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Short Communication

Synergy between baicalein and penicillins against penicillinase-producing *Staphylococcus aureus*

Minyi Qian, Shusheng Tang, Congming Wu, Yang Wang, Tao He, Tingting Chen, Xilong Xiao*

National Center for Veterinary Drug Safety Evaluation, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China

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ABSTRACT

The combination of baicalein (the active constituent of *Scutellaria baicalensis*) with penicillin G/amoxicillin showed potent synergy against 20 clinical penicillinase-producing *Staphylococcus aureus* strains including 10 isolates that were additionally methicillin-resistant (MRSA). The fractional inhibitory concentration (FIC) indices of penicillins + baiclein ranged from 0.14 to 0.38. Baicalein protected penicillins (penicillin G and amoxicillin) from penicillinase and increased the susceptibility of penicillinase-supplemented *S. aureus* ATCC 29213 in a dose-dependent manner. The inhibition of penicillinase activity by baicalein should be responsible for the synergism and protective effect. These findings offer us good evidence that the penicillins combined with baicalein showed potent synergistic activity against penicillinase-producing *S. aureus* and penicillinase-producing MRSA *in vitro* and might provide promising implications for clinical treatment of these bacterial infections.

Introduction

Staphylococcus aureus, an important pathogen in both human and veterinary medicine, is a common cause of tissue (skin and soft tissue) infections, respiratory diseases, and even food poisoning (Lowy, 1998; Padmanabhan and Fraser, 2005; Peton and Le Loir, 2014). The main treatment of choice for *S. aureus* infection is β-lactams including penicillins. However, in most countries, penicillinase-producing *S. aureus* strains are becoming extremely common and the resistance rate to penicillins in *S. aureus* has increased dramatically, from less than 5% in the 1940s to presently 84.7% in Spain, 90.4% in China, etc. (Chambers, 2001; Wang et al., 2015; Argudin et al., 2014). The production of penicillinase is a critical mechanism in staphylococcal resistance to penicillinase-sensitive penicillins and can also enhance the resistance of methicillin-resistant *S. aureus* (MRSA) (Franciolli et al., 1991). The use of combinations of β-lactams and β-lactamase inhibitors to combat penicillinase has been successful in the treatment of infections caused by penicillinase-producing staphylococcal strains (Maddux, 1991). However, the frequent use of β-lactamase inhibitors may be responsible for the frequent appearance and prevalence of β-lactamase-producing strains (Blazquez et al., 1993; Chaibi et al., 1999; Zhao et al., 2002).

One strategy used to overcome the evolution of these β-lactamase-producing strains is to combine the β-lactams with natural products that can restore the antibacterial activity or reverse the resistance of these resistant isolates (Wagner and Ulrich-Merzenich, 2009). Several natural products are candidates for new antimicrobial substances, including epigallocatechin gallate (EGCg) (Zhao et al., 2002, 2003). Baicalein is one of the main constituents of *Scutellaria baicalensis*, which is a popular herb that is used to treat bacterial/viral infections and enhance the human immune system in China. There were limited reports which showed that baicalein exhibited synergism against MRSA and MSSA combined with tetracycline, ciprofloxacin and β-lactams (Chan et al., 2011; Fujita et al., 2005; Liu et al., 2000). However, the mechanism of the synergy is yet unknown. The aim of this study was to investigate the combined effects of baicalein with penicillins on penicillinase-producing *S. aureus in vitro* and to explore the possible mechanism of this action.

* Corresponding author. Tel.: +86 10 62733377; fax: +86 10 62731032.
E-mail address: xiaoxl58@126.com (X. Xiao).

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Materials and methods

Chemicals and bacterial strains

Baicalein (purity ≥ 98%) and penicillinase were purchased from the National Institutes for Food and Drug Control, China. Penicillin G and amoxicillin were obtained from commercial sources (Sigma–Aldrich, Beijing, China). The reference strain *S. aureus* ATCC 29213 was purchased from the American Type Culture Collection (ATCC). The 14 animal-derived *S. aureus* strains were obtained from our laboratory, collected from diseased pigs ($n = 8$) and bovines ($n = 6$), while 6 human *S. aureus* isolates were provided by several hospitals in China, which were collected from clinical cases (Table 1). All the isolates were *blaZ* positive and 10 of them were also *mecA*-positive MRSA strains, which were confirmed by PCR using the primers as described previously (Martineau et al., 2000; Kohner et al., 1999).

Susceptibility testing and combined effects of penicillins and baicalein

Minimum inhibitory concentrations (MICs) were determined using the microdilution method, according to the recommendations of CLSI document VET01-A4 (CLSI, 2013). The *S. aureus* ATCC 29213 was used as the quality control strain. The combined effects of penicillins and baicalein were tested with the checkerboard method (Norden et al., 1979). Their effects were evaluated with the fractional inhibitory concentration index (FICI), which were interpreted as synergic when FICI values were ≤0.5 (Odds, 2003).

Protection of penicillins from penicillinase by baicalein

To confirm that baicalein protects penicillins from penicillinase, cultures of the susceptible *S. aureus* strain ATCC 29213 (5×10^4 cfu) were inoculated with serial two-fold dilutions of penicillins containing commercial penicillinase and various concentrations of baicalein (0, 8, 16, or 32 μg/mL). The MICs were determined after the samples were cultured at 35 °C for 24 h. Baicalein was considered to confer a protective effect if the MIC of penicillin + baicalein against ATCC 29213 was lower than that of penicillin without baicalein.

Inhibition of penicillinase by baicalein

The inhibition of penicillinase by baicalein was detected with an iodometric assay (CCP, 1999). Before the assay, penicillinase (10,000 U/mL) was pre-incubated at 35 °C for 18 h in MHB containing different concentrations (64, 32, 16, or 8 μg/mL) of baicalein, and then excess penicillin and iodide was added subsequently. Sodium thiosulfate ($Na_2S_2O_3$) was used to titrate the remaining iodide, and the volume of $Na_2S_2O_3$ consumed was recorded which was related to the inhibition activity of baicalein. Clavulanic acid, a classical β-lactamase inhibitor, was assayed as the positive control under the same conditions.

Results and discussion

Efficacy of penicillins alone and combined with baicalein against penicillinase-producing S. aureus *strains*

All clinical *S. aureus* isolates used in this study were resistant to penicillin G (MIC ≥ 4 μg/mL) and showed low susceptibility to amoxicillin (MIC ≥ 4 μg/mL). Although baicalein alone has low antimicrobial activity (128 μg/mL) against *S. aureus*, it enhanced the antibacterial activity of penicillins against penicillinase-producing *S. aureus* (Table 1). The combination of penicillins with baicalein (8, 16, or 32 μg/mL) had a synergistic effect against all the tested penicillinase-producing *S. aureus* strains including MRSA (FICI = 0.14–0.38). Previous studies showed that EGCg and galangin combined with β-lactams acted synergistically against penicillin-resistant *S. aureus* (Eumkeb et al., 2010; Zhao et al., 2002). The baicalein and galangin shared similar chemical structures within the flavonoid, while EGCg belonged to catechin and the key pharmacophores of these compounds needs to be further confirmed. Although the MICs observed for penicillin G were still above the breakpoints even in presence of the highest baicalein concentration tested here, penicillins combined with baicalein showed potent synergistic activity against these penicillinase-producing *S. aureus*. Moreover, further studies concerning the pharmacokinetics (PK) and pharmacodynamics (PD) relationship *in vivo* are also needed to establish a rational dosage regimen that would ensure the clinical effectiveness of the combinations.

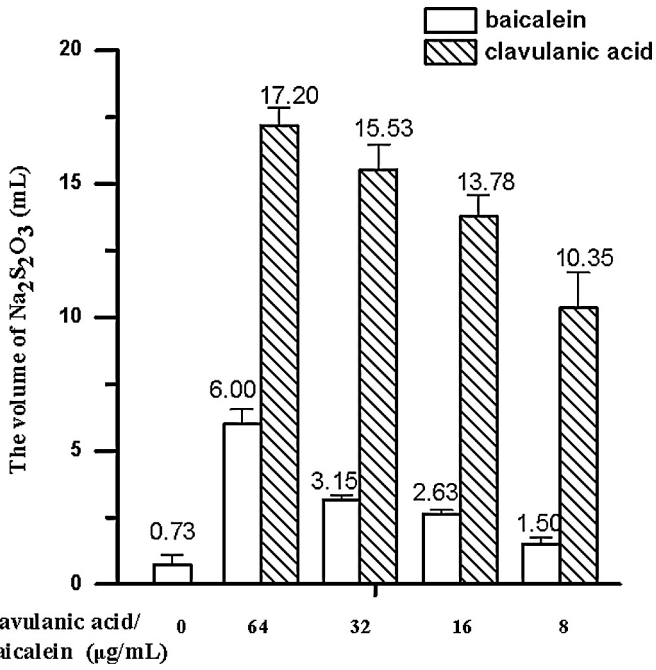

Fig. 1. Inhibition of penicillinase activity by baicalein and clavulanic acid. Penicillinase (10,000 U/mL) was incubated with baicalein or clavulanic acid (64, 32, 16, and 8 μg/mL) at 35 °C for 18 h. Penicillioic acid, the product of penicillin hydrolyzed by penicillinase, was detected using the iodometric assay, and measured indirectly by the volume of $Na_2S_2O_3$ consumed.

Protection of penicillins from penicillinase by baicalein

The MIC of penicillin G against *S. aureus* ATCC 29213 increased from 0.125 to 256, 64, 16, or 4 μg/mL in the presence of 7.5, 5, 2.5, or 1.25 U/mL penicillinase, respectively. In contrast, baicalein protected penicillin G from penicillinase and increased the susceptibility of penicillinase-added *S. aureus* ATCC 29213 to penicillin in a dose-dependent manner. For instance, the MIC of penicillin G decreased from 64 μg/mL to 16, 8, or 4 μg/mL in the presence of 8, 16, or 32 μg/mL baicalein, respectively, with 5 U/mL penicillinase. Similarly, baicalein potentiated the antibacterial activity of amoxicillin against penicillinase-supplemented *S. aureus* ATCC 29213 in a dose-dependent manner (data not show).

Inhibition of penicillinase activity by baicalein

Baicalein inhibited the activity of penicillinase in a dose-dependent manner. As shown in Fig. 1, 6.00, 3.15, 2.63, or 1.50 mL of $Na_2S_2O_3$ (0.01 mol/L) was required during the titration