http://pmjournal.ir Original Article

Spring 2023, Volume 8, Issue 29 (25-30)

Personalized Medicine for Antibiotics: Pharmacological Displacement of Thiocolchicosidum as Antimicrobial Agent

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DOI: 10.22034/pmj.2023.705460

Submitted: 2023-02-18
Accepted: 2023-05-23

Keywords:
Bacterial Resistance
Pharmacology
Thiocolchicosidum

Abstract :
Due to the increasing level of bacterial antibiotic resistance (AB), it is now required to modify the dosage for customized medication using therapeutic drug monitoring. The creation of a novel treatment for clinical use, such as situations of bacterial resistance, has been hailed as a feasible, affordable, and quick alternative by the pharmaceutical industry. Therefore, the current research sought to examine the myorelaxant Thiocolchicosidum's antibacterial activity against bacterial strains. Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 25933, and Pseudomonas aeruginosa ATCC 27853 were used as the bacteria in an in vitro experimental study, along with the protocols for antibacterial activity screening, minimum inhibitory concentration (MIC), and characterization of antibacterial activity. Thiocolchicosidum, at levels varying from 0.48 to 1000 µg/mL, was the chemical. The only bacterial strains that showed any sensitivity to the myorelaxant were E. coli and P. aeruginosa, both of which had MICs of 500 µg/mL and 1000 µg/mL, respectively. Thiocolchicosidum demonstrated a bacteriostatic effect in the antimicrobial characterization test. Therefore, despite the fact that this medication is already considered safe for human use, no discernible antibacterial effects were shown in common bacterial strains. Therefore, research is required to determine how it differs from other microbes, such as various kinds of bacteria, fungus, and protozoa, in order to rule it out as a potential antibiotic material for use in industry.

INTRODUCTION

A new era of advancement in the management of bacterial diseases in individuals, agriculture, and animals was ushered in with the development of antibiotics (1). However, the emergence of multi-drug-resistant bacteria (MDR), which have considerably grown in recent years owing to antibiotics' improper handling and have become a worldwide public health concern (2), has made the use of antibiotics problematic. Since more than 70% of microorganisms are tolerant to all or part of the known antibiotics, either new antibiotic types must be developed or very toxic "last-line" antimicrobial medicines must be used in antimicrobial therapy to treat bacteria effectively, especially in critically sick patients (3). According to the research, without the development of novel compounds, antimicrobial medication resistance illnesses are predicted to cause 10 million deaths globally annually by 2050 and cost near USD 100 trillion (4).

The World Health Organization (WHO) has created a variety of measures, including regulations on the marketing, administration, and dose of antibiotics (5,6), to prevent this rising incidence of MDR illnesses. Treatment failures are produced as a result of the fact that the majority of dosages are presently administered consistently to patients without consideration for the severity of the illness or the clinical picture (7,8). This might result in sub-therapeutic or toxic doses. The use of therapeutic drug monitoring (TDM), which tracks pharmacokinetic (PK) changes to quantify medications with a narrow therapeutic index (TI) but substantial toxicity, is one of the answers.

Dual or mass-coupled chromatographic procedures using a range of detectors, such as UV or fluorescence detectors, as well as immunoassays are examples of monitoring approaches (10,11). The US Food and Drug Administration (FDA) has given its approval to several of these methods (12). However, these are costly procedures that need for specialized labs and

qualified staff.

The desire for more potent pharmacological options for clinical use and the awareness of certain medications' pleiotropic effects lead to the repositioning of pharmaceuticals, which involves using an already-known and sold drug in an unfamiliar clinical setting. The cheaper cost and previous understanding of toxicological characteristics in the body of a person are the two benefits of this technique that may be highlighted in this situation (13). Inhibitors of HMG-CoA reductase and their possible uses for neurological protection and sepsis therapy should be brought up to clarify this situation (14).

A concerning problem for world health is the rise of bacterial antibiotic resistance. The primary root of this issue is determined to be the exaggerated and ineffective administration of antibiotic medication. Given the above, there is a need for fresh antibacterial pharmaceutical treatments, and medication repurposing seems to be a workable, quick, and affordable technique in comparison to traditional drug development. The fact that this gadget received greater attention during the COVID-19 epidemic is also lauded, however, the first publication on the subject was written by researchers, and the number of articles continued to rise (15).

Therefore, the necessity for more investigations from the standpoint of medication reuse is warranted given the urgency of finding novel antibacterial medicines, the rising detection of drug-resistant strains, and the rise in complications from infectious illnesses. Due to the prospect of a novel antimicrobial agent, this research intends to evaluate how the myorelaxant Thiocolchicosidum interacts with bacteria.

METHODS

Place of the search

The experiments were conducted at the Parsian BioProducts Company (PBP) of Iran's microbiology facility.

Chemicals utilized

An injectable Thiocolchicosidum mixture (Sanofi Aventis®) with a concentration of 2 mg/ml was utilized as the study's subject in order to conduct the tests. Additionally, utilized were sterilized purified water, gentamicin as a diluent, and a control group.

Microorganisms

Four typical ATCC strains of bacteria, including *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 25933, and Pseudomonas aeruginosa ATCC 27853*, had their sensitivity assessed. Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), and Brain Heart Infusion Broth (BHI Broth) were the culture media employed.

Microorganism inoculum

The bacteria had previously been cultured in sterilized BHI broth and maintained there for 24 hours at a temperature of 37 °C. Following this time, the suspension was introduced to culture using the streak depletion technique on petri dishes that included sterile Mueller Hinton Agar and cultured for an additional 24 hours. For the delivery of 0.5 McFarland turbidity (1 x 108 CFU/mL), small quantities of the produced bacterial suspension were collected, injected, and blended in a tube that included sterile saline. The administration was then validated with the use of a tubi-dimeter.

Antibacterial activity testing

The underlying idea behind the disk-diffusion approach is to apply a paper filter to the agar that has been saturated with bacterial solution in various concentrations. 6mm diameter discs with the following test material concentrations were used for this: 2000, 1000, 500, 250, and 125 µg/mL; distilled water was employed in a volume of 10 µL of solvent. Also used as a positive control were disks that included the commercially available antimicrobial (ATM) GEN - Gentamicin 10 µg (CinnaColon, Iran).

Using a sterile swab, the bacterial inoculum was put to the agar's surface and distributed over the bottom of the petri dish on four occasions at a 45-degree angle before being applied to the plate's margins and rotating the plate multiple times at a 60-degree angle. The previously sterilized disks were repositioned on the plate using sterile tweezers. The tests were completed in triplicate and stored for 24 hours at 35 °C in an oven.

Minimum Inhibitory Concentration (MIC)

Using sterile pipettes and tips, microdilution was carried out on a 96-well plate. 100µl of Mueller-Hinton broth was poured into each well. In order to dilute the test material, 100 µl of the solution was collected from the initial well and homogenized before another 100 µl was taken out for the subsequent well. All wells of lines A, B, and C underwent this procedure, yielding the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, 1.9, 0.9, and 0.48 µg/mL. For the common antibiotic, same procedure was carried out in well E. The next step was to introduce 10 µL of bacterial inoculum. The diluent (distilled water)-only test and sterility check were carried out. 24 hours were spent with the plates in an oven set to 35°C. A 20µL reagent containing sodium resazurin (0.01% w/v) was used in the colourimetric assay (SIGMA), which produced the reading.

Antibacterial Activity Evaluation

By planting, in Müeller-Hinton Agar, 10 µL aliquots

of the dilutions equal to the MIC and two subsequently higher (2xMIC and 4xMIC), where practicable, of the material inside of the wells of the microdilution plates, the requirement of antibacterial activity was satisfied. These concentrations just above the MIC are enough to show whether the substance exhibits bactericidal or bacteriostatic activity, with the bacteriostatic impact being demonstrated as there is the growth of bacteria in the previously delimited microdilution plate wells. Following planting, the plates will spend 24 hours in a bacteriological oven at 37 °C. The lowest concentration that prohibits observable bacterial growth or permits the production of a maximum of three Colony Forming Units (CFU) will be referred to as the Minimum Bactericidal Concentration (MBC). In triplicate, the tests will be carried out.

Statistic evaluation

Three duplicates of each experiment were carried out. With the aid of the GraphPad Prism® 5.0 program (GraphPad Software, San Diego), the data were authorized for statistical analysis. The acquired data were reported as mean + the standard deviation after being submitted for examination of variance (ANOVA). When $p < 0.05$, differences were computed and assessed using the paired t-test.

RESULTS

Screening for bacteria

None of the bacterial strains utilized in the research showed any evidence of the development of a microbial development inhibition halo during the process of testing for antibacterial activity (Table 1). Gentamicin was utilized as a method control, and it was shown that the strains of S. aureus ATCC 25923, E. coli ATCC 25922, P. mirabilis ATCC 25933, and P. aeruginosa ATCC 27853 formed growth inhibition zones in amounts of 22mm, 17mm, 20mm, and 22mm, respectively. As a negative control, a solution containing DMSO and Tween 80 was employed; no halo formation of microbial growth suppression was seen in this solution.

Minimum inhibitory concentration

The *Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853* strains showed some sensitivity vis-à-vis the myorelaxant, when they drank the testicles of microdilution, with a minimum inhibitory concentration of 500 µg/mL and 1000 µg/mL, respectively. The results can be seen in Table 2. The findings of the other isolates in the microdilution test for *P. mirabilis and S. aureus* are shown in Table 2. This compound redox reaction was seen in every well that was going to receive the test medication, suggesting that the bacterium was active.

Antibacterial action characterization

Following microdilution experiments, the antibacterial activity of Thiocolchicosidum was characterized by the drug-sensitive microorganisms P. aeruginosa and E. coli. This was accomplished by seeding aliquots of the wells according to the amounts of MIC, 2 x MIC, and 4 x MIC (which is restricted to the maximum concentration of 1,000 µg/mL) and incubating them for 24 hours under optimum conditions. As a result, it was discovered that bacterial growth was plentiful in the culture media on the plate at all doses tested, indicating that the test substance's antibacterial activity was bacteriostatic (Table 2).

DISCUSSION

A global public health issue that is having an impact on the economy and healthcare systems is the rise of MDR infections (16). Mismanagement of antibiotics has led to the use of very toxic compounds with limited therapeutic indices, which has affected not only the health sector but also agriculture, livestock, and the pharmaceutical business (17). For this reason, many techniques have been put into place, such as figuring out the proper dose for patients using TDM, finding antibiotics in food (mostly in chicken, meat, milk, and honey), and quantifying pharmaceutical company effluents (18).

Myorelaxant Thiocolchicosidum, which is produced using alkaloid colchicine, has the potential to be repositioned pharmacologically, notably in cancer (19). Using the downregulation of the NF-κB pathway and its associated gene products, investigations reveal a capacity to inhibit osteoclast-genesis produced by cancers of the breast and multiple myeloma cell lines as well as an anticancer impact (20).

There haven't been any bacteriology studies that look at the potential of Thiocolchicosidum, however, as of yet (21). It's intriguing to look at this drug's potential against germs since its safety in therapeutic use is well known. Due to the widespread usage of bacterial illnesses in modern society, particularly in less developed nations, antibiotic use is on the rise and sometimes pointless, leading to the development of bacterial resistance (22).

Acetylsalicylic acid, also known as ASA and fluoxetine are two medications with antibacterial effects that have been studied in scientific research (23). According to researchers, the in vitro antimicrobial activity of AAS results from a reduction in bacterial polysaccharide formation, which has an impact on the proliferation of these microorganisms (24). Fluoxetine inhibits conventional as well as resistant isolates of S. aureus at concentrations of 256 and 102 µg/mL in vitro, respectively. The MIC of fluoxetine against both susceptible and resistant isolates of P. aeruginosa was 161µg/mL, while against E. coli, it was 102 µg/mL

Table 1. The diameter of the zones where bacterial growth was inhibited by Thiocolchicosidum, gentamicin, and control strains Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus.

Diameter of the Growth Inhibition Halo (mm)							
Strains	**Thiocolchicosidum (µg/mL)**					**Gentamicin**	***C**
	2000	**1000**	**500**	**250**	**125**	**30 µg**	
***Staphylococcus aureus* ATCC 25923**	U#	U#	U#	U#	U#	22	U#
***Escherichia coli* ATCC 25922**	U#	U#	U#	U#	U#	17	U#
***Proteus mirabilis* ATCC 25933**	U#	U#	U#	U#	U#	20	U#
***Pseudomonas aeruginosa* ATCC 27853**	U#	U#	U#	U#	U#	22	U#

***C – solvent/diluent control: Discs impregnated with a solution of DMSO (10%) and Tween 80 (2%); #U: it was not possible to visualize the formation of a halo of inhibition of bacterial growth at the concentration of the substance used in the dif-disc method.**

Table 2. Thiocolchicosidum and gentamicin›s MIC and MBC values for the strains of Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Proteus mirabilis, respectively.

Strains	**Thiocolchicosidum (µg/mL)**			**Gentamicin (µg/mL)**	***C1**	****C2**	*****C3**
	MIC	Effect	MBC	MIC			
***Staphylococcus aureus* ATCC 25923**	+	U#	U#	<1	+	-	+
***Escherichia coli* ATCC 25922**	500	Bacteriostatic	U#	1	+	-	+
***Proteus mirabilis* ATCC 25933**	+	U#	U#	16	+	-	+
***Pseudomonas aeruginosa* ATCC 27853**	1000	Bacteriostatic	U#	<1	+	-	+

***C1 – microbial growth control: wells containing mueller-hinton broth and bacterial inoculum, in the absence of DMSO (10%), Tween 80 (2%), thiocolchicoside or gentamicina; **C2: Culture medium sterility control: wells containing mueller-hinton broth, in the absence of bacterial inoculum, DMSO (10%), Tween 80 (2%), thiocolchicoside or gentamicina; ***C3 – solvent/diluent control: wells containing mueller-hinton broth, DMSO (10%), Tween 80 (2%) and bacterial inoculum, in the absence of thiocolchicoside or gentamicina; #U: Indeterminate for thiocolchicoside concentrations used in the assay; (-): inhibition of bacterial growth; (+): presence of bacterial growth; MIC: Minimum Inhibitory Concentration; CBM: Minimum Bactericidal Concentration.**

(25).

The information gathered during this inquiry sheds light on the use of muscle relaxants in the context of antibiotic treatment (26). Thiocolchicosidum has not previously been documented in the literature in relation to strains of bacteria in vitro, and this investigation showed a mild antibacterial effect against the strains under examination (27, 28). Thus, from the perspective of repositioning pharmaceuticals for novel therapeutics against infectious pathogens, these data will direct future research (29, 30).

Despite having cytotoxic effects, this medication had no discernible antibacterial effects on the ATCC strains utilized in the study. More research is still required to determine how this medication affects other microbes, including fungus, protozoa, and other bacterial species.

Acknowledgements

The authors would like to thank the staff members of the Biotechnology Research Center of the Parsian BioProducts Company (PBP) in Iran. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data generated or analyzed during this study are included in this article.

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