Inhibition of streptolysin O by allicin – an active component of garlic

Mohsen Arzanlou[1] and Shahab Bohlooli[2]

Correspondence
Mohsen Arzanlou
m.arzanlou@arums.ac.ir

[1]Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

[2]Department of Pharmacology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

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Streptolysin O (SLO) is a potent cytolytic toxin produced by almost all strains of group A streptococci and is considered an important virulence factor for this organism. In this study we investigated the effect of allicin and aqueous garlic extracts on the haemolytic activity of SLO. All tested materials potentially inhibited the SLO haemolytic activity. Allicin neutralized SLO in a dose- and time-dependent manner. A 15 min incubation of SLO with 35 μg allicin totally inhibited the haemolytic activity of SLO [IC_{50} (concentration necessary to reach half maximum inhibition)=5.97 μg]. The inhibitory activity of an old extract of garlic was equipotent to pure allicin (IC_{50}=6.27 μg; $P<0.05$). In contrast, fresh extract of garlic inhibited the SLO haemolytic activity at lower concentrations (IC_{50}=1.59 μl; 1.9 μg allicin). The inhibitory effect of the allicin was restored by addition of reducing agent DTT at 2 mM, suggesting that allicin likely inhibits the SLO by binding to the cysteine residue in the binding site. These results indicate a new activity for allicin and allicin may be a potential alternative drug against streptococcal diseases.

INTRODUCTION

Streptococcus pyogenes (group A streptococci; GAS) is a highly versatile pathogen that causes a wide variety of important human diseases, ranging from localized infections, such as pharyngitis, erysipelas and cellulites, to life-threatening invasive diseases like streptococcal toxic shock syndrome and necrotizing fasciitis (Martin & Green, 2006; Steer *et al.*, 2007). GAS infections are associated with non-suppurative immune-mediated complications such as post-streptococcal glomerulonephritis, acute rheumatic fever and post-streptococcal reactive arthritis (Martin & Green, 2006; Steer *et al.*, 2007). This organism has a large armoury of virulence factors responsible for this broad range of human diseases (Cunningham, 2008). The strains differ in the expression of this wide range of virulence factors. However, some virulence factors are highly conserved and expressed by almost all strains. The potent streptococcal cytolytic toxin streptolysin O (SLO) is among such factors (Muller-Alouf *et al.*, 1997; Shiseki *et al.*, 1999). SLO contributes to the pathogenesis of streptococcal infections by direct toxic effects on human cell types, and by inducting the production and release of pro-inflammatory factors.

In vitro studies demonstrated that SLO kills a variety of human cell types by disruption of the biological membranes, at lethal concentrations (Alouf & Palmer, 1999), and recently it has been reported that SLO triggers the apoptotic death of human leukocytes, at sublethal concentrations (Timmer *et al.*, 2009). Purified SLO elicits high amounts of pro-inflammatory cytokines by immune cells and potentiates inflammatory responses, *in vitro* and *in vivo* (Hackett & Stevens, 1992; Shanley *et al.*, 1996). In parallel, isogenic SLO-deficient mutants showed a reduced ability to elicit the production of pro-inflammatory factors compared to parent strains (Ruiz *et al.*, 1998). Administration of SLO in animal models produces severe pathophysiological effects. In rabbits, intravenous administration of SLO caused blood vessel contraction, increased capillary permeability, massive intravascular thrombosis, dermal necrosis, cardiotoxicity and death (Alouf, 1980). Limbago and colleagues reported that SLO-deficient GAS was attenuated and mice infected with the stable *slo* mutant exhibited a significant decrease in mortality rates compared to mice infected with wild-type GAS (Limbago *et al.*, 2000). The data indicate that SLO has great potential as a drug target for the treatment of streptococcal infections.

SLO belongs to a family of pore-forming toxins referred to as thiol-activated toxins or recently as cholesterol-dependent cytolysins (Palmer, 2001). All toxins in this family consist of a single polypeptide chain, the length of which ranges from 471 amino acid residues with pneumolysin (Walker *et al.*, 1987) to 571 amino acid residues with SLO

Abbreviations: GAS, group A streptococci; HU, haemolytic unit; IC_{50}, concentration necessary to reach half maximum inhibition; RBC, red blood cell; SLO, streptolysin O.