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Original article

Enhancement of the antibiotic activity of aminoglycosides by alpha-tocopherol and other cholesterol derivates

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ABSTRACT

Alpha-tocopherol, one of the most abundant isoforms of vitamin E, is a biologically active liposoluble vitamin and potent antioxidant. It occurs naturally in foods of plant and animal origin. Because of its lipophilic character, it can cause perturbations in the bacterial cell membrane, resulting in damage to components essential for the integrity of the membrane, thereby allowing an increase in permeability. This is the first report of the modulatory effect of alpha-tocopherol in multiresistant bacteria. We evaluated alpha-tocopherol against multiresistant strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, and determined if there was any similarity with the mechanism of modulatory action of cholesterol and ergosterol. When combined with aminoglycosides in a microdilution broth assay, alpha-tocopherol possibly acted through a lipophilic action on the cell envelope, modulating more effectively *P. aeruginosa* and *E. coli*, when compared with *S. aureus*.

1. Introduction

Currently, bacterial infections are the focus of public health, due mainly to the significant growth of bacterial resistance, making it increasingly necessary to develop new drugs and efficacious techniques for treatment [1–4].

Infections caused by *Staphylococcus aureus* are the most common, proving more difficult to treat due to bacterial resistance to various antibiotics, which is responsible for causing different types of intoxications and a variety of infections of the skin, superficial and subcutaneous, post-surgical infections, osteomyelitis, pneumonia, abscesses, endocarditis and bacteremia [5–7]. The species *Pseudomonas aeruginosa* is the principal cause of hospital infections, attacking the skin, urinary tract, ears and eyes, and possess toxins and enzymes in their structure that significantly increase their virulence, making it resistant to antibiotics [8]. *Escherichia coli* is the most common species of the genus *Escherichia*; it is associated with serious infections of the urinary tract, meningitis and gastrenteritis [6,8].

The phenomenon of bacterial resistance is due mainly to the intensive utilization of antimicrobials, which exerts a growing selective pressure, leading to the development of adaptation and resistance of bacteria to the principal antibiotics, thereby resulting in a vertical and horizontal transfer of these characteristics by various mechanisms [4].

Liposoluble compounds are cited as modifiers of plasma membrane permeability in bacteria [9–11]. Thus, alpha-tocopherol with its liposoluble nature can alter the fluidity of the bacterial membrane, making it more susceptible to penetration by various substances, particularly antibiotics.

Alpha-tocopherol (Fig. 1) is one of the most abundant isoforms of vitamin E, and it is the most biologically active and potent antioxidant, and the most studied isoform of this liposoluble vitamin [12,13]. The growing interest in this vitamin E isoform is due to its biological activities, especially as an antioxidant agent, where it is able to slow aging and protect organisms from non-transmissible chronic diseases, such as Parkinson's, Alzheimer's, infectious and rheumatic states, cancer and cardiovascular diseases [12,14,15]. Recently, vitamin E has been studied as a modulator of cell signaling and gene transcription [14], and also as an agent capable of inhibiting the growth of malignant cells [16].

Vitamin E occurs naturally in foods of plant origin, in dark-red vegetables, oily seeds, vegetable oils and wheat germ, and of animal origin such as egg yolk and liver [14].

Cholesterol is a lipid component necessary for the normal functioning of the body. It performs an important structural function in the plasma membrane and in the membranes of

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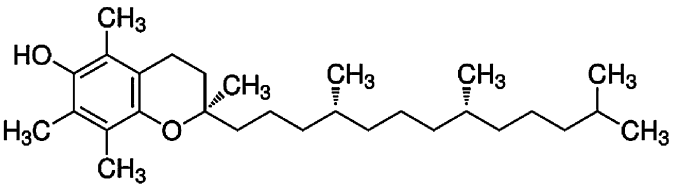

Fig. 1. Structural formulae of alpha-tocopherol.

organelles, and participates in the synthesis of bile acids necessary for the uptake of fat and liposoluble vitamins by the intestine and in the production of steroid hormones and vitamin E [17,18].

The cytoplasmic membrane of bacteria does not possess cholesterol or ergosterol, a derivative of cholesterol and lipid component of the membrane in fungi. They were both studied because they are complex substances of the steroid lipid type, [19–21], where they can affect the mosaic fluid nature of the bacterial membrane, modifying its fluidity, thereby possibly influencing the action of cholecalciferol on the bacterial plasma membrane.

Bacteria are simple organisms found in the majority of natural environments, and the bacterial cell displays various structures. One essential structure of bacteria is the cytoplasmic membrane, which is responsible for innumerable functions, including DNA replication, secretion of enzymes, biosynthesis of components, transport of solutes and energy production [9,22]. The cell wall is a structure that confers rigidity to many bacteria, and in accordance with its constitution, bacteria are divided into two classes: gram-positive and gram-negative, the difference being mainly in their permeability properties and in the surface components [6,9,22].

The aim of this study was to evaluate the modifying effect of alpha-tocopherol combined with aminoglycosides, against the multiresistance of strains of *S. aureus*, *P. aeruginosa* and *E. coli*, utilizing increasing subinhibitory concentrations of the vitamin, besides determining any similarity with the mechanism of modulatory action of cholesterol and ergosterol.

2. Materials and methods

2.1. Bacterial material

The bacteria used in the minimal inhibitory concentration (MIC) test were the standard strains of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442, *E. coli* ATCC 10536, and *K. pneumonia* ATCC 4362. To evaluate the modulatory activity of the extract, the following multi-resistant bacterial strains were used, isolated from clinical environments: *P. aeruginosa* 03, *E. coli* 27 and *S. aureus* 358, with the resistance profile demonstrated in Table 1 in the MIC *test.* All strains were obtained from the Laboratory of Clinical Mycology – UFPB. All strains were maintained on heart infusion agar slants (HIA, Difco Laboratories. Lawrence, USA) and prior to assay, the cells were grown overnight at 37oC in brain heart infusion (BHI, Difco Laboratories, Lawrence, USA).

Table 1
Bacterial resistance profile against antibiotics.

Bacteria	Source	Resistance profile
Escherichia coli 27	Surgical wound	Ast,Ax,Ami,Amox,Ca,Cfc,Cf, Caz,Cip,Clo,Im,Can,Szt,Tet,Tob
Staphhylococcus aureus 358	Surgical wound	Oxa,Gen,Tob,Ami,Can,Neo,Para, But,Sis,Net
Pseudomonas Aeruginosa 03	Urinary sample	Cpm,Ctz,Imi,Cip,Ptz,Lev,Mer,Ami

Ast: aztreonan; Amx: amoxacilin; Amp: ampicilin; Ami: amicilin; Amox: amoxilin, Ca: cefadroxil; Cfc: cefaclor; Cf: cefalotin; Caz: ceftazinidime; Cip: ciprofloxacin; Clo: clorafenicol; Imi: imipenem; Kan: kanamycin; Szt: sulfametrim; Tet: tetraciclin; Tob: tobramycin; Oxa: oxacilin; Gen: gentamicin; Neo: neomycin; Para: paramomycin; But: butirosin; Sis: sisomycin; Net: netilmycin; Com: cefepime; Ctz: ceftazidime; Ptz: piperacilin-tazobactam; Lev: levofloxacin; Mer: meropenem.

2.2. Drugs

2.2.1. Liposoluble vitamins

The vitamin E ((+) – α-tocopherol from vegetable oil) was obtained from Sigma Chemical Co., St. Louis, USA. Stock solutions were prepared in 1 mL of dimethylsulfoxide (DMSO), at a concentration of 100 mg/ml, after which they were diluted to a concentration of 1024 μg/mL in distilled water, except menadione which was diluted in DMSO. Thus, a DMSO control was included to determine any possible interference with the results.

2.2.2. Sterols

Cholesterol and ergosterol were obtained from Sigma Chemical Co., St. Louis, USA. Stock solutions were prepared in 2 mL of DMSO/Tween 80 at a concentration of 200 mg/ml, after which they were diluted to 1024 μg/mL in distilled water.

2.2.3. Antibiotics

The drugs used in the tests were the aminoglycosides amikacin, neomycin and gentamicin (Sigma Co., St. Louis, USA). All drugs were diluted in sterile water, to concentration 5000 μg/mL.

2.3. Antifungal and modulatory activity

The minimal inhibitory concentration (MIC) was determined by the broth microdilution assay. MIC is defined as the lowest concentration at which no microbial growth is observed [23]. MIC was determined using a fungal suspension of 10^5 CFU/mL in 10% brain heart infusion (BHI) broth. In 96-well microdilution plates, 100 μL of inoculum were added to each well followed by 100 μL of a serially diluted solution of (+) – α-tocopherol, cholesterol or ergosterol starting at a concentration of 1024 μg/ml. The final concentrations varied from 1024 to 8 μg/ml. The plates were incubated for 24 h at 35 °C. Fungal growth was determined by turbidity [24]. The potential of the vitamin, cholesterol and ergosterol as modifiers of antifungal resistance was determined as proposed by Coutinho et al. (2008) with modifications. The solution of vitamin was tested at three subinhibitory concentrations (MIC/8, MIC/4 and MIC/2). In a microdilution plate, 100 μL of BHI with bacterial inoculum and liposoluble vitamins were added to each well, and 100 μL of antimicrobial drugs were then added at concentrations varying 1024–0.5 μg/mL. The plates were incubated for 24 h at 37 °C.

3. Results

This is the first report of the antibacterial activity and potentiation of aminoglycosides by alpha-tocopherol, against multiresistant bacteria. To date, there has been no report of the utilization of alpha-tocopherol as a modulator of antibiotics, concomitant with other drugs.

There is a increasing search for new substances with antimicrobial activity. In the last decades, among the pharmacological activities, antimicrobials have been exhaustively studied, due to the worsening of resistance to antimicrobials in bacterial populations, mainly of hospital origin [25].

A solution of alpha-tocopherol was tested in broth microdilution assay to determine the minimal inhibitory concentration (MIC) against standard bacterial strains, and no clinically relevant antibacterial activity was observed, with all results being ≥1024 μg/mL (Table 2).