

Full Length Research Paper

Antibacterial activity of quercetin on oral infectious pathogens

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The aim of this study was to evaluate the antimicrobial activity of quercetin against pathogens of the main oral infectious diseases which include caries, periodontitis and oral mucosa infectious diseases associated microorganisms. Agar diffusion assay was adopted to observe the effects of quercetin on the growth of 11 main oral pathogenic microorganisms, including *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus sobrinus*, *Actinomyces viscosus*, *Actinomyces naeslundii*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Candida albicans*. The antibacterial activity of quercetin was determined in form of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) using agar dilution assay. In all experiments, results obtained indicated that quercetin had inhibitory effects on *S. mutans* with MIC of 2 mg/ml and MBC of 8 mg/ml, *S. sobrinus* with MIC of 1 mg/ml and MBC of 8 mg/ml, *L. acidophilus* with MIC of 2 mg/ml and MBC of 16 mg/ml, *S. sanguis* with MIC of 2 mg/ml and MBC of 16 mg/ml, *A. actinomycetemcomitans* with MIC of 1 mg/ml and MBC of 8 mg/ml, *P. intermedia* with MIC of 4 mg/ml and MBC of 16 mg/ml, respectively. Thus, it could be concluded that quercetin had different antibacterial activities against oral bacteria, in which the quercetin showed better antibacterial effect on caries-related bacteria, and could be encouraged for further development in caries prevention and treatment.

Key words: oral bacteria, quercetin, antibacterial, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

Oral cavity harbors a complex microbial community consisting of over 600 different nonharmful/commensal microbial species together, in form of commonly known as "dental plaque", with a limited number of specified pathogens including cariogenic and periodontogenic bacteria (Paster et al., 2001; Aas et al., 2005). Under certain pre-disease condition, the pathogens within the microbial community overgrow and are responsible for the development of oral infectious diseases such as dental caries and periodontitis (Marsh, 1994). Dental caries and periodontitis are two of the most prevalent chronic infectious oral diseases and causing a serious health problem if left untreated, such as high risks of cardiovascular diseases (McNicol and Israels, 2010),

osteomyelitis and pneumonia (Feres and Figueiredo, 2009). The prevention of oral infectious diseases has attracted great attention for several decades. However, the concept of using mechanical means to remove or chemical agent to kill all the bacteria in oral community has its limitations, because the mechanical removal of bacteria could not alter the disease-associated micro-environment for pathogens adhering to tooth or mucosa surfaces, and the use of chemical agents would disturb oral flora balance which might cause the second infection (Screenivasan and Gaffar, 2002). Furthermore, the synthetic drugs have been associated with severe side effects on human health. Due to these facts, it is urgent to explore an alternative antibacterial drug, which can affect the oral pathogenic bacteria with less drug-resistance, for rebuilding/maintaining a "healthy" microbial community (Valiathan, 1998). Recently, there has been a growing interest in the investigation and introduction of medicinal

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plants with various biological activities at the aspect of new drug development because of the advantages of ample materials source, ease of use, good efficacy and small side effects (Majorie, 2009). Quercetin is a natural extract of flavonoids which widely exist in many parts of plants (Toda et al., 1989; Dixon et al., 1983). Many studies have demonstrated that it had obvious pharmaceutical effects of anti-tumor (Feng et al., 2011), anti-oxidation (Chondrogianni et al., 2010), anti-inflammatory (Yamaguchi and Weitzmann, 2011), cardiovascular protection (Perez-Vizcaino and Duarte, 2010) and anti-viral (Mahmood et al., 1996). Recently, it has been confirmed that quercetin have ability to inhibit the acidogenicity of *Streptococcus mutans* (Gregoire et al., 2007). In this study, we aimed to evaluate the antibacterial ability of quercetin on the main oral infectious diseases-associated pathogens including cariogenic, periodontogenic and mucosa infectious micro-organisms.

MATERIALS AND METHODS

Bacterial stains and growth conditions

Well-defined, commonly used laboratory strains of 11 standard test bacterial species were kindly donated by the State Key Laboratory of Oral Disease, Sichuan University as follows: *S. mutans* ATCC25175, *S. aobrinus* 6715, *S. sanguis* ATCC10556, *L. acidophilu* ATCC4356, *A. naeslundii* ATCC12140, *A. viscosus* ATCC19246, *P. gingivalis* 381, *P. intermedia* ATCC25611, *F. nucleatum* 10953, *A. actinomycetemcomitans* 29523, *C. albicans* ATCC10691. *C. albicans* was resuscitated in BA agar media at 37°C in an aerobic atmosphere (5% CO₂) and other bacteria were resuscitated in BHI agar media at 37°C in an anaerobic atmosphere (80% N₂, 10% CO₂ and 10% H₂) for 48 h. Then typical colonies were selected and re-grown in fresh BA broth under aerobic condition and BHI broth under anaerobic condition at 37°C overnight. The concentrations of these bacterial suspensions were adjusted at 1×10⁹ CFU/ml for later experiment.

Preparation of quercetin solution

In order to examine the antibacterial activity of quercetin on oral pathogenic microorganisms, the quercetin purchased from Sigma was dissolved in dimethyl sulphoxide (DMSO, Sigma) and kept in -20°C overnight. Subsequently, PBS was added into the quercetin stock solutions to prepare the treatment solutions of various concentrations with low DMSO ratio (20%).

Antibacterial activity evaluation of quercetin on the growth of bacteria

In order to observe the antibacterial activity of quercetin on the 11 kinds of oral pathogenic microorganisms, agar well diffusion method was carried out in this study. 2 ml previously prepared bacterial suspension was mixed respectively with 18 ml agar culture media to make the agar medium containing 1×10⁹ CFU/ml of bacteria and four respective wells were drilled in these agar media using sterile punch. After addition of 10 µl of 4 mg/ml quercetin solution, PBS as negative control, 20% DMSO as solvent control and chlorhexidine (0.05%) as positive control in one well respectively, these bacterial

agar media were incubated in at 37°C in either aerobic or anaerobic atmosphere for 48 h. The area surrounding the well without bacteria growth was measured to determine antibacterial activity observed. All assays were performed in triplicate and repeated at least three times. The growths of those strains affected by quercetin were selected for further experiments.

Assay of MIC and MBC

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of quercetin on oral bacteria were assessed by agar dilution technique using the National Committee for Clinical and Laboratory Standards (NCCLS 1997) method. A series two-fold dilution quercetin agar media was prepared ranging from 0.5 - 32 mg/ml and 5 µl of 1×10⁸ CFU/ml bacterial suspension previously prepared was dropped on the each drug-agar medium to measure the MICs, The plates which show no colony growth after 24 h incubation were defined as MICs and selected. 0.1 ml of sterile distilled water was added to these plates and transferred to fresh medium with no quercetin and the lowest concentration without bacterial growth after fresh medium re-growth was MBCs. The negative, positive and solvent controls were described as before. And all experiments were done in triplicate and repeated at least three times.

RESULTS

In this study, we adopted agar well diffusion method to roughly evaluate the inhibitory effect of quercetin on main oral pathogenic microorganisms and further determine the MIC and MBC of these selected bacteria using agar dilution assay. Among the 11 oral microorganisms tested, we observed the inhibitory effect on the growth of 6 bacterial strains (Table 1). Table 2 shows the antibacterial activity of quercetin on the selected bacteria with MIC ranging from 1 - 4 mg/ml and MBC ranging from 8 - 16 mg/ml.

DISCUSSION

Many natural plants and their extracts have been used for medical purpose for centuries and their biological and pharmacological effects have attracted more and more investigations in recent years (Moerman, 1996; Cisowska et al., 2011). Flavonoids, a main bioactive compounds in many natural products, such as green tea (Xu et al., 2010, 2011), cranberry (Yamanaka et al., 2007), and *Rubus ulmifolius* (Martini et al., 2009), have been demonstrated to be effective antimicrobial substances against a wide array of microorganisms, possibly due to their ability to disrupt microbial membrane and form complex with extracellular and soluble proteins (Tsuchiya et al., 1996; Cushnie and Lamb, 2005). Quercetin, one kind of flavonoid, has been widely investigated with bioactivity to inhibit Gram-positive and Gram-negative bacteria through the inactivating extracellular proteins (Martini et al., 2004; Koo and Jeon, 2009).

More than 600 microbial species habitants in oral cavity

Table 1. The inhibitory of 4 mg/ml quercetin on the growth of 11 main oral pathogenic microorganisms.

Bacterial strain	Drug solution	Positive control	Negative control	Solvent control
<i>S. mutans</i>	+	+	-	-
<i>S. sanguis</i>	+	+	-	-
<i>S. sobrinus</i>	+	+	-	-
<i>A. viscosus</i>	-	+	-	-
<i>A. naeslundii</i>	-	+	-	-
<i>L. acidophilu</i>	+	+	-	-
<i>P. gingivalis</i>	-	+	-	-
<i>F. nucleatum</i>	-	+	-	-
<i>A. actinomycetemcomitans</i>	+	+	-	-
<i>P. intermedia</i>	+	+	-	-
<i>C. albicans</i>	-	+	-	-

+ : inhibitory zone; - : without inhibitory zone.

Table 2. MIC and MBC of quercetin against oral bacteria.

Bacterial strain	MIC(mg/ml)	MBC(mg/ml)
<i>S. mutans</i>	2	8
<i>S. sobrinus</i>	1	8
<i>S. sanguis</i>	2	16
<i>L. acidophilu</i>	2	16
<i>P. gingivalis</i>	4	16
<i>A. actinomycetemcomitans</i>	2	8

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

form commensal microflora in normal condition to participate the role of "health keeper", including cariogenic, periodontogenic and mucoa infection-associated microorganisms (Dewhirst et al., 2010). Under certain conditions, these pathogens overgrew and started the pathogenesis (He et al., 2009). It has been under more and more investigations to explore plant products and their biological compounds to control the flora microbiosystem balance in purpose of reestablishing and maintaining oral health and overcoming the shortage and side effect of mechanical and chemical compounds. In this study, we selected several oral pathogenic bacteria species inhibited by quercetin among 11 microorganisms and validated the antibacterial ability of quercetin. The results showed that the cariogenic bacteria were more sensitive to quercetin than periodontitis-associated bacteria with relatively low MIC and MBC value, which requires almost 4 - 8 times of MIC. Our results suggest that quercetin could be an alternative for exploring new natural drug in use of caries treatment. It has been demonstrated that the antibacterial mechanism of quercetin probably depended on disruption of the membrane and inactivation of extracellular proteins by forming irreversible complexes but the exact mechanism remains unclear (Cowan, 1999). It is proposed to work on the acidogenesis-relating gene and find the expression

protein differences under biofilm condition after quercetin dealing.

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