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Ciprofloxacin in Combination with Metronidazole

Summary: Ciprofloxacin has a reduced activity against anaerobic pathogens. Therefore, a combination of ciprofloxacin with an antimicrobial agent active against anaerobes, such as metronidazole, seems to be interesting for the treatment of mixed aerobic/anaerobic infections. High metronidazole concentrations (10 mg/l or 40 mg/l) neither affected the bactericidal efficacy of ciprofloxacin on aerobic pathogens, such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus faecalis,* nor on the anaerobic pathogens *Clostridium perfringens* and *Clostridium difficile,* as demonstrated by kill-kinetic curves. The same high concentrations, as well as lower therapeutically achievable concentrations (2 mg/l or 5 mg/l) of metronidazole in combination with ciprofloxacin were slightly more potent for the tested clostridia than ciprofloxacin or metronidazole alone.

Zusammenfassung: *Ciprofloxacin in Kombination mit Metronidazol.* Ciprofloxacin weist gegenüber anaeroben Keimen eine herabgesetzte Aktivität auf. Insbesondere bei aeroben/anaeroben Mischinfektionen ist die Kombination von Ciprofloxacin mit einem Anaerobier-aktiven Therapeutikum wie z. B. Metronidazol wichtig. Wie sich anhand von Bakterizidiekinetiken zeigen ließ, wird weder die bakterizide Effizienz von Ciprofloxacin auf aerobe pathogene Keime, wie z. B. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* und *Enterococcus faecalis,* noch auf anaerobe Pathogene, wie z. B. *Clostridium perfringens* und *Clostridium difficile,* durch hohe Metronidazolkonzentrationen (10 mg/l oder 40 mg/l) beeinträchtigt. Sowohl in hohen als auch in therapeutisch erreichbaren Metronidazolkonzentrationen erweist sich die Kombination mit Ciprofloxacin als aktiver auf die getesteten Clostridium-Stämme als die Substanzen alleine.

Introduction

Ciprofloxacin, a new fluoro-4-quinolone, is highly effective against gram-positive and gram-negative aerobic microorganisms. However, ciprofloxacin exhibits minor activity against anaerobic pathogens (1), as do other quinolones. Especially in mixed aerobic/anaerobic infections, a combination of ciprofloxacin with an antimicrobial agent active against anaerobes, such as metronidazole, seems desirable. In order to exclude possible antagonism, we studied the bactericidal efficacy of ciprofloxacin in combination with metronidazole on aerobic and anaerobic pathogens using kill-kinetic curves.

Materials and Methods

Strains: Various clinical isolates of aerobic and anaerobic pathogens were used. These were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Clostridium perfringens, Clostridium difficile.*
Antibiotics: Ciprofloxacin and metronidazole were donated by Bayer AG, Leverkusen, FRG.
In-vitro acitivity: The minimal inhibitory concentrations (MIC) for ciprofloxacin were assayed on Wilkens Chalgren agar (GIBCO, Eggenstein, FRG) according to the NCCLS methods (2). The results are given in Table 1.

Table 1: MICs of ciprofloxacin and metronidazole against the test strains (agar dilution test).

	Ciprofloxacin (mg/l)	Metronidazole (mg/l)
Enterococcus faecalis	1	> 256
Staphylococcus aureus	0.25	> 256
Pseudomonas aeruginosa	0.5	> 256
Escherichia coli	≤ 0.06	> 256
Clostridium difficile	0.5	4
Clostridium perfringens	0.25	4

Kill-kinetic studies: Kill-kinetic curves are assessed with the following regimens: growth control (without antibiotic) ciprofloxacin at ½, 1 or 2 × the MICs for the respective microorganisms, 2 mg/l or 5 mg/l of metronidazole or ciprofloxacin at ½ the MIC or 1 × the MIC with 2 mg/l or 5 mg/l metronidazole, ciprofloxacin at 2 × the MIC plus 10 mg/l of metronidazole, ciprofloxacin at 2 × the MIC and 40 mg/l of metronidazole.
For aerobic organisms, with brain-heart-infusion (BHI) broth was inoculated in an overnight BHI-broth culture to achieve a final concentration of 10^6 CFU/ml. The test broths were usually subcultured at 0, 2, 4, 6 and 24 h of incubation. Colony counts were performed by removing 0.5 ml aliquots and by making serial dilutions in sterile saline, which were then plated onto Columbia agar (GIBCO, FRG) with 5% horse blood.
The kill-kinetic curves were established by plotting the colony counts versus time. Kill-kinetics were determined in at least three independent experiments in order to verify reproducibility.
For clostridia kill-kinetics were determined using an open bench technique under a continuous oxygen-free nitrogen gas flow in thioglycollate broth at 35° C. The antibiotic regimens were as mentioned above. Clostridia were grown anaerobically overnight in Rosenow broth (Friesenius, München, FRG) at 35° C. The test vials were inoculated with these starter cultures to give a final concentration of 10^6 CFU/ml. Aliquots were removed at various times (at 0, ½, 1, 1½, 2, 4, 6 and 24 h of incubation) with a syringe, and appropriate dilutions were made anaerobi-

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