International Journal of Oral Biology, Vol. 33, No. 4, December 31 2008, p. 213~216

International Journal of Oral Biology

Antimicrobial Activity of Continentalic Acid from *Aralia cordata* Against *Enterococcus* Strains

Seung-Il Jeong[1,2], Yeon-Hee Yun[1], Shin-Moo Kim[3], Kui-Hyun Yoon[4], and Kang-Ju Kim[1]*

[1]*Department of Oral Microbiology, School of Dentistry and Biomaterial Implant Research Institute, Wonkwang University, Iksan, 570-749, Korea*
[2]*Jeonju Biomaterials Institute, Jeonju, 561-360, Korea*
[3] *Department of Clinical Pathology, Wonkwang Health Science College, Iksan, 570-750, Korea*
[4] *Department of Laboratory Medicine, Wonkwang Univ. Sanbon Hospital, Gunpo, 435-040, Korea*

(Received December 5, 2008 ; Revised December 12, 2008 ; Accepted December 19, 2008)

Continentalic acid (CA, (-)-pimara-8(14), 15-diene-19-oic acid) was isolated from the roots of *Aralia cordata* (*Araliaceae*) using bioassay-guided fractionation of a crude chloroform extract. The antibacterial activity of CA against *Enterococcus faecalis* and *Enterococcus gallinarium* was estimated by determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). CA exhibited potent activity against standard vancomycin-resistant enterococci (VRE) and vancomycin-susceptible enterococci (VSE), with MICs and MBCs values between 4 and 8 μg/mL and 4 and 16 μg/mL, respectively. This compound exhibited potent activity against strains of VRE, which are highly resistant to clinically useful antibiotics. These findings suggest that continentalic acid may be useful in controlling enterococcal infection.

Key words : *Aralia cordata*; continentalic acid; vancomycin-resistant enterococci (VRE); minimum inhibitory concentrations; minimum bactericidal concentrations

Introduction

Enterococci are occurring in opportunistic infections involving the oral cavity. Due to the low sensitivity to antimicrobial agents, enterococci may be selected in root canals undergoing standard endodontic treatment and significantly contribute to endodontic treatment failures (Dahlen *et al*., 2000). The patients with refractory periodontitis constituted a heterogeneous group based on their subgingival microbiota including *Enterococcus faecalis* (Colombo *et al*., 1998).

Enterococci may acquire resistance against vancomycin during treatment with vancomycin for diseases caused by methicillin-resistant *Staphylococcus aureus* (MRSA) infections. The rapid emergence of vancomycin-resistant entrococci (VRE) is believed to be caused by the excessive use of vancomycin. VRE has been emerging worldwide as one of the most important hospital and community pathogens. Therefore, the emergence of these resistant bacteria has created a major concern and an urgent need for new antibacterial agents(American Society for Microbiology, 1995; Davis, 1994; Spratt, 1994). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because many plants have defense systems against phytopathogenic bacteria. Medicinal plants that have been used for a long time may be good sources of safe antibacterial agents (Sinclair, 1998). There were some trials about the dental use of herbs such as chemotherapeutics (Baek, 2007; Kim *et al*., 2007).

During the course of our continuing screening studies for natural antibacterial agents, we recently found that the roots of *Aralia cordata* (*A. cordata*) have antibacterial activity against VRE (Hur *et al*., 2004; Jeong *et al*., 2006; Kim *et al*., 2004; Kim *et al*., 2005). The root of *Aralia cordata* Kitagawa (*Araliaceae*), known as 'Dokwhal' in Korea has

*Corresponding author: To whom correspondence should be addressed. Tel.: 82-63-850-7157, 5992; Fax.: 82-63-850-7158 E-mail: kjkimom@wonkwang.ac.kr

been used widely in oriental folk medicine as analgesia, antirheumatic, neuralgia and as a cure for arthralgia, rheumatism, lumbago and lameness (Kim *et al*., 1998). In this present study, bioassay-guided fractionation and purification of the active chloroform soluble extract resulted in the isolation of the continentalic acid (CA) as active principles.

Continentalic acid ((-)-pimara-8(14),15-diene-19-oic acid) is a major component isolated from the roots of *A. cordata*, which has been used as analgesic and anti-inflammatory agents (Okuyama *et al*., 1991; Han *et al*., 1983). Phytochemically, *A. cordata* contains diterpenes, polyacetylenes, lipid glycerol and sterols (Dang *et al*., 2005). However, limited information is available on the antibacterial activity of *A. cordata*. The purpose of this study was to investigate the antimicrobial activity of continentalic acid from *Aralia cordata* against *Enterococcus*, a opportunistic pathogen involving oral cavity.

Materials and Methods

Extraction and Isolation

The extraction and isolation of active principles from the root of *A. cordata* were conducted according to our previous report (Jeong *et al*., 2006). In brief, the root of *A. cordata* was extracted with methanol and partitioned chloroform, ethyl acetate and butanol. The active chloroform soluble extract yielded a diterpenic acid as the active principles after chromatography in silica gel and 1H (GPC). The structure of the compound was determined by its physico-chemical and spectral data, which are in agreement with those reported in literature (Han *et al*., 1983).

Bacteria

Enterococcus faecalis CDC-286 (*vanA*), *Enterococcus faecalis* CDC-583 (*vanB*), *Enterococcus gallinarium* CDC-45 (*vanC*) and *Enterococcus faecalis* ATCC 29212 (VSE) from Centers for Disease Control and Prevention (CDC) were used in this study.

Screening

The confirmation of the strain was done by detection of vancomycin resistance gene (*van*) with PCR analysis. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined in triplicate using microdilution techniques according to current Clinical and Laboratory Standards Institute (CLSI) (formerly the national Committee for Clinical Laboratory Standards [NCCLS]) recommendations (Wikler *et al*., 2008).

Results

In order to identify herbs with antibacterial properties against VRE, medicinal herb traditionally used for gasterointestinal infection in Korea was tested against VRE. Among the herbal extracts tested, *A. cordata* extracts (methanol extraction, chloroform fraction, ethyl acetate fraction, and butanol fraction) showed some antimicrobial activities against *Enterococcus faecalis* and *Enterococcus gallinarium* as determined by the agar dilution method. As shown in Table 1, the chloroform soluble fraction demonstrated a higher inhibitory activity (MICs/MBCs, 32/32 μg/mL) than ethyl acetate (MICs/MBCs, 32/64 μg/mL), methanol (MICs/MBCs, 64/64 μg/mL) and butanol (MICs/MBCs, 128/128 μg/mL) soluble fractions against VRE. Therefore, the chloroform soluble fraction was further subjected to silica gel column chromatography in an attempt to isolate the active guided fractionation. From these active fractions, a white compound was purified by repeated silica gel column chromatography followed by prep-HPLC. The compound was characterized by 1D-NMR and 2D-NMR, which gave a spectrum that matched perfectly with that of the previously described continentalic acid (Han *et al*., 1983). Table 2 shows the MICs/MBCs of vancomycin and CA against VRE. CA exhibited potent activity against standard

Table 1. Antibacterial activity of extracts and fractions from *A. cordata* against *E. faecalis* CDC-286 and *E. faecalis* CDC-583.

sample	MIC(μg/mL)	MBC(μgmL)
Methanol extract	64	64
Chloroform soluble fraction	32	32
Ethyl acetate soluble fraction	32	64
Butanol soluble fraction	128	128

VRE: vancomycin-resistant enterococcus.
MIC: minimum inhibitory concentration.
MBC: minimum bactericidal concentration.

Table 2. Antibacterial activity of continentalic acid isolated from roots of against standard VSE and 3 VRE strains (μg/mL).

Strains	Continental acid		Vancomycin	
	MIC	MBC	MIC	MBC
E. faecalis CDC-286 (*vanA*)	8	16	256	512
E. faecalis CDC-583 (*vanB*)	8	16	32	64
E.gallinarium CDC-45 (*vanC*)	8	16	16	16
E. faecalis ATCC29212(VSE)	8	8	4	4

VRE: vancomycin-resistant enterococcus.
VSE: vancomycin-susceptible enterococcus.
van: vancomycin-resistant gene.

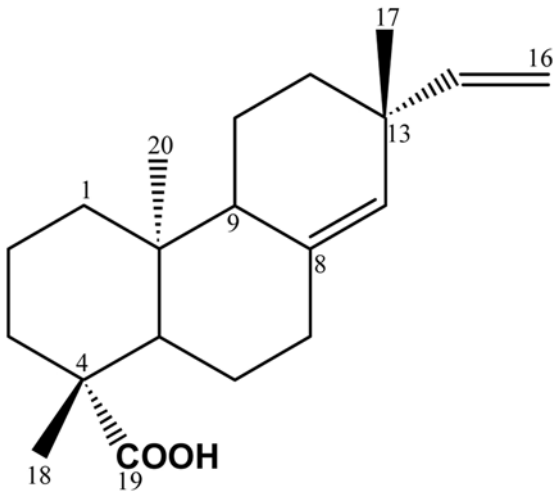

Fig. 1. Chemical structure of the continentalic acid isolated from *A. cordata*. ^{13}C-NMR (125MHz, $CDCl_3$) δ 184.6 (C-19), 147.4 (C-15), 138.1 (C-8), 128.2 (C-14), 113.1 (C-16), 56.3 (C-9), 50.8 (C-5), 44.3 (C-4), 39.4 (C-13), 38.7 (C-10), 38.2 (C-1), 36.6 (C-3), 36.0 (C-7), 31.8 (C-12), 29.6 (C-18), 29.4 (C-17), 24.3 (C-6), 19.8 (C-11), 19.4 (C-2), 14.0 (C-20).

VRE and vancomycin-susceptible enterococcus (VSE), with an MICs and MBCs values of 4 to 8 and 4 to 16 μg/mL, respectively.

Discussion

Strains of enterococcus are capable of causing a variety of opportunistic infection, such as periodontology, endodontology nephrology, hematology and oncology patients (Dahlen *et al*., 2000; Colombo *et al*., 1998; Moellering, 1992).

In order to identify herbs with antibacterial properties against VRE, medicinal herb traditionally used for gasterointestinal infection in Korea was tested against VRE. Among the herbal extracts tested, *A. cordata* extracts (methanol extraction, chloroform fraction, ethyl acetate fraction, and butanol fraction) showed some antimicrobial activities against *Enterococcus faecalis* and *Enterococcus gallinarium* as determined by the agar dilution method. As shown in Table 1, the chloroform soluble fraction demonstrated a higher inhibitory activity (MICs/MBCs, 32/32 μg/mL) than ethyl acetate (MICs/MBCs, 32/64 μg/mL), methanol (MICs/MBCs, 64/64 μg/mL) and butanol (MICs/MBCs, 128/128 μg/mL) soluble fractions against VRE. Therefore, the chloroform soluble fraction was further subjected to silica gel column chromatography in an attempt to isolate the active guided fractionation. From these active fractions, a white compound was purified by repeated silica gel column chromatography followed by prep-HPLC. The compound was characterized by 1D-NMR and 2D-NMR, which gave a spectrum that matched perfectly with that of the previously described continentalic acid (Han *et al*., 1983). Table 2 shows the MICs/MBCs of vancomycin and CA against VRE. CA exhibited potent activity against standard VRE and vancomycin-susceptible enterococcus (VSE), with an MICs and MBCs values of 4 to 8 and 4 to 16 μg/mL, respectively. CA has been reported to have various biological activities including anti-inflammatory activity, cytotoxic and COX-2 inhibitory activity (Han *et al*., 1983; Lee *et al*., 2006). In conclusion, these results suggest that CA from *A. continentalis* might be an effective inhibitor of VRE. However, for medicinal purposes, the safety and toxicity of this CA need to be addressed.

Conclusion

These findings suggested that continentalic acid might be useful in controlling enterococcal infection.

Acknowledgements

This paper was supported (in part) by Wonkwang University, 2006.

References

American Society for Microbiology (ASM) Task force on antibiotic resistance. Report of the ASM task force on antibiotic resistance. Antimicrob Agents Chemother. 1995; 39: 2-23.

Baek DH. Screening of antimicrobial activity among the therapeutic herbal extracts on dental pathogens. Int J Oral Biol. 2007;32:75-8.

Colombo AP, Haffajee AD, Dewhirst FE, Paster BJ, Smith CM, Cugini MA, Socransky SS. Clinical and microbiological features of refractory periodontitis subjects. J Clin Periodontol. 1998;25:169-80.

Dahlen G, Samuelsson W, Molander A, Reit C. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. Oral Microbiol Immunol. 2000;15: 309-12.

Dang NH, Zhang X, Zheng M, Son KH, Chang HW, Kim HP, Bae K, Kang SS. Inhibitory constituents against cyclooxygenases from *Aralia cordata* Thunb. Arch Pharm Res. 2005;28: 28-33.

Davis J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994;264: 375-81.

Han BH, Han YN, Han KA, Park MH, Lee EO. Studies on the anti-inflammatory activity of *Aralia continentalis* (I). Arch Pharm Res. 1983;6: 17-23.

Hur JM, Yang CH, Han SH, Lee SH, You YO, Park JC, Kim KJ. Antibacterial effect of *Phellinus linteus* against *methicillin-resistant Staphylococcus aureus*. Fitoterapia. 2004;75:603-5.

Jeong SI, Han WS, Yun YH, Kim KJ. Continentalic acid from *Aralia continentalis* shows activity against methicillin-resistant *Staphylococcus aureus*. Phytother Res.

2006;20:511-4.
Kim KJ, Kim JH. Anti-proliferative and anti-telomerase activity of *Curcuma* rhizome extract on oral squamous cell carcinoma and osteosarcoma cells. Int J Oral Biol. 2007;32:135-41.
Kim, KJ, Yu HH, Jeong SI, Cha JD, Kim SM, You YO. Inhibitory effects of *Caealpinia sappan* on growth and invasion of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 2004; 91: 81-7.
Kim KJ, Yu HH, Cha JD, Seo SJ, Choi NY, You YO. Antibacterial activity of *Curcuma longa* L. against methicillin-resistant *Staphylococcus aureus*. Phytother Res. 2005; 19: 599-604.
Kim JS, Kang SS. Saponins from the aerial parts of *Aralia continentalis*. Nat Prod Sci 1998;4: 45-50.
Lee IS, Jin W, Zhang X, Hung TM, Song KS, Seong YH, Bae K. Cytotoxic and COX-2 inhibitory constituents from the aerial parts of *Aralia cordata*. Arch Pharm Res. 2006; 29: 548-55.
Moellering RC. Emergence of enterococcus as a significant pathogen. Clin Infect Dis. 1992; 14: 1173-8.
Okuyama E, Nishimura S, Yamazaki M. Analgesic principles from *Aralia cordata* Thunb. Chem Pharm Bull 1991; 39: 405-7.
Seong CN, Shim ES, Kim SM, Yoo JC. Prevalence and characterization of vancomycin-resistant enterococci in chicken intestines and humans of Korea. Arch Pharm Res.2004; 27: 246-53.
Sinclair S. Chinese herbs: a clinical review of *Astragalus, Ligusticum*, and *Schizandrae*. Altern Med Rev.1998; 3: 338-44.
Spratt BG. Resistance to antibiotics mediated by target alterations. Science. 1994;264: 388-93.
Wikler MA, Bush K, Cockerill FR, Dudley MN, Eliopoulos GM, Hardy DJ,Hecht DW, Hindler JF, Patel JB, Powell M, Turnidge JD, Weinstein MP, Zimmer BL, Ferraro MJ, Swenson JM. Performance standards for antimicrobial susceptibility testing. Eighteenth Informational Supplement. M100-S18. pp 54-56. Clinical and Laboratory Standards Institute (CLSI). Wayne, PA, USA, 2005.