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Research Article

# Quercetin Reduces *Staphylococcus aureus* Interaction With Neutrophils

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ABSTRACT
Flavonoids, including quercetin, have been reported to modulate the ability of *Staphylococcus aureus* to adhere to host tissue without exhibiting direct antibacterial activity. In the present study, we evaluated the interaction of *S. aureus* pretreated with 40 µg/mL of quercetin with neutrophils to assay oxidative burst stimulation, using luminol-amplified chemiluminescence. *S. aureus* pre-incubated with subinhibitory concentration of quercetin induced significantly less light emission by neutrophils than did untreated bacteria. The results of the present study demonstrate that quercetin decreases *S. aureus* uptake by neutrophils.

KEYWORDS: Quercetin, *Staphylococcus aureus*, neutrophils, oxidative burst.

## INTRODUCTION

*Staphylococcus aureus* produces a wide variety of molecules that are important in the interaction with the host organism. Many surface proteins are covalently anchored to the bacterial cell-wall peptidoglycan through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases termed sortases[1]. Two sortase isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in *Staphylococcus aureus* (Mazmanian et al., 2001)[2]. The SrtA isoform plays a critical role in the pathogenesis of gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue and others virulence associated proteins to cell wall peptidoglycans[3]. Proteins such as protein A and fibronectin-binding proteins are attached to the cell wall by this type of reaction[2,4].

It has been suggested that sortase inhibitors should act as anti-infective agents and disrupt the pathogenesis of bacterial infections without affecting microbial viability[5]. Therefore, inhibitors of SrtA might be promising candidates for the treatment and/or prevention of gram-positive bacterial infections. Since sortases were identified, there have been several investigations for sortase inhibitors of natural and synthetic origin[6-9].

Flavonoids represent a large group of plant phenols. These compounds can be classified into flavones, flavonols, flavanones, isoflavones, and anthocyanidins. Naturally occurring flavonols have been reported to inhibit the adhesion of *S. aureus* to fibrinogen via inhibition of sortases and preliminary findings on the influence of structure on activity suggest that the co-occurrence of a hydroxyl group at C-3 of ring C and meta-hydroxy groups at C-2´ and C-4´ of the B ring is required for appreciable sortase inhibition[10].

Sortase is essential for the functional assembly of surface proteins and for the pathogenesis of *S. aureus*. The effect of polyphenols on staphylococcal adherence to eukaryotic cells has been reported[11,12], but the influence of theses compounds on bacterial interaction with phagocytes is also an important task. This study investigated the influence of pretreatment of *S. aureus* with subinhibitory concentration of quercetin on phagocytosis by neutrophils.

## MATERIALS AND METHODS

Chemicals and Culture Media: Quercetin (Q0125), DMSO (D8779), Ficoll-PaqueTM (D1047), oyster glycogen, luminol and Zymosan A were obtained from Sigma Chemicals Co. (St. Louis, MO, USA), Mueller-Hinton broth was purchased from Difco (Detroit, MI, USA).

Evaluation of antibacterial activity: The susceptibility of *S. aureus* ATCC 25923 (Fundação Oswaldo Cruz, RJ, Brazil) to quercetin was performed by the microdilution method as recommended by the Clinical and Laboratory Standards Institute[13]. Quercetin was dissolved in DMSO to give a stock solution of 10 mg/mL. The stock solution was diluted serially in Mueller-Hinton broth and dilutions from 0.46 to 120 µg/mL were inoculated with *S. aureus* ($10^8$ colony-forming units/mL). The final concentration of DMSO in the assay did not affect bacterial growth. All tests were performed in triplicate with controls containing standard antimicrobials. The concentration of quercetin selected for test was 40 µg/ mL, which was found to inhibit the bacterial growth by 50% relative to the quercetin-free control after 24 h of incubation.

Chemiluminescence Assay: Bacteria were grown overnight at 37°C in Mueller-Hinton broth either in the presence or in the absence of quercetin (40 µg/mL),

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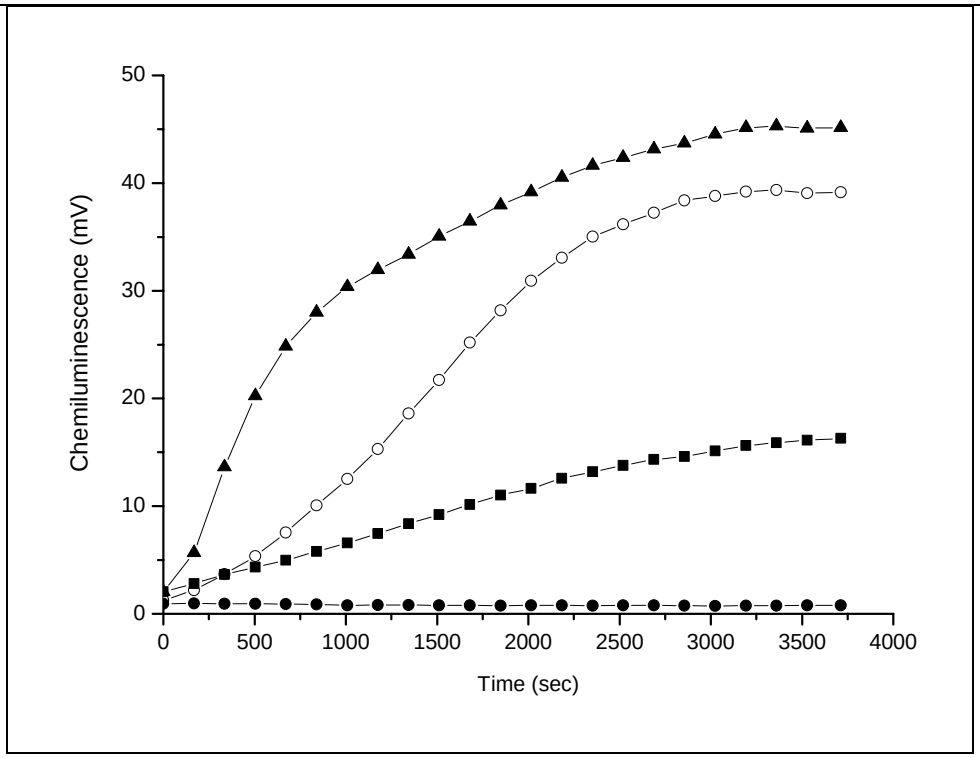


Figure 1. Luminol-enhanced chemiluminescence of neutrophils: (▲) zymosan (1mg/mL) only; (●) phosphate-buffered saline without calcium only as the background; (■) quercetin-treated bacteria; (○) bacteria without treatment. Results are the mean of at least three independent experiments (P = 0.000422)

washed three times in Dulbecco's phosphate buffered saline without calcium (PBS-D), and the suspension was adjusted photometrically to 2 X $10^8$ colony-forming units/mL. The phagocytic process was evaluated using luminol-enhanced chemiluminescence assay[14] to follow the respiratory burst of neutrophils exposed to staphylococci grown with or without quercetin in the medium. Rat peritoneal granulocytes were obtained by intraperitoneal injection of 10 mL of oyster glycogen solution 0.5% (w/v). Peritoneal exudate cells (PECs) were collected 12h later with PBS-D containing 10 IU heparin/mL. The cells were washed twice and carefully layered onto 5 mL of Ficoll-PaqueTM and centrifuged for 30 min at 700 g. The granulocytes were washed twice with Dulbecco's phosphate buffered saline and adjusted to a concentration of 2.0 x $10^6$ cells/mL. The proportion of neutrophils in the PECs was determined by cell staining with May-Grünwald-Giemsa. Cell preparations contained over 95% neutrophils. Neutrophis (2.0 x $10^6$ cells/mL) and luminol (2 x $10^{-5}$ M) were added to a tube containing PBS-D. This vial was placed in a lightproof chamber of a BioOrbit model 1251 luminometer (Turku, Finland) and the carousel was rotated to bring the sample in line with the photomultiplier tube, to record background activity. The stimulus, consisting of 1mg/mL Zymosan A or bacteria, was added to the suspension of neutrophils at a final volume of 1.0 mL. The chemiluminescence response was expressed as the integrated area (IA) below the resulting chemiluminescence curve recorded over a period of 0 to 60 min, calculated by the program Multiuse 2.0. This integrated light emission was used as the analytical parameter. All experiments were carried out in triplicate and repeated at least three times. Statistical difference between groups was determined by one-way analysis of variance (ANOVA). A difference was considered statistically significant when the P-value was $<0.05$.

## RESULTS AND DISCUSSIONS

Flavonoids are a family of plant-derived compounds with potentially exploitable activities, including direct antibacterial activity, synergism with antibiotics, and suppression of bacterial virulence[15]. Currently, there have researches in the literature describing the potential bacterial antiadhesion activity exhibit by flavonoids[9-11]. In the present study, interaction between *S. aureus* pretreated with 40 µg/mL of quercetin (MIC > 120 µg/mL) and neutrophils was investigated in vitro by luminol-enhanced chemiluminescence. Luminol is membrane permeable and thus excited by both intra- and extracellular produced reactive oxygen species formed by phagocytes[16]. The chemiluminescence response to the bacteria treated with quercetin (IA = 60276 ± 6188 mV) was significantly lower than that obtained with the untreated bacterial cells (IA = 88965 ± 8488mV) ($p<0.05$) (Figure 1). The decrease in the response of the neutrophils can be explained by inhibition of the synthesis or expression of adhesion factor that mediate interactions with phagocytes. This study does not have the proposal to identify mechanisms by which quercetin is able to reduce the bacterial uptake by neutrophils.

*S. aureus* expresses a lot of cell surface-associated proteins (adhesins) that interact with various components of extracellular matrix of eukaryotes. *S. aureus* microbial surface components recognizing adhesive matrix molecules (MSCRAMMs family) are involved in the first step of *S. aureus* infections. MSCRAMMs recognize extracellular matrix components such as fibrinogen and fibronectin, and are anchored to the bacterial wall peptidoglycan by a mechanism that involves the enzyme sortase[17, 18].

The correlation between antibacterial activity and membrane interference has previously been described for sophoraflavonone G (flavanone)[19], catechins (flavan-3-ols)[20], and galangin (flavonol)[21]. Even though quercetin at a low level has little effect on *S. aureus* viability, the knowledge that this flavonol is able to affect the adherence of staphylococci to cell surfaces may represent an advance for its potential application in the prevention of infections, considering that bacterial adherence is the initial event in the pathogenesis of bacterial infection. As both adherence and phagocytosis occur in association with polymorphonuclear leucocytes during infection, from this experiment we conclude that quercetin, paradoxically, may contribute to staphylococcal pathogenesis during the inflammatory stages of staphylococcal disease by decreasing the bacterial uptake by phagocytes.

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REFERENCES

1. Paterson GK, Mitchell TJ. The biology of Gram-positive sortase enzymes. *Trends of Microbiology* 2004; 12: 89-95.
2. Mazmanian SK, Ton-That H, Scheneewind, O. Sortase-catalysed anchoring of surface proteins to the cell wall of *Staphylococcus aureus. Molecular Microbiology* 2001; 40:1049-1057.
3. Weiss WJ, Lenoy E, Murphy T, Tardio L. Effect of *srtA* and *srtB* gene expression on the virulence of *Staphylococcus aureus* in animal models of infection. *Journal of Antimicrobial Chemotherapy* 2004; 53: 480-486.
4. Oh KB, Oh MN, Kim JG, Shin SD, Shin J. Inhibition of sortase-mediated *Staphylococcus aureus* adhesion to fibronectin via fibronectin-binding protein by sortase. *Applied Microbiology and Biotechnology* 2006; 70: 102-106.
5. Maresso AW, Schneewind O. Sortase as a target of anti-infective therapy. *Pharmacogical Reviews* 2008; 60:128-141.
6. Chenna BC, Shinkre BA, King JR, Lucius AL, Narayana SVL, Velu SE. Identification of novel inhibitorys of bacterial surface enzyme *Staphylococcus aureus* Sortase A. Bioorg Med Chem Lett 2008; 18: 380 – 385.
7. Jang KH, Chung SC, Shin J, Lee, SH, Kim, TI, Lee, HS, Oh, KB. Aaptamines as sortase A inhibitors from the tropical sponge Aaptos aaptos. *Bioorganic & Medicinal Chemistry Letters* 2007; 17: 5366 – 5369.
8. Park BS, Kim JG, Kim MR, Lee SE, Takeoka GR, Oh KB, Kim JH. Curcuma longa L. constituents inhibit sortase A and *Staphylococcus* aureus cell adhesion to fibronectin. *Journal of Agricultural and Food Chemistry* 2005; 53: 9005 – 9009
9. Oh I, Yang W, Chung S, Kim T, Oh K, Shin J. *In vitro* sortase A inhibitory and antimicrobial activity of flavonoids isolated from the roots of *Sophora flavescens*. *Archives of Pharmacal Research* 2011; 34: 217-222.
10. Kang SS, Kim JG, Lee TH, Oh KB. Flavonols inhibit sortases and sortase mediated *Staphylococcus aureus. Biologycal & Pharmaceutical Bulletin* 2006; 29: 1751 – 1755.
11. Parkar SG, Stevenson DE, Skinner MA. The potential influence of fruit polyphenols on colonic microflora and human gut health. *International Journal of Food Microbiology* 2008; 124: 295-298.
12. Wells CL, Jechorek RP, Kinneberg KM, Debol SM, Erlandsen SL. The isoflavone genistein inhibits internalization of enteric bacteria by cultured Caco-2 and HT-29 enterocytes. *Journal of Nutrition* 1999; 29: 634–640.
13. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, Approved standard M7-A5. 5 Ed, Villanova, PA: NCCLS, 2002.
14. Nomura S, Kuroiwa A, Nagayama A. Changes of surface hydrophobicity and charge of *Staphylococcus aureus* treated with sub-MIC of antibiotics and their effects on the chemiluminescence response of phagocytic cells. Chemoterapy 1995; 41: 77-81.
15. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents* 2011, 38: 99-107.
16. Lundqvist H, Dânmark S, Johansson U, Gustafsson H, Öllinger K.. Evaluation of electron spin resonance for studies of superoxide anion production by human neutrophils interacting with *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Journal of Biochemical and Biophysical Methods* 2008; 70: 1059-1065.
17. Heilmann C. Adhesion mechanisms of staphylococci. *Advanses in Experimental Medicine and Biology* 2011; 715:105-123.
18. Mazmanian SK, Ton-That H, Schneewind O. Sortase-catalysed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. *Molecular Microbiology* 2001; 40: 1049-1057.
19. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology* 1996; 50: 27–34.

20. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. *Biochimica et Biophysica* Acta 1993; 1147: 132–136.
21. Cushnie TP, Hamilton VE, Chapman DG, Taylor PW, Lamb AJ. Aggregation of Staphylococcus aureus following treatment with the antibacterial flavonol galangin. *Journal of Applied Microbiology* 2007; 103:1562-1567.