International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 11 (1): 67-71, 2015
ISSN 1811-7775

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.67.71

Flouroquinolones-induced Antibacterial Activity Atteneuation by Pretreatment with Vitamin B_{12}

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ARTICLE INFO
Article History:
Received: November 24, 2014
Accepted: January 05, 2015

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ABSTRACT
Fluoroquinolones antibiotics action involves interfering with bacterial DNA replication and transcription leading to increased oxidative stress and bacterial cell death. Vitamin B_{12}, on the other hand, has been shown to possess DNA protective and antioxidant properties. In this study, the possible interactive effect of Vitamin B_{12} on fluoroquinolones antibiotics-induced cytotoxicity against several reference bacteria was investigated. Standard bacterial strains included *E. coli* ATTC 35218, *Staphylococcus aureus* ATTC29213, *Pseudomonas aeruginosa* ATTC 9027, *S. epidermidis* ATTC 12228, *A. baumannii* ATTC 17978, *P. mirabilis* ATTC 12459 and *Klebsiella pneumoniae* ATTC 13883. The antibacterial activity of fluoroquinolones (ciprofloxacin and levofloxacin), with or without pretreatment of bacterial cells by vitamin B_{12} was assessed using the disc diffusion method and by measuring the Minimum Inhibitory Concentration (MIC) and zones of inhibition of bacterial growth. All of the tested bacterial strains were sensitive to both ciprofloxacin and levofloxacin. When pretreated with Vitamin B_{12}, all bacterial strains showed significantly smaller zones of inhibition and larger MIC values compared ciprofloxacin or levofloxacin alone. In conclusion, results indicate the possible antagonistic properties for Vitamins B_{12} when it is used concurrently with flouroquinolones.

Key words: Ciprofloxacin, levofloxacin, vitamin B_{12}, antimicrobial susceptibility, MIC

INTRODUCTION

Fluoroquinolones group of antibiotics is active against Gram-positive and Gram-negative bacteria. They are commonly used in the treatment of multiple infections such as urinary tract infections, chronic bacterial prostatitis, acute uncomplicated cystitis and acute sinusitis (Al-Soud and Al-Masoudi, 2003). The mechanism of antibacterial action of fluoroquinolones is not fully understood, however, it begin by interfering with the replication and transcription of DNA via inhibiting bacterial DNA gyrase/topoisomerase II and DNA topoisomerase IV, thus, preventing bacterial DNA from unwinding and duplication (Oliphant and Green, 2002). Practically, complexes of quinolone-enzyme-DNA are formed leading the generation of "Cellular poisons" and cell death (Chen *et al*., 1996; Drlica and Zhao, 1997). Antibiotics including flouroquinolones were shown to induce antibacterial activity via induction of oxidative stress (Becerra and Albesa, 2002; Albesa *et al*., 2004). For example, singlet oxygen ($^1O_2$) and superoxide anion (O_2^-) which are major reactive oxygen species, were shown to be generated by ciprofloxacin (Umezawa *et al*., 1997). Furthermore, many side effects of flouroquinolones such as phototoxicty and tendinopathies were correlated with the generation reactive oxygen species (Umezawa *et al*., 1997; Pouzaud *et al*., 2004).

Vitamin B_{12} (cobalamin), on the other hand, is a micronutrient that plays a crucial function in multiple biological processes. It is required for folate metabolism and for nucleotide biosynthesis where it acts as a coenzyme (Reynold, 2006). Vitamin B_{12} is found in either the adenosylcobalamin (Adocb1) form or methylcobalamin (Mecb1) forms. The Mecb1 form is important for activation of Methionine Synthase (MS) which is required for

the production of both methionine and its by-product S-adenosylmethionine (SAM) (Neil and Marsh, 1999). The SAM is required for the methylation of several macromolecules including DNA and RNA. Disruption of SAM pathway impairs both DNA biosynthesis and its methylation (Lucock, 2000; Stover, 2004). In addition, Vitamin B_{12} was shown to possess antioxidant properties (McCaddon *et al*., 2002; Birch *et al*., 2009). Recently, a number of studies pointed to a potential protective role of Vitamin B_{12} on toxicity induced by chemicals and drugs. For example, Vitamin B_{12} supplementation was shown to lower micronuclei frequency and to recover the proliferation potential of the ribavirin-treated cells (Joksic *et al*., 2006). Additionally, arsenic (Majumdar *et al*., 2009), paclitaxel (Alzoubi *et al*., 2014) and pioglitazone (Alzoubi *et al*., 2012), related oxidative DNA damage were significantly decreased after Vitamin B_{12} supplementation.

Fluoroquinolones treatment failure was reported in elderly patients taking multivitamins preparations (Mallet and Huang, 2005). In a recent study, it was shown that ciprofloxacin alters the antibacterial activity of two major antioxidants, namely, Vitamins E and C (Masadeh *et al*., 2012). Given that fluorquinolones work by induction of oxidative damage in bacteria (Becerra and Albesa, 2002; Albesa *et al*., 2004) and the known antioxidant activity of Vitamin B_{12} (McCaddon *et al*., 2002; Birch *et al*., 2009; Alzoubi *et al*., 2012, 2014), it is possible that Vitamin B_{12} attenuates the antibacterial activity of flouroquinoles. Therefore, in this study, the possible interaction between Vitamin B_{12} and fluoroquinolones was evaluated. Results of this study could be of clinical significance due to the common use of vitamin supplementation, especially, Vitamin B_{12} with antibiotics.

MATERIALS AND METHODS

Microbial culture and growth conditions: Antibacterial activity of fluoroquinolones/Vitamin B_{12} combinations were evaluated against different reference bacteria including *E. coli* ATTC 35218, *Staphylococcus aureus* ATTC29213, *Pseudomonas aeruginosa* ATTC 9027, *S. epidermidis* ATTC 12228, *A. baumannii* ATTC 17978, *P. 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, Md, USA). When ready for batch susceptibility testing, samples were thawed. Minimum Inhibitory Concentrations (MICs) were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI., 2009).

Antimicrobial susceptibility test: Antibiotic solutions were prepared on the day of use according to the manufacturer's recommendations. A wide range of fluoroquinolones concentrations were tested against different organisms. Serial 2 fold dilutions were added to molten BBL Muller-Hinton Gold II agar from BBL Microbiology Systems. After slight cooling and drying of the plates, a steer replicator was used to place aliquots containing approximately 5×10^4 CFU per drop for each tested bacterial strain. The plates were incubated at 37°C and read 24 h later. In some experiments, where ciprofloxacin at 100 μg mL^{-1} was combined with Vitamin B_{12}, Vitamin B_{12} was added to the media at a final concentration of 100 μM (Solovieva *et al*., 2007, 2008; Saito *et al*., 2009) Results (mean of 3 independent experiments) were recorded by measuring the zones of growth inhibition surrounding the antibiotic containing discs.

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined by serial dilution method according to the National Committee for Clinical Laboratory Standards (CLSI., 2009). Briefly, drugs were serially diluted and added to plates containing molten BBL Muller-Hinton Gold II agar (BBL Microbiology Systems). Thereafter, plates were slightly cooled and dried. Then, using an a steer replicator, aliquots containing about 5×10^4 CFU per drop of different bacterial strains were placed in each plate. After an 18 h 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 were detected. Plates were read in duplicate and the highest MIC values were recorded. The breakpoints indicated in the tables of the National Committee for CLSI (CLSI., 2009), were used to determine susceptibility and resistance.

Statistical analysis: Analysis was performed using GraphPad Prism software (version 4.0, GraphPad software, LA jolle, CA). One-way ANOVA followed by Tukey's post-test was used to determine if there is a statistically significant difference. The p-values of <0.05 were considered significant.

RESULTS

In this study, the possible interactive effect of Vitamin B_{12} with antibacterial activity of ciprofloxacin or levofloxacin against various species of reference bacteria, namely, *E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *A. baumannii*, *P. mirabilis* and *K. pneumoniae* were investigated. Results shown in Table 1 revealed that ciprofloxacin or levofloxacin induced antibacterial activity against tested reference bacteria. A zone of inhibition of 15 mm was selected to represent susceptibility of bacteria to each drug. When bacteria were treated with combination of ciprofloxacin or levofloxacin with Vitamin B_{12}, the zones of inhibition of the combination were significantly lower than those of ciprofloxacin or levofloxacin alone for all tested bacterial strains (Table 1).

Next, the minimal inhibitory concentrations of ciprofloxacin or levofloxacin alone and in combination with B_{12} were measured. As shown in Table 2, pretreatment of various reference bacteria cells with Vitamin B_{12} largely inhibited antibacterial activity of ciprofloxacin or levofloxacin. This is indicated by significantly higher MIC values (Table 2) for the combination of any of Vitamin B_{12} and ciprofloxacin or levofloxacin as compared to either alone.

Table 1: Comparison between the zones of inhibition of ciprofloxacin or levofloxacin alone and ciprofloxacin or levofloxacin in the presence of Vitamin B_{12} against standard bacterial strains

	Zones of inhibition (mm)*			
Standard bacterial strains	Ciprofloxacin	Ciprofloxacin+Vitamin B_{12}	Levofloxacin	Levofloxacin+Vitamin B_{12}
E. coli	26.7±0.6	14.3±0.6	26.7±0.6	14. 7±0.6
S. aureus	21.0±1.0	11.3±0.6	24.7±0.6	12.7±1.5
P. aeruginosa	23.3±0.6	12.3±0.6	9.3±0.6	4.0±1.0
S. epidermidis	21.7±0.6	10.3±0.6	13.7±0.6	7.7±0.6
A. baumannii	17.7±0.6	10.7±0.6	21.7±0.6	11.3±0.6
P. mirabilis	18.7±0.6	9.7±0.6	23.3±0.6	12.3±0.6
K. pneumonia	12.0±1.0	9.7±0.6	20.7±1.2	11.3±0.6

*Zones of inhibition values for ciprofloxacin or levofloxacin alone were significantly ($p<0.05$) lower than those of combination of ciprofloxacin or levofloxacin with Vitamin B_{12} for all tested bacterial strains. Results are presented as Mean±SD of 3 independent experiments

Table 2: Comparison between the minimum inhibitory concentrations of ciprofloxacin or levofloxacin alone and ciprofloxacin or levofloxacin in the presence of Vitamin B_{12} against standard bacterial strains

	MIC ($\mu g\ mL^{-1}$)*			
Standard bacterial strains	Ciprofloxacin	Ciprofloxacin+Vitamin B_{12}	Levofloxacin	Levofloxacin+Vitamin B_{12}
E. coli	0.04±0.02	0.17±0.07	1.12±0.14	1.75±0.00
S. aureus	0.12±0.00	0.41±0.14	1.42±0.00	2.00±0.00
P. aeruginosa	0.08±0.04	0.21±0.07	2.00±0.00	2.50±0.00
S. epidermidis	0.17±0.07	0.41±0.14	1.58±0.14	2.25±0.00
A. baumannii	0.25±0.00	0.41±0.14	1.41±0.14	2.25±0.00
P. mirabilis	0.21±0.07	0.41±0.14	1.12±0.14	2.00±0.00
K. pneumonia	0.17±0.07	0.41±0.14	1.67±0.14	2.25±0.00

*MIC values for ciprofloxacin alone were significantly ($p<0.05$) lower than those of combination of ciprofloxacin or levofloxacin alone and ciprofloxacin or levofloxacin in the presence of Vitamin B_{12} for all tested bacterial strains. Results are presented as Mean±SD of 3 independent experiments

DISCUSSION

This study shows the inhibition of the antibacterial activity of flouroquinolones antibiotics, namely, ciprofloxacin and levofloxacin when bacteria are pretreated with Vitamin B_{12}. These results were generated using wide range of standard bacterial strains. These results could be of importance when ciprofloxacin or levoflxacin are used together with Vitamin B_{12} for bacterial infections.

Current results show the efficacy of ciprofloxacin and levofloxacin on variety of bacterial strains including *E. coli*, *S. Aureus*, *P. aeruginosa*, *S. epidermidis*, *A. baumannii*, *P. mirabilis* and *K. pneumonia*. In accordance, the susceptibility of these bacterial strains to ciprofloxacin or levofloxacin was previously shown (Campoli-Richards *et al*., 1988; Masadeh *et al*., 2012; Furqan and Paracha, 2014). Additionally, the crucial role for reactive oxygen species in the actions of ciprofloxacin as antibacterial agent against various bacterial species including *P. aeruginosa*, *E. coli* and *S. aureus* were indicated in a number of previous studies (Umezawa *et al*., 1997; Becerra and Albesa, 2002; Albesa *et al*., 2004; Masadeh *et al*., 2012). Furthermore, common reactive oxygen species scavengers, such as Vitamin E and C, were shown to attenuate the antibacterial activity of ciprofloxacin (Masadeh *et al*., 2012). Ciprofloxacin was shown to induce reactive oxygen species production of during its course of action against bacterial strains including *E. coli*, *Enterococcus faecalis* and *S. aureus* (Albesa *et al*., 2004). Furthermore, ciprofloxacin-sensitive microorganisms were shown to possess elevated levels of intracellular superoxide as compared to the resistant ones (Becerra and Albesa, 2002). Additionally, application of exogenous Vitamin C or glutathione to *E. coli* resulted in reduced antibacterial activity of ciprofloxacin which was the result of scavenging superoxide anions and hydrogen peroxide species (Goswami *et al*., 2006).

Current results indicate that combining ciprofloxacin with Vitamin B_{12} results in inhibition of the antibacterial activity of ciprofloxacin against a panel of reference bacterial strains. To our knowledge, this is the first report of such effect or drug-drug interaction. Results thus could point out that simultaneous ciprofloxacin use along with Vitamin B_{12} might negatively interact with the antibacterial activity of this antibiotic. Therefore, the use of Vitamin B_{12} use might need to be monitored in patients who are taking ciprofloxacin.

The mechanism for this interactive effect of ciprofloxacin and Vitamin B_{12} is unknown. The bactericidal action of ciprofloxacin is exerted by inhibition of bacterial DNA gyrase, type II topoisomorase (Gellert, 1981; Gootz *et al*., 1990). However, a number of other effects for quinolones were reported including the ability of inhibiting the growth of various other cell types (Forsgren *et al*., 1987; Oomori *et al*., 1988; Nordmann *et al*., 1989; Lawrence *et al*., 1993, 1996), via interference with cell cycle, reduction of cell size, (Forsgren *et al*., 1987) inhibition of de novo pyrimidine synthesis (Forsgren *et al*., 1987) and oxidative stress (Gurbay and Hincal, 2004; Goswami *et al*., 2006).

Vitamin B_{12} is known to modulate DNA repair mechanisms and oxidative stress responses through enhancing methionine synthase activity that is linked to glutathione synthesis-a major intracellular antioxidant (McCaddon and Hudson, 2007; Al-Maskari *et al*., 2012). In addition, Vitamin B_{12} was shown to protect against DNA damage induced by a number of drugs via its protective effect against oxidative

DNA damage (Alzoubi *et al*., 2012, 2014). Given the importance of reactive oxygen species, energy metabolism, mitochondrial functions for the antibacterial action of floroquinolones (Umezawa *et al*., 1997; Becerra and Albesa, 2002; Albesa *et al*., 2004; Masadeh *et al*., 2012), it is possible that these mechanisms play a role in the observed inhibition of the antibacterial activity of ciprofloxacin by Vitamin B_{12}. To this end, the possibility of interaction of vitamin B_{12} with ciprofloxacin or levofloxacin exists. Future studies are needed to indicate the exact mechanism by which Vitamin B_{12} interferes with floroquinolones action.

CONCLUSION

In conclusion, the antibacterial action of ciprofloxacin or levofloxacin is inhibited when they are combined with Vitamin B_{12}. The significance of this observation comes from the wide use of quinolones antibiotic and their great therapeutic value. Thus, investigations of the clinical consequences of simultaneous use of Vitamin B_{12} with flouroquinolones antibiotics, namely, ciprofloxacin and levofloxacin in patients being treated against bacterial infections are recommended.

ACKNOWLEDGMENTS

We would like to acknowledge Jordan University of Science and Technology, Irbid, Jordan, for their support of this project through providing sabbatical leave to Dr. Masadeh.

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