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Investigation of the Antituberculosis Effectiveness of Escitalopram, a Selective Serotonin Reuptake Inhibitor

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Research Article

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ABSTRACT

Escitalopram is an antidepressant drug known as a selective serotonin reuptake inhibitor. Among the antidepressants, the most prominent group in terms of their antimicrobial effects are Serotonin Reuptake Inhibitors and tricyclic antidepressants. Many studies have shown that antidepressant drugs have antibacterial activity. Increasing resistance problems have led researchers to investigate the effectiveness of agents without antibacterial activity in primary care. In this study, the antituberculosis effect of escitalopram and its effectiveness on primary antituberculosis drugs isoniazid and rifampicin were tested on Mycobacterium tuberculosis ATCC strains and 18 clinical isolates (15 multidrug-resistant and three susceptible). In this study, the active ingredient escitalopram alone or in combination with antibiotics did not exhibit any antituberculosis effect. The minimum inhibitory concentration was>256 µg/ml for all ATCC and clinical isolates studied. In addition, no antagonistic or synergistic effects were found in our study to determine the effectiveness of escitalopram on isoniazid and rifampicin, which are primary antituberculosis drugs.

1. Introduction

Today, the increasing problem of bacterial resistance has led researchers to discover new drugs and examine the effects of agents without antibacterial activity in the first line. Many antidepressant drugs known as selective serotonin reuptake inhibitors (SSIR) may have antibacterial and antifungal effects as secondary effects [1,2]. Escitalopram (Cipralex, Lexapro) is a United States Food and Drug Administration (FDA)-approved antidepressant widely used in the treatment of major depressive disorder (MDD) and anxiety disorder [3]. Escitalopram increases serotonin levels by blocking serotonin reuptake into the presynaptic neuron thus increasing human serotonin levels [4]. However, it has a highly selective, strong, and dose-dependent inhibitory effect on transport [5]. Compared to existing SSRIs, it has the highest selectivity for the serotonin transporter (SERT) [6]. Therapeutic blood levels of escitalopram are generally in the range of 20-80 µg/L but may reach up to 80-200 µg/L in the elderly, patients with hepatic dysfunction, poor CYP2C19 metabolizers, or after acute overdose [7]. Previous studies have shown that escitalopram may have an antibacterial effect, especially on Gram-positive bacteria [8,9].

According to the data from the World Health Organization (WHO), tuberculosis was at the top of the list of diseases that cause deaths caused by infection in 2023 [10]. Isoniazid, rifampicin, streptomycin, and ethambutol, known as primary antituberculosis agents, are the first-line drugs of treatment. Reports of resistant cases have begun to be made even for newly approved agents such as bedaqulin and linezolid [11]. The problem of increasing resistance is important in the treatment of tuberculosis, as in all bacterial groups. Therefore, new-generation drug pioneers are needed. However, this process requires long and costly research. For this reason, it is also important to investigate the effectiveness of different drug-active ingredients. Therefore, we aimed to investigate the in vitro effectiveness of escitalopram on Mycobacterium tuberculosis (Mtb) standard strains and clinical isolates in our study.

2. Material and Method

Bacterial strains: In this study, M. tuberculosis H37RV (ATCC 27294), ATCC35822 (Isoniazid (INH)-resistant), ATCC35838 (Rifampicin (RIF)-resistant), ATCC35820 (Streptomycin (STR)-resistant), and ATCC35837 (Ethambutol (EMB)-resistant) standard strains and culture collection of Akdeniz University Tuberculosis Studies Application and Research Center were used. 18 clinical isolates (15 multidrug-resistant (MDR) and three isolates that were susceptible to all primary antitubercular drugs) were tested.

Chemical substances: Primary antituberculosis drugs, isoniazid (INH) and rifampicin (RIF) (Sigma-Aldrich, Germany), were tested in the study. Both antibiotics were purchased in commercial powder form. Stock solutions of 4096

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µg/ml were prepared with the recommended solvents (RIF-ethanol/INH-distilled water) and stored at -80℃ until use.

The active ingredient Escitalopram was purchased in commercial powder form (Abdi İbrahim, Istanbul). It was prepared in dimethyl sulfoxide (DMSO) (Merck, Germany) at a concentration of 4096 µg/ml and sterilized by membrane filtration. It was stored at -80℃ until use.

Griess reagent was prepared by mixing 1 part 50% (v/v) hydrochloric acid (HCl) (Merck, Germany), 2 parts 0.2% (w/v) sulfanilamide (Merck, Germany), and 2 parts 0.1% (w/v) n -1-naphthyl-ethylenediamine dihydrochloride (Sigma-Aldrich, Germany) [12].

Preparation of media: AYC.2.1 broth medium (AYC Medikal, Antalya) containing 5% sheep serum was used in our study. The media was prepared according to the manufacturer's instructions. To determine the nitrate reductase activity of the isolates, potassium nitrate (KNO_3) was added to the medium at a concentration of 1,000 µg/ml.

Preparation of Bacterial Inoculum: Inoculums of bacterial strains were prepared using fresh cultures grown in Löwenstein Jensen (LJ) medium (Merck, Germany). After the colonies were transferred from fresh cultures to tubes containing 8-10 sterile glass beads and 3-5 ml physiological saline, the tubes were tightly closed and wrapped with parafilm. Then, it was mixed by vortexing for 1 minute and the tubes were kept at room temperature for 1 hour to prevent aerosol formation. The supernatant was transferred to another tube and each isolate was adjusted to McFarland no 1 standard [13]. Prepared bacterial suspensions were AYC2.1 broth medium with KNO_3 added. It was diluted 1:10 with the medium [14].

Determination of Minimum Inhibitory Concentration (MIC) (Isoniazid/ Rifampicin/ Escitalopram): In our study, U-bottom 96-well microplates were used. Using the nitrate reductase activity of Mycobacterium tuberculosis, the MIC values of the isolates were determined by the Nitrate reduction assay (NRA) method described in previous studies [14]. 50 µl of AYC2.1 broth medium supplemented with KNO_3 was added into 96-well U-bottom microplates. After the addition of the medium, the concentrations of escitalopram, INH, and RIF in the wells were tested separately in the range of 256-0.03 µg/ml (MIC initial concentration for susceptible isolates was in the range of 16 µg/ml-0.03 µg/ml; for resistant isolates, it was 256 µg/ml-0.5 µg/ml range). 50 µl of the prepared bacterial suspensions (1:10 dilution) were inoculated into all wells except the sterility well, and the plates were incubated at 37 ℃ for 7 days. After incubation, 50 µl of Griess reagent was added to the growth control wells. If an instantaneous purple/violet colour transformation was observed in the control wells, Griess reagent was added to the test wells, and MIC values were determined.

Determination of INH and RIF Efficacy in the Presence of Escitalopram: Determination of INH and RIF effectiveness in the presence of escitalopram was carried out in two stages. In the first stage, it was adjusted into AYC2.1 broth medium supplemented with KNO3 at a level of 80 µg/L, which is the recommended therapeutic blood concentration of escitalopram, and distributed into the wells as 50 µl. INH and RIF antibiotics were tested separately for all isolates in the range of 256-0.03 µg/ml. In the second stage, to determine its effectiveness in case of acute overdose and accumulation in the blood, it was distributed to the wells by adjusting the blood concentration to 10 times (800 µg/L), and INH and RIF were tested for all isolates in the same MIC ranges. After inoculum and incubation (the same inoculum and conditions were applied for MIC determination), MIC values were determined by adding Griess reagent to the test wells upon seeing an instantaneous purple/violet colour transformation in the control wells.

3. Results and Discussion

The escitalopram MICs results for all M. tuberculosis ATCC and clinical isolates studied were determined to be >256 µg/ml.

In addition, no antagonistic or synergistic effects were found in our study to determine the effectiveness of escitalopram on INH and RIF, which are primary antituberculosis drugs. No significant decrease was observed between the MIC values found against primary antituberculosis drugs alone and the MIC results in studies conducted with escitalopram in combination. MIC results are shown in Table 1 and Table 2.

Table 1. Rifampicin and escitalopram combination MIC results

Isolates	RIF MIC	80 µg/L Escitalopram +	800 µg/L Escitalopram+
	µg/ml	RIF	RIF
ATCC27294 H37RV	0,25	0,25	0,125
ATCC35838 RIF	64	64	64
ATCC35822 INH	0,25	0,25	0,25
ATCC35837 EMB	<0,015	<0,015	<0,015
ATCC35820 STM	0,25	0,015	0,06
MDR1	128	128	128
MDR2	128	128	128
MDR3	128	128	128
MDR4	128	128	128
MDR5	64	64	64
MDR6	128	128	128
MDR7	16	16	16
MDR8	256	256	128
MDR9	256	128	128
MDR10	256	256	256
MDR11	256	128	128
MDR12	64	32	32
MDR13	32	32	32
MDR14	256	256	256
MDR15	>256	>256	>256
Susceptible 1	0,25	0,125	0,006
Susceptible 2	0,03	0,015	<0,015
Susceptible 3	0,03	0,03	<0,015

Table 2. Isoniazid and escitalopram combination MIC results

Isolates	INH MIC	80 µg/L Essitalopram+	800 µg/L Essitalopram+
	µg/ml	INH	INH
ATCC27294 H37RV	0,06	0,06	0,06
ATCC35838 RIF	0,03	0,06	0,03
ATCC35822 INH	4	4	2
ATCC35837 EMB	<0,015	< 0,015	< 0,015
ATCC35820 STM	0,06	0,06	0,03
MDR1	4	4	4
MDR2	4	4	4
MDR3	32	16	32
MDR4	8	8	4
MDR5	4	4	4
MDR6	4	4	4
MDR7	4	4	4
MDR8	4	16	4
MDR9	4	16	4
MDR10	8	16	8
MDR11	4	16	4
MDR12	4	4	4
MDR13	4	4	4
MDR14	8	32	16
MDR15	8	32	16
Susceptible 1	0,03	0,06	0,03
Susceptible 2	0,03	0,06	0,03
Susceptible 3	0,03	0,0125	0,03

4. Conclusion

Today, thousands of patients die each year from infection-related causes worldwide and in our country as a result of the rapidly increasing resistance problem caused by infectious microorganisms. Unfortunately, new drug strategies cannot be developed at the same pace, exacerbating the problem. Although the contribution of new technologies and systems such as modelling can help drug design processes, studies investigating the effectiveness of existing drugs against infections beyond their primary effects are extremely valuable. Especially in many disease cases, changes in the person's mood lead to the addition of psychrotrophic drugs to the treatment process.

Studies with many pharmaceutical active substances classified as psychotropic have shown high antibacterial and antifungal activity, especially in the group classified as SSIR. Notably, they are especially effective against microorganisms such as multi-drug resistance M. tuberculosis, Mycoplasma, and Salmonella, which are much more difficult to treat [1, 2, 8, 15].

Escitalopram, which we used in our study, is a drug in the SSIR group and is widely used as an antidepressant. There are previous studies on escitalopram showing that it has antimicrobial activity. However, in vivo studies and clinical trials are needed to evaluate the effectiveness of these drugs in treatment [16]. There is no evidence in the literature of escitalopram's antitubercular activity. This led us to choose escitalopram in our study. In our study, no antituberculosis activity was found when escitalopram was used alone. However, in the results of our study in combination with INH and RIF, which are the primary antituberculosis agents in the treatment of tuberculosis, an increase or decrease in MIC values was observed for some isolates. Due to the natural structure of the tuberculosis bacillus, differences could be observed even in the methods recommended as reference methods, which caused us not to consider these results as meaningful. The low number of isolates is a limitation of our study. Therefore, studies on more isolates are necessary. Although drug strategies in the treatment of tuberculosis are a very long and difficult road, it is extremely important to investigate the antituberculosis activities of drugs with different primary effects. Therefore, further studies are needed in the field.

Declaration

Author Contribution: Conceive-K.Y.; Design-K.Y.; Supervision-K.Y; Experimental Performance, Data Collection and/or Processing-K.Y.; Analysis and/or Interpretation-K.Y.; Literature Review-K.Y.; Writers-K.Y.; Critical Reviews–K.Y.

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