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In Vitro Activities of Panduratin A against Clinical *Staphylococcus* Strains▽

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In vitro antistaphylococcal activities of panduratin A, a natural chalcone compound isolated from *Kaempferia pandurata* Roxb, were compared to those of commonly used antimicrobials against clinical staphylococcal isolates. Panduratin A had a MIC at which 90% of bacteria were inhibited of 1 μg/ml for clinical staphylococcal isolates and generally was more potent than commonly used antimicrobials.

Staphylococci are frequently refractory to many new and commonly used antimicrobial agents and have become a problem in recent years (8, 12, 17). Methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) infections have emerged as a worldwide problem, and clinical strains of MRSA exhibit reduced susceptibility to antimicrobial agents (18). Moreover, coagulase-negative staphylococci are well established due to nosocomial bacteremia and indwelling medical device-associated infection, showing increased multidrug resistance (1, 14). Thus, the identification of novel agents effective in inhibiting these strains has gained renewed urgency (7). In addition, there is renewed interest in plants with antimicrobial properties as a consequence of current problems associated with the use of antibiotics (4, 9). Panduratin A, a natural chalcone compound isolated from the rhizome of fingerroot (*Kaempferia pandurata* Roxb.), has been reported to possess antibacterial activity against *Prevotella intermedia*, *Prevotella loescheii*, *Porphyromonas gingivalis*, *Propionibacterium acnes*, and *Streptococcus mutans*, as well as antibiofilm activity against multispecies oral biofilms in vitro (6, 13, 15, 16). However, antimicrobial activities of panduratin A against other pathogenic bacteria, such as staphylococci, have not yet been investigated.

In this study, we compared the in vitro activities of panduratin A against MRSA, methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), and methicillin-susceptible coagulase-negative staphylococci (MSCNS) with those of treatments with available antimicrobial agents, such as ampicillin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, tetracycline, thymol, and vancomycin.

Clinical MRSA (n = 27), MSSA (n = 27), MRCNS (n = 28), and MSCNS (n = 26) were obtained from the Research Institute of Bacterial Resistance, College of Medicine, Yonsei University, South Korea. The clinical *Staphylococcus* strains were collected in 2008 from patients at a Korean tertiary-care hospital. The strains were isolated from body fluids, blood, genital secretions, pus, or sputum, of the patient. The species were identified by conventional methods (2) or by using the Vitek system (bioMerieux SA, Marcy l'Etoile, France) according to the manufacturer's instructions. Reference strains *S. aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 from the American Type Culture Collection (Rockville, MD) were included as controls.

Panduratin A (FIG. 1) was isolated in pure form from an ethanol extract of *Kaempferia pandurata* Roxb. according to the method of Park et al. (13). Panduratin A was dissolved in 10% dimethyl sulfoxide (DMSO) to obtain a 1,024-μg/ml stock solution. Ampicillin, erythromycin, gentamicin, tetracycline, thymol, and vancomycin were purchased from Sigma-Aldrich. Co. (St. Louis, MO). Levofloxacin and oxacillin were purchased from Sigma-Fluka Co. (Steinheim, Germany), and linezolid was provided by Dong-A Pharmaceutical Co. (Seoul, South Korea). Stock solutions of commercial antimicrobial agents were prepared according to the manufacturer's instructions.

In vitro susceptibility tests were performed in a 96-well microtiter plate to determine MICs of panduratin A and other antimicrobial agents against 108 isolates of clinical staphylococci using standard broth microdilution methods with an inoculum of 5×10^5 CFU/ml, according to the guidelines of CLSI standard M7-A6 (3). A twofold dilution of panduratin A stock solution or other antimicrobial agent preparation was mixed with the test organisms (5×10^5 CFU/ml) in Mueller-Hinton broth (MHB) medium (Difco Becton Dickinson, Sparks, MD). Column 12 of the microtiter plate contained the highest concentrations of panduratin A or other antimicrobial agents, and column three contained the lowest concentrations of panduratin A or other antimicrobials agents. Column 2 served as the positive control for all samples (only medium and inoculum or antimicrobial agent-free wells), and column 1 was the negative control (only medium, no inoculum, and no antimicrobial agent). Microtiter plates were incubated aerobically at 37°C for 24 h. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in the complete inhibition of visible growth.

Panduratin A was diluted in 10% DMSO, followed by twofold dilutions in the test wells; thus, the final concentration of DMSO would be serially decreased. We examined the effect of DMSO on the growth and viability of all staphylococci

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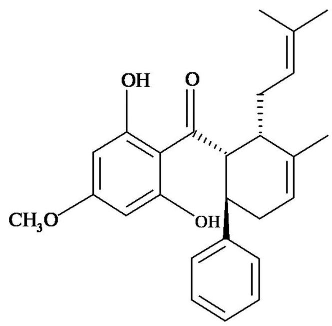

FIG. 1. Structure of panduratin A.

tested. DMSO at ≤10% was found not to affect growth or viability of the staphylococci tested. These results suggest that DMSO had no effect on activity and that all the antimicrobial activity was due to panduratin A.

Minimal bactericidal concentrations (MBCs) were determined for each antimicrobial agent per *Staphylococcus* strain as outlined for MICs (5). Briefly, medium (approximately 100 μl) from each well showing no visible growth was spread onto MHA (MHB supplemented with 1.5% bacterial agar) plates. Wells in column 2, the positive controls (antimicrobial agent-free wells), and wells in column 1, growth-negative controls, were included for the MBC test. Plates were incubated at 37°C for 24 h or until growth was seen in the growth-positive control plates. MBC was defined as the lowest concentration of antimicrobial agent at which all bacteria in the culture are killed or the lowest concentration at which no growth occurs on MHA plates (5, 10).

Table 1 shows the MICs and MBCs of panduratin A in comparison to those of ampicillin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, tetracycline, thymol, and vancomycin for clinical staphylococci isolates. In this study, all isolates were susceptible to panduratin A, with MICs of ≤2 μg/ml. In our previous report (13), the MIC of panduratin A against *P. gingivalis*, *P. loescheii*, and *S. mutans* was 4 μg/ml while that of panduratin A against *P. intermedia* and *P. acnes* was 2 μg/ml (6, 13, 15). These results show that panduratin A has activities against clinical staphylococci stronger than those against *P. gingivalis*, *P. loescheii*, and *S. mutans* and comparable or equal to those against *P. intermedia* and *P. acnes*. Moreover, panduratin A has the capability of preventing the biofilm formation of primary multispecies oral bacteria (*Actinomyces viscosus*, *S. mutans*, and *Streptococcus sanguis*) in vitro (16). This report suggests that panduratin A might also have the ability to inhibit staphylococcal biofilm formation. Hence, future research is necessary to determine the inhibition activity of panduratin A against staphylococcal biofilm formation.

In this study, all isolates of MRSA, MSSA, MRCNS, and MSCNS were resistant to ampicillin. However, all isolates of MRSA, MSSA, MRCNS, and MSCNS were inhibited by ≤2 μg/ml of panduratin A. MICs of panduratin A against all isolates tested were much lower than those of thymol (≤512 μg/ml), which has been reported to possess antistaphylococcal activity (11). Moreover, most isolates of MRSA were resistant to erythromycin, gentamicin, levofloxacin, oxacillin, and tetracycline. Although all isolates of MRSA were inhibited by ≤2 μg/ml of linezolid, these MICs of ≤2 μg/ml were still higher than that of panduratin A or vancomycin, which inhibited the growth of all isolates of MRSA with MICs of ≤1 μg/ml.

The majority of MSSA isolates were susceptible to erythromycin (MIC at which 90% of bacteria were inhibited [MIC_{90}] = 32 μg/ml), gentamicin (MIC_{90} = 64 μg/ml), levofloxacin (MIC_{90} = 8 μg/ml), linezolid (MIC_{90} = 4 μg/ml), oxacillin (MIC_{90} = 1 μg/ml), tetracycline (MIC_{90} = 8 μg/ml), and vancomycin (MIC_{90} = 0.5 μg/ml). However, the MIC_{90} of panduratin A was 1 μg/ml. These results indicate that panduratin A has stronger antistaphylococcal activity against MSSA isolates than erythromycin, gentamicin, levofloxacin, linezolid, or tetracycline.

The MRCNS isolates were also resistant to most of the antimicrobial agents tested. All MRCNS isolates were inhibited by ≤2 μg/ml of vancomycin and ≤4 μg/ml of linezolid. The MIC range and MIC_{90} of panduratin A for MRCNS isolates was 0.125 to 2 μg/ml and 1 μg/ml, respectively. These results indicate that antistaphylococcal activity of panduratin A against MRCNS is equal to that of vancomycin and stronger than that of linezolid.

Finally, the MIC range of panduratin A against MSCNS isolates (0.063 to 2 μg/ml) was narrower than those of erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, and tetracycline. Vancomycin had the narrowest range of MIC (0.063 to 1 μg/ml) against MSCNS isolates. Interestingly, the MIC_{90} of vancomycin against MSCNS isolates was the same as the MIC_{90} of panduratin A against MSCNS isolates.

The range of MICs of panduratin A for MRSA and MSSA were very narrow at 0.5 to 1 μg/ml and 0.5 to 2 μg/ml, respectively. In contrast, the ranges of panduratin A MICs for MRCNS and MSCNS were large at 0.125 to 2 μg/ml and 0.063 to 2 μg/ml. These results could be interpreted to mean that MRSA and MSSA are composed of only one species of *Staphylococcus*, *S. aureus*, whereas MRCNS and MSCNS are composed of different species of *Staphylococcus*: *S. hominis*, *S. epidermidis*, *S. haemolyticus*, *S. simulans*, and *S. sciuri*. Yong et al. (17) reported that the ranges of MICs for DA-7867, a novel oxazolidinone, for MRSA and MSSA were broader than those for MRCNS and MSCNS. Moreover, the ranges of MICs for CG400549, a novel FaI inhibitor, for MRSA and MSSA were very narrow at 0.12 to 0.5 μg/ml and 0.12 to 1 μg/ml, respectively. In contrast, the ranges of CG400549 MICs for MRCNS and MSCNS were broad at 0.12 to 16 μg/ml and 0.5 to 8 μg/ml (18). Thus, the MICs of panduratin A for MRSA, MSSA, MRCNS, and MSCNS were in agreement with other reports. In addition, the MIC of panduratin A against *P. intermedia* was 2 μg/ml, whereas that against *P. loescheii* was 4 μg/ml. They belong to the same genus, *Prevotella*, but are different species (13). Thus, coagulase-negative staphylococci (MRCNS and MSCNS) have a wider MIC dispersion with panduratin A than that of coagulase-positive staphylococci (MRSA and MSSA).

The in vitro MBCs of panduratin A with an endpoint after 24 h demonstrated that panduratin A was able to kill staphylococcus strains with MBCs of ≤8 μg/ml for MRSA, MSSA, and MRCNS. On the other hand, panduratin A can kill MSCNS with MBCs of ≤4 μg/ml. These results were similar to the MBCs of vancomycin against the clinical staphylococcal strains (Table 1). These panduratin A MBC results suggest that panduratin A may be as bactericidal as vancomycin. In addition, the MBC of panduratin A against a *P. gingivalis*, *P.*

TABLE 1. Comparative in vitro activities of panduratin A and other antimicrobial agents against clinical staphylococcal isolates

Staphylococcal group (n^a) or antimicrobial agent	MIC (μg/ml)			Susceptibility (%)[b]			MBC (μg/ml)		
	Range	50%	90%	S	I	R	Range	50%	90%
MRSA (27)									
Ampicillin	16–128	16	64	0	—	100	32–512	64	256
Erythromycin	16–64	32	64	0	0	100	32–256	128	256
Gentamicin	0.5–64	32	64	15	22	63	2–256	64	128
Levofloxacin	0.5–256	8	128	18	0	82	0.5–512	16	512
Linezolid	0.5–2	1	2	100	—	—	8–16	8	16
Oxacillin	32–64	64	64	0	—	100	256–512	256	512
Tetracycline	0.5–64	16	32	30	0	70	2–512	64	512
Thymol	64–128	128	128	—	—	—	256–512	512	512
Vancomycin	0.25–1	0.5	0.5	100	0	0	0.5–8	1	2
Panduratin A	0.5–1	0.5	1	—	—	—	2–8	2	4
MSSA (27)									
Ampicillin	0.5–64	32	32	0	—	100	1–256	64	128
Erythromycin	0.5–65	0.5	32	56	4	40	1–256	4	128
Gentamicin	0.125–64	4	64	52	0	48	0.5–256	8	128
Levofloxacin	0.125–64	0.125	8	81	4	15	0.125–16	1	8
Linezolid	0.25–8	1	4	96	—	—	2–32	8	32
Oxacillin	0.125–32	0.125	1	89	–	11	1–128	16	64
Tetracycline	0.5–32	4	8	70	19	11	1–64	8	32
Thymol	64–128	128	128	—	—	—	128–512	256	512
Vancomycin	0.125–2	0.25	0.5	100	0	0	0.25–8	1	2
Panduratin A	0.5–2	0.5	1	—	—	—	1–8	2	4
MRCNS (28)									
Ampicillin	0.5–128	16	64	0	—	100	2–>512	32	256
Erythromycin	0.5–32	16	32	11	15	74	1–128	32	128
Gentamicin	0.125–64	8	64	45	7	48	0.25–128	8	128
Levofloxacin	0.25–8	0.25	4	26	15	59	0.5–32	4	16
Linezolid	0.125–4	0.25	2	100	—	—	0.5–8	2	4
Oxacillin	0.125–64	64	64	26	—	74	1–256	128	256
Tetracycline	0.25–64	1	64	18	7	75	0.5–128	4	128
Thymol	32–512	64	128	—	—	—	64–512	256	512
Vancomycin	0.125–2	0.125	2	100	0	0	0.5–8	2	4
Panduratin A	0.125–2	0.25	1	—	—	—	1–8	2	4
MSCNS (26)									
Ampicillin	0.5–128	2	16	0	—	100	1–256	4	128
Erythromycin	0.5–128	0.5	16	50	23	27	1–256	2	128
Gentamicin	0.125–64	0.125	32	73	0	27	0.25–64	1	64
Levofloxacin	0.25–8	0.25	0.5	96	0	4	0.5–32	1	4
Linezolid	0.125–16	0.25	4	96	—	—	0.25–32	2	4
Oxacillin	0.125–32	0.125	0.5	96	—	4	0.25–128	2	8
Tetracycline	0.5–128	0.5	32	69	19	12	1–256	8	128
Thymol	4–128	64	64	—	—	—	64–512	256	512
Vancomycin	0.063–1	0.25	1	100	0	0	0.125–4	1	4
Panduratin A	0.063–2	0.5	1	—	—	—	0.125–4	1	4

[a] *n*, no. of isolates tested.
[b] S, susceptible; I, intermediate; R, resistant; —, CLSI breakpoint is not available.

loescheii, and *S. mutans* was 8 μg/ml, and the MBC of panduratin A against *P. intermedia* and *P. acnes* was 4 μg/ml (6, 13, 15). Panduratin A has been reported to have the ability to reduce the biofilm of multispecies oral bacteria in vitro (16). It would be interesting to evaluate the antibiofilm activity of panduratin A in reducing staphylococcal biofilms. Further work toward these objectives may resolve these issues.

In conclusion, panduratin A is an antimicrobial agent with high in vitro activities against clinical MRSA, MSSA, MRCNS, and MSCNS, including organisms resistant to other antimicrobials. These results suggest that panduratin A should undergo further testing to assess its potential for the treatment of diseases caused by staphylococci. Obviously, toxicity studies, animal model studies, and human clinical trials will determine whether in vitro microbiological results translate into a useful drug for treating human infections.

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