SHORT COMMUNICATION

Inhibitory Effect of Newly-Synthesized Chalcones on Hemolytic Activity of Methicillin-Resistant Staphylococcus aureus

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Abstract
Pathogenicity of methicillin-resistant Staphylococcus aureus (MRSA) is associated with a broad spectrum of virulence factors, amongst which is α-hemolysin. The aim of this study was to investigate the effect of three newly-synthesized chalcones (1,3- Bis-(2-hydroxy-phenyl)-propanone, 3-(3-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propanone and 3-(4-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propanone) on α-hemolysin production of clinical isolates of MRSA. Subinhibitory concentrations of the tested compounds reduced hemolytic activity of MRSA strains, with almost complete abolishment of hemolysis at concentrations in the range of 1/2–1/4 x MIC (25–12.5 μg/ml). In conclusion, newly-synthesized chalcones tested in this study showed potent inhibitory activity on α-hemolysin production of multiresistant and genetically diverse MRSA strains.

Key words: α-hemolysin, chalcones, MRSA

Pathogenicity of methicillin-resistant Staphylococcus aureus (MRSA) is directly associated with a broad spectrum of virulence factors, amongst which is α-hemolysin (i.e. Hla or α-toxin). It is generally considered that α-hemolysin plays a central role in the pathogenesis of staphylococcal infections, especially in pulmonary infections caused by these bacteria (Bubeck Wardenburg et al., 2007; Burlak et al., 2007; Montgomery et al., 2008). Approximately one half of staphylococcal necrotizing pneumonia cases affecting previously healthy adults and children are caused by community-associated MRSA strains (Ragle and Bubeck Wardenburg, 2009). Besides direct lysis of the pulmonary cells, α-hemolysin also activates alveolar macrophages or monocytes, induces massive polymorphonuclear leukocyte influx into lung parenchyma with subsequent degranulation and destruction of microvascular endothelium and adjacent tissues, and induces platelet-neutrophil co-aggregation leading to the host response to toxin that contribute to the severity of lung destruction (Parimon et al., 2013). Such complex pathogenicity and occurrence of multiresistant strains urge the development of antimicrobial compounds that selectively target virulence factors. In past years, several studies investigated the inhibitory activity of various compounds against extracellular virulence factors of S. aureus (Escaich, 2008; Cegelski et al., 2008). Previously we have reported antimicrobial activity of three newly-synthesized chalcones against clinical isolates of MRSA, and inhibitory effect on the expression of virulence factors related to the early step of bacterial invasion-adherence (i.e. biofilm formation, glycocalyx production and adherence to human fibronectin) (Božić et al., 2014). Therefore, the aim of this study was to investigate the effect of 1,3- Bis-(2-hydroxy-phenyl)-propanone (O-OH), 3-(3-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propanone (M-OH) and 3-(4-Hydroxy-phenyl)-1-(2-hydroxy-phenyl)-propanone (P-OH) on α-hemolysin production of clinical isolates of multiresistant and genetically diverse strains of MRSA.

Antibacterial activity of chalcones was tested against 20 clinical isolates of MRSA isolated from blood (4), wound (6), sputum (3), endotracheal tube (2), abdominal drain (1), nose (1), skin (1), urine (1) and external.
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Identification of the isolates and methicillin resistance were determined by VITEK 2 test cards GP and AST-P580 (bioMérieux, France) and confirmed by PCR for nuc (Brakstad et al., 1992) and mecA (Bignardi et al., 1996) genes. Genotyping (SCCmec, agr, pvl and spa typing) of MRSA was performed according to previously described protocols (Boye et al., 2007; Lina et al., 2003; Harmsen et al., 2003). The spa types were clustered into spa clonal complexes (CCs) using the algorithm based upon repeat pattern (BURP) with Ridom Staph-Type 1.4 software (http://www.ridom.de). The multiresistance profile of MRSA strains was determined by VITEK 2 test card AST-P580 and further supplemented with disc diffusion test according to CLSI guidelines (CLSI, 2007).


**Table I**

<table>
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<th>CC type</th>
<th>spa type</th>
<th>No* of strains</th>
<th>SCCmec type</th>
<th>agr type</th>
<th>PVL</th>
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and P-OH was tested in the two-fold higher concentrations than O-OH chalcone (50.0–6.2 µg/ml), corresponding the same range of MIC’s (1/2 × MIC – 1/16 × MIC). Hemolytic activity of MRSA strains cultivated with 1/2 × MIC and 1/4 × MIC of M-OH was in the range of 7.1–18.8% of the positive control (p < 0.01; p < 0.001), and cultivation with 1/8 × MIC lead to the increase of the hemolysis up to 40.7% of the positive control (p < 0.05). The lowest applied concentration of M-OH (1/16 × MIC, 6.2 µg/ml) did not reduce the hemolytic activity of MRSA (p > 0.05). Statistically significant inhibition of hemolytic activity of MRSA strains cultivated with P-OH chalcone occurred only in concentrations of 1/2 × MIC (7.7%; p < 0.01) and 1/4 × MIC (43.8%; p < 0.05) (Fig. 2). Chalcones themselves did not perform any hemolytic activity or induced hemolysis of rabbit erythrocytes in the same range of MIC’s (1/2 × MIC – 1/16 × MIC; p > 0.05).

Antistaphylococcal activity of various chalcones is strongly correlated with their chemical characteristics. It is generally considered that lipophilicity of the ring A (Nowakowska, 2007) and presence of free hydroxyl group/s at various positions of the ring B are necessary for antistaphylococcal activity of these compounds (Sato et al., 1996; Alcaraz et al., 2000; Kromann et al., 2004; Talia et al., 2011). Three compounds investigated in our study carrying free hydroxyl group at various positions of the B ring exerted strong anti-MRSA activity, with 1,3- Bis-(2-hydroxy-phenyl)-propenone (O-OH) bearing the hydroxyl group at the position 2 of ring B as the most active one. These results are in accordance with the findings of other investigators (Alcaraz et al., 2000; Kromann et al., 2004). Subinhibitory concentrations of the tested compounds also inhibited hemolytic activity of MRSA strains in a dose dependent manner, with almost complete abolishment of hemolysis at concentrations in the range of 1/2–1/4 MIC (25–12.5 µg/ml). Inhibition of hemolytic activity of α-hemolysin was dose dependent. Chalcone with free hydroxyl group at the position 2 of ring B was the most active one, since the lowest applied concentration of this compound (1/16 × MIC, 3.1 µg/ml) exerted statistically significant inhibition of hemolysis. Higher concentrations of other tested compounds exerted similar inhibitory effect, with partial or complete recovery of hemolytic activity of α-hemolysin when applied in the lowest concentrations (1/16 × MIC, 6.2 µg/ml). Previously it has been reported that subinhibitory concentrations of chalcones with similar chemical structure isolated from natural sources, like licochalcone A and E, inhibit α-hemolysin production (Qiu et al., 2010a; Zhou et al., 2012). Licochalcone A possess anti-MRSA activity in the range of MIC’s from 2.0–8.0 µg/ml (Fukai et al., 2002; Qiu et al., 2010a), and inhibitory effect on α-hemolysin production in the range of 1/2–1/8 × MIC (Qiu et al., 2010a). Licochalcone E was found to be the most active licochalcone against S. aureus, since the inhibitory effect was detected in the range of MIC’s from 1.0–4.0 µg/ml, and inhibition of α-hemolysin production in the range of 1/4–1/16 × MIC (Zhou et al., 2012).

Precise mechanism of antimicrobial activity of chalcones and inhibition of α-hemolysin synthesis has not been clarified yet. Several different mechanisms could be involved, like direct enzyme inhibition, inhibition
of DNA or RNA synthesis (Mori et al., 1987; Ohemeng et al., 1993) or interference with energy metabolism by inhibition of NADH-cytochrome c reductase, reducing the energy required for active uptake of various metabolites and biosynthesis of macromolecules (Haraguchi et al., 1998; Cushnie and Lamb, 2011). Licochalcone A (Qiu et al., 2010a) or thymol (Qiu et al., 2010b) perform inhibitory effect on α-hemolysin synthesis through inhibition of agrA transcription, one of the components of the agr global regulatory system of S. aureus. Besides influence on agr global regulatory system, licochalcone E also directly inhibits expression of hla-gene encoding α-hemolysin synthesis (Zhou et al., 2012). Accordingly, it is possible that chalcones also inhibit production of α-hemolysin through mechanisms proposed by Qui et al. and Zhou et al. Further studies are necessary to elucidate the precise mechanisms of inhibitory effect of tested chalcones on hemolytic activity of α-hemolysin.

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Literature


