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Ciprofloxacin-Induced Antibacterial Activity Is Attenuated by Phosphodiesterase Inhibitors

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ABSTRACT

Background: Ciprofloxacin is a commonly used antibiotic for urinary tract infection that interacts with bacterial topoisomerases leading to oxidative radicals generation and bacterial cell death. Phosphodiesterase inhibitors (PDEis), on the other hand, are commonly used drugs for the management of erectile dysfunction. The group includes agents such as sildenafil, vardenafil, and tadalafil.
Objectives: We investigated whether PDEi could interfere with the antibacterial activity of ciprofloxacin.
Methods: PDEis were tested in several reference bacteria, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Acinetobacter baumannii, Proteus mirabilis, and Klebsiella pneumoniae utilizing a standard disc diffusion method and measuring both zones of inhibition and MIC.
Results: Results from both assays indicated that ciprofloxacin demonstrates potent activity against the tested reference bacteria. Additionally, when bacteria were treated with a combination of ciprofloxacin and sildenafil, tadalafil, or vardenafil, the zones of the combination inhibition were significantly reduced, whereas the MIC values were significantly greater than those of ciprofloxacin alone for all tested bacterial strains. In an attempt to examine the mechanism by which PDEis interfere with the action of ciprofloxacin, we utilized the in vitro *E coli* DNA gyrase cleavage assay. The results showed that PDEi drugs had no effect on ciprofloxacin's inhibition of *E coli* gyrase activity.
Conclusions: Pretreatment of various reference bacterial cells with PDEis largely inhibited the antibacterial activity of ciprofloxacin.

Introduction

Phosphodiesterase inhibitors (PDEis) are a widely used group of oral therapy for erectile dysfunction. This group is selective for cyclic guanosine monophosphate-specific phosphodiesterase (PDE) type 5 present in corpora cavernosa.[1] The group has 3 major members: sildenafil, vardenafil, and tadalafil.[2] These agents differ in their degree of selectivity in inhibiting PDE isoenzymes, in their pharmacokinetic profiles, in their drug-food interactions, and in their adverse effects.[1,3] These agents have been shown to possess antioxidative or oxidative stress-protective properties.[4–5]

Ciprofloxacin is a fluoroquinolone antibiotic that possesses strong activity against gram-negative bacteria. Ciprofloxacin is commonly used for the treatment of a number of infections such as acute uncomplicated cystitis, urinary tract infections, acute sinusitis, and chronic bacterial prostatitis.[6] The mechanism of antibacterial action of quinolone, including ciprofloxacin, involves interfering with replication and transcription of DNA via inhibiting bacterial DNA gyrase/topoisomerase II and DNA topoisomerase IV, and further preventing DNA of bacteria from unwinding and duplicating.[7] Thus, complexes of quinolone-enzyme-DNA are formed, leading to the production of cellular poisons and cell death.[8]

Microbiologic studies of various bacteria ascertain the presence of the guanosine monophosphate-PDE system in bacteria,[9] which could represent a possible pharmacologic target for sildenafil and similar agents in bacteria.[10] Moreover, a previous study[11] showed that coadministration of ciprofloxacin and clarithromycin

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significantly increased sildenafil bioavailability in human beings. This could point to a possible interaction with antibiotic agents that are commonly administrated concomitantly with these agents. We evaluated, for the first time, the possible interaction among members of the PDEi group and ciprofloxacin. The results of our study could be of clinical significance due to the common use of PDEis, especially, sildenafil, when antibiotics are used for treatment of urinary tract infection.

Materials and Methods

Chemicals

Ciprofloxacin used in this study was donated by Al-Hikma Pharmaceuticals (Amman, Jordan). Sildenafil was obtained from Sigma-Aldrich Corporation (St Louis, Missouri). Vardenafil and tadalafil were obtained from Orchid Chemical Supplies Ltd (Hangzhou, China). All drugs were used as raw material.

Microbial culture and growth conditions

Antibacterial activity of ciprofloxacin and/or PDEi combinations were evaluated against different reference bacteria, including *Escherichia coli* ATTC 35218, *Staphylococcus aureus* ATTC29213, *Pseudomonas aeruginosa* ATTC 9027, *Staphylococcus epidermidis* ATTC 12228, *Acinetobacter baumannii* ATTC 17978, *Proteus mirabilis* ATTC 12459, and *Klebsiella pneumoniae* ATTC 13883. The organisms were stored at –70°C in trypticase-soy broth and 20% glycerol (BBL Microbiology Systems, Cockeysville, Maryland). When ready for batch susceptibility testing, samples were thawed. MICs were determined in accordance with the Clinical and Laboratory Standards Institute.[12]

Antimicrobial susceptibility test

Antibiotic solutions were prepared on the day of use according to the manufacturer's recommendations. A wide range of ciprofloxacin concentrations were tested against different organisms. Serial 2-fold dilutions were added to molten BBL Muller-Hinton Gold II agar (BBL Microbiology Systems). After slight cooling and drying of the plates, a steers replicator was used to place aliquots containing approximately 5×10^4 CFU per drop for 4 test strains. The plates were incubated at 37°C and read 24 hours later. In experiments where 0.1 μg/mL ciprofloxacin was combined with PDEi, PDEis were added to the media at a final concentration of 100 μM. Results (ie, the mean of 3 independent experiments) were recorded by measuring the zones of growth inhibition surrounding the antibiotic-containing discs. The breakpoints indicated in the tables of the Clinical and Laboratory Standards Institute guidelines[12] were used to determine susceptibility and resistance.

Determination of MIC

The MICs were determined by serial dilution method as described previously.[13] Briefly, drugs were serially diluted and added to 96-well plates that were prepared by

dispensing into each well 100 μL of an appropriate medium (BBL Muller-Hinton Gold II agar; BBL Microbiology Systems) and 20 μL inoculum (containing about 5×10^4 CFU). After an 18-hour incubation period at 37°C, plates were read. MIC is defined as the lowest concentration at which no growth, a faint haze, or fewer than 3 discrete colonies was detected. Plates were read in duplicate and the highest MIC value was recorded.

E coli DNA gyrase cleavage assay

The effect of PDEis on antigyrase activity of ciprofloxacin was examined using the *E coli* DNA gyrase cleavage assay as described by the manufacturer (Inspirals, Norwich, United Kingdom). In brief, DNA gyrase was incubated with 0.5 μg supercoiled pBR322 in a reaction volume at 37°C for 1 hour in the presence of 0.1 μg/mL ciprofloxacin and/or different PDEis (100 μM). SDS and proteinase K (0.2% and 0.1 μg/mL final concentrations, respectively) were added before a further incubation at 37°C for 30 minutes. About 10 μL reaction mixture was electrophoresized using 1% agarose and bands were visualized using ethidium bromide.

Statistical analysis

Analysis was performed using GraphPad Prism software (version 4.0, GraphPad Software, La Jolla, California). One-way ANOVA followed by Tukey's posttest were used to determine if there was any statistically significant difference. P values < 0.05 were considered significant.

Results

We investigated the possible attenuating effect of a PDEi on the antibacterial activity of ciprofloxacin against various species of reference bacteria, namely, *E coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *A baumannii*, *Proteus mirabilis*, and *K pneumoniae*. Inhibition zones suggested in the Clinical and Laboratory Standards Institute guidelines were considered representative of bacterial susceptibility to the compounds.[12] **Table I** shows that ciprofloxacin possessed significant antibacterial activity against the reference bacteria that were tested, except for *A baumannii* and *K pneumonia,* which showed a zone of inhibition in the intermediate and resistant ranges. When reference strains were treated with a combination of ciprofloxacin with sildenafil, tadalafil, or vardenafil, the zones of inhibition of the combination were significantly lower than those of ciprofloxacin alone for all tested bacterial strains (**Table I**).

Table I
Comparison among the zones of inhibition (mm) of ciprofloxacin alone and ciprofloxacin in the presence of sildenafil, tadalafil, or vardenafil against standard bacterial strains

Standard bacterial strains	Zones of inhibition (mm)*			
	Ciprofloxacin	Ciprofloxacin + sildenafil	Ciprofloxacin + tadalafil	Ciprofloxacin + vardenafil
Escherichia coli	26.7 (0.6)	11.3 (1.5)	11.0 (1.0)	11. 7 (0.6)
Staphylococcus aureus	21 (1.0)	9. 7 (1.2)	9. 7 (0.6)	9.3 (1.5)
Pseudomonas aeruginosa	23.3 (0.6)	11 (1.0)	10. 7 (0.6)	7.0 (2.0)
Staphylococcus epidermidis	21. 7 (0.6)	10.3 (1.2)	10.3 (0.6)	11.3 (0.6)
Acinetobacter baumannii	17. 7 (0.6)	8.3 (0.6)	7. 7 (0.6)	8.3 (0.6)
Proteus mirabilis	18. 7 (0.6)	8. 7 (0.6)	8. 7 (0.6)	7. 7 (0.6)
Klebsiella pneumoniae	12.0 (1.0)	4. 7 (0.6)	6. 7 (0.6)	5. 7 (0.6)

* The zones of inhibition values for ciprofloxacin alone were significantly ($P < 0.05$) lower than those of combination of ciprofloxacin with sildenafil, tadalafil, or vardenafil for all tested bacterial strains. Results are presented as mean (SD) of 3 independent experiments.