Effects of chloramphenicol and ciprofloxacin on the selected functions of rabbit phagocytic cells

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Abstract

Chloramphenicol and ciprofloxacin belong to the antimicrobials used in veterinary medicine for, among others, rabbit otitis, respiratory or skin diseases treatment. Chloramphenicol binds to the 50S subunit of the ribosome, inhibiting thereby bacterial protein synthesis, however it is known to disturb also mammalian mitochondrial protein synthesis. Ciprofloxacin belongs to fluoroquinolones, which are able to accumulate inside the phagocytic cells, reaching far more higher intra- than extracellular concentrations. Our aim was to compare the influence of the drugs on the basic functions of phagocytic cells isolated from rabbit blood.

The study was carried out in vitro with pure, water soluble chloramphenicol (Sigma) and pure ciprofloxacin (Polfa, Grodzisk) at the concentrations of 5, 10, 30 μg/ml. The blood was obtained from the marginal ear vein and the polymorphonuclear cells (PMNs) were isolated by gradient centrifugation. The cells were incubated for 24 h at the presence of the drugs. Then the following parameters were assessed: PMN viability by quantitation of the ATP present, signaling the presence of metabolically active cells, PMN cell ability to zymozan particles phagocytosis and their metabolic activity determined by nitrotetrazolium blue (NBT) reduction after phorbol myristate acetate (PMA) stimulation. Both antibiotics exhibited modulatory effects on the two last parameters.

Key words: *chloramphenicol, ciprofloxacin, rabbit polymorphonuclear cells, zymosan phagocytosis, respiratory burst activity, phagocytic cell viability.*

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Introduction

Antibiotics, except desired antimicrobial activity, are also known to be able to modulate some immune cell functions. As proper status of the immunocompetent cells is vital for the process of healing, it is important to find the possible influence of antimicrobial drugs on immune cell functioning at the concentrations used during treatment.

Chloramphenicol and ciprofloxacin belong to the antimicrobials used in veterinary medicine for, among others, rabbit otitis, respiratory or skin diseases treatment. Chloraphenicol binds to the 50S subunit of the ribosome, thereby inhibiting bacterial protein synthesis, however it is known to disturb also mammalian mitochondrial protein synthesis. Ciprofloxacin belongs to fluoroquinolones, which mode of action depends upon blocking bacterial DNA replication by binding itself to DNA gyrase and causing double-stranded breaks in the bacterial chromosome. Fluoroquinolones are able to accumulate inside the phagocytic cells, reaching far more higher intra- than extracellular concentrations.

Our aim was to compare the *in vitro* influence of the drugs on the basic functions of polymorphonuclear (PMN) cells isolated from rabbit blood.

Material and Methods

In the study the Principles of laboratory animal care and the national laws on the protection of animals were followed

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(Opinion and Approval of Local Committee of Ethics Nr. 490/2004).

Antimicrobial drugs

Water soluble chloramphenicol (CAP, Sigma) and pure ciprofloxacin (CIP, Polfa-Grodzisk) at the concentrations of 5, 10 and 30 µg/ml in RPMI 1640 (BioMed, Poland) were used.

Phagocytic cells

Blood of three adult, 5 kg weighing rabbits (*Oryctolagus cuniculus*), was taken into heparinised containers from the marginal ear vein. Leucocytes were isolated by density gradient centrifugation on Gradisol G (1.115 g/ml, Aqua-Medica, Łódź). The cell viability tested by the dye exclusion method (0.1 % of trypan blue) was ≥97%. Then the neutrophil number was adjusted to the appropriate concentration in RPMI 1640 supplemented with 0.1% of inactivated FCS (foetal calf serum, Gibco, England) and left in 96-well microtiter plates (Nunc) for adherence. Then the supernatant with non-adherent cells was removed and 100 µl of CIP or CAP at the appropriate concentrations or RPMI 1640 to the control wells were added and the plate was left for 24 h, 39°C, 5% CO_2, in dark.

Cell viability assay

Cell viability was assessed with the use of the luminescent CellTiter-Glo® test (Promega). The principle of the assay is to determine the number of viable cells on quantitation of the ATP present, which signals the presence of metabolically active cells. Assay procedure was conduced according to the producer protocol. Shortly, to the incubated cells (5×10^5 cell/ml) lysing Reagent® was added and the plate was mixed for 2 min in orbital shaker to induce the cell lysis. Then the plate was incubated at the room temperature for 10 min to stabilize the luminescent signal and the reading was made on luminometer Lumistar Optima (BMG Labtech).

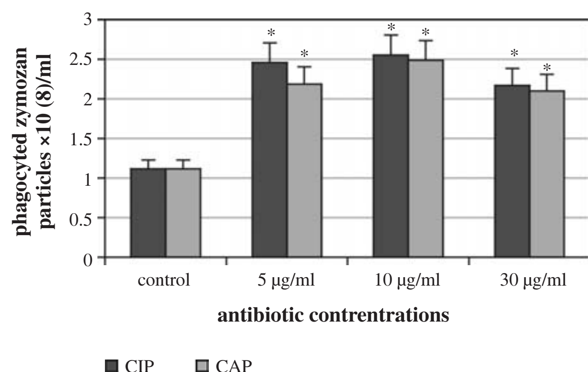

Fig. 1. The influence of ciprofloxacin and chloramphenicol on dyed zymozan phagocytosis by rabbit blood PMN cells ($\bar{x}$±SD, n=5, *P<0.05)

Leucocyte phagocytosis assay

The assay measures the uptake of neutral red dyed zymozan (Sigma) by phagocytic cells. The zymozan particles (5×10^8 particles/ml) were added to the cells (5×10^6 cell/ml) and incubated with addition of autological serum for 30 min, at the room temperature. Then the supernatant was removed and the cells were washed several times with PBS. The series of standards of dyed zymozan were added and the plate was spinned at 60 g for 5 min. Acifided ethanol was used to resolubilize the remaining dye and after 10 min the plate was read at 450 nm (BioRad 550).

Leucocyte metabolic activity assay

Metabolic activity was measured by the respiratory burst activity (RBA) with oxygen burst activator PMA (phorbol myristate acetate, Sigma). Shortly, to the cells (5×10^6 cell/ml) 0.1% NBT (nitrotetrazolium blue, Sigma) solution in RPMI 1640 without phenol red (BioMed, Poland) was added with 1 µg/ml of PMA. After 30 min incubation at the room temperature, while NBT was reduced to an insoluble blue formazan, supernatant was removed and the adherent cells were fixed with ethanol. Plates were left to dry. Measurement was performed on a microplate reader BioRad 550 at 630 nm after 2M KOH and DMSO addition, with 9 sec mixing time.

Statistical analysis

Statistical analysis was performed by one-way ANOVA at significance level $P<0.05$. When significant differences were detected, Duncan's test was used to compare the experimental groups to the control.

Results

Cell viability assay

We found no statistically significant influence of the drugs at any used concentrations on the phagocytic cell viability after 24 h incubation period (data not shown).

Zymosan phagocytosis of PMNs

We observed stimulation of zymosan particle phagocytosis at each tested concentrations, both CIP and CAP, in comparison to the control cells incubated in antibiotic-free medium. The highest amount of phagocyted particles were found in cells incubated in the presence of 10 µg/ml of the drugs, while slightly lower amount in 30 µg/ml (Fig. 1).

Respiratory burst activity of PMNs

As the respiratory burst activity is one of the mechanisms allowing to destroy phagocyted microorganisms, the effects

of the clinically achievable concentrations of CIP and CAP were tested. We found that all tested drug concentrations stimulated the production of superoxide radicals measured by NBT reduction to blue formazan. The highest values of OD were achieved with cells incubated in the presence of 10 µg/ml of the drugs (Fig. 2).

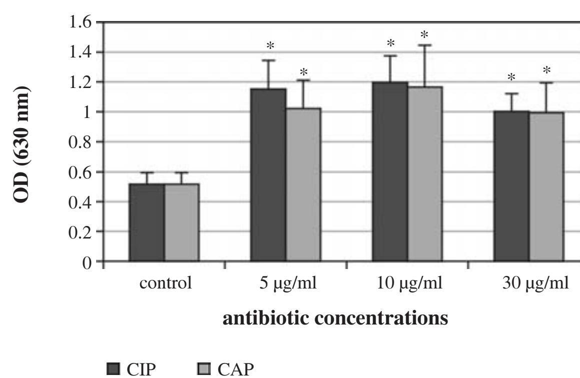

Fig. 2. The influence of ciprofloxacin and chloramphenicol on respiratory burst activity of rabbit blood PMN cells ($\bar{x}$±SD, n=6, *P<0.05)

Discussion

Phagocytic cells play a crucial role in the first line defence against invading microorganisms. It is well documented, that both antibiotics, CIP and CAP, are able to accumulate inside the phagocytic cells, reaching higher intra- than extracellular concentrations. Fluoroquinolones are known to achieve 5-18 fold higher intracellular concentrations [1, 2] and their ability to accumulate is dependent on the type of exposed cell and probably the maturation stage of the cell [3]. They do not accumulate in blood isolated monocytes, but they are able to concentrate in neutrophils and tissue macrophages. Chloramphenicol is also able to accumulate in polymorphonuclear leucocytes and rabbit macrophages reaching 2-5 fold higher amounts inside the cells [4].

Such ability to achieve high concentrations inside the phagocytic cells without the negative influence on the phagocyting and killing activity of those cells makes possible to destroy efficiently pathogens having a potency to live inside the cell after phagocytosis. As there are data indicating that some antibiotic adversely affect phagocytic functions [5, 6], it is very important to determine the possible effects of CIP and CAP on PMNs activity at clinically achievable concentrations.

In our study CIP at all tested concentrations changed respiratory burst activity of the rabbit blood PMN cells. There are some reports on the effects of ciprofloxacin and other fluoroquinolones on phagocytic activity of mammalian PMN cells, but the data are conflicting. Similar stimulatory effects were seen in the studies on human neutrophils, manifested as increase of phagocytic and killing ability or enhancement of respiratory burst activity after incubation with CIP and other fluoroquinolones [7-9]. Also in the *in vitro* study by Hoeben et al. on bovine PMNs low concentrations of some fluoroquinolones stimulated chemiluminescence of the cells after PMA stimulation, higher ones caused return of the parameter to the control level, while very high concentrations, 100-1000 µg/ml, had the inhibitory effects [6]. The observed stimulatory effects can be partially explained by ciprofloxacin ability to significant potentate of cytosolic free calcium level, which is a factor of key function in the mechanism of the respiratory burst [7, 8, 10].

On the other hand, Szczypka and Obmińska-Domoradzka found, that ciprofloxacin given to mice at the therapeutic doses, caused decrease of phagocytic and killing activity of peritoneal macrophages. Moreover stimulatory effects of LPS on macrophages were inhibited or completely blocked by CIP [11]. While Desnottes, Forsgren et Bergkvist and Delfino et al. found no effects of the drug at the therapeutically relevant concentrations on phagocytic activity, chemiluminescence nor killing activity of human neutrophils after *in vitro* exposure [12-14].

As some cases of inhibitory activity of CAP on phagocytosis were described [6, 10, 15, 16], our aim was to check the possible negative effects of the drug on rabbit blood PMN cells at clinically achievable concentrations. We found that CAP, similarly to CIP, stimulated zymozan phagocytosis and respiratory burst activity with no influence on cell viability. Also Briheim and Dahlgren observed stimulatory effects of CAP at 0.1-10 µg/ml on metabolic activity of human PMNs, revealed as formylmethionyl-leucyl-phenylalanine induced chemiluminescence, however higher concentrations of the drug lacked that stimulatory activity [17]. On the contrary, in high CAP concentrations, decrease of phagocytic activity, superoxide anion generation and chemiluminescence of bovine PMN cells were seen [6, 10, 16]. Probably, in high concentrations, CAP can cause the loss of pseudopodia and inhibition of the assembly of actin monomers into filaments, being important for degranulation and maintenance of pseudopodia. Moreover the inhibition of myeloperoxydase or NADPH-oxidase activity is suggested [6, 10, 17]. However in the tested concentrations no negative influence of the drug was seen, on the contrary, stimulation of studied parameters was recordered.

In summary, tested CIP and CAP concentrations had no influence on the viability of rabbit blood PMN cells and stimulated both phagocytosis and respiratory burst activity of the cells. However the drugs are also used locally in form of drops or ointment directly on skin, ear or eye, so other than tested concentrations can be temporarily reached. Further research into modulatory activity of CIP and CAP with use of wider range of drug concentrations on rabbit PMNs would be interesting.

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