Effect of cephapirin and mecillinam on the phagocytic and respiratory burst activity of neutrophil leukocytes isolated from bovine blood

H. DOSOGNE*
D. HOEBEN*
C. BURVENICH* &
J. A. C. M. LOHUIS†

**Department of Veterinary Physiology, Biochemistry and Biometrics, University of Ghent, Faculty of Veterinary Medicine, Salisburylaan 133, B-9820 Merelbeke, Belgium, †Pharma Division, Intervet International bv, Wim de Körverstraat 35, PO Box 31, 5830 AA Boxmeer, The Netherlands*

Dosogne, H., Hoeben, D., Burvenich, C., Lohuis, J. A. C. M. Effect of cephapirin and mecillinam on the phagocytic and respiratory burst activity of neutrophil leukocytes isolated from bovine blood. *J. vet. Pharmacol. Therap.* **21,** 421–427.

Antimicrobial therapy is the most commonly used treatment of bacterial infections in dairy cows. Polymorphonuclear neutrophil leukocytes (PMN) play an important role in the first line defence against invading bacteria and it is important that the function of PMN is not compromised by antibiotics. We investigated the *in vitro* effect of cephapirin, a first generation cephalosporin, and mecillinam, an amidinopenicillin with activity against mainly Gram-negative bacteria, on phagocytosis and respiratory burst activity of PMN isolated from bovine blood. After *in vitro* incubation of PMN with different concentrations of the antibiotics, phagocytosis was evaluated by flow cytometry and respiratory burst activity was evaluated by registration of chemiluminescence (CL) with a luminometer. None of the investigated concentrations of cephapirin and mecillinam had an effect *in vitro* on phagocytosis of *Escherichia coli* by PMN. At high concentrations (100 and 1000 μg/mL), cephapirin and mecillinam reduced the respiratory burst activity of PMN. Part of these suppressive effects could be ascribed to oxidant scavenging. Inhibitory effects of cephapirin were stronger than mecillinam. In conclusion, cephapirin and mecillinam did not seem to affect antibacterial activity of PMN isolated from bovine blood *in vitro* at therapeutic concentrations.

(Paper received 6 February 1998; accepted for publication 24 June 1998)

Prof. Dr Christian Burvenich, Department of Veterinary Physiology, Biochemistry and Biometrics, University of Ghent, Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke, Belgium (E-mail: christian.burvenich@rug.ac.be)

INTRODUCTION

Cephapirin and mecillinam are broad-spectrum antibiotics for the treatment of bacterial infections in dairy cows. Cephapirin belongs to the cephalosporins and has high activity against Gram-positive bacteria and moderate activity against Gram-negative Enterobacteriaceae. Mecillinam belongs to the amidinopenicillins and is mainly active against a broad range of Enterobacteriaceae. Several investigations have demonstrated that certain antibiotics exert a significant effect *in vitro* on leukocyte antimicrobial activity (Nickerson *et al.*, 1986). Phagocytosis and the production of reactive oxygen species (respiratory burst) by PMN are important aspects of non specific defence of cows against infections. Several PMN functions were found to be impaired in cows shortly after parturition and this impairment was related to an increased susceptibility to infections, e.g. acute mastitis caused by environmental pathogens (Burvenich *et al.*, 1994). Therefore, it seems important that treatment of periparturient dairy cows does not compromise the animal's natural defence mechanism. *In vitro* experiments can provide information on the possible side-effects of drugs on bovine blood PMN functions.

MATERIALS AND METHODS

Animals

Eight healthy Holstein-Friesian cows from the Ghent University dairy herd were used as a source of blood PMN. The cows were between 21 and 35 days after parturition and in their first to sixth lactation. The selected animals showed no signs of periparturient diseases. Milk production of the cows was between 30 and 56 L/day.

Isolation of PMN

Peripheral blood (40 mL) was aseptically collected from the jugular vein in vacutainer tubes (Laboratoire EGA, Nogent-le Roi, France) containing 125 I.U. heparin as anti coagulant.

Polymorphonuclear leukocytes were isolated from the blood according to Hoeben *et al*. (1997a, b). The final cell pellet was resuspended in 1 mL Dulbecco's phosphate buffered saline (DPBS, Gibco BRL, Life Technologies, Paisley, UK). For CL assays, 1 mg/mL gelatin was added. The cell concentration was determined with an electronic cell counter (Coulter Electronics Ltd. Luton, Beds., UK) and leukocytes were differentiated microscopically after staining with Hemacolor® (Merck Diagnostica, Darmstadt, Germany). On average, 95% of the isolated cells were neutrophils with a viability of 98%. For the CL assays the cell concentration was adjusted to 5×10^6 viable PMN/mL and for the phagocytosis assays to 2×10^6 PMN/mL DPBS and stored on ice until use.

Drugs

Stock solutions of 100 mg/mL cephapirin and mecillinam (Intervet International, Boxmeer, The Netherlands) were prepared in sterile, pyrogen free water, diluted to working solutions and used immediately or stored at –80 °C and used within 14 days. The stock solutions were diluted with DPBS to working solutions of 1000, 100, 10, 1 and 0.1 μg/mL.

Effect of antibiotics on phagocytosis

Phagocytosis was tested according to a method described by Saad & Hageltorn (1985) with minor modifications. *Escherichia coli* P4 (Dr J. Bramley, University of Vermont, Burlington) grown in brain heart infusion broth (BHI, DIFCO, Detroit, MI, USA) was labelled with fluorescein isothiocyanate (FITC isomer I, Sigma Chemicals, Bornem, Belgium) (Gelfand *et al*., 1976). Isolated PMN (2×10^5cells/mL, final concentration) were incubated with FITC-labelled *E. coli* P4 (2×10^6CFU/mL, final concentration), 5% (v/v) pooled bovine serum and 0.1–1000 μg/mL antibiotics. The sham treatment of PMN consisted of identical incubations, except that no antibiotics were present in the incubation medium. The FITC fluorescence of 3000 PMN per sample, selectively gated in the forward–side scatter dot plot was measured on a logarithmic scale from 1 to 10 000 by flow cytometry (FACScan, Becton Dickinson, Aalst, Belgium) with a 530–560 nm filter while 460 V was applied on the photomultiplier. Percent fluorescence and mean fluorescence intensity (MFI) were calculated from the measurements with the CellQuest® software (Becton Dickinson). The percentage PMN with a fluorescence higher than autofluorescence, due to attachment or ingestion of FITC-labelled bacteria, was used for estimation of percentage phagocytosis whereas the MFI-values were a parameter for the number of ingested bacteria per cell. An example of a flow cytometric analysis of phagocytosis is presented in Fig. 1.

Effect of antibiotics on respiratory burst

Chemiluminescence assay

Luminol-dependent cellular chemiluminescence (CL) was used to measure the respiratory burst activity of PMN isolated from the blood of eight cows after stimulation with phorbol 12-myristate 13-acetate (PMA) (Sigma). A stock solution of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (Sigma) (0.1 M) was stored at –20 °C and immediately before use thawed and diluted to a concentration of 1 mM with DPBS. A stock solution of PMA was prepared by dissolving 200 μg of PMA in 1 mL of DMSO and stored at –20 °C. The CL assay was performed at 37 °C with a microtitreplate luminometer (LB96P, EG & G Berthold GmbH & Co., D-75312 Bad Wildbad, Germany). The total volume of the reaction medium was 200 μL. The cell suspension with a final concentration of 2×10^6 cells/mL was preincubated with DPBS (sham treatment) and different concentrations of the drugs during 30 min at 37 °C. After this preincubation, 0.3 mM luminol and 200 ng/mL of PMA were added and the CL reaction was registered in duplicate immediately for 30 min. The area

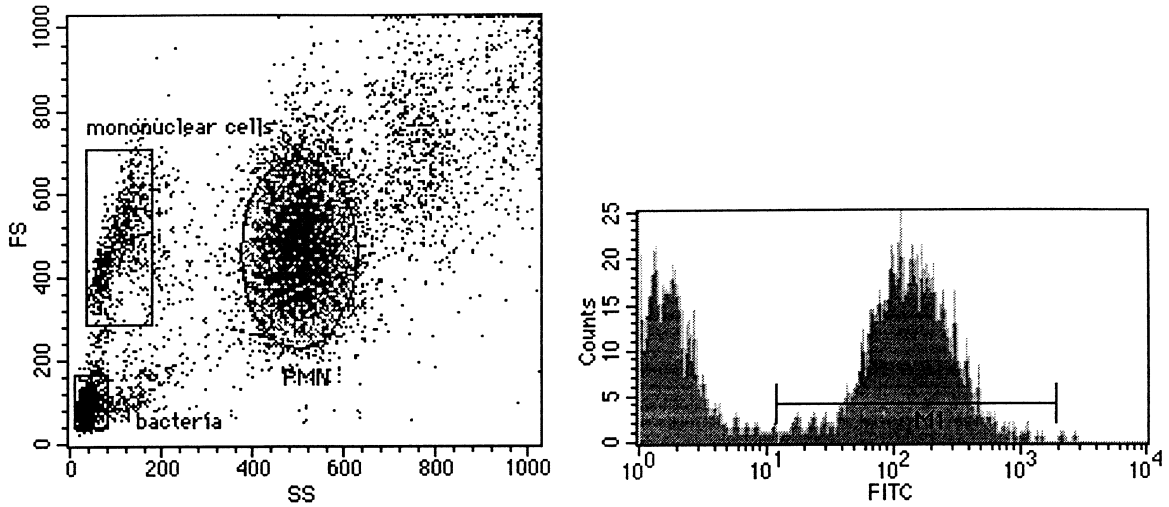

Fig. 1. Flow cytometric analysis of phagocytosis of polymorphonuclear leukocytes (PMN) isolated from bovine blood. Left: forward scatter (FS) – side scatter (SS) dot plot in which PMN are selectively gated. Right: green fluorescence of fluorescein isothiocyanate (FITC) of the PMN gated in the FS–SS dot plot. The gate M1 is used to determine both percentage fluorescence (% PMN with at least one phagocytosed bacteria) and mean fluorescence intensity of the PMN positive for phagocytosis.

under the curve (*AUC*) was calculated for the registered impulse rates over the whole 30 min period and a comparison was made for drug-incubated cells vs. cells treated with sham. This quotient was multiplied by 100 and formed the CL index.

Effect of antibiotics on chemiluminescence in a cell-free system

The CL in a cell-free system was measured in duplicate according to the method of Briheim & Dahlgren (1987) modified by Hoeben *et al.* (1997a, b). Briefly, PMN isolated from the blood of eight cows were pooled and sonicated according to Hoeben *et al.* (1997a, b). This procedure results in destruction of the NADPH-oxidase enzyme but leaves the myeloperoxidase enzyme, which is present in the granules, intact. After this sonication, the suspension was centrifuged and the supernatant (25 μL), which originated from the PMN of eight cows, was used for further experiments. This supernatant was incubated with 0.1 mM luminol and 0.1 mM H_2O_2 in the presence or absence of the antibiotics, and CL was measured.

Effect of antibiotics on myeloperoxidase activity

The effect of the antibiotics on myeloperoxidase (EC 1.11.1.7) (MPO) activity was measured in duplicate according to a method of Somersalo *et al.* (1990), modified by Hoeben *et al.* (1997a, b). This method is based on the oxidation of *ortho*-dianisidine by PMN extract containing H_2O_2. Twenty-five microlitres of the supernatant after sonication of isolated PMN pooled from eight cows, DPBS, and antibiotics (20 μL) were preincubated at 37 °C for 5 min. After this preincubation, 0.1 mM H_2O_2 and 0.8 mM *ortho*-dianisidine were added, and the absorption was measured in a spectrophotometer (Multiskan Plus Type 314; Labsystems Oy, Helsinki, Finland) at 450 nm.

Effect of antibiotics on chemiluminescence from hypochlorite and luminol

The CL from hypochlorite and luminol was measured in duplicate according to Hoeben *et al.* (1997a, b).

Effect of antibiotics on generation of superoxide anions by stimulated PMN

The effect of antibiotics on the production of superoxide radicals by PMN stimulated by PMA was measured by means of the cytochrome *c* reduction test as described by Hoeben *et al.* (1997a, b).

Statistical analysis

Statistical analysis of the PMN FS, CL and phagocytosis data was performed for each antibiotic using a two-way analysis of variance (Snedecor & Cochran, 1967). The concentration of antibiotics was a fixed factor, the cows ($n = 8$) were randomized factors, and their interaction term was the error term. Comparisons of means were performed *via* least significant differences (Snedecor & Cochran, 1967). A logarithmic transformation of the absolute values of the CL data of the calculated *AUC* was used. A Bartlett's test of equal variances was used to study the equality of variances of the different groups. The results of this test allowed us to use the analysis of variance. Statistical analysis of the results from the cell-free assay ($n = 2$: duplicate measurements), the MPO assay ($n = 2$), the assay with luminol and sodium hypochlorite ($n = 3$: triplicate measurements), and the cytochrome *c* reduction test ($n = 3$) was performed for each antibiotic by a one-way analysis of variance. For statistical analysis, a logarithmic transformation of the absolute values of the results from the different assays for oxygen radical production was used. Comparison of means was performed *via* least significant differences. A Bartlett's test of equal variances was used to study the equality of variances. The Statistix® program package (v. 4.0 1992, Analytical Software, Tallahassee, FL, USA) was used. Significant differences were determined at $P < 0.05$*, $P < 0.01$** and $P < 0.001$***.

RESULTS

Effect of antibiotics on polymorphonuclear leukocyte cell size

The forward scatter (FS) registrated by flow cytometry was used as a measure for cell size of the PMN selected in the forward–side scatter dot plot. There were no significant effects of antibiotic treatment of PMN on FS but cephapirin seemed to have a slight decreasing effect on PMN cell size (Fig. 2). There was a non significant difference between FS of sham and 1000 μg/mL cephapirin-treated PMN of 4%.

Effect of antibiotics on polymorphonuclear leukocyte phagocytosis

There was no significant difference between sham treatment and any of the different doses of mecillinam on percentage fluorescence (Fig. 3) or MFI (Fig. 4) of PMN isolated from bovine blood. At 1000 μg/mL, cephapirin decreased the percentage phagocytosis by 4% and MFI of PMN by 8% ($P < 0.05$). The difference of percentage phagocytosis of PMN between sham and 100 μg/mL cephapirin treatment was 5% ($P < 0.05$).

Effect of antibiotics on respiratory burst activity of polymorphonuclear leukocytes

Effect of antibiotics on cellular chemiluminescence

Cephapirin and mecillinam did not affect CL at concentrations up to 100 μg/mL. At a concentration of 1000 μg/mL, both drugs induced significant ($P < 0.001$) inhibitory effects on the cellular CL of bovine PMN. Mecillinam decreased the reaction by 30%, whereas cephapirin induced an inhibition of 40% (Figs 5 and 6).

Effect of antibiotics on chemiluminescence in a cell-free system

The effects of both drugs on CL were more pronounced in the cell-free system than in the cellular system (Figs 5 and 6).

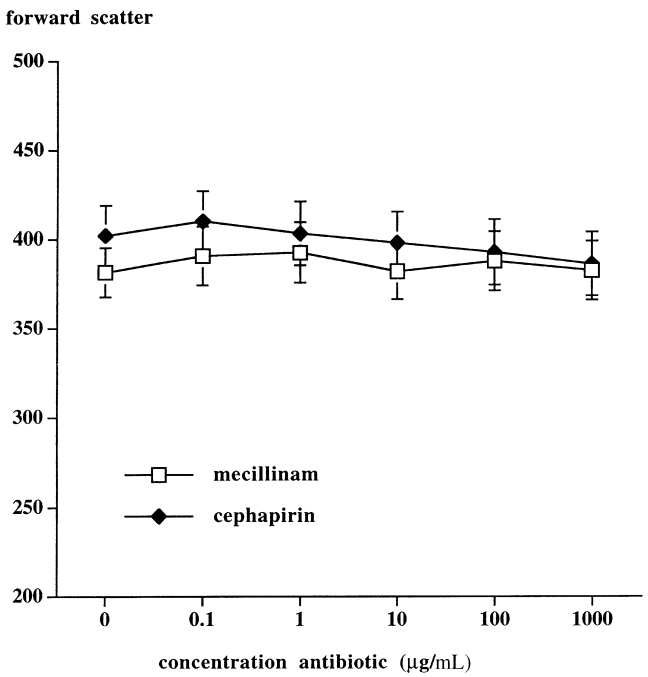

Fig. 2. *In vitro* effect of mecillinam and cephapirin on the forward scatter (FS) of isolated blood polymorphonuclear leukocytes measured by flow cytometry. Values are means ± SEM for PMN isolated from the blood of 8 cows.

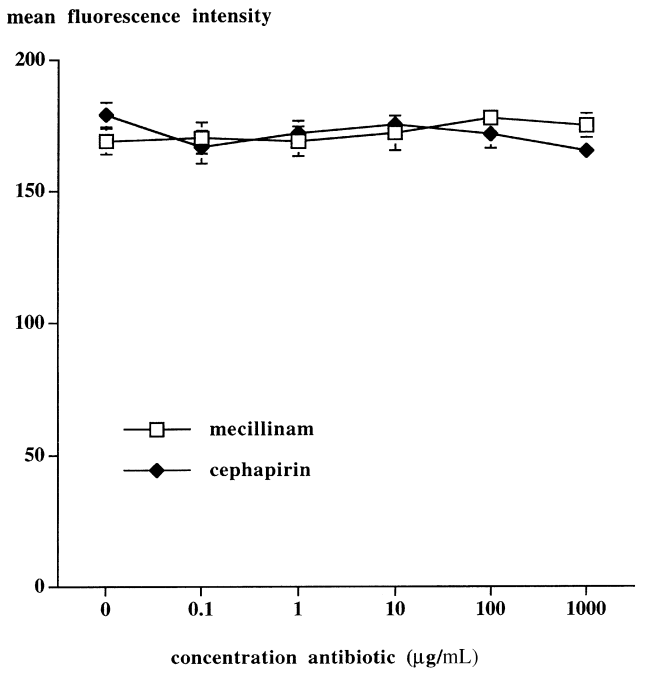

Fig. 4. *In vitro* effect of mecillinam and cephapirin on the mean fluorescence intensity (MFI) of phagocytic polymorphonuclear leukocytes (PMN) measured as the fluorescence of PMN selectively gated in the forward scatter–side scatter dot plot after incubation with FITC-labelled *E. coli*. Values are means ± SEM for PMN isolated from the blood of 8 cows.

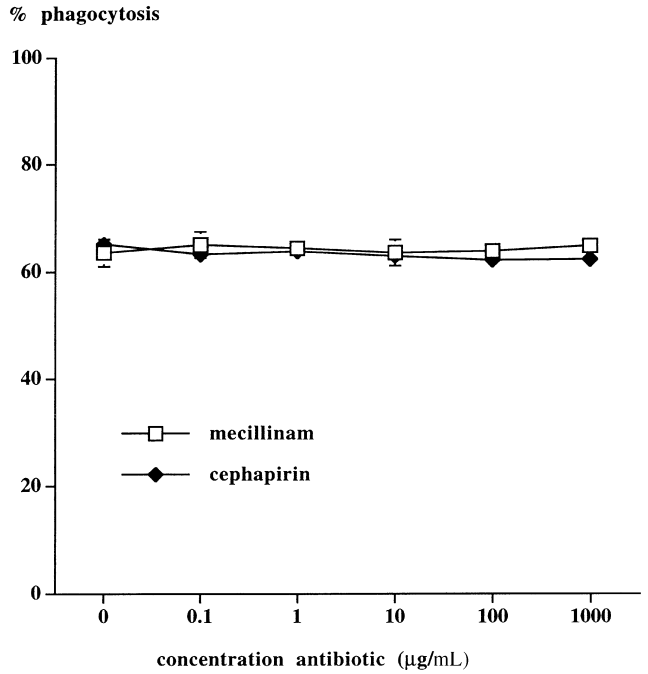

Fig. 3. *In vitro* effect of mecillinam and cephapirin on the percentage phagocytic polymorphonuclear leukocytes (PMN) measured as the fluorescence of PMN selectively gated in the forward scatter–side scatter dot plot after incubation with FITC-labelled *E. coli* (% fluorescence). Values are means ± SEM for PMN isolated from the blood of 8 cows.

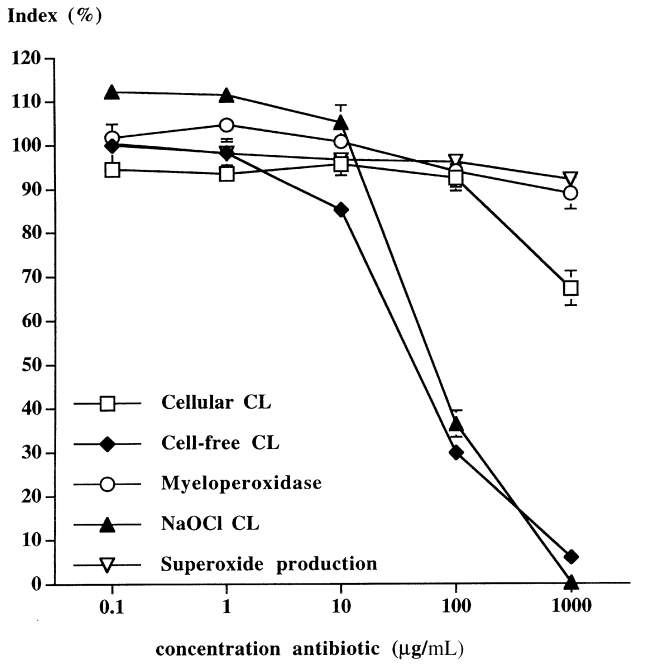

Fig. 5. Effect of mecillinam on cellular chemiluminescence (CL), cell-free CL, myeloperoxidase activity, CL of luminol and hypochlorite, and production of superoxide radicals. Data are expressed as percentage of sham treatment. Values are means ± SEM for PMN isolated from the blood of 8 cows.

Mecillinam reduced cell-free CL at a concentration of 10 µg/mL by 15% ($P < 0.01$), at 100 µg/mL by 70% ($P < 0.001$), and at 1000 µg/mL by 94% ($P < 0.001$). Cephapirin induced even more pronounced effects. Cell-free CL was decreased by 22% at a

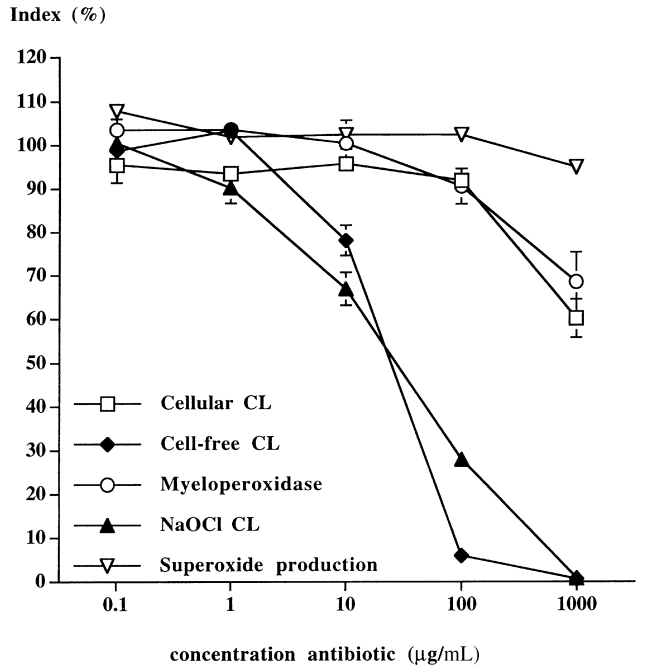

Fig. 6. Effect of cephapirin on cellular CL, cell-free CL, myeloperoxidase activity, CL of luminol and hypochlorite, and production of superoxide radicals. Data are expressed as percentage of sham treatment. Data are means ± SEM for PMN isolated from the blood of 8 cows.

concentration of 10 µg/mL ($P < 0.01$), by 94% at 100 µg/mL ($P < 0.001$), and by 99% at a concentration of 1000 µg/mL ($P < 0.001$).

Effect of antibiotics on myeloperoxidase activity

As in the cell-free system, cephapirin induced the most pronounced effects of both drugs (Figs 3 and 4). Only at a concentration of 1000 µg/mL significant effects were observed. Mecillinam reduced the reaction by 12% ($P < 0.05$) whereas cephapirin induced a significant effect at 1000 µg/mL with an inhibition of 32% ($P < 0.01$).

Effect of antibiotics on chemiluminescence of luminol and sodium hypochlorite

The CL reaction of luminol and sodium hypochlorite was significantly reduced by both drugs (Figs 5 and 6). Mecillinam decreased the reaction at a concentration of 100 µg/mL by 64% ($P < 0.001$) and at 1000 µg/mL by 99.75% ($P < 0.001$). Cephapirin induced significant inhibitory effects at the three highest concentrations ($P < 0.001$): a decrease of 33% at 10 µg/mL, a decrease of 72% at 100 µg/mL, and a decrease of 99.25% at 1000 µg/mL.

Effect of antibiotics on the production of superoxide radicals

The production of superoxide radicals was significantly ($P < 0.05$) reduced by mecillinam at a concentration of 1000 µg/mL by 8%. Cephapirin decreased the production at a concentration of 1000 µg/mL by 5% ($P < 0.01$) and increased the production significantly ($P < 0.01$) by 8% at a concentration of 0.1 µg/mL (Figs 5 and 6).

DISCUSSION

It is well known that PMN function is impaired in dairy cows shortly after parturition and that this is an important aspect of increased susceptibility to acute mastitis (Burvenich *et al.*, 1994). Whereas antimicrobial therapy, e.g. with cephapirin, is a successful treatment of cows at drying off (Owens *et al.*, 1994; Owens & Ray, 1996), this effectiveness may be less evident for treatment of early lactation cows with acute *E. coli* mastitis. Nevertheless, antibiotic treatment is still the most frequently used therapy at this moment. Therefore, it is very important not to further suppress the already impaired non specific host defence mechanism by antimicrobial treatment because this may decrease the cow's chances of recovery. *In vitro* experiments may provide valuable information on the possible side-effects of antibiotics on non specific host defence mechanisms. In the present experiments, the effect of antibiotics on phagocytosis of *E. coli* and respiratory burst activity of PMN was studied independently: chemiluminescence of PMN was stimulated by PMA which is a direct activator of NADPH-oxidase, the key enzyme of the respiratory burst whereas phagocytosis of *E. coli* by PMN was investigated by flow cytometry. Both PMN functions are very important for the defence of cows against intramammary infections by *E. coli* during the early postpartum period: significant correlations between severity of mastitis and decreased PMN phagocytosis (Dosogne *et al.*, 1997) and respiratory burst activity (Heyneman *et al.*, 1990) have been demonstrated.

There were no significant effects of cephapirin and mecillinam on the FS of circulating PMN, indicating that the two tested antibiotics had no effect on PMN cell size. There was no significant effect of cephapirin and mecillinam on PMN phagocytosis *in vitro* in any of the tested concentrations in our experiments. Indeed, the lack of effect of both antibiotics on the FS of PMN suggests that phagocytosis would also be unaffected because many drugs that inhibit phagocytic activity also induce abnormal changes in morphology *in vitro* (Ziv *et al.*, 1983; Nickerson *et al.*, 1986; Hoeben *et al.*, 1997b) and *in vivo* (Paape *et al.*, 1990). Our experiment adds evidence to the general conclusion that β-lactam antibiotics do not interfere with PMN phagocytosis at therapeutic concentrations (Ziv *et al.*, 1983; Paape *et al.*, 1991). The slight reduction of PMN cell size and phagocytosis by 1 mg/mL penicillin (Hoeben *et al.*, 1997b) and by 1 mg/mL cephapirin in our experiments could be explained by increased osmolarity of the incubation medium.

Phagocytosis of *E. coli* by PMN was reduced by 4% with 1 mg/mL cephapirin. The *in vitro* treatment of PMN isolated from bovine milk with 1 mg/mL cephapirin reduced phagocytosis of *Staphylococcus aureus* by 13% (Paape *et al.*, 1991) whereas *in vivo* intramammary treatment of cows with 300 mg benzathine cephapirin reduced the ingestion index of milk PMN by 10%

(Lintner & Eberhart, 1990). The higher reduction of phagocytosis by milk PMN could be explained by the fact that milk PMN have a decreased phagocytic capacity compared to blood PMN (Paape *et al.*, 1979). There is also a difference in surface expression of receptors between blood and milk PMN (Paape *et al.*, 1996) which could explain a difference in sensitivity to external circumstances. The difference in the pathogen and technique used in these assays could also be an explanation for the different results.

The effects of mecillinam on the respiratory burst were significantly more pronounced in the cell-free system compared to the cellular assay. This was most likely due to the poor penetrability of β-lactam antibiotics into PMN (Van den Broek, 1989; Tulkens, 1991). The results of the cytochrome *c* reduction test demonstrate a decreased production of superoxide radicals at high (1 mg/mL) mecillinam concentrations which suggests an effect on the activity of NADPH oxidase. The results of the hypochlorite assay indicate scavenging of oxygen radicals by mecillinam. Scavenging effects have been mentioned in the case of other penicillins such as penicillin G and ampicillin (Briheim & Dahlgren, 1987; Gunther *et al.*, 1993; Hoeben *et al.*, 1997b). Luminol-dependent CL is a suitable tool to study H_2O_2 production of PMN (Faulkner & Fridovich, 1993), an effect that can not be verified in the cell-free and the MPO systems because in these systems H_2O_2 is added externally. Results from the luminol-dependent CL assay indicate a reducing effect of high (1 mg/mL) mecillinam concentrations on H_2O_2 production by PMN. The effect on the MPO assay was small, indicating a slight and probably unimportant effect on the MPO activity, but rather a scavenging effect on reactive oxygen species. The most pronounced effects were noted when luminol was added to the incubation mixture. Mecillinam may react with luminol thereby reducing its availability to react with oxygen radicals. Similar results were observed with penicillin G (Briheim & Dahlgren, 1987; Hoeben *et al.*, 1997b).

The results of cephapirin were comparable with those of mecillinam but the effects of cephapirin were more pronounced except in the cytochrome *c* reduction assay. Cephapirin showed a yellow colour at the highest concentration. This yellow colour induced an increased absorption of light, which has earlier been noted with oxytetracycline and doxycycline (Hoeben *et al.*, 1997a, b). This colour interfered with the blue light that was emitted by luminol at a wavelenght of 425 nm. However, this was a slight effect only occurring at the highest concentration. The more pronounced effects in the cell-free system compared with the cellular assay may be explained by the poor penetrability of cephalosporins into PMN (Van den Broek, 1989; Tulkens, 1991). Our results suggest that cephapirin may act as a scavenger of free oxygen radicals. There was also a marked effect in the MPO assay at the highest concentration. This supports the concept of scavenging, but a direct effect on the activity of the MPO enzyme may also be involved. This was previously reported for cefdinir and ceftiofur (Labro *et al.*, 1994; Hoeben *et al.*, 1997b). Effects on the NADPH-oxidase and on the production of superoxide radicals are rather doubtful. As with Na^+-ceftiofur and -cefdinir, interference with the interaction between luminol and the MPO-H_2O_2-halide system may play a role (Labro *et al.*, 1994; Hoeben *et al.*, 1997b). The stronger reduction of PMN respiratory burst activity than phagocytosis by cephapirin is consistent with the higher reduction of the killing index of milk PMN by *in vivo* cephapirin treatment of cows when compared to the ingestion index (Lintner & Eberhart, 1990). This is also in agreement with the more pronounced effects on PMN respiratory burst activity than on phagocytosis by other antibiotics (Hoeben *et al.*, 1997b) and may reflect a general difference in sensitivity of these PMN functions to external circumstances.

In this study, we investigated the *in vitro* effect of antibiotics on functions of PMN isolated from the blood of healthy cows. Of course the situation during therapy of bacterial infections is more complicated. First, PMN functions are often reduced during bacterial infections (Heyneman *et al.*, 1990; Kremer *et al.*, 1993; Dosogne *et al.*, 1997), and therefore it seems appropriate to study effects of antibiotics on PMN isolated from diseased cows. Moreover, antibiotics may influence virulence factors and cell wall properties of bacteria, which may favour the phagocytosis and killing of bacteria.

In conclusion, mecillinam and cephapirin did not seem to exert any important adverse effect on cell size or phagocytosis of *E. coli* of PMN isolated from bovine blood at concentrations up to 1000 μg/mL and did not affect the respiratory burst activity of PMN at concentrations up to 100 μg/mL. A decrease of the respiratory burst activity was observed at 1000 μg/mL with a more pronounced effect of cephapirin but scavenging of oxygen radicals has to be kept in mind during interpretation of the test results.

ACKNOWLEDGMENTS

This study was supported by the Belgian Ministry of Agriculture (grant No. D1/2–5741 A). The excellent technical assistance of E. Vander Elstraeten, L. De Bruyne and M.R. De Smet is greatly appreciated. The authors also appreciate the assistance of M.T. Ysebaert with statistical analysis of the data.

REFERENCES

Briheim, G. & Dahlgren, C. (1987) Influence of antibiotics on formyl-methionyl-leucyl-phenylalanine-induced leukocyte chemiluminescence. *Antimicrobial Agents and Chemotherapy*, **31**, 763–767.

Burvenich, C., Paape, M.J., Hill, A.W., Guidry, A.J., Miller, R.H., Heyneman, R., Kremer, W.D.J. & Brand, A. (1994) Role of the neutrophil leukocyte in the local and systemic reactions during experimentally induced *E. coli* mastitis in cows immediately after calving. *The Veterinary Quarterly*, **16**, 45–50.

Dosogne, H., Burvenich, C., van Werven, T., Roets, E., Noordhuizen-Stassen, E.N. & Goddeeris, B. (1997) Increased surface expression of CD11b receptors on polymorphonuclear leukocytes is not sufficient to sustain phagocytosis during *Escherichia coli* mastitis in early post-partum dairy cows. *Veterinary Immunology and Immunopathology*, **60**, 47–59.

Faulkner, K. & Fridovich, I. (1993) Luminol and lucigenin as detectors for O_2^-. *Free Radical Biology and Medicine*, **15**, 447–451.

Gelfand, J.A., Fauci, A.S., Green, I. & Frank, M.M. (1976) A simple method for the determination of complement receptor-bearing mononuclear cells. *Journal of Immunology*, **116,** 595–599.

Gunther, M.R., Mao, J. & Cohen, S. (1993) Oxidant-scavenging activities of ampicillin and sulbactam and their effects on neutrophil functions. *Antimicrobial Agents and Chemotherapy*, **37,** 950–956.

Heyneman, R., Burvenich, C. & Vercauteren, R. (1990) Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced *Escherichia coli* mastitis in cows. *Journal of Dairy Science*, **73,** 985–994.

Hoeben, D., Burvenich, C. & Heyneman, R. (1997a) Influence of antimicrobial agents on bactericidal activity of bovine milk polymorphonuclear leukocytes. *Veterinary Immunology and Immunopathology*, **56,** 271–282.

Hoeben, D., Dosogne, H., Heyneman, R. & Burvenich, C. (1997b) Influence of antibiotics on phagocytosis and respiratory burst activity of isolated circulating bovine polymorphonuclear leukocytes. *European Journal of Pharmacology*, **332,** 289–297.

Kremer, W.D.J., Noordhuizen-Stassen, E.N., Grommers, F.J., Daemen, A.J.J.M., Brand, A. & Burvenich, C. (1993) Blood polymorphonuclear leukocyte chemotaxis during experimental *Escherichia coli* bovine mastitis. *Journal of Dairy Science*, **76,** 2613–2618.

Labro, M.T., Benna, J.E., Charlier, N., Abdelghaffar, H. & Hakim, J. (1994) Cefdinir (CI-938), a new oral amino-2-thiazolyl cephalosporin, inhibits human neutrophil myeloperoxidase in the extracellular medium but not the phagolysosome. *Journal of Immunology*, **152,** 2447–2455.

Lintner, T.J. & Eberhart, R.J. (1990) Effects of antibiotics on phagocyte recruitment, function, and morphology in the bovine mammary gland during the early nonlactating period. *American Journal of Veterinary Research*, **51,** 533–542.

Nickerson, S.C., Paape, M.J., Harmon, R.J. & Ziv, G. (1986) Mammary leukocyte response to drug therapy. *Journal of Dairy Science*, **69,** 1733–1742.

Owens, W.E., Nickerson, S.C., Washburn, P.J. & Ray, C.H. (1994) Prepartum antibiotic therapy with a cephapirin dry-cow product against naturally occurring intramammary infections in heifers. *Zentralblatt für Veterinärmedizin B*, **41,** 90–100.

Owens, W.E. & Ray, C.H. (1996) Therapeutic and prophylactic effect of prepartum antibiotic infusion in heifers. *Zentralblatt für Veterinärmedizin B*, **43,** 455–459.

Paape, M.J., Lilius, E.M., Wiitanen, P.A., Kontio, M.P. & Miller, R.H. (1996) Intramammary defense against infections induced by *Escherichia coli* in cows. *American Journal of Veterinary Research*, **57,** 477–482.

Paape, M.J., Miller, R.H. & Ziv, G. (1991) Pharmacologic enhancement or suppression of phagocytosis by bovine neutrophils. *American Journal of Veterinary Research*, **52,** 363–366.

Paape, M.J., Nickerson, S.C. & Ziv, G. (1990) In vivo effects of chloramphenicol, tetracycline, and gentamicin on bovine neutrophil function and morphologic features. *American Journal of Veterinary Research*, **51,** 1055–1061.

Paape, M.J., Wergin, W.P., Guidry, A.J. & Pearson, R.E. (1979) Leukocytes, second line of defense against invading mastitis pathogens. *Journal of Dairy Science*, **62,** 135–153.

Saad, A.M. & Hageltorn, M. (1985) Flow cytometric characterization of bovine blood neutrophil phagocytosis of fluorescent bacteria and zymosan particles. *Acta Veterinaria Scandinavica*, **26,** 289–307.

Snedecor, G.W. & Cochran, W.G. (1967) *Statistical Methods*. Iowa State University Press, Ames.

Somersalo, K., Salo, O.P., Bjorksten, F. & Mustakallio, K.K. (1990) A simplified Boyden Chamber Assay for Neutrophil Chemotaxis based on Quantitation of Myeloperoxidase. *Analytical Biochemistry*, **185,** 238–242.

Tulkens, P.M. (1991) Target drugs for the treatment of intracellular infections. *Flemish Veterinary Journal*, **62,** 163–168.

Van den Broek, P.J. (1989) Antimicrobial drugs, microorganisms, and phagocytes. *Reviews of Infectious Diseases*, **11,** 213–245.

Ziv, G., Paape, M.J. & Dulin, A.M. (1983) Influence of antibiotics and intramammary antibiotic products on phagocytosis of *Staphylococcus aureus* by bovine leukocytes, *American Journal of Veterinary Research*, **44,** 385–388.