Effect of glutathione on antimicrobial activity of levofloxacin

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ABSTRACT

Overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms and this lead to search for agents that may be a solution. Fluoroquinolones are a group of antibiotics widely used because of their broad spectrum activity against both Gram-positive and Gram-negative bacteria.

In this study, we report the effect of glutathione on the antibacterial action of levofloxacin on E. coli and S. aureus as levofloxacin is an important and commonly used member of the fluoroquinolones antibiotics. It inhibit DNA topoisomerase II and DNA topoisomerase IV activities, eventually leading to bacterial cell death. In addition, an increase of reactive oxygen species in the bacterial cells in response to levofloxacin has been shown.

Keywords: Fluoroquinolones, side effects, microbial activity and antioxidants

1. INTRODUCTION

Antibiotics are weapons of choice in fight against infectious bacterial diseases. Fluoroquinolones are anti-microbial agents, with broad spectrum bactericidal activity against both Gram-positive, Gram-negative bacteria, anaerobic bacteria, and even Mycobacterium (Shenoy, et al., 2011). The mechanism of antibacterial action of quinolones is not completely understood; however, it has been proposed that the initial event is the inhibition of DNA synthesis by interference with the nick sealing activity of DNA topoisomerase II (DNA gyrase) and DNA topoisomerase IV. In the presence of these antibiotics, the enzyme is trapped on the DNA, resulting in the formation of quinolone-enzyme-DNA complexes, and the subsequent release of DNA ends from this complex leads to the generation of “cellular poison” which ultimately leads to cell death (Kumar et al., 2011). They have useful pharmacokinetic properties, achieve high tissue and serum levels, and have chemical and biological stability. Several fluoroquinolones have been developed, and many derivates have been synthesized to improve bactericidal and metabolic properties (Alba et al., 2008). Fluoroquinolones are well tolerated in patients but their usages have been associated with some adverse effects, including gastrointestinal discomfort, cutaneous reactions e.g. phototoxicity, juvenile joint toxicity and adverse central nervous system effects. Although the incidence of these side effects is relatively low, the high prescription rates of these antibiotics may pose
serious health effects on the general population (Naeem et al., 2016). Fluoroquinolones, including levofloxacin, have been demonstrated to stimulate the production of reactive oxygen species (ROS) in bacterial cells. Reactive oxygen species are reactive by-products formed by the partial reduction of molecular oxygen. Redox cycling of various chemical substances, including fluoroquinolones, affects the reactive oxygen species produced by cells during the oxidation process (Goswami et al., 2006).

Fluoroquinolones are known to induce the formation of singlet oxygen and superoxide anion, which are responsible for the phototoxic effect of the fluoroquinolones. A number of diverse cellular processes that lead to cell death are also mediated through ROS (Kohanski et al., 2010).

Antioxidant systems prevent the uncontrolled formation of free radicals, and inhibit ROS and its reaction with biological structures. Antioxidant molecules, for example reduced glutathione, act against several oxidant compounds, such as hydrogen peroxide superoxide anion, hydroxyl radical and reactive species of carbon (Manfredini et al., 2005). The small molecules as glutathione and cysteine can reduce a wide range of oxidized proteins, and protect against direct and indirect oxidation of lipid membranes and proteins as an adaptive response to increased basal oxidative damage caused by superoxide anion (Cexiong et al., 2009). Glutathione can also be oxidized spontaneously in the presence of ROS and thus neutralize them by its antioxidant capacity. Furthermore, glutathione protects cells from the effects of the free radicals generated during metabolism and is considered to be a biological marker of the levels of antioxidant activity (Pa´ez et al., 2010).

Aim of the work:
To determine whether the addition glutathione can modify the susceptibility of S. aureus and E. coli to levofloxacin.

2. MATERIAL AND METHODS

Bacterial strains: Five urine samples were collected from patients. The specimens were processed according to standard microbiological methods. Two clinical bacterial isolates were obtained and identified by conventional techniques [Koneman, 2006]

Antimicrobial susceptibility test:
It was done for the two isolates (S. aureus and E. coli) by disk diffusion method against levofloxacin (Oxoid). Procedures were performed and results were interpreted according to the Clinical and Laboratory Standard Institute guidelines (CLSI, 2016).

Table 1. Zone diameter interpretive charts inhibition measurements for S. aureus (CLSI, 2016)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc content</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>5µg</td>
<td>≤15</td>
<td>16-18</td>
<td>≥ 19</td>
</tr>
</tbody>
</table>

Table 2. Zone diameter Interpretive standards for disc diffusion susceptibility testing for E. coli (CLSI, 2016)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc content</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>5µg</td>
<td>≤13</td>
<td>14-16</td>
<td>≥ 17</td>
</tr>
</tbody>
</table>
Determination of the minimum inhibitory concentration (MIC) in the presence of glutathione

The effect of exogenous glutathione on the antibacterial activity of levofloxacin was investigated in two clinical bacterial isolates (S. aureus and E. coli) which were provided by Urology department at Menoufiya University Hospital. The determination of the MIC for levofloxacin was performed using the broth macrodilution test, according to the Clinical and Laboratory Standards Institute (CLSI, 2016).

Preparation of antioxidant: antioxidant was freshly prepared before use. Stock solutions (10mM) of glutathione was prepared in sterile distilled water.

Preparation of antibiotic dilution range: Dilution ranges for levofloxacin (0.03µg/ml - 128 µg/ml) for levofloxacin-glutathione.

Preparation of inoculum: The inocula were adjusted to 10⁵ CFU (equal to 0.5 McFarland standard).

Inoculation

Sufficient 75 × 12 mm sterile capped tubes were arranged in two rows for each antibiotic to cover the range of antibiotic dilutions chosen in duplicate. One ml volumes of levofloxacin dilution in broth were transferred to the tubes. A final inoculum of 10⁶CFU/ml was required and therefore suspensions were diluted 1:100 in broth medium for preparing the antibiotic dilutions. One ml aliquots of test organism to one set of tubes and 1 ml of control organism to the other. Contents of the tubes were mixed thoroughly, incubated for 18-20 hours at 35°C.

Reading and interpretation: the MIC endpoint was read as the lowest concentration of antibiotic that prevented bacterial growth after 18 h of incubation was the MIC, both in the presence and absence of glutathione.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>≥ 4</td>
<td>2</td>
<td>≤ 1</td>
</tr>
</tbody>
</table>

Table 3. Levofloxacin susceptibility of S. aureus by MIC tube dilution method (CLSI, 2016)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>≥ 2</td>
<td>0.25–1</td>
<td>≤ 0.12</td>
</tr>
</tbody>
</table>

Table 4. Levofloxacin susceptibility of E. coli by MIC tube dilution method (CLSI, 2016)

3. RESULTS AND DISCUSSION

Regarding antibiotic susceptibility of isolated S. aureus and E. coli, both isolates were sensitive to levofloxacin. In S. aureus, the values of MIC obtained for levofloxacin was 1µg/ml. When the sensitivity to antibiotics was determined in the presence of glutathione, there were no significant changes in the MIC (Table 1).

In E. coli, the values of MIC was 0.12 µg/ml for levofloxacin. In the presence of glutathione, there were no significant changes in the MIC (Table 2).
This study showed that the antibacterial activity of levofloxacin was not affected by the presence of glutathione as it was previously shown that synthetic quinolone antibiotics promoted the formation of the hydroxyl radical that contributed to cell death (Kohanski et al., 2007), and it was proposed that oxidative damage contributes to bacterial cell death following gyrase poisoning with an oxygen-dependent death pathway appearing to amplify the primary effect on gyrase (Dwyer et al., 2007) and glutathione was chosen because it is a scavenger of ROS which has been shown to be involved in protecting the cell either directly or indirectly. This might constitute an adaptive response to oxidative damage, which is known to increase in the presence of the antibiotic (Prinz et al., 1997; Pomposiello & Demple, 2002).

A previous study conducted on E. coli suggested that glutathione modulates the effect of antibiotics (Goswami & Jawali, 2007). These authors reported a reduction in MIC for ampicillin and penicillin, from 8 to 4 mg mL⁻¹ and from 64 to 48 mg mL⁻¹, respectively. Also, this result came into line with (Goswami et al., 2011), as they reported that GSH can act as an important modulator of antibiotic susceptibility for bacteria as it augments the efficacy of β-lactams such as penicillin and ampicillin on E. coli cells which become more susceptible towards them in the presence of GSH (Goswami, et al., 2011).

### 4. CONCLUSION

On the basis of our studies it can be concluded that antibacterial action of therapeutically relevant antibiotics could be augmented by the presence of antioxidants like GSH and these findings are of immense values for further investigations surrounding the intake of antioxidants on antibacterial effect of different antibiotics for treatment of various infections are warranted in future.

### 5. REFERENCES


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**Table 5. Effect of addition of glutathione on the susceptibility of *S. aureus* and *E. coli* to levofloxacin**

<table>
<thead>
<tr>
<th>MIC (µg/ml)</th>
<th><em>S. aureus</em> MIC (µg/ml)</th>
<th><em>E. coli</em> MIC (µg/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>1</td>
<td>0.12</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Levofloxacin and glutathione</td>
<td>1</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

P >0.05 = not significant