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New roles of fluoxetine in pharmacology: Antibacterial effect and modulation of antibiotic activity

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ABSTRACT

The antimicrobial activity of psychotropic drugs, especially those of the class of mainly phenothiazines has been previously reported. Other drugs, including verapamil and trifluoperazine demonstrated to be effective against multidrug-resistant strains. Selective serotonin reuptake inhibitors (SSRIs) are antidepressant drugs that have presented significant activity against resistant bacterial resistance, but the antibacterial effect as well the antibiotic modulating properties of fluoxetine remain to be elucidated. Therefore, the present study aimed to evaluate *in vitro,* the antibacterial effect and the antibiotic modulating activity of fluoxetine against standard and multiresistant bacterial strains. The microorganisms used were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. For the antibacterial tests, 10 mg fluoxetine hydrochloride were and diluted in 1 mL of dimethyl sulfoxide (DMSO) and then diluted in sterile distilled water to a concentration of 1024 μg/mL. To determine the Minimum Inhibitory Concentrations (MICs), the drugs were diluted to concentrations ranging from 512 to 0.5 μg/mL in 96-well microdilution plates. The evaluation of the modulatory activity of fluoxetine was performed by combining this drug with the following antibiotics: Erythromycin, Gentamicin, Imipenem, Norfloxacin and Tetracycline at subinhibitory concentrations (MIC/8). Our results demonstrated that the MIC fluoxetine were 256 and 102 μg/mL against standard and resistant strains of *S. aureus*, respectively. The MIC of fluoxetine against both standard and resistant strains of *P. aeruginosa* was 161 μg/mL and against *E. coli*, the MIC of fluoxetine was 102 μg/mL for both standard and resistant strains, demonstrating that this drug present significant antibacterial activity. The association of fluoxetine with gentamicin and erythromycin *P. aeruginosa* and *E. coli* presented synergistic effects, demonstrating that this drug can selectively modulate the activity of antibiotics of clinical use. In conclusion, fluoxetine presented significant antibacterial effect and potential antibiotic modulating activity against multiresistant bacteria. Therefore, additional studies are needed to characterize the antimicrobial properties of this drug, as well as the clinical implications of its use in the treatment of infections by resistant microorganisms.

1. Introduction

The development of penicillin, in the middle of the last century, represented a revolution in the treatment of infections. Thus, several infections that in the past represented some of the main causes of illness and death, are now successfully treated with antibiotics. However, The indiscriminate use of antibiotics has generated a selective pressure, stimulating the microorganisms to develop mechanisms of defense against antibiotics, and therefore, reducing the effectiveness of several drugs [1,2].

Bacterial resistance consists of set of adaptations that allow the bacteria to overcome the harmful and lethal effects of antimicrobials, providing an environment that allows them to maintain their multiplication rate even with high concentrations of antibiotics [3,4]. This phenomenon usually occurs by one of the following 4 mechanisms:1) inactivation or modification of the drug by hydrolysis; 2) modification of the target by altering the affinity of the antibiotic with the binding site; 3) alteration of the membrane permeability, avoiding the entry of the drug into the bacterium and 4) expression of efflux pumps, which eliminate the antibiotic from the intracellular environment [5,6].

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The intrinsic resistance (also known as innate resistance) occurs naturally in the microorganism, during successive replications, through spontaneous mutations, which alter the DNA sequences, giving the microbe resistance to one or more drugs. On the other hand, acquired resistance results from physiological changes in specific genes, such as mutation and selection, or due to gene acquisition or transfer by one of the following processes: conjugation, transduction and transformation. Such mechanisms can be established within the same population or in different populations, guaranteeing genetic variability and making antibiotic action in these microorganisms [7–9].

The expression of efflux pumps is considered the main mechanism of multidrug resistance (MDR), because it avoids the action of most antibiotics. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are bacteria that have several efflux systems and studies indicate that inhibiting efflux pumps in these microorganisms can restore the effects of antimicrobial agents [10]. Therefore, the search for drugs that act as inhibitors of efflux pumps can contribute to the therapy of infections by bacteria resistant to multiple drugs. Thus, the research with drugs that already have clinical use can optimize this process, since these drugs already have several established pharmacological parameters, including pharmacokinetic characteristics and toxicological profile [11].

The antibacterial properties of large group of non-antibiotic drugs has reported, including: non-steroidal anti-inflammatory drugs (NSAIDS), cytostatic drugs and psychotropic drugs. Among the psychotropics, promising results have been obtained with phenothiazines, especially with the selective serotonin reuptake inhibitors such as sertraline (SSRIs), fluoxetine and paroxetine [12]. However, the antibacterial effect as well the antibiotic modulating properties of fluoxetine remain to be elucidated. Therefore, the present study aimed to evaluate *in vitro,* the antibacterial effect and the antibiotic modulating activity of fluoxetine against standard and multiresistant bacterial strains.

2. Materials and methods

2.1. Test compound

The anxiolytic drug Fluoxetine Hydrochloride, manufactured by *Teuto*® was used in this study.

2.2. Bacterial lineages

The microorganisms used in the tests were obtained from the Laboratory of Microbiology and Molecular Biology (LMBM) of the Regional University of Cariri (URCA). Standard strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 25923, and multi-resistant strains of *Escherichia coli* 06, *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* 24 were used in this study. The resistance profile of the microorganisms is shown in Table 1 [13].

Table 1
Origin of bacterial strains and resistance profile of bacteria to antibiotics.

Bacterium	Source	Resistance profile
Escherichia coli 06	Urine culture	Cf, Cef, Ca, Cro
Staphylococcus aureus 10	Rectal Swab	Ca, Cef, Cf, Oxa, Pen, Amp,Amox, Cip,Lev, Asb, Amc, Cla, Azi
Pseudomonas aeruginosa 24	Catheter tip	Ctz, Imi, Cip, Ptz, Lev, Mer

Amp- Ampicilin; Asb- Ampicilin + Sulbactam; Amox-Amoxicilin; Amc-Amoxicilin + Clanulanic Acid; Azi- Azithromycin; Ca- Cefadroxil; Cf-Cefalothin; Cef- Cephalexin; Cla- Clarithromycin; Cro- Ceftriaxone; Ctz-Ceftazidime; Cip- Ciprofloxacin; Imi- Imipenem; Oxa- Oxacilin; Lev-Levofloxacin; Mer- Meropenem; Pen- Penicilin; Ptz- Piperacilin + Tazobactam.
Fonte: Lima et al. (2016).

2.3. Preparation of the test solution

To prepare the starting solution, 10 mg of fluoxetine hydrochloride were weighed and diluted in 1 mL of dimethyl sulfoxide (Dmso-Merck, Darmstadt, Germany). This solution was diluted in sterile distilled water to reach the concentration of 1024 μg/mL, which was used in the tests.

2.4. Determination of the minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by the broth microdilution method, as recommended by NCCLS M7-A6 (CLSI, 2008). A bacterial sample was withdrawn from previously cultured Petri dishes and diluted in test tubes containing 3 mL of 0.9% saline. The turbidity of each tube was compared to the turbidity of the McFarland 0.5 scale (1×10^8 CFU/mL). This procedure was done in triplicate for each bacterium. The MIC was defined as the lowest concentration at which no microbial growth was observed. Briefly, 1.5 mL of a solution, composed of 1350 μL of 10% BHI and 150 μL of the bacterial suspension, was prepared in *Eppendorf*® tubes. A sterile 96-well microdilution plate was filled in the numerical sense, by adding 100 μL of the distribution solution into each well. Then, serial dilutions were performed with 100 μL of the test (fluoxetine) solution, to obtain concentrations ranging from 512 to 0.5 μg/mL, until the penultimate cavity and the last cavity was destined to control microbial growth. The plates were then incubated at 35 °C for 24 h and the MICs were determined using 20 μL of resazurin as indicator, 1 h before the readings. Of note, resazurin shows distinct colorations in its oxidized and reduced forms. The form added in the wells was oxidized (blue). In the wells, where bacterial growth exceeded a cell density above 10^6 CFU/mL, resazurin was reduced to pink [14].

2.5. Evaluation of the modulating effect of fluoxetine on antibiotic activity

To evaluate the potential of fluoxetine as a modifier of antibiotic resistance, the method proposed by Coutinho et al. (2008) [15] was used. The substance was tested at a subnibitory concentration (MIC/8). The distribution medium was prepared with 10% BHI +150 μL of the bacterial suspension + the fluoxetine solution reaching 1.5 mL. As control, 1.5 mL of a solution containing only 10% BHI +150 μL of the microbial suspension was used. The microdilution plate was filled alphabetically by adding 100 μL of the dispensing solution into each well (1: 1 ratio) with 100 μL of the drug (antibiotic) until the penultimate cavity. Then, the plates were incubated at 37 °C for 24 h. The whole procedure was done in triplicate. The reading was performed as described for the determination of the MIC.

2.6. Statistical analysis

Data were analyzed by two-way ANOVA followed by Tukey's test using GraphPad Prism software (GraphPad, San Diego, CA). Differences with $p < 0.05$ were considered significant.

3. Results and discussion

The minimum inhibitory concentration of fluoxetine hydrochloride for *Staphylococcus aureus* was 256 and 102 μg/mL against standard and resistant strains, respectively. Against both standard and resistant strains of *Pseudomonas aeruginosa*, fluoxetine had a MIC of 161 μg/mL and against *Escherichia coli*, the MIC of fluoxetine was 102 μg/mL for both standard and multidrug resistant strains (Table 2). Together, these data demonstrated that fluoxetine hydrochloride presented potent antibacterial effects *in vitro*.

Fluoxetine belongs to the group of selective serotonin reuptake inhibitors (SSRIs). It has been shown that these drugs present significant antimicrobial activity against Gram positive bacteria, but are