Fluroquinolones-induced Antibacterial Activity Attenuation by Pretreatment with Vitamin B₁₂

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ABSTRACT

Fluroquinolones antibiotics action involves interfering with bacterial DNA replication and transcription leading to increased oxidative stress and bacterial cell death. Vitamin B₁₂, on the other hand, has been shown to possess DNA protective and antioxidant properties. In this study, the possible interactive effect of Vitamin B₁₂ on fluroquinolones antibiotics-induced cytotoxicity against several reference bacteria was investigated. Standard bacterial strains included E. coli ATCC 35218, Staphylococcus aureus ATCC29213, Pseudomonas aeruginosa ATCC 9027, S. epidermidis ATCC 12228, A. baumanii ATCC 17978, P. mirabilis ATCC 12459 and Klebsiella pneumoniae ATCC 13883. The antibacterial activity of fluroquinolones (ciprofloxacin and levofloxacin), with or without pretreatment of bacterial cells by vitamin B₁₂ 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 B₁₂, 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 B₁₂ when it is used concurrently with fluroquinolones.

Keywords: Ciprofloxacin, levofloxacin, vitamin B₁₂, antimicrobial susceptibility, MIC

INTRODUCTION

Fluroquinolones 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-Scud 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 fluoroquinolones were shown to induce antibacterial activity via induction of oxidative stress (Becerra and Albesa, 2002; Albesa et al., 2004). For example, singlet oxygen (O₂⁺) and superoxide anion (O₂⁻) which are major reactive oxygen species, were shown to be generated by ciprofloxacin (Umezawa et al., 1997). Furthermore, many side effects of fluoroquinolones such as phototoxicity and tendinopathies were correlated with the generation reactive oxygen species (Umezawa et al., 1997; Pouzaud et al., 2004).

Vitamin B₁₂ (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 B₁₂ is found in either the adenosylcobalamin (AdoCbl) 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 (Lucocq, 2000; Stover, 2004). In addition, Vitamin B₁₂ was shown to possess antioxidant properties (McCadden et al., 2002; Birch et al., 2009). Recently, a number of studies pointed to a potential protective role of Vitamin B₁₂ on toxicity induced by chemicals and drugs. For example, Vitamin B₁₂ 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 B₁₂ 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 fluoroquinolones work by induction of oxidative damage in bacteria (Becerra and Albasa, 2002; Albasa et al., 2004) and the known antioxidant activity of Vitamin B₁₂ (McCadden et al., 2002; Birch et al., 2009; Alzoubi et al., 2012, 2014), it is possible that Vitamin B₁₂ attenuates the antibacterial activity of fluoroquinolones. Therefore, in this study, the possible interaction between Vitamin B₁₂ and fluoroquinolones was evaluated. Results of this study could be of clinical significance due to the common use of vitamin supplementation, especially, Vitamin B₁₂ with antibiotics.

MATERIALS AND METHODS

Microbial culture and growth conditions: Antibacterial activity of fluoroquinolones/Vitamin B₁₂ combinations were evaluated against different reference bacteria including E. coli ATCC 35218, Staphylococcus aureus ATCC29213, Pseudomonas aeruginosa ATCC 9027, S. epidermidis ATTC 12228, A. baumannii ATCC 17978, P. mirabilis ATCC 12459 and Klebsiella pneumoniae ATCC 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 5×10⁶ 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 B₁₂, Vitamin B₁₂ was added to the media at a final concentration of 100 μM (Soloviova et al., 2007, 2008; Sato 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 5×10⁶ 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 B₁₂ 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 B₁₂, 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 B₁₂ were measured. As shown in Table 2, pretreatment of various reference bacteria cells with Vitamin B₁₂ 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 B₁₂ 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 B₁₂ against standard bacterial strains

<table>
<thead>
<tr>
<th>Standard bacterial strains</th>
<th>Ciprofloxacin (mm)</th>
<th>Ciprofloxacin+Vitamin B₁₂ (mm)</th>
<th>Levofloxacin (mm)</th>
<th>Levofloxacin+Vitamin B₁₂ (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>26.7±0.6</td>
<td>14.3±0.6</td>
<td>26.7±0.6</td>
<td>14.3±0.6</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>21±0.0</td>
<td>11.3±0.6</td>
<td>21±0.0</td>
<td>11.3±0.6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>23±0.6</td>
<td>12.3±0.6</td>
<td>23±0.6</td>
<td>12.3±0.6</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>21.7±0.6</td>
<td>10.3±0.6</td>
<td>21.7±0.6</td>
<td>10.3±0.6</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>17.7±0.6</td>
<td>10.7±0.6</td>
<td>17.7±0.6</td>
<td>10.7±0.6</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>18.7±0.6</td>
<td>9.7±0.6</td>
<td>18.7±0.6</td>
<td>9.7±0.6</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>12.0±1.0</td>
<td>9.7±0.6</td>
<td>20.7±1.2</td>
<td>11.3±0.6</td>
</tr>
</tbody>
</table>

*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 B₁₂ 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 B₁₂ against standard bacterial strains

<table>
<thead>
<tr>
<th>Standard bacterial strains</th>
<th>Ciprofloxacin (µg mL⁻¹)</th>
<th>Ciprofloxacin+Vitamin B₁₂ (µg mL⁻¹)</th>
<th>Levofloxacin (µg mL⁻¹)</th>
<th>Levofloxacin+Vitamin B₁₂ (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.04±0.02</td>
<td>0.17±0.07</td>
<td>1.12±0.14</td>
<td>1.75±0.00</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.01±0.00</td>
<td>0.41±0.14</td>
<td>1.42±0.00</td>
<td>2.06±0.90</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.08±0.04</td>
<td>0.21±0.07</td>
<td>2.00±0.00</td>
<td>2.50±0.00</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>0.17±0.07</td>
<td>0.41±0.14</td>
<td>1.58±0.14</td>
<td>2.25±0.00</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>0.25±0.00</td>
<td>0.41±0.14</td>
<td>1.41±0.14</td>
<td>2.25±0.00</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>0.21±0.07</td>
<td>0.41±0.14</td>
<td>1.12±0.14</td>
<td>2.00±0.00</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>0.17±0.07</td>
<td>0.41±0.14</td>
<td>1.67±0.14</td>
<td>2.25±0.00</td>
</tr>
</tbody>
</table>

*MIC values for ciprofloxacin alone were significantly (p<0.05) lower than those of combination of ciprofloxacin or levofloxacin alone or ciprofloxacin or levofloxacin in the presence of Vitamin B₁₂ 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 fluoroquinolones antibiotics, namely, ciprofloxacin and levofloxacin when bacteria are pretreated with Vitamin B₁₂. These results were generated using wide range of standard bacterial strains. These results could be of importance when ciprofloxacin or levofloxacin are used together with Vitamin B₁₂ 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 B₁₂ 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 B₁₂ might negatively interact with the antibacterial activity of this antibiotic. Therefore, the use of Vitamin B₁₂ use might need to be monitored in patients who are taking ciprofloxacin.

The mechanism for this interactive effect of ciprofloxacin and Vitamin B₁₂ is unknown. The bactericidal action of ciprofloxacin is exerted by inhibition of bacterial DNA gyrase, type II topoisomerase (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 Hineal, 2004; Goswami *et al.*, 2006).

Vitamin B₁₂ 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 B₁₂ 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 fluoroquinolones (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 fluoroquinolones 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 fluoroquinolones antibiotics, namely, ciprofloxacin and levofloxacin in patients being treated against bacterial infections are recommended.

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REFERENCES


