THE CYTOTOXICITY OF SUILYSIN, THE PORE-FORMING TOXIN OF STREPTOCOCCUS SUIS ON THE CENTRAL NERVOUS SYSTEM CELLS

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ABSTRACT Streptococcus suis (S. suis)-induced meningitis is a catastrophic neurological disorder with high morbidity rates. Several studies have mentioned that Suilysin (SLY), the only toxin of S. suis, interacts with the cells in either human or swine Central Nervous System (CNS) cells and trigger destructing inflammatory events on them. However, the pathogenesis of cytotoxicity is currently being an unresolved question. This review highlights current literatures emphasizing the role of SLY in causing cytotoxicity of Brain Microvascular Endothelial Cells (BMEC), Choroid Plexus Epithelial Cells (CPEC), and astrocytes, along with the proposed pathological mechanisms. SLY has been considered to be the key player in various pathological mechanisms related to CNS cell death, including alteration of tight junction, barrier integrity, inflammatory response, and activation of RhoA GTPase as the newly recognized mechanism. These findings should be taken into consideration in order to design novel therapeutic options for S. suis meningitis. Although the exact mechanism of SLY-induced cytotoxicity is still pausible, the possible neurobiological pathways may further help to dissect the biological role of SLY during S. suis meningitis.

KEYWORDS Suilysin, Streptococcus suis, Cytotoxicity, Central Nervous System, Meningitis

Summary
This review highlights the cytotoxicity of Suilysin to the cells of CNS, such as brain microvascular endothelial cells, choroid plexus epithelial cells, and astrocytes.

Introduction
Streptococcus suis (S. suis)-induced meningitis is the main serious clinical manifestation of S. suis infection that is currently being put under the spotlight according to its increasing number of cases and its ability to induce morbidity among survivors such as sensorineural hearing loss [1-3]. Along with that, the number of S. suis cases has notably increased during the past few years, with the highest prevalence rate in the Southeast Asia region, where there is a high rate of swine consumption [3]. Initiating the framework and future directions for controlling the S. suis meningitis should be initiated by studying the pathogen’s way to induce pathological lesions. The S. suis bacteria is an important gram-positive bacterial pathogen that has raised attention among researchers because of its role in causing serious infections in humans. Almost all of the approximately 1,300 serotyped S. suis instances of infection in humans is caused by S. suis serotype 2 (SS2), with over 70% of those showing clinical indications of meningitis. In addition, this pathogen is the leading cause of adult bacterial meningitis in Vietnam, Thailand, and Hong Kong [4]. It has equipped with various virulence factors. Some in vitro and in vivo studies have mentioned Suilysin, the only cytotoxic virulence factor of S. suis, to play an important role in the pathogenesis of S. suis infection and inflammatory re-
Sulysin and Its Intention to Trigger Brain Inflammation

Sulysin (SLY) is an extracellular protein encoded by the sly gene [7]. The sly gene has been found in the majority of European and Asian S. suis invasive serotype 2 strains [8]. It was first recognized as a surface protein from the bacterium S. suis belonging to hemolysin in 1994 [9]. SLY protein has a molecular weight of 54 kDa. It consists of 497 amino acids which have cholesterol-dependent properties that are cytotoxic to epithelial, endothelial, neutrophil, and macrophage cells, as well as bactericidal and antiphagocytic [6, 10]. The CDC protein structure generally consists of four domains. Domains 1 and 3 are known as the N-terminal portion, domain 2 is known as the connection domain, and domain 4 is known as the C-terminal domain. The four domains have their respective functions. Domains 1 to 3 have no continuity in their primary sequence [7]. Domain 4 contains Tryptophan (Trp)-Rich Motif, a highly conserved undecapeptide region. The Trp-rich motif is a structural determinant for the binding of the CDC protein to the target cell membrane [11]. SLY is known to be the most influential virulence factor in epidemic strains of S. suis, especially the highly virulent strain that causes Streptococcal Toxic Shock-Like Syndrome (STTLS) [6, 12].

An experimental study conducted by Takeuchi et al. (2014) was done to examine the SLY neurotoxicity to the brain tissue. Mice inoculated with wild-type ST1 strain S. suis showed neurological clinical manifestations of grasping weakness and paralysis of the extremities. Mice inoculated with sly-knockout strain S. suis only showed manifestations of lethargy and ruff fur without the involvement of other clinical alterations. Mice inoculated with wild-type ST1 S. suis on other studies also showed significant signs of neurotoxicity, such as hyperexcitation, bending head to one side, swivel gait, and some other locomotor problems [13]. These findings were the rationale for the next studies, which observed the number and density of the inflammation cells through microbiological and histopathological examinations. The density of the bacteria in the blood and brain were significantly higher in the wild-type ST1 group compared to the sly-knockout group after 72 hours of the onset of infection. Mice inoculated with ST104 strain S. suis did not show any inflammation or bleeding in the brain tissue. The number of inflammatory cells and their location were further examined using samples from the brain parenchyma. These findings suggest that SLY induces neurotoxicity through the induction of brain inflammation, initiated by the accumulation of inflammation cells in meninges and ventricles, eventually leading to the destruction of more centrally located physiological structures of the brain [6].

Based on the onset of infection, SLY was not indicated to have major parts of the central nervous system (CNS) invasion in the early phase of the inflammation: 24 hours after the inflammation onset, mice inoculated with strains ST1 and ST104 S. suis both showed CNS trophism. Nevertheless, bacterial density in the brain was subsequently higher in the mice with ST1 (more SLY) S. suis compared to ST104 [6]. ST1 was more likely to cause meningitis compared to ST104. These findings accumulatively indicate that SLY contributes to making a more virulent strain of S. suis through the induction of longer, more severe, and uncontrollable inflammation mechanisms in the brain whilst facilitating the bacteria to survive from the immune system of the brain.

Role of SLY on CNS Cells

As higher levels of inflammation are identified in cells treated with S. suis bacteria which have more abundant levels of SLY, it brings out a propensity that SLY has an important role in meningitis-induced S. suis, the well-known severe clinical manifestation of human S. suis infection. These hypotheses have been evaluated in either in vivo or in vitro studies.

In most cases of bacterial meningitis, the Brain Microvascular Endothelial Cells (BMEC) seems to be the primary site of the breakdown of the Blood-Brain Barrier (BBB) [19]. Several studies are proving that SLY-positive strains exhibited cytotoxic effects on BMEC, either in porcine or humans [14, 15]. Moreover, it seems that the cell wall components of S. suis have no additive cytotoxic effect on the cells [14]. The epithelium of the choroid plexus constitutes the fundamental basis for the Blood Cerebrospinal Fluid Barrier (BCSFB). Tenenbaum et al. (2005) revealed that the administration of SLY-producing strains of S. suis isolates was more toxic for the cultured Porcine Choroid Plexus Epithelial Cells (PCPEC) than the SLY-negative strain. The study also highlighted a significant reduction of transepithelial resistance and elevation of paracellular [3H]-mannitol flux of cell line treated with S. suis isolates, indicating an increase of tight-junction permeability [17]. Massive cell death in the choroid plexus will ultimately result in a leaky BCSFB, thus allowing chemicals to enter the ventricular system and then alter the brain parenchyma. Recently, S. suis-induced cytotoxicity was analyzed in human meningeal cells and astrocytes, showing that S. suis was not cytotoxic for meningeal cells, but to some extent, for astrocytes. Because the ST1 and ST7 strains produced larger levels of cytotoxicity than the SLY-negative strain LPH4, the S. suis-induced astrocyte cell death has been attributed to SLY-induced cytotoxicity [18].

Proposed Mechanism of SLY-induced CNS Cells Cytotoxicity

The proposed model of action of SLY has been considered as a “multi-hit” activity that may include simple lysis of host cells, interference with immune cell function, and induction of inflammatory cytokine [12, 20]. Before exaggerating its effect on the cells, there are some factors determined to be able to affect the susceptibility of epithelial cells regarding the effects of SLY. They are composed of membrane-bound SLY, the cell’s cholesterol content, and the cell’s resealing capacity [21]. Most studies that concentrate on evaluating the cytotoxic effect of SLY use Lactate Dehydrogenase (LDH) as the indicator of cytotoxicity.
were caused by Tumor Necrosis Factor (TNF-α) meningitis [27].

The Blood-Brain Barrier (BBB) at the brain endothelium and the tight junction proteins will further incite an excessive influx of causing loss of epithelial barrier integrity [26]. Disruption of SLY was the virulence factor to Bercier (2020) revealed that SLY is the increase of Matrix Metalloproteinases (MMPs) activities which further will contribute to the breakdown of BCSFB [24]. The MMPs, particularly MMP-9 and MMP-3, also have a pivotal role in the breakdown of BBB due to its ability to cause degradation of the extracellular matrix [28, 29]. Moreover, SLY seems to suppress the production of Tissue Inhibitor of Metalloproteinases (TIMPs), the enzymes that act as an outstanding inhibitor of MMP [30]. MMP-3 expression was also upregulated after cytokine exposure in mouse brain astrocytes [31].

**The SLY-induced Alteration of Tight Junction, Barrier Integrity, and Inflammatory Response**

The Blood-Brain Barrier (BBB) at the brain endothelium and the BCSFB have been discussed as potential brain entrance routes for S. suis. At these barriers, direct interaction along with cytotoxicity caused by SLY towards host cells are justified. It has been revealed that SLY’s direct cytotoxic effects on brain endothelium have been linked to disruption of barrier integrity and inflammatory response [14, 22]. In several studies, it has been proven that the increased permeability of brain endothelial cells and PCPEC were caused by Tumor Necrosis Factor (TNF-α) [23, 24]. These SLY-induced destructive events are currently being considered as one of the underlying mechanisms of cytotoxicity.

Previously, it was already known that S. suis target massive rearrangement of three important tight junction proteins; Zonula Occludin (ZO)-1, occludin, and claudin-1 of PCPEC in vitro without knowing what virulence factor was considered to be the main culprit [25]. After some years, a recent study by Bercier (2020) revealed that SLY was the virulence factor to be responsible for the disruption of occludin and ZO-1, thus causing loss of epithelial barrier integrity [26]. Disruption of tight junction proteins will further incite an excessive influx of Polymorphonuclear Neutrophils (PMNs) from systemic circulation to the brain, which is being a critical point in bacterial meningitis [27].

Related to barrier dysfunction, increasing production of TNF-α after induction of SLY is also a matter of interest due to its ability to further evokes different inflammatory processes. TNF-α causes a drastic decrease in transepithelial electrical resistance of PCPEC as the permeability of the cells will be elevated afterwards. Another inflammatory pathway triggered by TNF-α is the increase of Matrix Metalloproteinases (MMPs) activities which further will contribute to the breakdown of BCSFB [24]. The MMPs, particularly MMP-9 and MMP-3, also have a pivotal role in the breakdown of BBB due to its ability to cause degradation of the extracellular matrix [28, 29]. Moreover, SLY seems to suppress the production of Tissue Inhibitor of Metalloproteinases (TIMPs), the enzymes that act as an outstanding inhibitor of MMP [30]. MMP-3 expression was also upregulated after cytokine exposure in mouse brain astrocytes [31].

**Suilysin and RhoA-GTPase**

Recent literature stated the involvement of cytoskeleton structure in the specific SLY-induced cytotoxicity [16]. It provides us with a new insight after the correlation between S. suis treatment and cytoskeletal component alteration was proven in some earlier studies [25]. Actin microfilaments were required for S. suis invasion of PBMEC, but not microtubular cytoskeletal components, active bacterial RNA, or protein synthesis [15]. The change of actin cytoskeleton, however, has opened our insights of SLY-induced cytotoxicity by another novel mechanism mediated by RhoA GTPase.

SLY roles in RhoA activation and the change of cytoskeleton structure both use its property of cholesterol-dependent. A study by Lv et al. (2014) combined human Brain Microvascular Endothelial Cells (hBMEC) with SLY-cholesterol with a mass ratio of 1:1 and 1:5 for 10 minutes. The mixture of SLY-cholesterol with a ratio of 1:1 was able to activate RhoA, unlike the ratio of 1:5 in which were unable to activate RhoA. These findings indicate that RhoA activation by SLY is cholesterol-dependent [16]. Based on current literature, cholesterol-dependent RhoA activation by SLY can be assumed due to the formation of the pores on the cell membrane.

RhoA activation can be done by a number of receptors. These include the Receptor of Tyrosine Kinases Family (RTK), G-Protein Coupled Receptor (GPCR), and cytokine/ion channel receptor [32]. TrkB, a receptor in the RTK group, can bind with BDNF. Once it binds with BDNF, the anti-apoptotic pathway will be activated, resulting in the increase of cell survival [33].

RhoA GTPase plays a role in cell regulation by regulating actin cytoskeleton through the fusion of stress fibres (actin–myosin filaments) with focal adhesion, gene expression, and enzymatic activities. Events related to actin cytoskeleton remodelling include neuronal wiring – particularly axonal guidance –, phagocytosis, cell migration, cell polarity, and intercellular interactions. Some signal transduction pathways are known to be related to actin cytoskeleton rearrangement. These pathways include FAK, PI3K, Src kinase, and Rho GTPase. Up to today, pneumolysin is the only member of CDC protein known to use Rho GTPase in intact cells to alter the physiology of the actin cytoskeleton [34, 35].

The major downstream effector of RhoA GTPase is Rho kinase (ROCK)/1,2. Following RhoA activation, ROCK activates several intracellular signalling cascades, which leads to cellular death. ROCK triggers Myosin Light Chain (MLC) phosphorylation. This phosphorylation gives rise to the formation of actomyosin contractile [36]. RhoA-ROCK activation also stabilizes actin filaments and alters the cytoskeleton conformation by inducing kinase-dependent phosphorylation of LIM [37]. Another ROCK substrate triggered after RhoA-ROCK activation is

*Figure 1. SLY-induced RhoA Activation.*

Despite the exact mechanism for SLY cytotoxicity to CNS cells remaining unclear, there are some studies that revealed possible explanations.
Collapsin Response Mediator Protein-2 (CRMP-2). CRMP-2 is a protein that can bind with microtubules. CRMP-2 phosphorylation due to ROCK inhibits its ability to bind with tubulin, resulting in neuronal growth cone collapse [38]. ROCK activation also gives rise to phosphorylation and stimulation of dual protein/lipid Phosphatase and Tensin Homolog (PTEN) activities. PTEN is a tumour suppressor protein that can inhibit the growth and survival of cells [39].

Another result of RhoA/ROCK activation is the activation of cytosolic Phospholipase A2 (cPLA2). cPLA2 enzyme is a lipase with a molecular weight of 85 kDa. cPLA2 enzyme mediates inflammation and cellular death. RhoA inhibition lowers cPLA2 activation by 66%. The active form of cPLA2 will then translocate from the cytosol into the cells and organelles, hydrolyzing glycerophospholipid to produce arachidonic acid and eicosanoid. These events eventually lead to the induction of caspase activation and intrinsic apoptosis of the cell [40]. The formation of mitochondrial reactive oxygen species (ROS), which contributes to cellular apoptosis, is also known to be cPLA2-dependent [41]. The pathogenesis mechanism summary of cell death related to SLY and RhoA GTPase pathway can be seen in Figure 1. RhoA GTPase is involved in gene expression and enzymatic activities by its role of apoptosis regulation, cell cycle, accumulation of ROS, membrane trafficking, proliferation, gene transcription, neuronal morphology, plasticity, and neuronal migration [32, 42-44].

The established cytotoxicity of SLY on CNS cells is an important finding in neuroscience. The mechanism of action that includes neuroinflammatory pathway, tight junction disruption, and RhoA-GTPase activation should be taken into consideration in order to design novel therapeutic options for S. suis meningitis. These findings underline the possible neurobiology mechanisms and may further help to dissect the biological role of SLY during S. suis meningitis.

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Conflict of interest:

There are no conflicts of interest to declare by any of the authors of this study.

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