Effect of glutathione on antimicrobial activity of levofloxacin

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ABSTRACT

Overuse of antibiotics has become the major factor for the emergence anddissemination of multi-drug resistant strains of several groups of microorganisms andthis lead to search for agents that may be asolution,Fluoroquinolones are a group of antibiotics widelyused 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 members of the fluoroquinolonesantibiotics.Itinhibit DNA topoisomerase II and DNA topoisomerase IV activities, eventually leading tobacterial 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 fightagainst infectious bacterial diseases.Fluoroquinolonesare anti-microbial agents, withbroad spectrum bactericidal activity against both Gram- positive, Gram-negative bacteria, anaerobic bacteria, and even *Mycobacterium***(Shenoy, et al., 2011)** .

The mechanism ofantibacterial action of quinolones is not completely understood;however, it has been proposed that the initial event isthe inhibition of DNA synthesis by interference with thenick 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 theformation of quinolone-enzyme-DNA complexes, and thesubsequent release of DNA ends from this complex leads tothe generation of "cellular poison" which ultimately leads tocell death**(Kumar et al.,2011)**.

They haveusefulpharmacokinetic properties, achieve high tissue and serum levels, and have chemical and biological stability.Severalfluoroquinolones have been developed, andmany derivates have been synthesized to improve bactericidaland metabolic properties **(Alba et al., 2008).**Fluoroquinolones are well tolerated in patients but their useshave been associated with some adverse effects, includinggastrointestinal discomfort, cutaneous reactions e.g.phototoxicity, juvenile joint toxicity and adverse centralnervous system effects.Although the incidenceof these side effects is relatively low, the high prescriptionrates of these antibiotics may pose

serious health effects on thegeneralpopulation**(Naeem et al., 2016).**
Fluoroquinolones, including levofloxacin,have been demonstrated to stimulate the production of reactiveoxygen species (ROS) in bacterial cells. Reactiveoxygen species are reactive by-products formed by the partialreduction of molecular oxygen. Redox cycling of various chemical substances, including fluoroquinolones, affects the reactiveoxygen species produced by cells during the oxidation process **(Goswami et al., 2006).**
Fluoroquinolones are known to induce the formationof singlet oxygen and superoxide anion, which are responsible for the phototoxic effect of the fluoroquinolones. A number of diversecellular processes that lead to cell death are also mediatedthrough ROS **(Kohanski et al., 2010)**
Antioxidant systems prevent the uncontrolledformationof free radicals, and inhibit ROS and its reaction withbiological structures.. Antioxidant molecules, for example reduced glutathione, actagainst 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 rangeof oxidized proteins, and protect against direct and indirectoxidation of lipid membranes and proteins as an adaptiveresponse to increased basal oxidative damage caused by superoxide anion**(Cexiong et al., 2009).** Glutathione can also be oxidized spontaneously in thepresence of ROS and thus neutralize them by its antioxidantcapacity. Furthermore, glutathione protects cells from theeffects of the free radicals generated duringmetabolism and isconsidered to be a biological marker of the levels of antioxidantactivity **(Pa´ez et al., 2010)**

Aim of the work:

To determinewhether 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)*

Antibiotic	***Disc content***	***Resistant***	***Intermediate***	***Sensitive***
Levofloxacin	***5µg***	***≤15***	***16-18***	***≥ 19***

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

Antibiotic	***Disc content***	***Resistant***	***Intermediate***	***Sensitive***
Levofloxacin	***5µg***	***≤13***	***14-16***	***≥ 17***

Determination of the minimum inhibitoryconcentration (MIC) in the presenceof 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 MenoufiyaUniversityHospital.The determination of the MIC for levofloxacin was performed using the brothmacrodilution test, according to the Clinical and LaboratoryStandards Institute **(CLSI, 2016)**.

Preparation ofantioxidant: 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 10^5 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 10^5CFU/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 thetubes 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 incubationwas the MIC, both in the presence and absenceof glutathione.

table3. Levofloxacin susceptibility of *S. aureus* by MIC tube dilution method (*CLSI, 2016*)

Antibiotic	Resistant	Intermediate	Sensitive
Levofloxacin	**≥ 4**	**2**	**≤1**

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

Antibiotic	Resistant	Intermediate	Sensitive
Levofloxacin	**≥ 2**	**0.25–1**	**≤ 0.12**

3.RESULTS AND DISCUSSION

Regarding antibiotic susceptibility of isolated *S. aureus and E-coli*,bothisolates were sensitive to levofloxacin.

In S. aureus, the values of MIC obtained for levofloxacin was1µg/ml. When the sensitivity to antibiotics was determinedin the presence of glutathione, there were no significantchanges in the MIC**(Table 1).**

In *E-coli*, the values of MICwas0.12 µg/ml for levofloxacin.

In thepresence of glutathione, there were no significant changes in the MIC **(Table 2).**

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

MIC(µg/ml)	***S. aureus*** **MIC** (µg/ml)	***E-coli*** **MIC** (µg/ml)	**P value**
Levofloxacin	1	0.12	P >0.05
Levofloxacin and glutathione	1	0.12	

P>0.05 =not significant

This study showed that the antibacterial activity of levofloxacin wasnot affected by presence of glutathione as it was previously shown that synthetic quinolone antibioticspromoted the formation of the hydroxyl radical that contributed to cell death **(Kohanski et al., 2007),** and it wasproposed that oxidative damage contributes to bactericidalcell death following gyrase poisoning with an oxygendependentdeath pathway appearing to amplify the primaryeffect 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 celleither directly or indirectly. This might constitute an adaptiveresponse to oxidative damage, which is known toincrease in the presence of the antibiotic **(Prinz et al., 1997;Pomposiello&Demple, 2002)**.

A previous study conducted on E. coli suggeststhat glutathione modulates the effect of antibiotics **(Goswami&Jawali, 2007).** These authors reported a reduction inMIC for ampicillin and penicillin, from 8 to 4 mgmL_1 andfrom 64 to 48 mgmL_1, respectively.also this result come in line **with (Goswami.,et al 2011)**. as they reported that GSH can act as an importantmodulator of antibiotic susceptibility for bacteria asitaugments the efficacy of β-lactams suchas penicillin and ampicillin on E. coli cellswhich become more susceptible towards themin presence of GSH**(Goswami.,et al 2011)**.

4.CONCLUSION

On the basis of our studies it can be concluded thatantibacterial action of therapeutically relevantantibiotics could be augmentedby the presence of antioxidants like GSH andthese findingsare of immense values for further investigations surrounding the intake ofantioxidants on antibacterial effect of differentantibiotics for treatment of various infections arewarranted in future.

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