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Pharmacological Repositioning of Thiocolchicoside: Antibacterial Evaluation *In vitro*

Dicla Aline Semedo da Veiga [a++], Hélida Maravilha Dantas e Sousa Almeida [b#], Emmanuel Florêncio Leite [c], Kevyn Gabriel Mascarenha [c], Lívia Maria das Chagas Rocha [c], Sara Lohanna Saraiva França [c] and Sávio Benvindo Ferreira [a†*]

[a] Academic Unit of Life Sciences (UACV), Teacher Training Center (CFP), Federal University of Campina Grande (UFCG), Cajazeiras, Paraíba-58900-000, Brazil.
[b] Postgraduate Program in Pharmaceutical Sciences, Health Sciences Center, Federal University of Rio Grande do Norte, Natal, University Campus, Lagoa Nova, Natal - RN, 59078-970, Brazil.
[c] Cajazeiras Health Technical School, Cajazeiras, Paraíba-58900-000, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DASV, EFL, KGM, LMCR and SLSF did the conceptualization and literature searches. Authors HMDSA and SBF did the formal analysis. Authors DASV, EFL, KGM, LMCR and SLSF did the study investigation. Authors HMDSA and SBF did the data validation. Authors DASV and HMDSA wrote the original draft of the manuscript. Author DASV, HMDSA and SBF wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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[++] Medical Student;
[#] Bachelor of Science in Nursing;
[†] Professor of Microbiology;
**Corresponding author: E-mail: savio.benvindo@professor.ufcg.edu.br, saviobenvindo@gmail.com;*

ABSTRACT

Pharmacological repositioning has been increasingly praised as a viable, low-cost and rapid alternative for the development of a new therapy for clinical application, such as cases of bacterial resistance. Therefore, the present study aimed to investigate the antibacterial action of the myorelaxant thiocolchicoside. For this, an in vitro experimental study was developed using the bacterial strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933 and *Pseudomonas aeruginosa* ATCC 27853, and the protocols: antibacterial activity screening, Minimum Inhibitory Concentration (MIC) and characterization of antibacterial activity. The substance was thiocolchicoside, in concentrations ranging from 0.48 to 1000 µg/mL. Low sensitivity of bacterial strains to the myorelaxant was observed, obtaining an MIC of 500 µg/mL for *E. coli* and 1000 µg/mL for *P. aeruginosa*, the only strains sensitive to the compound. In the antibacterial activity characterization test, thiocolchicoside showed bacteriostatic action. The conclusions this research indicate the need to studies are needed on its action in comparison with other microorganisms, such as other bacterial species, fungi and protozoa, in order to evaluate its effect on other pathogenic organisms.

Keywords: Antibacterial activity; bacterial resistance; pharmacological repositioning; thiocolchicoside.

1. INTRODUCTION

The repositioning of drugs, which consists of applying a drug that is already known and commercialized in a new clinical context, arises from the glimpse of the pleiotropic effects of some drugs and the need for increasingly effective pharmacological alternatives for clinical practice. In this scenario, how advantages of this route that can be exalted are the lower cost and prior knowledge about toxicological aspects in the human body [1]. To illustrate this context, mention should be made of HMG-CoA reductase inhibitors and their potential for neuroprotection and sepsis management [2].

The growing increase in bacterial drug resistance is a worrying issue in the context of global health. The exacerbated and inconsequential application of antibiotic therapy is identified as the main cause of this problem [3,4]. In view of the above, there is a need for new antibacterial pharmacological therapies and drug reuse seems to be a viable, fast and less costly strategy when compared to standard drug development [5,6]. It is also extolled that during the COVID-19 pandemic this device was more discussed, however, the first article on this topic was published by [7], Ashburn and Thor, and the number of articles increased after Langedijk et al. [8].

Thus, considering the urgency in the search for new antibacterial therapies, the increasing identification of drug-resistant strains and the increase in complications due to infectious conditions, the need for further studies from the perspective of drug reuse is justified. Therefore, this study aims to investigate the action of the myorelaxant thiocolchicoside against bacteria, raising the possibility of a new antimicrobial agent.

2. MATERIALS AND METHODS

2.1 Search Location

The tests were carried out in the Microbiology laboratory of the Teacher Training Center (CFP) of the Federal University of Campina Grande (UFCG), Cajazeiras campus.

2.2 Substances Used

To carry out the experiments, an injectable thiocolchicoside solution (Sanofi Aventis®) of 2mg/ml was used as the target of the investigation. Sterile distilled water, gentamicin as diluent and control group were also used, respectively.

2.3 Microorganisms

The sensitivity of four standard American Type Culture collection (ATCC) bacterial strains was evaluated: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933 and *Pseudomonas aeruginosa* ATCC 27853. The culture media used were Mueller-Hinton Agar (MHA), Müeller-Hinton Broth (MHB) and BHI Broth (Brain Heart Infusion Broth) (KASVI).

2.4 Bacterial Inoculums

The bacteria were previously incubated in sterile BHI broth and placed in an oven for 24 hours at 35 ± 2°C. After this period, the suspension was seeded on petri plates containing sterile Mueller Hinton Agar using the streak depletion method and incubated again for another 24 hours. Small volumes of the formed bacterial suspension were collected and inserted and homogenized in a tube containing sterile saline for the administration of 0.5 McFarland turbidity (1 x 10^8 CFU / mL), being verified with the aid of a tubidimeter.

2.5 Screening for Antibacterial Activity

The principle followed for the disk-diffusion method consists of applying a paper filter with bacterial solution at different concentrations on the agar. For this purpose, 6mm diameter discs received according to the following concentrations of the test substance: 2000, 1000, 500, 250, 125 µg/mL; distilled water, in a volume of 10 µL of solution. Also, disks containing the commercial antimicrobial (ATM) GEN - Gentamicin 10 µg (CECON) were considered as a positive control.

The bacterial inoculum was applied to the surface of the agar with the aid of a sterile swab and spread on the petri dish four times at a 45º angle, rotating the plate several times at an angle of 60º and ending with the contour on the edges of the agar. With the aid of sterilized tweezers, the previously treated disks were adjusted on the plate. The experiments were carried out in triplicate and conserved in an oven for 24 hours at 35ºC.

2.6 Minimum Inhibitory Concentration

Microdilution was performed in a 96-well plate, with the aid of sterilized pipettes and tips. In all wells, 100 µL of Mueller-Hinton broth was added. For the dilution of the test substance, 100µL of the solution was discarded and homogenized in the first well and 100µL removed for the next well. This process was carried out in all wells of lines A, B and C, obtaining the following concentrations: 1000; 500; 250; 125; 62.5; 31.25; 15.62; 7.8; 3.9; 1.9; 0.9; 0.48 µg/mL.

This sequence was repeated in well E, for the standard antibiotic. After that, 10 µL of bacterial inoculum was added. Sterility control and the test with only the diluent (distilled water) were performed. The plates were kept in an oven at a temperature of 35ºC for 24 hours. The reading occurred through the colorimetric assay with 20 µL of sodium resazurin solution (0.01%; w/v) (SIGMA).

2.7 Characterization of Antibacterial Activity

10µL aliquots of the dilutions corresponding to the MIC and two immediately higher (2xMIC and 4xMIC), when possible, of the contents of the wells of the microdilution plates, was met by sowing, in Müeller-Hinton Agar.

These concentrations immediately above the MIC are sufficient to demonstrate if characterization of antibacterial activity of substance is bactericidal or bacteriostatic, with the bacteriostatic effect being evidenced when there is bacterial growth in the microdilution plate wells delimited previously [9].

After sowing, the plates will be incubated in a bacteriological oven at 35 + 2ºC for 24 hours. In case of impediment the visible growth of bacteria or allows the formation of up to three Colony Forming Units (CFU), the activity is characterized as bactericidal. The Minimum Bactericidal Concentration (MBC) will be considered as the lowest concentration that shows these results. The experiments will be performed in triplicate.

2.8 Statistical Analysis

All experiments were performed in triplicate. The results were approved for statistical treatment using GraphPad Prism® 5.0 software (GraphPad Software, Inc., San Diego, CA). The data obtained were subjected to analysis of variance (ANOVA) and expressed as mean $\pm$ standard deviation. Differences were evaluated using the paired t-test and were calculated when $p < 0.05$.

3. RESULTS

3.1 Antibacterial Screening

In screening for antibacterial activity, the formation of a microbial growth inhibition halo was not observed for any of the bacterial strains used in the study (Table 1).

Table 1. Diameter of bacterial growth inhibition zones for thiocolchicoside, gentamicin and controll against strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933 and *Pseudomonas aeruginosa* ATCC 27853 strains

Strains	Diameter of the Growth Inhibition Halo (mm)						
	Thiocolchicoside (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*

As a method control, the antibiotic gentamicin was used, where the formation of growth inhibition zones was evidenced for the strains *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. mirabilis* ATCC 25933 e *P. aeruginosa* ATCC 27853 in the amount 22mm, 17mm, 20mm and 22mm respectively. A solution with DMSO and Tween 80 was used as a negative control, where no halo formation of microbial growth inhibition was observed.

3.2 Minimum Inhibitory Concentration

The strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 showed some sensitivity vis-à-vis the myorelaxant, when they drank the testicles of midrodilution, with a minimum inhibitory concentration of 500µg/mL and 1000 µg/mL, respectively. The results can be seen in Table 2.

The results of the other strains in the microdilution test are shown in the Table 2, for *P. mirabilis* and *S.aureus*, with resazurin redox reaction being observed in all wells destined to the test drug, indicating biological activity of the microorganism.

Table 2. MIC and MBC values for thiocolchicoside and gentamicin against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933 and *Pseudomonas aeruginosa* ATCC 27853 strains

Strains	Thiocolchicoside (µg/mL)			Gentamicin (µg/mL)	$^{*}C_1$	$^{**}C_2$	$^{***}C_3$
	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 antibacterial action for thiocolchicoside concentrations used in the assay; (-): inhibition of bacterial growth; (+): presence of bacterial growth; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration*

3.3 Characterization of Antibacterial Activity

The characterization of the antibacterial activity demonstrated by thiocolchicoside was performed for drug-sensitive strains, *P. aeruginosa* and *E. coli*, after microdilution assays.

For this, aliquots of the wells corresponding to the concentrations of MIC, 2 x MIC and 4 x MIC (limited to the highest concentration of 1,000 µg/mL) were seeded and incubated under favorable conditions for 24 hours. Thus, it was observed that, in all concentrations used, there was abundant bacterial growth in the plate with culture medium, characterizing the antibacterial effect presented by the test substance as bacteriostatic (Table 2).

4. DISCUSSION

Thiocolchicoside is a myorelaxant derived from the alkaloid colchicine, and is used as a myorelaxant, with potential for pharmacological repositioning, especially in oncology. Studies suggest an ability to suppress osteoclastogenesis induced by breast cancer and multiple myeloma cells, as well as an anticancer effect through the downregulation of the NF-κB pathway and its gene products [10,11].

However, until now, there is no knowledge of researches in bacteriology that investigate the potential of thiocolchicoside. As the safety of this drug in clinical practice is public knowledge, it is interesting to investigate its potential against bacteria. Due to the current scenario of dissemination of bacterial infections in world societies, especially in poorer countries, the use of antibiotics is increasing and sometimes inconsequential, which is causing and expanding bacterial resistance [12].

Some drugs, such as acetylsalicylic acid (ASA) and fluoxetine, already have antibacterial action explored in the scientific literature. Lee et al. [13], suggest that the in vitro antibacterial activity of AAS occurs from the decrease in bacterial production of polysaccharides, affecting the growth of these microorganisms. Fluoxetine has an in vitro inhibitory effect of 256 and 102 µg/mL against standard and resistant strains of S. aureus, respectively. Against standard and resistant strains of *P. aeruginosa* it was 161 µg/mL and against *E. coli*, the MIC of fluoxetine was 102 µg/mL [14].

The data obtained in this investigation bring to light the perspective of muscle relaxants from the point of view of antibiotic therapy. There is no previous information described in the literature about thiocolchicoside against bacterial strains in vitro, and this study demonstrated a weak antibacterial activity against the investigated strains. Thus, these results will guide future research from the point of view of repositioning drugs for new therapies against infectious agents.

Although this drug has cytotoxic action, no significant antibacterial effect was observed on the ATCC strains used in the study, but studies are still needed on its action against other microorganisms, such as other bacterial species, fungi and protozoa [15].

5. CONCLUSION

Repositioning studies were carried out in the field of microbiology with the myorelaxant thiocolchicoside, granting unprecedented results for researchers in the area, observing little sensitivity of the standard *E. coli* and *P. aeruginosa* strains to the tested drug, through the microdilution test. However, studies are needed that subject this drug to other microbial agents, such as fungi and protozoa.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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