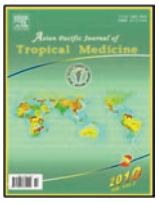
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



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Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin-resistant *Staphylococcus aureus*

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ARTICLE INFO

Article history:
Received 11 Novermber 2009
Received in revised form 7 December 2009
Accepted 20 December 2009
Available online 20 February 2010

Keywords:
Rosmarinus officinalis L.
Medicinal plants
Antibacterial activity
Herb-drug interactions
Plant extracts

ABSTRACT

Objective: To determine the antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) and to investigate the synergistic effects of this extract combined with cefuroxime against methicillin-resistant *Staphylococcus aureus* (MRSA). **Methods**: The inhibitory and bactericidal activities of rosemary ethanol extract, alone and in combination with cefuroxime, were studied. **Results**: The minimum inhibitory concentrations (MICs) of the ethanol extract of rosemary were in the range of 0.39-3.13 mg/mL. The minimum bactericidal concentrations (MBCs) were usually equal to or double that MICs. The antimicrobial activity of combinations of the ethanol extract of rosemary and cefuroxime indicated their synergistic effects against all MRSAs. **Conclusions**: The present work clearly demonstrates that rosemary has a key role in the elevation of susceptibility to β-lactams.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen in the past 2 decades. Therapeutic options for MRSA infection are very limited because most MRSA strains are resistant not only to β-lactams but also to multiple antimicrobial agents, such as macrolides, aminoglycosides, and fluoroquinolones [1-4]. Numerous researches have been carried out to study the potential antimicrobial activity of rosemary extracts [5-8] but attention has not been focused deeply on studying the herb-drug interaction between rosemary extracts and β-lactams against MRSA strains.

The purpose of the present work was to determine the antimicrobial activity of rosemary ethanol extract and to investigate the synergistic effects of this extract combined with ceforuxime against MRSA, thereby throwing light on the potential role of rosemary in increasing the effectiveness of antibiotics.

2. Materials and methods

2.1. Plant material and preparation of extract

Rosmarinus officinalis was harvested in the northern area of Palestine in June 2009. Air-dried and powdered leaves were extracted with 80% ethanol. After filtration of total extracts, the extracts were evaporated to dryness at 40 ℃ and weighed.

2.2. Bacterial strains

Five clinical MRSA isolates were used in the study. A standard bacteria strain of *Staphylococcus aureus* ATCC 25923 was also used as control.

2.3. Antibacterial activity

Antibacterial activity was determined by the well diffusion method according to the NCCLS [9]. Petri plates containing Mueller Hinton agar medium were seeded with a 24 h culture of the bacterial strains. Wells (6 mm diameter) were cut into the agar and 50 μL of the plant extracts were tested in a concentration of 100 mg/mL. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony-forming units (CFU)/mL. Incubation was performed at 37 ℃ for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well. A standard 30 μg cefuroxime disk was used as a positive control.

Minimum inhibitory concentrations (MICs) were determined by the microbroth dilution method described by the National Committee for Clinical Laboratory Standards [10]. The range

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of ceforuxime and plant extracts dilutions were 0.016 mg/mL to 0.000 125 mg/mL and 50 mg/mL to 0.195 mg/mL in Mueller-Hinton broth (Difco Laboratories), respectively. A final concentration of 1×10^5 CFU/mL of test bacteria was added to each dilution. The tubes were incubated at 37 ℃ for 48 h. MIC was defined as the lowest concentration of antimicrobial agent that inhibited bacterial growth, as indicated by the absence of turbidity. Each test included two growth controls consisting of the medium with the solvent and medium with bacterial suspension as well as sterility control. All tests were performed in duplicates.

Minimum bactericidal concentrations (MBCs) were determined by inoculating a 10 μL of medium from each of the wells from the MIC test which showed no turbidity onto a fresh drug-free agar plates. MBCs were defined as the lowest concentration of antimicrobial agent where was no bacterial growth on the plates.

2.4. Evaluation of synergy between plant extracts and antibiotics

This evaluation was done according to Muroi and Kubo [11]. Aliquots of 100 μL of bacterial cultures (10^5 CFU/ mL) were inoculated in Mueller-Hinton broth supplemented with ceforuxime at a concentration corresponding to 1/2 MIC with different concentrations of plant extracts. The concentration for plant extracts ranged from 1/32×MIC to 2×MIC, based on MIC values, that had previously been evaluated.

2.5. FIC testing

The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotic and extract combination permitting no visible growth of the test organisms on the plates [12]. The FIC value for each agent was calculated using the formula:

FIC(antibiotic)
= MIC of antibiotic in combination / MIC of antibiotic alone

FIC (extract)
= MIC of extract in combination / MIC of extract alone

Combinations were classified as synergistic, if the FIC indices were <1, additive if the FIC indices were = 1, indifferent if the FIC indices were between 1 and 2 and antagonistic if the FIC indices were >2 [13].

3. Results

Antimicrobial screening tests of the ethanol extract of rosemary was assayed *in vitro* by agar well diffusion method against 5 clinical MRSA isolates and *Staphylococcus aureus* ATCC 25923 (Table 1). Zones of inhibition ranged from 16 to >28 mm against all the test isolates. The significant antibacterial activity of the active plant extracts was comparable to the standard antimicrobics, ceforuxime (30 μg/disc).

The MICs of the extracts and the antibiotics varied between 0.000 125 mg/mL and 3.13 mg/mL (Table 1). Specifically, the MICs ranged from 0.39-3.13 mg/mL for rosemary on all of isolates tested. For the standard antibiotics, the ranges were 0.000 125-0.008 mg/mL for cefuroxime. The MBC of both rosemary extract and cefuroxime was usually equal to or double that MIC, except in the case of cefuroxime against MRSA-5 where the MBC was four times more than the MIC (Table 1).

Table 1
Antibacterial activity of cefuroxime and *Rosmarinus officinalis* extracts.

Test isolate	Cefuroxime (positive control)		*Rosmarinus officinalis* ethanol extract	
	30 μg per disk	MIC (mg/mL)	5 mg per well	MIC (mg/mL)
MRSA-1*	6	0.008	20	0.78
MRSA-2	11	0.001	16	1.56
MRSA-3	10	0.002	16	1.56
MRSA-4	10	0.002	17	3.13
MRSA-5	14	0.001	23	0.78
Staphylococcus aureus ATCC 25923	>26	0.000 125	>28	0.39

* Methicillin-resistant *Staphylococcus aureus*. Diameter of the zone of inhibition (mm) including the diameter of well (6 mm).

Table 2
Fractional inhibitory concentration (FIC) values for the combinations between cefuroxime and *Rosmarinus officinalis* extracts.

Test isolate	FIC (Cefuroxime)	FIC (Extract)	FIC Index	Interaction
MRSA-1	0.500	0.063	0.563	Synergy
MRSA-2	0.500	0.063	0.563	Synergy
MRSA-3	0.500	0.125	0.625	Synergy
MRSA-4	0.500	0.246	0.746	Synergy
MRSA-5	0.500	0.501	1.000	Synergy

The interpretations of the activity of rosemary extract combined with cefuroxime produced a remarkable synergistic activity against the tested 5 MRSA isolates (Table 2). The MICs of rosemary extracts for the MRSAs were decreased from the range of (0.78-0.049) to (0.49-0.39) mg/mL when these extracts were combined with cefuroxime at a concentration corresponding to 1/2 MIC.

4. Discussion

MRSA is resistant to virtually all kinds of β-lactams, and it thereby threatens the most potent antibiotics we have [1-4]. To recover the β-lactams efficiency, we investigated the antibacterial activity of rosemary extract, and cefuroxime alone and in combination on the susceptibility of MRSAs.

The results of disc diffusion support and extend previous findings that rosemary contains numerous biologically active compounds and some of these have been frequently used in folk medicine for their antimicrobial properties.

In addition, the MIC and MBC values support the findings of the diffusion method. The biological activity of rosemary

against the tested bacteria could be attributed to the presence of flavonoids, phenolic acids (caffeic, chorogenic and rosmarinic) and essencial oils (camphor and cineole) and diterpenes (carnosol) [14-16].

The area of concern is that MIC values of the active plant extracts obtained in this study were lower than the MBC values, suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration.

In the present study, rosemary exhibits remarkable synergistic activity in combination with cefuroxime, which is reflected by changes in the MIC values of the test MRSAs (FIC index range for synergism, 0.56 to 1.00). Although the level of antibiotic potentiation was low, the results seem promising considering that crude extracts were used. The potentiation is likely to have been much more pronounced if pure compounds were used.

Although the synergistic effects resulting from the combination of antibiotics with extracts were documented in the literature [17-19], the mechanism governing the joint action of rosemary extract components and antibiotics is still unknown. This may be due to the large number of different groups of chemical compounds present in rosemary extracts [14-16]. Biologically active components are believed to disturb permeability of the cytoplasm membrane and thereby facilitate the influx of antibiotics [20].

The results presented in this report highlight the potential of rosemary extract as a source of antibiotic resistance modifying compounds. Further work is presently under way to characterize the action mechanisms of these interesting compounds responsible for the synergistic activity against MRSAs.

Conflict of interest statement

We declare that we have no conflict of interest.

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