**In vitro** synergistic effects of baicalin with oxytetracycline and tetracycline against *Staphylococcus aureus*

**Pavel Novy**¹, Jan Urban², Olga Leuner¹, Jaroslav Vadlejch³ and Ladislav Kokoska¹,³*

¹Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamýcka 129, 165 21 Prague 6—Suchdol, Czech Republic; ²National Reference Laboratory for Disinfection and Sterilization, National Institute of Public Health, Srobarova 48, 100 42 Prague 10, Czech Republic; ³Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcka 129, 165 21 Prague 6—Suchdol, Czech Republic

*Corresponding author. Tel: +420-224382180; Fax: +420-234381829; E-mail: kokoska@its.czu.cz.

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**Objectives:** In this study we examined the **in vitro** synergistic effect of baicalin, the flavone constituent of *Scutellaria* spp., in combinations with oxytetracycline and tetracycline on the growth of *Staphylococcus aureus*.

**Methods:** The MICs were determined by the broth microdilution method and the effect of combinations was evaluated according to the sum of fractional inhibitory concentration indices (FICIs).

**Results:** Synergistic activity (FICI ≤ 0.5) was observed for combinations of baicalin with oxytetracycline or tetracycline against 4 of 10 strains tested, whereas the baicalin/oxytetracycline combination possessed the strongest synergistic effect (FICI = 0.418).

**Conclusions:** Baicalin acts synergistically with oxytetracycline and tetracycline, enhancing its antimicrobial activity against *S. aureus*, including methicillin- and tetracycline-resistant strains.

**Keywords:** antimicrobial agents, flavonoid, staphylococci, synergy

**Introduction**

*Staphylococcus aureus* has become one of the most serious human pathogens during recent decades owing to its acquisition of antibiotic resistance, whereas methicillin-resistant *S. aureus* (MRSA) is at the present time one of the most important causes of antibiotic-resistant healthcare-associated infections worldwide, resulting in prolonged hospital stays and increased mortality.¹ Because of its exceptional ability to also acquire resistance to other commonly used classes of antibiotics, such as aminoglycosides, fluoroquinolones, macrolides and tetracyclines, the continual development of new antibacterial compounds and, more importantly, new templates with novel modes of action, represented for example by synergistically acting plant-derived compounds, is urgently needed to eradicate the resistance of *S. aureus* and to slow down its spread and development.²

Among a number of potential candidates of this type of agent is baicalin (Figure 1), a flavonoid present in *Scutellaria baicalensis* Georgi. It exerts, along with other medicinal properties,³ remarkable synergistic antistaphylococcal activity with antibiotics. It has been reported to restore the effectiveness of β-lactam antibiotics against MRSA and other strains of β-lactam-resistant *S. aureus*, probably via its direct antibacterial action on cell growth or due to the inhibition of β-lactamase, or by interactions with penicillin binding proteins.⁴ Despite the fact that the synergy of baicalin with β-lactams against *S. aureus* has already been reported, its ability to potentiate antistaphylococcal activity in other antibiotics has not previously been scrutinized. Therefore, we decided to perform a screening test focused on determining its combined effect with representatives of the main antibiotic groups, namely β-lactams (ampicillin, cefamandole, oxacillin, penicillin), tetracyclines (doxycycline, minocycline, oxytetracycline, tetracycline), aminoglycosides (streptomycin), quinolones (ciprofloxacin), macrolides (erythromycin), lincosamides (lincomycin), glycopeptides (vancomycin) and sulphonamides (sulfanilamide), against three standard strains of *S. aureus*. Together with β-lactams, tetracyclines produced results worth further investigation (P. Novy and L. Kokoska, unpublished data). As a continuation of this work, the present study reports on a detailed examination of the synergistic effect of oxytetracycline and tetracycline with baicalin against four standard strains and six clinical isolates of *S. aureus*.

**Materials and methods**

**Chemicals**

Oxacillin, oxytetracycline and tetracycline were purchased from Sigma-Aldrich (Prague, CZ). DMSO, ethanol (Lach-Ner, Neratovice, CZ) and deionised water were used as solvents.
Determination of MICs

A broth microdilution method was used to determine the MICs of test antimicrobial agents following the guidelines described by the CLSI using 96-well microtitre plates. Briefly, the samples were 2-fold diluted in MHB (100 μL), inoculated with bacterial suspension to obtain the final concentration of 5×10⁵ CFU/mL and then incubated at 37°C for 24 h. The bacterial growth was measured as turbidity by a Multiscan Ascent Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA) at 405 nm. The MIC was defined as the lowest concentration of the compound that inhibited the growth of the test bacteria by ≥80%. Oxacillin and tetracycline were used as markers of methicillin and tetracycline resistance, respectively, and S. aureus ATCC 29213 was used as a reference strain for the standardization of antibiotic susceptibility testing. All tests were performed as three independent experiments, each carried out in triplicate.

Evaluation of combination effect

Initially, 2-fold serial dilutions of an antibiotic were tested in combination with 2-fold serial dilutions of baicalin, where individual MICs of each compound were used as starting concentrations. The most promising combinations were then selected for detailed testing, where 2-fold serial dilutions of antibiotics starting at quadruple concentration of their particular MICs were combined with constant concentrations of baicalin (100, 75 and 50 mg/L). The fractional inhibitory concentration (FIC) was calculated as the ratio of the MIC of agents A and B in combination with the MIC of agent A (or B) alone. The FIC index (FICI) was then calculated using the combined FICs of agents A and B. The obtained results were interpreted in accordance with the BSAC recommendation as follows: synergy (FICI ≤ 0.5); no interaction (FICI > 0.5–4); antagonism (FICI > 4).7

Results and discussion

The susceptibilities of S. aureus strains to the antibiotics tested, either alone or in the presence of baicalin, are summarized in Table 1. The results show that baicalin possesses only a weak antistaphylococcal effect (MICs ranging from 128–512 mg/L), but its addition resulted in significant MIC reduction in oxytetracycline and tetracycline. The combination of baicalin and oxytetracycline showed the strongest synergistic effect, whereas the addition of baicalin in concentrations of 75 and 50 mg/L resulted in an 8- and 4-fold reduction (FICI = 0.418 and 0.445), respectively, in oxytetracycline MIC against S. aureus ATCC 29213. The baicalin/oxytetracycline combination showed synergistic activity against 4 of 10 S. aureus strains tested, of which 1 was a MRSA strain and 1 was a TRSA strain. The baicalin/tetracycline combination had a synergistic effect against one S. aureus strain, where the addition of 50 mg/L baicalin resulted in a 4-fold reduction in the MIC of tetracycline (FICI = 0.445). In contrast to a report by Fujita et al.,6 describing that baicalin at concentrations below its MIC does not significantly affect the growth-inhibitory activity of tetracycline against MRSA, our results showed that 50, 75 and 100 mg/L baicalin (<1/2 MIC) caused a 2- to 8-fold reduction in the MIC of tetracycline against TRSA and MRSA strains (FICI = 0.516–0.793). Despite the differences in results obtained from both studies, which may be influenced by variations in experimental design (e.g. only two strains were tested in the above mentioned report), our findings suggest a certain degree of combined antistaphylococcal effect of baicalin and tetracycline against resistant strains. Combinations of baicalin with doxycycline and minocycline exerted no interaction when tested against three standard S. aureus ATCC strains (FICI = 0.73–1.5), while baicalin at concentrations below its MIC caused a 2-fold reduction in the MIC of doxycycline.

Our positive results on the synergy of baicalin with oxytetracycline or tetracycline can also be supported by the fact that the FICI of both combinations reached values of <0.6 for 75% of S. aureus strains tested, which would be assessed as a strong additive effect if evaluated according to the European Committee for Antimicrobial Susceptibility Testing criteria for synergy. Thus, although synergistic interactions of baicalin with oxytetracycline and baicalin with tetracycline were observed against 4 and 1 of 10 strains, respectively, we can conclude that baicalin significantly potentiated the antimicrobial effect of oxytetracycline and tetracycline against S. aureus. These results suggest baicalin is a promising compound for the development of new synergistically acting drugs with the potential to extend the pharmacological action of tetracyclines, which, as antistaphylococcal agents, are clinically applied in limited cases only (e.g. in the treatment of skin or skin structure infections due to S. aureus susceptible strains). In addition, since the effectiveness of oxytetracycline and tetracycline (FICI = 0.42–0.7) was significantly more improved than the action of doxycycline and minocycline (FICI = 0.75–1.25) when tested against S. aureus ATCC 29213, 25923 and 43300 (P. Novy and L. Kokoska, unpublished data), we estimate that baicalin has the ability to augment the antistaphylococcal effect of the short-acting tetracyclines to a much greater extent than the representatives of the long-acting tetracycline group.

Previously it has been suggested that baicalein, the aglycone of baicalin, may produce a similar effect on the cell wall as epigallocatechin gallate,8 which interferes with cell wall integrity through direct binding to peptidoglycan9 and exhibits the characteristics of a cell wall active agent against enterococci.10 Since both baicalin and baicalein possess a flavone structure and similar pharmacological activity, both have a weak antimicrobial effect against S. aureus and both exert synergistic antimicrobial
Antistaphylococcal synergistic effect of baicalin with tetracyclines

Table 1. In vitro inhibitory activity of baicalin in combination with oxytetracycline and tetracycline against *S. aureus*

<table>
<thead>
<tr>
<th><em>S. aureus</em> strain</th>
<th>B</th>
<th>OXA</th>
<th>OXY</th>
<th>TET</th>
<th>MIC (mg/L) of compound alone</th>
<th>OXY with B at following concentrations (mg/L):</th>
<th>TET with B at following concentrations (mg/L):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>75</td>
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<tr>
<td>ATCC 29213</td>
<td>256</td>
<td>0.25</td>
<td>1</td>
<td>0.5</td>
<td>0.125</td>
<td>0.516</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC 25923</td>
<td>256</td>
<td>0.125</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.516</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC 43300 (MRSA)</td>
<td>256</td>
<td>8</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.516</td>
<td>0.125</td>
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<tr>
<td>ATCC BAA-976</td>
<td>256</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.516</td>
<td>0.125</td>
</tr>
<tr>
<td>M02</td>
<td>256</td>
<td>0.125</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.516</td>
<td>0.125</td>
</tr>
<tr>
<td>M05</td>
<td>128</td>
<td>0.125</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.906</td>
<td>0.125</td>
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<tr>
<td>M238 (MRSA)</td>
<td>256</td>
<td>32</td>
<td>0.5</td>
<td>0.25</td>
<td>0.063</td>
<td>0.516</td>
<td>0.125</td>
</tr>
<tr>
<td>M003 (TRSA)</td>
<td>256</td>
<td>0.25</td>
<td>64</td>
<td>32</td>
<td>0.516</td>
<td>0.641</td>
<td>0.793</td>
</tr>
<tr>
<td>M079 (TRSA)</td>
<td>512</td>
<td>0.5</td>
<td>64</td>
<td>16</td>
<td>0.445</td>
<td>0.646</td>
<td>0.598</td>
</tr>
<tr>
<td>M926 (TRSA)</td>
<td>512</td>
<td>0.25</td>
<td>64</td>
<td>32</td>
<td>0.695</td>
<td>0.646</td>
<td>1.098</td>
</tr>
</tbody>
</table>

B, baicalin; OXA, oxacillin; OXY, oxytetracycline; TET, tetracycline. FICI: <0.5, synergy (bold font indicates synergistically acting combinations); >0.5–4, no interaction; >4, antagonism.

activity with tetracycline and β-lactams against *S. aureus* we can surmise that their modes of action could possibly be similar. In addition, since baicalein has previously been reported to restore the effectiveness of tetracycline against MRSA due to the inhibition of TetK-mediated tetracycline efflux and possibly inhibits TetM or some other pump, we can presume that also baicalin may act as an efflux pump inhibitor. Moreover, the synergistic effect of the baicalin/tetracycline combination against one TRSA strain tested in this study may indicate its selectivity for particular tetracycline resistances mechanisms, for instance, against a specific efflux pump. On the other hand, the fact that we did not observe any significant difference between the FICIs of effective combinations obtained against resistant and susceptible *S. aureus* strains indicates that baicalin does not affect resistance mechanisms of resistant strains but affects some other processes common to both susceptible and resistant *S. aureus* strains. In view of this, we suggest that baicalin may have a direct antimicrobial effect on cell growth and the synergistic activity of baicalin with tetracyclines is probably produced by a multitarget effect of combined compounds.

In conclusion, this is the first report on the synergistic effect of oxytetracycline and tetracycline with baicalin against *S. aureus*, including its MRSA and TRSA strains. Regarding the synergy mechanism of its antistaphylococcal effect, direct enhancement of antibiotic activity rather than a resistance reversal effect is probably responsible for the inhibition of resistant strains. In addition, because of its ability to potentiate the effect of tetracyclines against *S. aureus* and its low toxicity, baicalin seems to be an attractive candidate for the development of new synergistically acting antistaphylococcal drugs.

**Transparency declarations**

None to declare.

**References**


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