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NOTE

Quercetin 7-*O*-glutamate sensitizes *Escherichia coli* to vancomycin

Kwang-Su Park[1] · Woong Jung[2] · Youhoon Chong[1] · Mi Kyoung Kim[1]

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Abstract We have reported previously that conjugation of glutamic acid at the 7-*O* position of quercetin could significantly enhance its biological activity. In this study, as a part of our ongoing efforts to investigate the therapeutic potential of this novel quercetin–glutamate conjugate (**1**), we evaluated the effect of its combination with vancomycin against Gram-negative *Escherichia coli*, which are not normally affected by vancomycin alone. Quercetin conjugate **1** was shown to potentiate the antibacterial activity of vancomycin against *E. coli*, which could be attributed to its membrane perturbation activity, but not to facilitation of the efflux pump-mediated penetration of vancomycin.

Keywords Antibacterial · Conjugate · Gram-negative · Quercetin · Sensitization · Vancomycin

Introduction

Quercetin (Fig. 1), a representative flavonoid found in many plants including apples, peppers, grape skins, and red onions, is characterized by various health-promoting effects such as anti-proliferative, anti-inflammatory, antiviral, and antibacterial effects (Bischoff 2008). However, quercetin is also well known for its chemical and metabolic instability, which limits its therapeutic use. We have reported previously that the introduction of a functional promoiety to the phenolic hydroxyl group of quercetin results in its protection from chemical breakdown and metabolic changes (Kim et al. 2009, 2010, 2012; Cho et al. 2013; Kim et al. 2014, 2015). In particular, conjugation of glutamic acid at the 7-*O* position of quercetin provided the resulting quercetin conjugate, Q-7-*O*-Glu (**1**) (Fig. 1), with a significantly higher biological activity than quercetin (Kim et al. 2014).

Based on the various pharmacological activities of quercetin, it was naturally anticipated that Q-7-*O*-Glu (**1**) might also exhibit a wide range of biological activities, which spurred extensive investigation around the therapeutic uses of this novel quercetin conjugate. In this study, we exploited the ability of quercetin to increase the permeability of the bacterial membrane (Siriwong et al. 2016), which would sensitize Gram-negative bacteria to large antibiotics such as vancomycin.

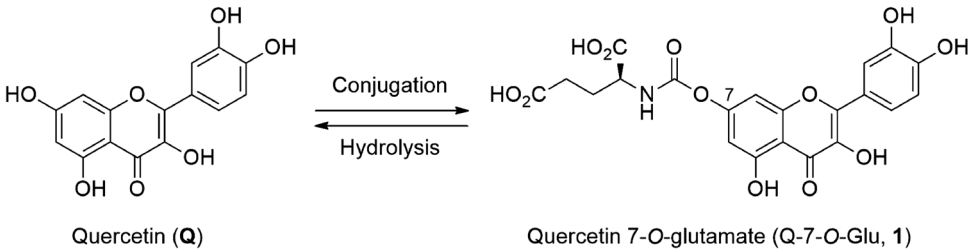
Vancomycin is famous for its antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA), which makes it a valuable asset in the endless battle against drug-resistant pathogens. However, vancomycin is not active against Gram-negative bacteria because of their characteristic outer membrane, which works as an effective barrier against this large antibacterial glycopeptide (Nikaido 2003). Considering global health threats imposed by the lack of treatment options for Gram-negative bacteria that are resistant to a wide spectrum of antibiotics, development of novel compounds effective against those bacteria is highly imperative. Nevertheless, insufficient understanding of the outer membrane permeability has

✉ Youhoon Chong
chongy@konkuk.ac.kr

✉ Mi Kyoung Kim
mkkim@konkuk.ac.kr

[1] Department of Integrative Bioscience & Biotechnology, Bio/Molecular Informatics Center, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea

[2] Department of Emergency Medicine, Kyung Hee University Hospital at Gangdong, Seoul 134-727, Republic of Korea



Quercetin (**Q**) Quercetin 7-*O*-glutamate (Q-7-*O*-Glu, **1**)

Fig. 1 Structures of quercetin (Q) and its 7-*O*-glutamate conjugate (Q-7-*O*-Glu, **1**)

hampered the campaign of novel drug discovery. Instead, significant efforts have been made to render Gram-negative bacteria susceptible to other clinically useful antibiotics including vancomycin (Bolla et al. 2011).

Since the impermeability of the bacterial outer membrane to vancomycin is the key factor for its lack of antibiotic activity against Gram-negative bacteria, the most straightforward way to solve this problem is to disrupt the bacterial membrane. Recently, Stokes et al. reported that the Gram-negative bacteria, *E. coli,* became susceptible to vancomycin through altering its outer membrane integrity by cold stress (Stokes et al. 2016). However, chemical entities with membrane-disrupting properties have rarely been reported. Quercetin is one of the membrane permeabilizers known to interact with bacterial membranes (Siriwong et al. 2016). However, the effect of a combination of quercetin and vancomycin against Gram-negative bacterial infections has not been reported. Therefore, in this study, we attempted to sensitize the Gram-negative bacteria, *E. coli*, to vancomycin by combining it with quercetin or its glutamate conjugate **1**.

Materials and methods

Chemicals

Quercetin 7-*O*-glutamate (Q-7-*O*-Glu, **1**) was prepared according to a previously reported method (Kim et al. 2014), and vancomycin hydrochloride hydrate was purchased from Sigma-Aldrich.

Bacterial strains

Wild-type *E. coli* strain MG1655 and its deletion mutants—Δ*acr*B (ΔB) and Δ*tol*C (ΔC)—were obtained from the Coli Genetic Stock Center (CGSC) at Yale University.

Antibiotic susceptibility testing

A broth dilution method was used to determine the antimicrobial susceptibility of wild-type (MG1655) and mutant (Δ*acr*B and Δ*tol*C) *E. coli* strains in Muller-Hinton broth to vancomycin in the absence and presence of quercetin conjugate (**1**). Antimicrobial susceptibility tests of wild-type and mutant *E. coli* strains to a combination of quercetin (256 μg/mL) or **1** (256 μg/mL) and six serial dilutions of vancomycin (512 μg/mL) were performed.

Results and discussion

First, antibacterial activities of vancomycin, quercetin, and **1** were evaluated in wild-type *E. coli* MG1655. In this strain, neither quercetin nor **1** was found to have bactericidal activity up to a concentration of 512 μg/mL (data not shown). On the other hand, as shown by Zhou et al. (2015), Gram-negative bacteria were not totally insensitive to vancomycin, as they were susceptible to high concentrations (Fig. 2). The weak bactericidal effect of vancomycin against wild-type *E. coli* MG1655 was observed at concentrations of 256 μg/mL and higher, while its minimum inhibitory concentration (MIC) for wild-type *E. coli* was higher than 512 μg/mL (VAN, Fig. 2). Combination of

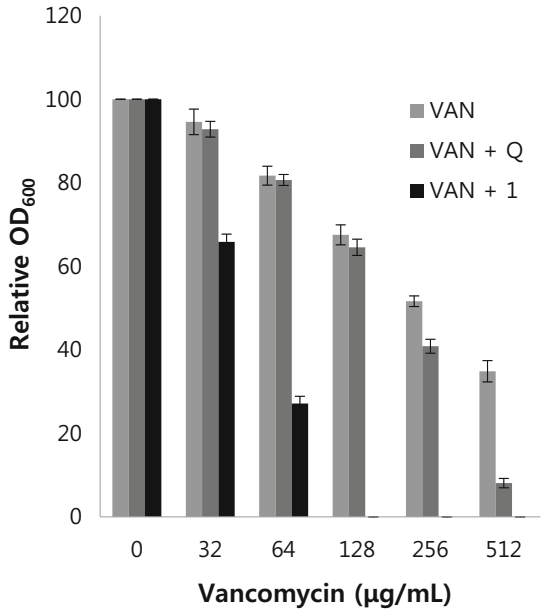


Fig. 2 Antibacterial activity of vancomycin (VAN) alone and vancomycin in combination with quercetin (VAN + Q) or Q-7-*O*-Glu (VAN + **1**) (256 μg/mL) against wild-type *E. coli* MG1655

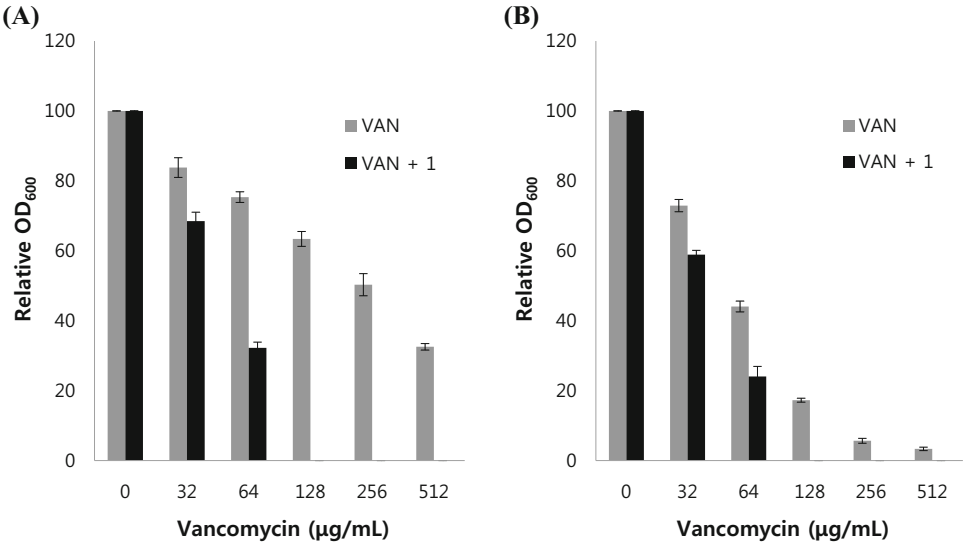


Fig. 3 Analysis of the potency of vancomycin (VAN) in combination with **1** (256 μg/mL) against (**A**) *E. coli* Δ*acr*B (ΔB) strain and (**B**) *E. coli* Δ*tol*C (ΔC) strain

vancomycin with 256 μg/mL of quercetin (VAN + Q, Fig. 2) did not result in a significant change in MIC of vancomycin. In contrast, the wild-type *E. coli* strain became more susceptible to vancomycin upon addition of Q-7-*O*-Glu (**1**) (256 μg/mL), and the MIC of vancomycin decreased to 128 μg/mL (VAN + **1**, Fig. 2). Therefore, as initially proposed, combination with **1** potentiated the antibacterial activity of vancomycin against *E. coli*, which might be attributed to perturbation of the outer bacterial membrane mediated by **1**.

On the other hand, vancomycin is also known to slip through an AcrAB–TolC system, a representative multidrug efflux pump of *E. coli* (Weeks et al. 2010; Krishnamoorthy et al. 2013), to accumulate inside the bacteria. Therefore, facilitation of efflux pump-mediated influx of vancomycin could be proposed as another mechanism of vancomycin potentiation, also mediated by **1**. According to this mechanism, defection of efflux pumps could lead to the abrogation of vancomycin-potentiating effect of **1**. In order to prove this hypothesis, we obtained *E. coli* strains lacking the key components of the AcrAB–TolC efflux pump, AcrB (ΔB) and TolC (ΔC), and evaluated their susceptibility to vancomycin (Fig. 3).

Compared with the wild-type *E. coli* (Fig. 2A), *E. coli* ΔB (Fig. 3A) and ΔC (Fig. 3B) strains did not show a decrease in susceptibility to vancomycin, which suggests that the efflux pump components AcrB and TolC are not related to penetration of vancomycin through bacterial cell membranes. Moreover, the *E. coli* ΔC strain was more sensitive than the wild-type *E. coli* to vancomycin (Fig. 3B), and this result indicates that TolC might be working as a barrier rather than a channel for intracellular accumulation of vancomycin. The differences between the vancomycin susceptibilities of these two mutant strains were abolished upon co-administration of vancomycin with **1** (256 mg/mL), and they became equally susceptible to vancomycin with an MIC of 128 μg/mL (Fig. 3A, B). Therefore, these results suggested that the vancomycin potentiation effect of **1** can hardly be attributed to facilitating penetration of vancomycin through the AcrB–TolC efflux pump of the Gram-negative bacteria.

Taken together, we have shown that the quercetin–glutamate conjugate **1** could potentiate the antibacterial activity of the large glycopeptide, vancomycin, against the Gram-negative bacteria, *E. coli*. The vancomycin potentiation activity of **1** could be attributed to the membrane perturbation activity, but not to facilitation of the efflux pump-mediated penetration of vancomycin. The detailed study of the mechanism by which **1** potentiates the activity of vancomycin against Gram-negative bacteria is underway and it will be reported in due course.

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References

Bischoff SC (2008) Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 11:733–740

Bolla J-M, Alibert-Franco S, Handzlik J, Chevalier J, Mahamoud A, Boyer G, Kieć-Kononowicz K, Pagès J-M (2011) Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. FEBS Lett 585:1682–1690

Cho SY, Kim MK, Park K-S, Choo H, Chong Y (2013) Quercetin-POC conjugates: differential stability and bioactivity profiles between breast cancer (MCF-7) and colorectal carcinoma (HCT116) cell lines. Bioorg Med Chem 21:1671–1679

Kim MK, Park K-S, Yeo W-S, Choo H, Chong Y (2009) In vitro solubility, stability and permeability of novel quercetin-amino acid conjugates. Bioorg Med Chem 17:1164–1171

Kim MK, Park K-S, Lee C, Park HR, Choo H, Chong Y (2010) Enhanced stability and intracellular accumulation of quercetin by protection of the chemically or metabolically susceptible hydroxyl groups with a pivaloxymethyl (POM) promoiety. J Med Chem 53:8597–8607

Kim MK, Park K-S, Chong Y (2012) Remarkable stability and cytostatic effect of a quercetin conjugate, 3,7-bis-O-pivaloxymethyl (POM) quercetin. ChemMedChem 7:229–232

Kim MK, Choo H, Chong Y (2014) Water-soluble and cleavable quercetin-amino acid conjugates as safe modulators for P-glycoprotein-based multidrug resistance. J Med Chem 57:7216–7233

Kim MK, Park K-S, Choo H, Chong Y (2015) Quercetin-POM (pivaloxymethyl) conjugates: modulatory activity for P-glycoprotein-based multidrug resistance. Phytomedicine 22:778–785

Krishnamoorthy G, Tikhonova EB, Dhamdhere G, Zgurskaya HI (2013) On the role of TolC in multidrug efflux: the function and assembly of AcrAB-TolC tolerate significant depletion of intracellular TolC protein. Mol Microbiol 87:982–997

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656

Siriwong S, Teethaisong Y, Thumanu K, Dunkhunthod B, Eumkeb G (2016) The synergy and mode of action of quercetin plus amoxicillin against amoxicillin-resistant *Staphylococcus epidermidis*. BMC Pharmacol Toxicol 17:39

Stokes JM, French S, Ovchinnikova OG, Bouwman C, Whitfield C, Brown ED (2016) Cold stress makes *Escherichia coli* susceptible to glycopeptide antibiotics by altering outer membrane integrity. Cell Chem Biol 23:267–277

Weeks JW, Celaya-Kolb T, Pecora S, Misra R (2010) AcrA suppressor alterations reverse the drug hypersensitivity phenotype of a TolC mutant by inducing TolC aperture opening. Mol Microbiol 75:1468–1483

Zhou A, Kang TM, Yuan J, Beppler C, Nguyen C, Mao Z, Nguyen MQ, Yeh P, Miller JH (2015) Synergistic interactions of vancomycin with different antibiotics against *Escherichia coli*: trimethoprim and nitrofurantoin display strong synergies with vancomycin against wild-type *E. coli*. Antimicrob Agents Chemother 59:276–281