Antimicrobial Activities of 2-Methyl-8-Hydroxyquinoline and Its Derivatives against Human Intestinal Bacteria

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The survival of bacterial microbiota in the human intestine is largely dependent on the equilibrium that it is able to establish with its environment. Loss of this equilibrium is associated with gastrointestinal disease in humans [Canche-Pool *et al*., 2008]. Humans interact with a great deal of microorganisms that are classified in two groups, beneficial and harmful intestinal bacteria. The gut microbiota contributes to the host's immune system development, angiogenesis, fat storage, and nutrition. Thus, the balance between potentially beneficial and non-beneficial bacteria is very important [Myllyluoma, 2007]. *Clostridium perfringens*, a Gram-positive, spore-forming, non-motile, anaerobic rod-shaped bacterium producing a variety of virulence factors, is one of the main causes of food-borne illnesses [Angelotti *et al*., 1962; Byrne *et al*., 2008]. In contrast, bifidobacterium and lactobacillus of the beneficial bacteria play important roles in the protection against infection, cleaning the inside of intestines, and supplying of nutrients [Kim *et al*., 2006b; Kim and Yi, 2008]. Recently, antibiotics such as ampicillin, erythromycin, tetracycline, and vancomycin have been used against the exogenous pathogens. Antibiotics, however, tend to disturb the health of the intestinal microorganisms [Van Den Bogaard Jr and Weidema, 2008]. Furthermore, over-applied antibiotics can cause damages, such as microorganism susceptibility, vomiting, diarrhea, and stomachache [Chen *et al*., 1989; Kim *et al*., 2006a]. Therefore, interest on the development of new effective techniques to modulate the intestinal bacteria has been growing. In our previous study, the antimicrobial activities of 8-quinolinol isolated from the roots of *Sebastiania corniculata* were evaluated for selective-growth inhibition toward the human intestinal bacteria [Kim *et al*., 2006b]. Therefore, we reasoned that 8-quinolinol could be a lead to the selective antimicrobial agent. In this regard, the growth inhibitory effects of the 8-quinolinol derivatives including 2-methyl-8-hydroxy-quinoline on the intestinal bacteria were evaluated with regard to their ability to inhibit the human intestinal bacteria.

Bacterial strains used in this study were *Bifidobacterium bifidum* ATCC 29521, *Bifidobacterium longum* ATCC 15707, *Clostridium difficile* ATCC 9689, *Clostridium perfringens* ATCC 13124, *Escherichia coli* ATCC 11775, *Lactobacillus acidophilus* ATCC 4356, and *Lactobacillus casei* ATCC 393. Stock cultures of these strains were stored routinely on the eggerth gagnon (EG) liver extract-Field's slants at −80°C, and subcultured on EG agar (Eiken Chemical, Tokyo, Japan), when required. The plates including the subculture of these strains were incubated for 2 days at 37°C in an anaerobic chamber (Hirayama, Tokyo, Japan) in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2. The bacteria were subsequently grown in the BHI broth (pH 7.6) and the MRS broth.

Growth responses varied according to the tested chemicals and dose, as well as the bacterial strains. Antimicrobial activities of the test samples were used for the paper disc agar diffusion method. To determine the effects of the chemicals on the growth inhibition of the evaluated bacteria, one loopful of bacteria was suspended in 1 mL sterilized physiological saline. An aliquot (0.1 mL) of the bacterial suspension was seeded on the EG agar. The desired dose of the sample was then dissolved in 0.1 mL methanol, which was subsequently applied to a paper disc (Advantec, diameter 8 mm and thickness 1 mm, Toyo Roshi, Japan) using a Drummond glass microcapillary tube. After evaporation of the solvents, the disc was then placed on the surface of the agar plates that had been inoculated with the test bacteria. All plates were then anaerobically incubated for 2 days at 37°C. The control samples exerted no adverse effects against the tested organisms. All growth inhibition tests were conducted in triplicate, and the antimicrobial activity was determined by assigning one of the following values

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Abbreviations: BHI, brain heart infusion; EG, eggerth gagnon; MRS, deMan rogosa sharpe

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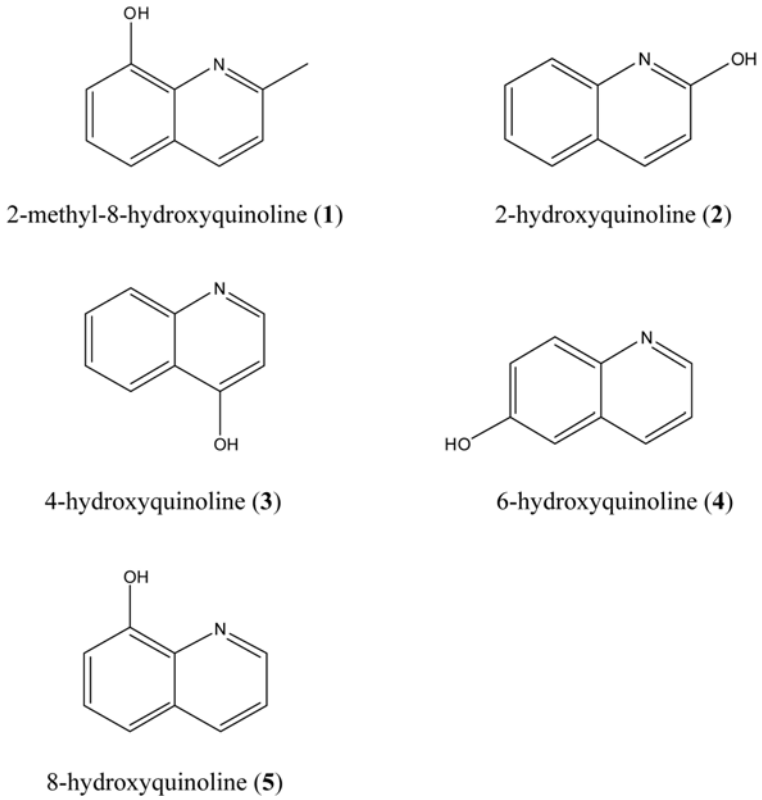

Fig. 1. Structures of 2-methyl-8-hydroxyquinoline and its derivatives.

based on the estimated size (diameter) of the zone of inhibition produced by the sample. The inhibitory responses were classified as previously described [Lim *et al*., 2007]: potent response (++++), zone diameter >30 mm; strong response (+++), zone diameter 21-30 mm; moderate response (++), zone diameter 16-20 mm; weak response (+), zone diameter 10-15 mm; and little or no response (-), zone diameter <10 mm.

In a test with *C. difficile*, 2-methyl-8-hydroxyquinoline produced strong and moderate inhibitions at 0.2 and 0.1 mg/disc, respectively. 2-Methyl-8-hydroxyquinoline also showed potent and strong growth inhibitions at 0.2 and 0.1 mg/disc and exhibited moderate and weak growth inhibitions at 0.05 and 0.02 mg/disc, respectively, against *C. perfringens*. In a test with *E. coli*, 2-methyl-8-hydroxyquinoline produced strong and moderate growth inhibitions at 1.0 and 0.2 mg/disc, respectively, but revealed weak growth inhibition at 0.1 mg/disc. Furthermore, 2-methyl-8-hydroxyquinoline had moderate and weak growth inhibitions against *B. longum* at 2.0 and 1.0 mg/disc, respectively, whereas no growth inhibition was observed against *B. bifidum*, *L. acidophilus*, and *L. casei*.

Study on the structure-activity relationship of 2-methyl-8-hydroxyquinoline derivatives revealed that the antimicrobial activity of 8-hydroxyquinoline against the seven intestinal bacteria was similar as that of 2-methyl-8-hydroxyquinoline. Table 1 shows the growth-inhibiting activities of 2-methyl-8-hydroxyquinoline and its derivatives against the intestinal bacteria. 8-Hydroxyquinoline exhibited strong and moderate growth inhibitions against *C. difficile* at 0.5 and 0.1 mg/disc, respectively, and exhibited weak growth inhibition at a low dose of 0.05 mg/disc. In the test with *C. perfringens*, 8-hydroxyquinoline showed potent and strong growth inhibitions at 0.5 and 0.1 mg/disc, respectively, and weak at 0.02 mg/disc. With *E. coli*, 8-hydroxyquinoline exhibited potent and strong growth inhibitions at 1.0 and 0.5 mg/disc, respectively, and moderate growth inhibition at 0.2 mg/disc. On the other hand, 6-, 4-, and 2-hydroxyquinoline showed no growth inhibition against all intestinal bacteria tested. In a similar study, Öllinger and Brunmark [1991] reported on the effect of hydroxyl substituent position on 1,4-naphthoquinone toxicity in the rat hepatocytes. Our results also showed that hydroxyl functional group in quinoline at different positions leads to increased or decreased growth inhibited activity against the intestinal bacteria. In particular, 2-methyl-8-hydroxyquinoline and 8-hydroxyquinoline the presence of the hydroxyl functional group at the C_8-position allowed effective growth inhibition against *C. difficile*, *C. perfringens*, and *E. coli*, whereas the methyl functional groups of 2-methyl-8-hydroxyquinoline derivatives

Table 1. Growth-inhibiting activities of 2-methyl-8-hydroxyquinoline and its derivatives against intestinal bacteria

Compound[c]	Dose (mg/disc)	Bacteria strain[a]						
		B. bifidum	*B. longum*	*C. difficile*	*C. perfringens*	*E. coli*	*L. acidophilus*	*L. casei*
2-Methyl-8-hydroxyquinoline	2.0	-[b]	++	++++	++++	+++	-	-
	1.0	-	+	++++	++++	+++	-	-
	0.5	-	-	++++	++++	++	-	-
	0.2	-	-	+++	++++	++	-	-
	0.1	-	-	++	+++	+	-	-
	0.05	-	-	+	++	-	-	-
	0.02	-	-	-	+	-	-	-
8-Hydroxyquinoline	2.0	-	++	++++	++++	++++	-	-
	1.0	-	+	+++	++++	++++	-	-
	0.5	-	-	+++	++++	+++	-	-
	0.2	-	-	++	+++	++	-	-
	0.1	-	-	++	+++	+	-	-
	0.05	-	-	+	++	-	-	-
	0.02	-	-	-	+	-	-	-
6-Hydroxyquinoline	2.0	-	-	-	-	-	-	-
4-Hydroxyquinoline	2.0	-	-	-	-	-	-	-
2-Hydroxyquinoline	2.0	-	-	-	-	-	-	-

[a]Cultured on EG agar at 37°C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2. [b]Inhibitory zone diameter >30 mm, ++++; 21-30 mm, +++; 16-20 mm, ++; 10-15 mm, +; and <10 mm, -. [c]Each assay was determined in triplicate.

appeared ineffective against *C. difficile*, *C. perfringens*, and *E. coli*.

Recently, 1,4-naphthoquinone derivatives, quinoline, and quinolinol isolated from medicinal plants have been studied as antimicrobial agents [Cho *et al.*, 2005; Kim *et al.*, 2006b; Lim *et al.*, 2007]. Furthermore, they have been used in medicines and food industries as antifungal, antibacterial, and antiprotozoic drugs [Musiol *et al.*, 2005]. Quinaldine is a simple derivative of a heterocyclic compound quinoline. Quinaldine has been widely used in industries as an anti-malaria drug, in manufacturing dyes, as food colorant (e.g. quinoline yellow) and a pH indicator [Mohan *et al.*, 1999; Rodrigues and de Dominguez, 2007]. Many studies have been performed to develop novel chemotherapeutic agents from quinolines. However, in spite of the potential physiological activities of the quinolines, little work has been carried out on the effect of the compounds on the growth of intestinal bacteria. Further studies on the quinoline derivatives, especially quinaldine, should be carried out with regard to the safety issues on humans, formulations for improving the antimicrobial stability and potency, and antimicrobial mode of action.

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