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Article

Vorinostat Pretreatment Enhanced Ciprofloxacin-Induced Antibacterial Activity

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Abstract: The mechanism of ciprofloxacin action involves interference with transcription and replication of bacterial DNA, which results in elevated oxidative stress, and bacterial cell death. Vorinostat was shown to induce oxidative DNA damage. In the current work, the possibility for interactive effect of vorinotat on ciprofloxacin-induced cytotoxicity against a number of reference bacteria was investigated. Standard bacterial strains were *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus epidermidis* ATCC 12228, *Acinetobacter baumannii* ATCC 17978, *Proteus mirabilis* ATCC 12459, *Klebsiella pneumoniae* ATCC 13883, methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), and *Streptococcus pneumoniae* (ATCC 25923). The antibacterial activity of ciprofloxacin with or without pretreatment of bacterial cells by vorinostat was examined using disc diffusion procedure and determination of the minimum inhibitory concentration (MIC) and zones of inhibition of bacterial growth. All tested bacterial strains showed sensitivity to ciprofloxacin. When pretreated with vorinostat, significantly larger zones of inhibition and smaller MIC values were observed in all bacterial strains compared ciprofloxacin alone. As a conclusion, current results showed the possible agonistic properties for vorinostat when it is used together with ciprofloxacin. Future research will be focus on molecular mechanisms possible for such interactive effect.

Keywords: ciprofloxacin; MIC; vorinostat; antimicrobial susceptibility

1. Introduction

Ciprofloxacin is the prototype member of the fluoroquinolones antibiotics group. It possesses both Gram-positive and Gram-negative activity. It is commonly used for the treatment of infections including urinary tract infections, chronic bacterial prostatitis, acute uncomplicated cystitis, and acute sinusitis [1]. The mechanism of action for the antibacterial properties of ciprofloxacin is not fully understood. Yet, the antibacterial action starts by interference with replication and transcription of DNA via inhibition of bacterial DNA gyrase/topoisomerase II and DNA topoisomerase IV, thus, prevention unwinding and duplication of bacterial DNA [2]. Eventually, quinolone-enzyme-DNA complexes are formed, which leads to "cellular poisons" generation and cell death [3,4]. Antibiotics including ciprofloxacin were shown to possess their antibacterial activity via induction of oxidative stress [5,6]. For instance, major reactive oxygen species including singlet oxygen ($^1O_2$) and superoxide anion (O_2) were shown to be generated by ciprofloxacin [7]. Moreover,

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multiple adverse effects for ciprofloxacin including phototoxicty and tendinopathies were associated with reactive oxygen species generation [7,8].

Vorinostat (suberoylanilide hydroxamic acid) is a derivative of hydroxamic acid that inhibits both histone deacetylases classes I and II [9]. It has been approved in the USA for patients with refractory and relapsed cutaneous T-cell lymphoma with persistent, progressive or recurrent disease on/or following two systemic therapies [10-12]. The mechanism for vorinostat antiproliferative effect involves inhibiting the activity of histone deacetylase, leading to the accumulation of acetylated proteins, such as histones [9,13]. Additionally, vorinostat was shown to induce DNA damage that is related to oxidative lesions generation [14-16]. We have recently shown that vorinostat induce oxidative chromosomal damage leading its mutagenic effect in blood lymphocytes (ref). Recently, we showed that the antibacterial activity of ciprofloxacin is altered by major antioxidants, such as, vitamins E and C[17], tempol, pentoxifylline and melatonin [18]. Given that ciprofloxacin acts by inducement of bacterial oxidative damage [5,6], and the known oxidative cell-damaging activity of vorinostat [19], it is likely that vorinostat pretreatmnet enhances ciprofloxacin antibacterial activity. Therefore, in this study, the possibility of interaction between vorinostat and ciprofloxacin was investigated.

2. Materials and Methods

Microbial culture and growth conditions

Antibacterial activity of combinations of ciprofloxacin/vorinostat were investigated against a panel of reference bacteria that included *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus epidermidis* ATCC 12228, *Acinetobacter baumannii* ATCC 17978, *Proteus mirabilis* ATCC 12459, *Klebsiella pneumoniae* ATCC 13883, methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), and *Streptococcus pneumoniae* (ATCC 25923). The microorganisms were stored in 20% glycerol and trypticase-soy broth at -70 °C (BBL Microbiology Systems, Md, USA). Samples were thawed when ready for batch susceptibility testing. The MICs were evaluated as per the Clinical and Laboratory Standards Institute [20].

Testing of antimicrobial susceptibility

On the day of use, solutions of antibiotic were prepared according to manufacturer's recommendations. A panel of ciprofloxacin concentrations was used to test for susceptibility of various microorganisms. Two folds serial dilutions were added to plates containing molten BBL Muller-Hinton Gold II agar (BBL Microbiology Systems). The plates were slightly cooled and dried. Thereafter, aliquots of around $5x10^4$ colony forming units per drop were added to each tested bacterial strain using a steer replicator. Plates were incubated at 37°C and read 24 hours later. In part of the experiments, combination of ciprofloxacin at 100µg/mL and vorinostat at 100µM were mixed with the agar [21-23]. The zones of growth inhibition surrounding the antibiotic containing discs were measured. Mean values of 3 independent experiments were recorded.

Minimum inhibitory concentration (MIC) Determination

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Serial dilution method was used for determination of MICs as per the National Committee for Clinical Laboratory Standards[20]. In Brief, drugs were serially diluted, and added to plates containing molten BBL Muller-Hinton Gold II agar (BBL Microbiology Systems). Then, plates were slightly cooled and dried. Thereafter, aliquots containing about $5x10^4$ colony forming units per drop of different bacterial strains were placed in each plate using an a steer replicator. Plates were read after an 18-hour incubation period at 37°C. The MIC was defined as the lowest concentration at which no growth, a faint haze or fewer than 3 discrete colonies were detected. Plates reading were carried out in duplicate and the highest MIC values were recorded. The breakpoints indicated in the tables of the National Committee for CLSI [20], were used to determine susceptibility and resistance.

Statistical Analysis:

Statistical analysis was carried out via GraphPad Prism software, version 4.0, LA jolle, CA. tests used was One-way ANOVA followed by Tukey's post-test. *P*-values of less than 0.05 were considered significant.

3. Results

In the current study, the possible interactive effect for vorinostat on ciprofloxacin antibacterial activity was investigated against various species of reference bacteria. Results (Table 1) showed that ciprofloxacin possessed antibacterial activity against several reference bacteria, namely, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *A. baumannii*, *P. mirabilis*, and *K. pneumoniae*. A 15 mm zone of inhibition was selected to indicate susceptibility of bacteria to tested agents. When bacteria were treated with both vorinostat and ciprofloxacin, the zones of inhibition of the combination were significantly larger than those of ciprofloxacin alone in all tested bacterial species (Table 1).

Table 1. A comparison between the zones of inhibition (mm) of ciprofloxacin (100 μg/mL) alone and ciprofloxacin in the presence of 100 μM of vorinostat against standard bacterial strains.

Standard Bacterial Strains	Zone of Inhibition (mm)*		
	Ciprofloxacin	Vorinostat	Ciprofloxacin + Vorinostat
Gram +ve:			
S. aureus	22.0±0.0	10.0±0.0	37.0±1.0
S. epidermidis	21.3±1.5	9.7±0.6	35.0±1.0
MRSA	10.7±0.6	4.3±0.6	19.3±1.5
S. pneumoniae	13.3±0.6	7.0±1.0	23.0±1.0
VRE	7.7±1.5	2.7±0.6	18.3±1.0
S. pyogenes	21.0±1.0	10.3±0.6	29.3±0.6
Gram –ve:			
E. coli	28.3±0.6	10.7±0.6	43.3±0.6
P. aeruginosa	23.3±0.6	10.0±0.0	38.0±1.0
P. mirabilis	19.7±0.6	7. 7±1.5	26.3±1.5
K. pneumoniae	22.3±0.6	5.3±0.6	29.0±1.0

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A. baumannii	13.0±1.7	4.3±0.6	19.0±1.0

* The zones of inhibition values for ciprofloxacin alone were significantly ($p < 0.05$) lower than those of combination of ciprofloxacin with vorinostat for all tested bacterial strains. Results are presented as mean ± SD of three independent experiments.

The minimal inhibitory concentrations (MICs) of ciprofloxacin alone and in combination with vorinostat were estimated. As Table 2 shows, pretreatment of reference bacteria with vorinostat enhanced the antibacterial activity of ciprofloxacin. This is shown by significantly smaller MIC values (Table 2) for the combination of all doses of vorinostat and ciprofloxacin as compared to either alone.

Table 2. A comparison between the minimum inhibitory concentrations (MIC; μg/mL) of ciprofloxacin alone and ciprofloxacin in the presence of 100 μM of vorinostat against standard bacterial strains

Standard Bacterial Strains	**MIC (μg/mL)***		
	Ciprofloxacin	**Vorinostat**	**Ciprofloxacin + Vorinostat**
Gram +ve:			
E. coli	0.031±0.00	33.33±14.43	0.006±0.007
S. aureus	0.052±0.018	83.33±14.43	0.003±0.001
S. epidermidis	0.083±0.036	100.00±25.00	0.005±0.002
MRSA	0.41±0.14	300.00±25.00	0.072±0.048
S. pneumonia	0.33±0.14	275.00±25.00	0.035±0.025
VRE	0.66±0.29	325.00±25.00	0.17±0.072
S. pyogenes	0.16±0.072	116.7±28.87	0.038±0.022
Gram –ve:			
P. aeruginosa	0.496±0.00	291.67±14.43	0.010±0.037
P. mirabilis	0.17±0.072	116.67±28.87	0.009±0.009
K. pneumonia	0.10±0.036	125.00±25.00	0.015±0.009
A. baumannii	0.496±0.00	308.33±14.43	0.17±0.00

* In each experiment, ciprofloxacin (100 μM) alone or in combination with a final concentration of 100 μM of vorinostat were added to agar right before they were added to plates for 24 hrs incubation period. The MIC values for ciprofloxacin alone were significantly ($p < 0.05$) higher than those of combination of ciprofloxacin and vorinostat for all tested bacterial strains. Results are presented as mean ± SD of three independent experiments.

4. Discussion

The current study indicates enhanced antibacterial activity of ciprofloxacin when pretreating bacteria with vorinostat. Current results were produced using a variety of standard bacterial strains. These results could be important if ciprofloxacin and vorinostat are used concurrently for bacterial infections associated with cancer chemotherapy.

These results indicate ciprofloxacin effectiveness on several bacterial strains such as *E. coli*, *S. Aureus*, *P. aeruginosa*, *S. epidermidis*, *A. baumannii*, *P. mirabilis*, and *K. pneumonia.* In agreement, ciprofloxacin susceptibility of these bacterial strains was previously shown [17,24,25]. Additionally, reactive

oxygen species had essential role in the antibacterial effect of ciprofloxacin against bacteria such as *P. aeruginosa, E. coli*, and *S. aureus*[5-7,17]. Moreover, common scavengers of reactive oxygen species, including vitamin E, vitamin C, vitamin B12 and other aniroxidants such as melatonin, tempol, and pentoxypheline were shown to reduce ciprofloxacin antibacterial activity [17]. During the course of its action against bacterial strains such as *E. coli, Enterococcus faecalis, and S. aureus,* ciprofloxacin systematically induced the production of reactive oxygen species[5]. Moreover, microorganisms that are sensitive to ciprofloxacin had elevated intracellular levels of superoxide as compared to ones that are resistant[6]. Treatment of *E. coli* with vitamin C or glutathione lead to reduced ciprofloxacin antibacterial activity, which was due to scavenging of hydrogen peroxide and superoxide anions species[26].

Results show that combination of ciprofloxacin and vorinostat results in enhancement of the antibacterial activity of ciprofloxacin against variety of reference bacteria. As per our information, this study represents the first report of such effect or drug-drug interaction. Current results thus could indicate that simultaneous use of ciprofloxacin along with vorinostat might positively interact with ciprofloxacin antibacterial activity. Thus, combined usage of vorinostat and ciprofloxacin might need to be monitored.

The mechanism for the observed interactive effect of ciprofloxacin and vorinostat is not known. Ciprofloxacin bactericidal effect is manifested via inhibition of bacterial DNA gyrase, type II topoisomorase [27,28]. However, multiple other effects for ciprofloxacin were reported including inhibiting the growth of various other cell types [29-33], through interference with cell cycle, reduction of cell size [33], inhibition of de novo pyrimidine synthesis [33], and oxidative stress [26,34].

Vorinostat was shown to induce DNA damage, which is related to the generation of oxidative lesions [14-16]. We have recently shown that vorinostat induce oxidative chromosomal damage leading its mutagenic effect in blood lymphocytes [19]. Given the importance of reactive oxygen species, energy metabolism, mitochondrial functions for the antibacterial action of floroquinolones [5-7,17], it is probable that these mechanisms has a role in the observed enhancement of ciprofloxacin antibacterial activity by vorinostat. Thus, a drug-drug interaction between vorinostat and ciprofloxacin is a possibility. More studies are required to find the exact mechanism whereby vorinostat interact with floroquinolones action.

5. Conclusions

Ciprofloxacin antibacterial action is enhanced when it is combined with vorinostat. The importance of such observation is related to the wide usage of quinolones antibiotic and their great therapeutic value. Thus, studying of the clinical consequences of simultaneous use of vorinostat with ciprofloxacin in patients being treated against bacterial infections is recommended.

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