Alpha mangostin vs Vancomycin resistant Enterococci (VRE) March 2005

Title: Antibacterial activity of [alpha]-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics.

Date: 3/1/2005; **Publication**: Phytomedicine: International Journal of Phytotherapy & Phytopharmacology; **Author**: Dharmaratne, H.R.W.

Abstract

[alpha]-Mangostin, isolated from the stem bark of Garcinia mangostana L., was found to be active against vancomycin resistant Enterococci (VRE) and methicillin resistant Staphylococcus aureus (MRSA), with MIC values of 6.25 and 6.25 to 12.5 [micro]g/ml, respectively. Our studies showed synergism between [alpha]-mangostin and gentamicin (GM) against VRE, and [alpha]-mangostin and vancomycin hydrochloride (VCM) against MRSA. Further studies showed partial synergism between [alpha]-mangostin and commercially available antibiotics such as ampicillin and minocycline. These findings suggested that [alpha]-mangostin alone or in combination with GM against VRE and in combination with VCM against MRSA might be useful in controlling VRE and MRSA infections.

Keywords: Mangostin; Garcinia mangostan; Antibacterial activity; Vancomycin resistant Enterococci; Synergy effect with antibiotics

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Introduction

Enterococci and Staphylococcus aureus are two of the leading causes of nosocomial infections in long-term healthcare facilities. Reports on vancomycin resistant Enterococci (VRE) and methicillin resistant Staphylococcus aureus (MRSA) infections in hospitals have been increasing worldwide in recent years (Emori and Gaynes, 1993; Leclercq and Courvalin, 1997; Murry, 1997; Moellering, 1998). There are a considerable number of reports on valuable trials carried out to control the infections caused by VRE (Garner, 1996; Slaughter et al., 1996; Montecalvo et al., 1999; Nourse et al., 2000) and MRSA (Coolson, 1995; Cox et al., 1995; Voss et al., 1994; Working Party Report, 1998; Kotilainen et al., 2001). However, further trials would be necessary to discover more reliable methods to adequately control VRE and MRSA infections. In this context, the use of natural products as anti-VRE and anti-MRSA agents would be a promising field on the pathway towards the prevention of VRE and MRSA infections. Further it would be very important to investigate the synergistic behavior of the active natural products with the commercially available antibiotics, with the hope of enhancing their activity.

Garcinia mangostana L. of the family Clusiaceae (Guttiferae) is a tree found in Sri Lanka and other South East Asian countries, which is very popular due to its delicious fruits. Treatment of diarrhea, dysentery, skin infections and as an anti-inflammatory agent are some of the medicinal application of this plant. Xanthones, terpenoids and sugars have been reported from the fruit hulls and leaves of G. mangostana, and some of them have shown a variety of biological activities (Suksamrarn et al., 2002; Mahabusakam and Wiriyachitra, 1987; Praveen et al., 1991). Among them antibacterial activity against MRSA of [alpha]-mangostin is significant (Iinuma et al., 1996). However, no work on anti-VRE activity of [alpha]-mangostin have been reported so far. Therefore, we investigated the anti-VRE activity of [alpha]-mangostin. Further, studies on the synergism between [alpha]-mangostin and commercially available antibiotics against VRE and MRSA strains were also carried out with the hope of prevention of VRE and MRSA infections.

Materials and methods

Extraction and isolation of [alpha]-mangostin and [beta]-mangostin

Stem bark of G. mangostana (1 kg) was dried, powdered and extracted with hexane, methylene chloride and methanol respectively. Silica gel column chromatography of (Fluka 6074, 1 particle size 0.063 [+ or -] 0.2 mm with hexane, methylene chloride and methanol as solvents) of the hexane extract (11.9 g) and methylene chloride extract (25 g) gave two major compounds, [alpha]-mangostin (11.6 g, 1.16%) and [beta]-mangostin (6.4 g, 0.64%) as yellow needles (Fig. 1). These structures were confirmed by the direct comparison with authentic samples and spectral data (Mahabusakam and Wiriyachitra, 1987; Praveen et al., 1991).

Antibiotics

Ampicillin (ABPC), gentamicin (GM), minocycline (MINO) and vancomycin hydrochloride (VCM) were used for the test of synergistic studies.

Test bacteria

VRE: Five strains of VRE (Enterococcus faecalis ATCC 51299, E. faecalis ATCC 51575, E. faecium ATCC 51559, E. faecium KIHC-237 and E. gallinarum KIHC-241) were used in this experiment. Three ATCC strains were purchased from American Type Culture Collection (ATCC). Two strains of E. faecium KIHC-237 and E. gallinarum KIHC-241 were supplied by Kobe Institute of Public Health. The genotypes of E. faecalis ATCC 51299, E. faecium KIHC-237 and E. gallinarum KIHC-241 were van B(+), van A(+) and van Cl(+), respectively. The genotypes of the other VRE such as E. faecalis ATCC 51575 and E. faecium ATCC 51559 were unknown. Minimum Inhibitory Concentrations (MIC) of the five strains of VRE to VCM were measured as 250, 32, 200, 200 and 16 [micro]g/ml, respectively.

[FIGURE 1 OMITTED]

VSE: Three strains of vancomycin-sensitive Enterococci (VSE) (E. faecalis IFO 12965, E. faecium IFO 3535 and E. faecalis ATCC 8459) were used in this experiment. These strains were purchased from the Institute for Fermentation of Osaka (IFO), Japan, and ATCC, respectively.

MRSA: Each of the 3 strains (total: 9 strains) of methicillin-resistant Staphylococcus aureus (MRSA) was kindly donated by Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka National Hospital and Kitano Hospital in 1997. MIC values of nine strains of MRSA to methicillin (DMPPC) were measured as 12.5, 400, 25, 12.5, 400, 1600, 25, 12.5 and 400 [micro]g/ml, respectively.

MSSA: Methicillin-sensitive Staphylococcus aureus (MSSA), Staphylococcus aureus IFO 13276, S. aureus IFO 12732 and S. aureus IFO 3060 were purchased from IFO and used in this experiment.

Broth

SCD broth (Nihon Pharm. Co., Ltd.) was used for pre-incubation of VRE, VSE, MRSA and MSSA. Mueller-Hinton (MH) Agar (Difco Co., Ltd.) was used for the measurement of MIC.

MIC

MIC values were determined by the agar dilution method (Goto et al., 1981) using micro-inoculators (Sakuma Seisakusho Co., Ltd., Tokyo).

Synergism between [alpha]-mangostin and the commercially available antibiotics

A solution (50% dimethylsulfoxide in water) of [alpha]-mangostin alone, and [alpha]-mangostin in combination with respective antibiotics were prepared by the doubling dilution method with sterilized water, and they were poured into sterilized plastic Petri dishes separately. Sterilized MH Agar 8 ml (MH Agar 9 ml was poured into [alpha]-mangostin alone or the antibiotic alone) was poured into the above Petri dishes and mixed. After cooling, the MICs of [alpha]-mangostin, the antibiotics alone and [alpha]-mangostin in combination with each antibiotic, were examined. Through out this experiment, the fraction inhibitory concentration (FIC) indices were calculated by the method used by Didry et al. (1993), and the interactive effects between [alpha]-mangostin and the antibiotics were examined.

Results

Antibacterial activities of [alpha]-mangostin and [beta]-mangostin against VRE and MRSA

Table 1 shows the anti-VRE activity of [alpha]-mangostin and [beta]-mangostin, and Table 2 shows the anti-VRE activity of [alpha]-mangostin and [beta]-mangostin, respectively.

[alpha]-Mangostin was found to be active against five strains of vancomycin-resistant Enterococci (VRE) and nine strains of methicillin-resistant Staphylococcus aureus (MRSA) with MIC values of 6.25 and 6.25 to 12.5 [micro]g/ml, respectively.

Synergism between [alpha]-mangostin and the commercial antibiotics

Since [alpha]-mangostin was found to be active against VRE and MRSA, we extended our investigation to study the synergistic effect between [alpha]-mangostin and commercially available antibiotics. FIC index calculations, which is a widely accepted method to evaluate in vitro synergistic studies between different antibacterial compounds were used in our experiments and the results are given in Fig. 2.

Synergism between [alpha]-mangostin and GM against VRE, and [alpha]-mangostin and VCM against MRSA also observed. In the above synergistic studies, the average of FIC indices were calculated as 0.451 [+ or -] 0.069 and 0.441 [+ or -] 0.131, respectively. Partial synergism between [alpha]-mangostin and ampicillin (ABPC), minocycline (MINO), fosfomycin (FOM) and VCM against VRE with FIC indices of 0.606 [+ or -] 0.328, 0.969 [+ or -] 0.217, 0.826 [+ or -] 0.286 and 0.508 [+ or -] 0.271 were observed, respectively.

[FIGURE 2 OMITTED]

[FIGURE 3 OMITTED]

Further, partial synergisms between [alpha]-mangostin and ABPC, GM, MINO and VCM against MRSA were also observed, and their FIC indices were calculated as 0.779 [+ or -] 0.343, 0.667 [+ or -] 0.359, 0.586 [+ or -] 0.303 and 0.504 [+ or -] 0.149, respectively

In VSE, synergism between [alpha]-mangostin and VCM was observed, and the FIC index was calculated as 0.378 [+ or -] 0.113. Partial synergisms between [alpha]-mangostin and the commercially available antibiotics ABPC, GM, MINO and FOM were observed, and their FIC indices were calculated as 0.836 [+ or -] 0.284, 0.500 [+ or -] 0.108, 0.750 [+ or -] 0.000 and 0.792 [+ or -] 0.191, respectively.

On MSSA, FIC indices between [alpha]-mangostin and the commercially available antibiotics ABPC, GM, MINO, FOM and VCM were observed, and their FIC indices were calculated as 0.635 [+ or -] 0.325, 0.428 [+ or -] 0.209, 0.750 [+ or -] 0.000, 0.625 [+ or -] 0.217 and 0.625 [+ or -] 0.000, respectively.

[FIGURE 4 OMITTED]

Synergism between [alpha]-mangostin and GM against 5 strains of VRE, and [alpha]-mangostin and VCM against 9 strains of MRSA were also tested by the evaluation method described by Williamson (2001). The results were shown in Figs. 3 and 4, respectively. Synergism between [alpha]-mangostin and GM against VRE, and [alpha]-mangostin and VCM against MRSA were reconfirmed by this method.

Discussion

In our present study, we have investigated the anti-VRE and anti-MRSA activities of [alpha]-mangostin and [beta]-mangostin, which are the major compounds present in the stem bark extracts of G. mangostana.

Furthermore, the synergistic effect between [alpha]-mangostin and the commercially available antibiotics were investigated. Previous work has also established its excellent activity against MRSA (Iinuma et al., 1996), and above finding led us to investigate its anti-VRE activity and synergism between [alpha]-mangostin and commercially available antibiotics against VRE and MRSA. Activity studies were evaluated by means of MIC. In our previous work on anti-VRE activity and synergistic studies, we reported remarkable anti-VRE and anti-MRSA activities of calozeyloxanthone isolated from Calophyllum moonii, an endemic species from Sri Lanka, against VRE and synergism with the commercially available antibiotics (Sakagami et al., 2002). Our present work on anti-VRE activity and synergistic studies of [alpha]-mangostin clearly shows that its activity is almost similar to that of calozeyloxanthone.

Extended antibacterial activity studies indicated that [alpha]-mangostin and [beta]-mangostin are inactive against gram negative bacteria, such as Escherichia coli IFO 3972. Proteus vulgaris IFO 3988, Serratia marcescens IFO 12648, E. coli O157 (ATCC 43888), Klebsiella pneumoniae IFO 13277 and Pseudomonas aeruginosa IFO 13275 (data not shown).

Our new findings suggested that [alpha]-mangostin alone or in combination with GM against VRE, and in combination with VCM against MRSA, might be useful in controlling VRE and MRSA infections, and should be investigated further in vivo models.

Table 1. MIC values of [alpha]-mangostin and [beta]-mangostin against 5 strains of VRE and 3 strains of VSE

	MIC ([micro]g/ml)		
	[alpha]-mangostin	[beta]-mangostin	Gentamicin
E. faecalis ATCC 51299 (VRE) (a)	3.13	25	>100
E. faecalis ATCC 51575 (VRE) (a)	3.13	25	>100
E. faecium ATCC 51559 (VRE) (a)	3.13	25	6.25
E. faecium KIHC-237 (VRE) (b)	3.13	25	6.25
E. gallinarum KIHC-241	6.25	25	3.13

(VRE) (b)			
E. faecalis IFO 12965 (VSE) (c)	6.25	25	12.5
E. faecium IFO 3535 (VSE) (c)	3.13	25	6.25
E. faecalis ATCC 8459 (VSE) (c)	3.13	25	6.25

(a) Purchased from American Type Culture Collection (ATCC).
(b) Supplied from Kobe Institute of Public Health.
(c) Purchased from Institute for Fermentation of Osaka (IFO), Japan.

Table 2. MIC values of [alpha]-mangostin and [beta]-mangostin against 9 strains of MRSA and 3 strains of MSSA

	MIC ([micro]g/ml)		
	[alpha]-mangostin	[beta]-mangostin	Gentamicin
MRSA-1 (a)	6.25	>100	25
MRSA-2 (a)	6.25	>100	3.13
MRSA-3 (a)	6.25	>100	1.56
MRSA-4 (b)	6.25	>100	3.13
MRSA-5 (b)	6.25	>100	6.25
MRSA-6 (b)	6.25	>100	0.2
MRSA-7 (c)	6.25	>100	0.2
MRSA-8 (c)	12.5	>100	6.25
MRSA-9 (c)	6.25	>100	>100
MSSA 1 (S. aureus IFO 13276) (d)	6.25	>100	0.2
MSSA 2 (S. aureus IFO 12732) (d)	6.25	>100	0.2
MSSA 3 (S. aureus IFO 3080) (d)	6.25	>100	0.2

(a) Donated from Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan.
(b) Donated from Osaka National Hospital, Japan.
(c) Donated from Kitano Hospital, Japan.
(d) Purchased from Institute for Fermentation of Osaka (IFO), Japan.

Received 6 May 2003; accepted 22 September 2003

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