTED AND *E. COLI*-INFECTED MICE

Marianna Szczypka, Bartosz Gawęda, Bożena Obmińska-Mrukowicz

Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Agricultural University, Wrocław, Poland

Key words: lymphocyte subsets, macrophages activity, marbofloxacin, mice

The studies were conducted on non-infected and *E. coli*-infected mice treated with marbofloxacin (Marbocyl 10 % inj.) administered intramusculary five times at 24-h intervals at a dose of 2 mg/kg. It has been found that marbofloxacin augments the percentage of phagocyting cells in non-infected mice, but does not change the number of phagocytized bacteria. However, marbofloxacin impaires the killing activity of peritoneal macrophages in non-infected mice (decreased nitric oxide production) and *E. coli*-infected mice (decreased percentage of NBT-positive cells). Moreover, marbofloxacin enhances the synthesis and release of interleukin-1 (IL-1), both in non-infected and *E. coli*-infected mice.

The administration of marbofloxacin has also been found to increase the percentage of immature $CD4^+CD8^+$ thymic cells and to decrease the percentage of double-negative ($CD4^-CD8^-$) and single-positive ($CD4^+$ and $CD8^+$) thymocytes. Administration of marbofloxacin does not change the percentage of T and B splenocytes and mesenteric lymph node cells in non-infected and *E. coli*-infected mice.

INTRODUCTION

Marbofloxacin is a newer fluoroquinolone derivative used in veterinary medicine to the treatment of bacterial infection in the dog, cat, cattle and pig. Like other fluoroquinolones it is bactericidal by inhibiting bacterial DNA-gyrase. Marbofloxacin shows a broad spectrum of activity against Gram-negative pathogens, especially *E. coli*, Gram-positive bacteria and *Mycoplasma spp.* [Spreng *et al.*, 1995]. Marbofloxacin is well absorbed after parenteral and oral administration, weakly bounds to plasma proteins, has a large volume of distribution and is excreted mostly in the urine [Schneider *et al.*, 1996].

The fluoroquinolones (ciprofloxacin, enrofloxacin, enoxacin, ofloxacin, pefloxacin, rufloxacin and grepafloxacin) show an immunotropic action, they can either stimulate or inhibit the functions of the immune system.

The fluoroquinolones at the therapuetic doses reach very high concentration (five to ten times higher than in the serum) in phagocytic cells including neutrophils and macrophages [Chateau & Caravano, 1993; Hawkins *et al.*, 1998].

Trials conducted *in vitro* show that fluoroquinolones did not affect the phagocytic activity of the neutrophils and macrophages [Delfino *et al.*, 1985; Forsgren & Bergkvist, 1985]. Moreover, the results obtained *in vivo* indicate that fluorinated quinolones are able to suppress the phagocytic and killing activity of leucocytes and can modulate the synthesis and release of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) by monocytes/macrophages [Bailly *et al.*, 1991; Szczypka & Obmińska-Domoradzka, 2002]. The fluoroquinolones exerted a modulating effect on the maturation and differentiation of lymphocytes resulting in an increased and/or decreased number of specific cluster differentiation antigens. The modulating effects depend on chemical structure, the dose administered as well as immunological status [Szczypka & Obmińska-Mrukowicz, 2003]. The fluoroquinolones also alter the production and/or release of the cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ) by T lymphocytes [Riesbeck *et al.*, 1989; Riesbeck & Forsgren, 1994]. There is limited information on the influence of marbofloxacin on the number and function of immunological cells.

The purpose of the present study was to determine effects of marbofloxacin on the activity of peritoneal macrophages and the surface marker expression of thymus, spleen and lymph node cells in non-infected and *E. coli*-infected mice.

MATERIALS AND METHODS

Animals. The studies were conducted on male and female Balb/c mice, each weighing 16–18 g, (8 weeks of age). The experimental animals were obtained from a breeding laboratory at the Medical University, 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 studies were performed on non-infected and *E. coli*-infected mice. Infection was induced by a single intravenous injection of *Escherichia coli* ATCC 25922 (Polish Collection

Author's address for correspondence: Marianna Szczypka, Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Agricultural University, Norwida 31, 50-375 Wrocław, Poland; e-mail: marianna@ozi.ar.wroc.pl

of Microorganisms, Wrocław, Poland) at a dose of 3×10^6 bacteria/mouse. The dose volume of *E. coli* suspension was 0.1 mL per animal.

Drugs and treatment. Marbofloxacin (Marbocyl®, 10% inj., Vetoquinol S.A. 70204 Lure Cedex, France, series AMM 676064 0) was administered intramusculary on five occasions at 24-h intervals at a dose of 2 mg/kg. The first dose of marbofloxacin was administered 4 h after injection of *E. coli*. The trials in control mice were conducted in parallel. The mice in control group received phosphate buffered saline solution (PBS) instead marbofloxacin. The dose volume of each dose of drug or PBS was 0.1 mL per animal. Each experimental group contained eight mice.

Measurements. The following indices were measured: (i) the phagocytic activity *ex vivo* of peritoneal macrophages against *Staphylococcus intermedius* (catalase- and coagulase-positive strain) was tested by the method of Doleżal *et al.* [1955]; (ii) the killing ability was determined by nitroblue-tetrazolium test (NBT, Sigma, St Louis, MO, USA) following the method of Park *et al.* [1968]; (iii) the killing ability by the production of nitric oxide (NO) in the culture supernatants of peritoneal macrophages stimulated *in vitro* with lipopolysaccharide from *E. coli* (LPS 055:B5, Sigma) was tested by the method of Stuehr and Marletta [1985]; (iv) the production of interleukin-1 (IL-1) in the culture supernatants of peritoneal macrophages stimulated *in vitro* with LPS from *E. coli* were determined by means of ELISA kit for determination of murine IL-1β (R&D systems, Minneapolis, USA); (v) CD subsets $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$ and $CD8^+$ in thymus and $CD19^+$, $CD3^+$, $CD4^+$ and $CD8^+$ in spleen and mesenteric lymph nodes were determined by flow cytometry using monoclonal antibodies (mAb) coupled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) (BioSource Int., Nivelles, Belgium) according to the manufacturer's instructions.

The phagocytic and killing ability of macrophages, the level of IL-1 and nitric oxide and CD subsets of thymocytes, splenocytes and lymph node cells were determined 24 h after the last injection of marbofloxacin.

Production of interleukin-1 (IL-1) and nitric oxide (NO). Mice were anaesthetized with halothane (Narcotan®, Leciva, Praha, Czech Republic). Peritoneal exudate macrophages were harvested in sterile, ice-cold phosphate buffered saline solution (PBS) with antibiotics (penicillin 10 U/mL and streptomycin 1 μg/mL, Sigma). Cells were washed and suspended in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS; Pro Animali, Wrocław, Poland), 10 mmol/L HEPES (Sigma), 2 mmol/L L-glutamine (Sigma) and antibiotics (pencillin 10 U/mL and streptomycin 1 μg/mL, Sigma), adjusted to a concentration of 1.5×10^6 cells/mL, dispensed in 100 μL volumes in 96-well flat bottom plate (Sarstedt Inc., Newton, USA). The medium with nonadherent cells was replaced after 3-h incubation at 37°C in normal atmosphere with 5% CO_2. Incubation was continued and the medium was replaced after 18 h by the medium without FCS, but containing LPS from *E. coli* (Sigma) at a concentration of 2.5 μg/mL. Each culture was tested in triplicate. After 24 h of incubation, supernatants were removed and stored at -70°C. A commercial ELISA kit (R&D Systems) was used to determine mouse IL-1β in macrophage culture supernatants, according to the manufacturer's instructions.

The nitric oxide (NO) release from stimulated, peritoneal macrophages was measured as nitrite [Stuehr & Marletta, 1985]. 50 μL aliquots of macrophage supernatant were incubated with an equal volume of Griess reagent: 1% sulfanilamide (Sigma-Aldrich Laborchemikalien, Seelze, Germany) 0.1% N-(1-Naphtyl)-etylendiamine dihydrochloride (Sigma-Aldrich Laborchemikalien, Seelze, Germany) and 2% H_3PO_4 (Chempur, Piekary Śląskie, Poland) for 5 min at room temperature. The absorbency of samples was measured at 550 nm wave length with the microplate autoreader. The concentration of nitrite of three replicates was calculated by reference to a standart serial dilution of sodium nitrite (Chempur, Piekary Śląskie, Poland) ranging from 0.3125 to 10 nmol/L.

Assay of thymocyte, splenocyte and lymphocyte of mesenteric lymph nodes subsets. Mice were anaesthetized with halothane 24 h after the final dose of marbofloxacin. The thymuses, spleens and mesenteric lymphonodes were removed and placed in disposable Petri dishes containing sterile, ice-cold PBS. The suspended cells were released from the lymphatic organs by passage through a nylon mesh and then centrifugaed on a layer of Ficoll400® (Pharmacia, Fine Chemicals AB, Uppsala, Sweden)/Uropolinum75%® (diatrizoate sodium and meglumine diatrizoate, Polpharma S.A., Starogard Gdański, Poland) at 1:3 ratio, density 1.071. After centrifugation at 4°C, cells were collected from the interphase and washed twice with PBS supplemented with 1% bovine serum albumine (BSA, Sigma) at 4°C. After the second wash, the cells were suspended in PBS with 1% BSA at 1×10^7 cells/mL. The viability of each cell suspension was determined by trypan blue dye exclusion. It was found at the level 90-98%. Cells were resuspended in 100 μL PBS buffer with 1% BSA and stained with FITC-labelled antibody to mouse $CD4^+$ clone: YTS 177.9 (lot: 14218-02S, BioSource) and PE-labelled antibody to mouse $CD8^+$ clone: KT15 (lot: 13927-03S, BioSource) or FITC-labelled antibody to mouse $CD3^+$ clone: KT3 (lot: 19887-01S, BioSource) and PE-labelled antibody to mouse $CD19^+$ clone: 6D5 (lot: 16249-02S, BioSource) in a dilution recommended by the producers. Cells were incubated at 4°C for 30 min, and washed three times with ice-cold PBS buffer. Fluorescence was analysed by a flow cytometer (FACS Calibur, Becton-Dickinson Biosciences, Heidelberg, Germany). Lymphocyte marker distribution was analysed with a CellQuest program.

Statistical analysis. The data collected in the study were analysed statistically using a t-test. The differences were considered significant at $p<0.05$.

RESULTS

The effects of marbofloxacin on the phagocytic and killing activity of murine peritoneal macrophages

Marbofloxacin administered five times at a dose of 2 mg/kg to mice augments the phagocytic ability of peritoneal macrophages resulting in an increased percentage of phagocyting macrophages but did not change the number of bacteria phagocytized by a single macrophage. No effect of marbofloxacin on the capability of peritoneal macrophages

to reduce NTB was observed. On the other hand, the administration of marbofloxacin decreased the production of NO by these cells in non-infected mice (Table 1).

The experimental infection with *E. coli* in mice increased the phagocytic activity of peritoneal macrophages against *Staphylococcus intermedius*, which is reflected in the increased percentage of phagocyting macrophages. Exposure to infection enhanced metabolic activity of macrophages, which was reflected in the increased percentage of these cells spontaneously reducing NBT leading to the appearance of black formazan deposits present in macrophages (% NBT+ cells). However, the production of NO by peritoneal macrophages was impaired in the *E. coli*-infected mice.

Treatment with marbofloxacin did not change the stimulatory effect of infection on the percentage of phagocyting macrophages, but partially diminished the macrophages ability to reduce NBT in *E. coli*-infected mice. Marbofloxacin also did not change the suppressive effect of infection on nitric oxide production by peritoneal macrophages in mice.

The effects of marbofloxacin on IL-1 production by peritoneal macrophages in mice

The infection did not affect the production of IL-1. Administration of marbofloxacin in non-infected and *E. coli*-infected mice enhances the synthesis and release of IL-1 by peritoneal macrophages stimulated *in vitro* with LPS (2.5 μg/mL), (Table 1).

The effects of marbofloxacin on thymocyte, splenocyte and mesenteric lymph node lymphocyte subsets

Marbofloxacin administered intramusculary five times at 24-h intervals at a dose of 2 mg/kg increased the percentage of immature $CD4^+CD8^+$ thymic cells (double-positive cells) with corresponding decreased in the percentage of double-negative thymocytes ($CD4^-CD8^-$ cells) and single-positive $CD8^+$ cells (Table 2). Diminution of the percentage of mature $CD8^+$ thymic cells by marbofloxacin leads to an increased $CD4^+/CD8^+$ ratio compared with the control group. Treatment with marbofloxacin did not affect the percentage of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ splenocytes and mesenteric lymph node cells (Table 2).

The experimental infection with *E. coli* in mice increased the percentage of double-positive $CD4^+CD8^+$ thymocytes with corresponding decreased in the percentage of mature $CD4^+$ thymic cells. Exposure to infection with *E. coli* did not affect the percentage of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ splenocytes and mesenteric lymph node cells.

The administration of marbofloxacin after infection with *E. coli* did not change the modulatory effect of infection on the percentage of $CD4^+CD8^+$ and $CD4^+$ thymic cells. The $CD4^+/CD8^+$ ratio was observed to decreased upon marbofloxacin administration in *E. coli*-infected.

The administration of marbofloxacin also did not change the surface marker expression of splenocytes and mesenteric lymph node cells in *E. coli*-infected mice.

TABLE 1. The activity of peritoneal macrophages in non-infected and *E.coli*-infected mice treated with marbofloxacin.

Index	Control	Marbofloxacin	*E. coli*	Marbofloxacin+*E. coli*
% of phagocyting cells	45.1±6.2	54.9±4.9 ○	55.6±7.8 ○	52.4±6.7 ○
number of phagocytized bacteria	7.0±1.4	8.0±1.3	7.3±1.3	7.1±1.2
% NBT-positive cells	10.3±2.8	12.0±2.8	20.3±6.1 ○	15.5±3.8 ○●
concentration of NO (nmol/L)	1.53±0.42	1.11±0.35 ○	0.96±0.29 ○	1.00±0.21 ○
concentration of IL-1 (pg/mL)	327.7±98.5	918.9±234.0 ○	477.5±298.3	1106.7±299.3 ○●

The mean value (n=8) and standard deviation. ○ $p<0.05$ as compared to the control group; ● $p<0.05$ as compared to the infected mice

TABLE 2. The percentage of lymphocyte subsets in non-infected and *E. coli*-infected mice treated with marbofloxacin.

Index	Control	Marbofloxacin	*E. coli*	Marbofloxacin+*E. coli*
Thymus%				
CD4-CD8-	18.87 ±3.23	15.52 ±1.24 ○	16.40 ±3.10	14.72 ±1.80 ○
% CD4+CD8+	63.31 ±3.41	68.58 ±1.83 ○	68.84 ±3.51 ○	70.32 ±2.34 ○
% CD4+	11.67 ±1.82	10.93 ±0.89	9.47 ±0.89 ○	8.79 ±0.52 ○□
% CD8+	6.15 ±0.84	4.77 ±0.49 ○	5.30 ±0.62	6.18 ±1.14 □
CD4+/CD8+	1.95 ±0.50	2.31 ±0.27	1.81 ±0.20	1.47 ±0.26 ○●□
Spleen				
% CD19+	41.91 ±7.37	51.03 ±3.19	47.27 ±3.65	52.45 ±2.57
% CD3+	22.48 ±6.02	20.29 ±4.06	18.79 ±3.52	17.81 ±4.61
% CD4+	19.15 ±3.85	17.12 ±2.79	17.51 ±3.09	16.48 ±3.78
% CD8+	5.89 ±1.53	6.64 ±0.82	5.80 ±0.51	6.99 ±1.81
CD4+/CD8+	3.34 ±0.45	2.58 ±0.23 ○	3.00 ±0.37	2.38 ±0.18 ○●
Mesenteric lymph nodes				
% CD19+	34.95 ±4.07	35.78 ±6.63	38.87 ±5.58	34.78 ±5.28
% CD3+	38.65 ±6.28	40.10 ±6.92	39.16 ±2.21	38.92 ±6.79
% CD4+	30.57 ±5.17	29.00 ±5.52	30.57 ±1.86	27.53 ±3.93
% CD8+	11.79 ±2.90	12.78 ±1.79	11.03 ±2.01	12.72 ±2.08
CD4+/CD8+	2.77 ±0.80	2.32 ±0.52	2.89 ±0.71	2.19 ±0.28 ●

The mean value (n=8) and standard deviation. ○ – $p<0.05$ as compared to the control group; ● – $p<0.05$ as compared to the infected mice; □ – $p<0.05$ as compared to marbofloxacin group

DISCUSSION

It is well known that fluoroquinolones *in vivo* may alter the phagocyting activity of neutrophils and monocyte/macrophages. The present trials conducted on mice confirm the stimulating effect of marbofloxacin on the phagocyting activity of peritoneal macrophages. Marbofloxacin treatment increased the percentage of phagocyting macrophages. Wong *et al.* [2000] also reported that the phagocyting activity of macrophages increases 7-fold in mice pretreated with three daily doses of liposome-encapsulated ciprofloxacin (45 mg/kg) compared to the untreated control group. On the other hand, the results obtained in previous experiment show that exposure to six therapeutic or five higher than therapeutic doses of flumequine, norfloxacin, enrofloxacin and ciprofloxacin decreased the phagocyting ability of peritoneal macrophages resulting in a decreased percentage of phagocyting cells and the number of phagocytized bacteria by a single macrophage [Szczypka & Obmińska-Domoradzka, 2002]. Recently, Azuma *et al.* [2001] have reported that ofloxacin, lomefloxacin, tosufloxacin, fleroxacin, sparfloxacin and levofloxacin significantly inhibited phagocytosis of *E. coli* by macrophages. It has been found different effect of fluoroquinolones on the killing ability of phagocyting cells. Flumequine, norfloxacin, enrofloxacin and ciprofloxacin administered orally six times at the therapeutic doses inhibited the killing ability of peritoneal macrophages in mice, which is expressed as the decreased capability of these cells to reduce NBT [Szczypka & Obmińska-Domoradzka, 2002]. Treatment with marbofloxacin did not change the percentage of NBT-positive macrophages in non-infected mice, but partially decreased the macrophages ability to reduce NBT in the *E. coli*-infected mice.

NBT reduction test enables evaluating the ability of phagocyting cells to produce the oxygen radicals which are toxic for bacteria [Rosen, 1993]. Spehner *et al.* [1996] have shown that marbofloxacin administered five times in calves caused a moderate decrease of oxygen burst in peripheral blood neutrophiles. On the other hand, Azuma *et al.* [2001] reported that ofloxacin, lomefloxacin, tosufloxacin and levofloxacin were able to increase the production of hydrogen peroxide, while fleroxacin and sparfloxacin did not. It has been found that fluorinated quinolones exert a modulating effect on the production of NO by peripheral macrophages. The results obtained in previous experiment indicate that the administration of flumequine, norfloxacin, enrofloxacin and ciprofloxacin, irrespective of the dose applied, induced synthesis of NO by peripheral macrophages previously stimulated *in vitro* with lipopolysaccharide from *E. coli* (LPS) at a concentration of 2.5 μg/mL [Szczypka & Obmińska-Domoradzka, 2002]. Similarly Wong *et al.* [2000] have shown that *Staphylococcus auresus*-infected macrophages incubated with liposomes containing subinhibitory concentrations of ciprofloxacin (0.05 to 0.25 μg/mL) increased (up to 40 μmol/L) the production of NO_2^-, which is the end product of NO synthesis. The results in the present study show that marbofloxacin decreased the production of NO by peritoneal macrophages in non-infected and *E. coli*-infected mice. NO is able to modulate the activity of cyclooxygenase-2 (COX-2), and leads to augmentation in the level of prostaglandin E (PGE) in macrophages [Marnett *et al.*, 2000; Perkins & Kniss, 1999]. It has been found that PGE can modulate properties of phagocyting cells, *i.e.* suppress the oxidative burst in leucocytes [Ottonello *et al.*, 1995] and synthesis of interleukin-1 and tumor necrosis factor-α by monocytes/macrophages [Beyaert & Fiers, 1998; Colotta *et al.*, 1998]. These results suggest that quinolones can differentially affect phagocytosis and killing ability of macrophages.

It is very likely that the modulating action of fluoroquinolones on the phagocytic activity of monocytes/macrophages can be attributed to the activation of monocytes/macrophages and T cells through the cytokine cascade such as Il-1, Il-2 and TNF-α.

Recently, trials conducted *in vivo* and *in vitro* have indicated that fluoroquinolones are able to modulate the production and/or release of the interleukin-1. This relationship is dose-dependent. It has been found that flumequine administered at the therapeutic doses (15 mg/kg) stimulated the production of IL-1β by murine peritoneal macrophages, whereas norfloxacin (15 mg/kg), enrofloxacin (5 mg/kg) and ciprofloxacin (15 mg/kg) did not change the synthesis nor the release of this cytokine. Five times higher doses of those fluoroquinolones decreased IL-1β production by murine peritoneal macrophages [Szczypka & Obmińska-Domoradzka, 2002]. Stunkel *et al.* [1991] demonstrated that ciprofloxacin at the concentrations of 0.3–10 μg/mL increased the levels of IL-1 in the culture supernatants of adherence-enriched mouse macrophages, but not in freshly isolated human monocytes. On the other hand, Bailly *et al.* [1993] noted a decrease in the release of IL-1 *in vitro* by LPS-stimulated monocytes at ciprofloxacin concentrations higher than 25 g/mL. Ono *et al.* [2000] also noted that ciprofloxacin, grepafloxacin and ofloxacin at the therapeutic concentrations did not change or increased synthesis and release of IL-1, but at higher concentrations than therapeutic those drugs suppressed the production of IL-1.

The clinical results indicate that ciprofloxacin treatment *in vivo* increased the *ex vivo* capacity of LPS-stimulated human monocytes to produce of Il-1 [Bailly *et al.*, 1991]. The data presented in this study demonstrate that *in vivo* marbofloxacin induced the production of IL-1 by peritoneal macrophages in the non-infected and *E. coli*-infected mice.

The administration of fluoroquinolones (ciprofloxacin, pefloxacin, ofloxacin) modulates not only the production of IL-1 but also the production of TNF-α, the effect is dose-dependent [Bailly *et al.*, 1993, Gollapudi *et al.*, 1993].

Purswani *et al.* [2002] demonstrated that ciprofloxacin at the dose higher than therapeutic significantly attenuated the production of TNF- and IL-12 as a response after LPS challenge. In adddition, ciprofloxacin significantly increased serum IL-10 concentration but had little or no effect on IL-6 or IL-1β serum concentration.

A number of investigators have reported that fluoroquinolones (ciprofloxacin, enoxacin, norfloxacin, ofloxacin and pefloxacin) induced the synthesis and release of IL-2 by human peripheral blood T cells stimulated *in vitro* with phytohaemagglutinin (PHA). At a therapeutic concentration of 5–20 μg/mL quinolones increased IL-2 production, but at a high concentration of 80 μg/mL hyperproduction of IL-2 was observed [Riesbeck *et al.*, 1989]. The stimulating effect of fluoroquinolones on the synthesis and release of IL-2 by T lymphocytes can act on all subpopulations of T cells.

IL-2 is a major factor regulating growth, proliferation and activation of T lymphocytes [Thorpe, 1998].

Earlier studies by the same authors indicate that fluoroquinolones (flumequine, norfloxacin, enrofloxacin and ciprofloxacin) are able to modulate the number of specific cluster differentiation antigens (CD) on the surface of thymocytes, T splenocytes and lymph node T cells [Szczypka & Obmińska-Mrukowicz, 2003]. The modulating effect of fluoroquinolones depends on the chemical structure, the dose administered as well as the immunological status. The present study indicates that the administration of marbofloxacin at the therapeutic dose (2 mg/kg) increases the percentage of immature $CD4^+CD8^+$ thymic cells with corresponding decreases in the percentage of single-positive $CD8^+$ cells. A similar effect of flumequine at five times higher than therapeutic doses (75 mg/kg) on the thymic cells subpopulations was observed. It has been found that the treatment with marbofloxacin did not change the surface marker expression of splenocytes and mesenteric lymph node cells in non-infected and *E. coli*-infected mice. Gollapudi *et al.* [1992] reported that rufloxacin did not change the percentage of $CD4^+$ and $CD8^+$ human lymphocytes. Gollapudi *et al.* [1993] noted that rufloxacin administered once daily at a dose of 50 mg/kg or ciprofloxacin administered twice daily at a dose of 40 mg/kg for 10 consecutive days did not alter the percentage of helper (L3T4) and suppressor (LYT2) T splenocytes and the helper/suppressor ratio in *Bacteroides fragilis*-infected mice.

CONCLUSIONS

In conclusion, it can be stated that marbofloxacin at a dose of 2 mg/kg administered five times is able to modulate the activity of peritoneal macrophages and the differentiation of intrathymic T lymphocytes in non-infected and *E. coli*-infected mice.

REFERENCES

1. Azuma Y., Shinohara M., Wang P.L., Ohura K., Quinolones alter defense reactions mediated by macrophages. Int. Immunopharmacol., 2001, 1, 179–187.
2. Bailly S., Fay M., Ferrua B., Gougerot-Pocidalo M.A., Ciprofloxacin treatment *in vivo* increases the *ex vivo* capacity of lipopolysaccharide-stimulated human monocytes to produce IL-1, IL-6 and tumor necrosis factor-alpha. Clin. Exp. Immunol., 1991, 85, 331–334.
3. Bailly S., Fay M., Gougerot-Pocidalo M.A., Effects of antibiotics on production of cytokines by human monocytes. Pathol. Biol., 1993, 41, 838–844.
4. Beyaert R., Fiers W., Tumor necrosis factor and lymphotoxin, 1998, *in:* Cytokines (ed. A.R. Mire-Sluis, R. Thorpe). Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, pp. 335–360.
5. Chateau M.T., Caravano R., Rapid fluorometric measurement of the intracellular concentration of ciprofloxacin in mouse peritoneal macrophages. J. Antimicrob. Chemother., 1993, 31, 281–287.
6. Colotta F., Ghezzi P., Mantovani A., Interleukin 1, 1998, *in:* Cytokines (eds. A.R. Mire-Sluis, R. Thorpe). Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, pp. 1-18.
7. Delfino D., Bonina L., Berlinghieri M.C., Mastroeni P., Effects of a new quinoline derivative, ciprofloxacin, on some professional phagocytic cell functions. Chemioterapia, 1985, 4, 463–466.
8. Doleżal M., Walker R., Bąk S., Wpływ antybiotyków na fagocytozę w chirurgicznych chorobach. Pol. Prz. Chir., 1955, 12, 1171–1174 (in Polish).
9. Forsgren A., Bergkvist P.I., Effect of ciprofloxacin on phagocytosis. Eur. J. Clin. Microbiol., 1985, 4, 575–578.
10. Gollapudi S.V., Chuah S.K., Harvey T., Thadepalli H.D., Thadepalli H., *In vivo* effects of rufloxacin and ciprofloxacin on T-cell subsets and tumor necrosis factor production in mice infected with *Bacteroides fragilis*. Antimicrob. Agents Chemother., 1993, 37, 1711–1712.
11. Gollapudi S.V., Perumal V., Thadepalli H., Effect of rufloxacin on *in vitro* proliferation and differentation of human mononuclaer cells. J. Antimicrob. Chemother., 1992, 29, 669–676.
12. Hawkins E.C., Boothe D.M., Guinn A., Aucoin D.P., Nguyen J., Concentration of enrofloxacin and its active metabolit in alveolar macrophages and pulmonary epithelial lining fluid of dogs. J. Vet. Pharmacol. Ther., 1998, 21, 18–23.
13. Marnett L.J., Wright T.L., Crews B.C., Tannenbaum S.R., Morrow J.D., Regulation of prostaglandin biosynthesis by nitric oxide is revealed by targeted deletion of inducible nitric-oxide synthase. J. Biol. Chem., 2000, 275, 13427–13430.
14. Ono Y., Ohmoto Y., Ono K., Sakata Y., Murata K., Effect of grepafloxacin on cytokine production *in vitro*. J. Antimicrob. Chemother., 2000, 46, 91–94.
15. Ottonello L., Morone M.P., Dapino P., Dallegri F., Cyclic AMP-elevating agents down-regulate the oxidative burst induced by granulocyte-macrophage colony-stimulating factor (GM-CSF) in adherent neutrophils. Clin. Exp. Immunol., 1995, 101, 502–506.
16. Park B.H., Fikrig S.M., Smithwick E.M., Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet, 1968, 2, 532–534.
17. Perkins D.J., Kniss D.A., Blockade of nitric oxide formation down-regulates cyclooxygenase-2 and decreases PGE2 biosynthesis in macrophages. J. Leukoc. Biol., 1999, 65, 792–799.
18. Purswani M.U., Eckert S.J., Arora H.K., Noel G.J., Effect of ciprofloxacin on lethal and sublethal challenge with endotoxin and on early cytokine responses in a murine *in vivo* model. J. Antimicrob. Chemother., 2002, 50, 51–58.
19. Riesbeck K., Andersson J., Gullberg M., Forsgren A., Fluorinated 4-quinolones induce hyperproduction of interleukin 2. Proc. Natl. Acad. Sci. USA, 1989, 86, 2809–2813.
20. Riesbeck K., Forsgren A., Increased interleukin 2 transcription in murine lymphocytes by ciprofloxacin. Immunopharmacology, 1994, 27, 155–164.
21. Rosen, F., Immunodeficiency, 1993, *in:* Immunology (eds. I.M. Roitt, J. Brostoff, D.K. Male). Mosby-Year Book Europe Limited, St. Louis, Baltimore, Boston, Chicago, London, Philadelphia, Sydney, Toronto, pp. 18.1–18.10.
22. Schneider M., Thomas V., Boisrame B., Deleforge J., Pharmacokinetics of marbofloxacin in dogs after oral

and parenteral administration. J. Vet. Pharmacol. Ther., 1996, 19, 56–61.
23. Spehner V., Aberlin S., Biichle S., Seilles E., Study of the membrane oxidative response of phagocytes after treatment with marbofloxacin in healthy cattle. C.R. Seances Soc. Biol. Fil., 1996, 190, 633–639.
24. Spreng M., Deleforge J., Thomas V., Boisrame B., Drugeon H., Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use against canine and feline isolates. J. Vet. Pharmacol. Ther., 1995, 18, 284–289.
25. Stuehr D.J., Marletta M.A., Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to *Escherichia coli* lipopolysaccharide. Proc. Natl. Acad. Sci. USA, 1985, 82, 7738–7742.
26. Stunkel K.G., Hewlett G., Zeiler H.J., Ciprofloxacin enhances T cell function by modulating interleukin activities. Clin. Exp. Immunol., 1991, 86, 525–531.
27. Szczypka M., Obmińska-Domoradzka B., Wpływ fluorochinolonów na aktywność makrofagów otrzewnowych myszy. Med. Wet., 2002, 58, 68–73 (in Polish).
28. Szczypka M., Obmińska-Mrukowicz B., Comparative effects of fluoroquinolones on subsets of T lymphocytes in normothermic and hyperthermic mice. J. Vet. Pharmacol. Therap., 2003, 26, 253–258.
29. Thorpe R., Interleukin 2, 1998, *in:* Cytokines (ed. A.R. Mire-Sluis, R. Thorpe). Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, pp. 19–34.
30. Wong J.P., Schnell G., Simpson M., Saravolac E., Effects of liposome-encapsulated ciprofloxacin on phagocytosis, nitric oxide and intracellular killing of *Staphylococcus aureus* by murine macrophages. Artif. Cells Blood Substit. Immobil. Biotechnol., 2000, 28, 415–428.