Short Communication

Streptococcal meningitis reveals the presence of residual streptococci and down-regulated aquaporin 4 in the brain

Tatsuya Nakayama

Received: 14 May 2021 / Revised: 29 August 2021 / Accepted: 16 September 2021 / Published online: 25 September 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
The pathology of streptococcal meningitis is poorly understood, even though streptococcal infection induces meningitis. The aim of this study was to clarify the relationship between streptococcal meningitis and aquaporin 4 (AQP4) in the mouse brain. After Streptococcus suis infection, the streptococcal number was calculated, and AQP4 mRNA expression in the brain was quantified at 2 and 7 days after infection. At 7-day post-infection, mice with neurological symptoms showed significantly higher S. suis levels in the brain than mice without neurological symptoms. AQP4 expression was significantly decreased in mice with neurological symptoms than in mice without neurological symptoms. Image analysis demonstrated that S. suis progressed to invade the white matter. Pathological analysis revealed that infected mouse brains had higher inflammation and neurological damage scores than uninfected mouse brains. Therefore, mice with neurological symptoms caused by streptococcal meningitis had high S. suis levels in the brain and reduced AQP4 expression.

Keywords
Streptococcal meningitis · Streptococcus suis · Aquaporin 4 · Neurological symptoms

Streptococcus suis is a gram-positive, facultative anaerobic bacterium. Approximately 35 S. suis serotypes have been reported (De-Greeff et al. 2002). Serotype 2 is the most virulent serotype and is frequently isolated in swine and humans. Several virulence-associated genes have been reported, such as muramidase-released protein (Smith et al. 1992), extracellular protein factors (Smith et al. 1993), and suilysin (Lun et al. 2003). The primary infection routes of S. suis are infection of a wound (Gottschalk et al. 2010) or the gut from consuming raw pork (Nakayama et al. 2013). Several descriptions of human clinical manifestations of S. suis infection have been published (Werheim et al. 2009). According to our epidemiological study, approximately 20% of patients had diarrhea and altered consciousness, and hearing loss is a unique characteristic of this infection (Kerdzin et al. 2011). Although mouse models have been used as infectious experimental models to examine the responses of mice to cytokines and chemokines produced during S. suis infection (Domínguez-Punaro et al. 2008), the relationship between streptococcal meningitis and brain pathology has not been adequately studied. Almost all studies have only mentioned meningitis after detecting S. suis in the brain. Aquaporins (AQPs) are membrane proteins involved in water transport within the body (Verkman et al. 2000). AQP4 has been identified in the brain and participates in water homeostasis (Iacovetta et al. 2012). The astrocyte plasma membrane domains that ensheath the cerebral microvessels are enriched in AQP4 water channels, which are strongly implicated in brain (Papadopoulos and Verkman 2007, Tang et al. 2013). However, the relationship between AQP4 expression and streptococcal meningitis remains unknown. Our aim was to clarify the relationship between streptococcal meningitis and AQP4 in the mouse brain.

The S. suis 31533 strain was provided by the National Institute of Animal Health in Japan. Todd-Hewitt broth (Difco Laboratories, Detroit, MI, USA) was used for streptococcal cultures. 22 female specific pathogen-free A/J mice (7–9 weeks) (SLC, Shizuoka, Japan) were acclimated under standard laboratory conditions and provided free access to rodent chow and water. Animal studies were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Osaka University.

Communicated by Erko Stackebrandt.

* Tatsuya Nakayama
t-nakayama@hiroshima-u.ac.jp

1 Graduate School of Integrated Sciences for Life, Hiroshima University, 1-4-4 Kagamiyama, Higashihiroshima, Japan
(Osaka, Japan), and all animal experiments were approved by the Animal Welfare Assurance of Compliance H-21-09-0.

500 µL of streptococcal suspension (5.0 × 10⁹ colony forming units (CFU) of *S. suis*) or the control solution (sterile PBS) were administered to mice via intraperitoneal injection. Animals in a septic state lose the ability to maintain their body temperature, and a decrease in body temperature beyond a certain point (a decrease of 6 °C from normal body temperature) has been correlated with death in several infectious disease models (Offert et al. 2000). Therefore, mouse body temperature was measured to determine the clinical endpoints.

Three and five mice with neurological symptoms were sacrificed at 2 and 7 days after infection, respectively, and three non-infected mice were sacrificed as a control using 100 µL of Ketalar solution, composed of 10 mL Ketalar (Daiichi-Sankyo, Tokyo, Japan) mixed with 2.2 mL of 2% Selactar (Bayer Health Care, Leverkusen, Germany). The brains were aseptically excised and transferred to 500 µL of sterile PBS in a cell strainer (BD Biosciences, San Jose, CA, USA) and homogenised using the rubber tip of a syringe bar (Terumo, Tokyo, Japan). The homogenised solution was used for RNA extraction. Serial dilutions of 10 µL of the homogenate in PBS were spread onto sheep blood agar plates and incubated at 37 °C for 24 h. Streptococcal colonies were counted and expressed as CFU g⁻¹ for brain samples.

RNA was extracted as previously described (Nakayama et al. 2010). Briefly, PBS-Trizol (Invitrogen, Carlsbad, CA, USA) and chloroform (Wako, Osaka, Japan) were used in the present study. Standard DNA amounts covered and stained with haematoxylin and eosin (H&E). The stained tissue was assessed using inflammatory and neuronal damage scores at 7-day post-infection, according to a previously described method (Wellmer et al. 2001). The tissue was sectioned and stained with haematoxylin and eosin (H&E). Finally, the aqueous mounting medium Permafluor (Thermo Fisher Scientific, Waltham, USA) was added to the specimens. All slides were examined under a fluorescence microscope. A histopathological analysis was conducted to clarify the inflammatory and neuronal damage scores in 7-day post-infection, according to a previously described method (Wellmer et al. 2001). The tissue was sectioned and stained with haematoxylin and eosin (H&E). The stained tissue was assessed using inflammatory and neuronal damage scores. The data are presented as the mean values and standard errors of the mean, and were generated using Student’s *t* test in Fig. 1a, b and the Mann–Whitney test in Fig. 1c.

After measuring the number of bacteria in the brain after infection, we detected 4.5 × 10⁶ and 10 CFU/g in mice with neurological symptoms and 8.6 × 10⁵ and 2.4 × 10² CFU/g in mice without neurological symptoms at 2 and 7 days after infection, respectively (Fig. 1a). There was no difference in the number of bacteria between mice with and without neurological symptoms 2 days after infection; however, the difference was apparent at 7 days after infection. We quantified AQP4 expression after infection and detected 59 and 29 copy numbers in mice with neurological symptoms and 3.7 × 10⁷ and 1.2 × 10⁵ copy numbers in mice without neurological symptoms at 2 and 7 days after infection, respectively (Fig. 1b). AQP4 expression was significantly decreased after infection and was significantly lower in mice with neurological symptoms than in mice without neurological symptoms at 2 and 7 days after infection.
Streptococcal localisation in the brain 7 days after infection was observed, and the bacteria were localised in the lateral ventricles in the brains of mice that exhibited neurological symptoms. No *S. suis* was detected in the brains of non-infected mice or mice without neurological symptoms (Fig. 2a). H&E staining results showed leucocyte aggregation in the lateral ventricles of mice with neurological symptoms 7 days after infection, which was consistent with the localisation of the bacteria in the lateral ventricles (Fig. 2b). The brains were analysed based on inflammatory...
and neurological damage scores 7 days after infection. Pathological analysis based on H&E staining showed that mouse brains with and without neurological symptoms had inflammation and neurological damage scores, whereas the brains of uninfected mice had no scores (Table 1).

Streptococcal infection can cause meningitis (brain oedema) and neurological symptoms (Dutkiewicz et al. 2018); however, the pathogenesis of neurological symptoms is not fully understood. When mice become infected and develop neurological symptoms, they never recover. Although almost all mice that exhibited neurological symptoms died of sepsis and bacteraemia, a few mice survived for more than 6 months following the onset of neurological symptoms.

Immunohistochemistry and histopathological analyses revealed that *S. suis* was localised to the lateral ventricles in the white matter of the brain, and lymphocyte aggregation in mice that exhibited neurological symptoms. Previous studies have reported that brain abnormalities are associated with the development of sensorineural hearing loss caused by ependymoma, which is derived from ependymal cells traversing the central nervous system (Morris et al. 2009) and may develop in response to cytomegalovirus infection (Matsuno et al. 2014). Cytomegalovirus infections in the developing brain may result in abnormalities, such as mental retardation, microcephaly, chorioretinitis, seizures, intracranial calcification, and neurological disorders, including hearing loss. These infections are commonly found in the periventricular white matter region (Moinuddin et al. 2003).

Several patients with *S. suis* exhibit a unique effect of hearing loss (Kerdsin et al. 2011). Thus, detectable *S. suis* levels in the lateral ventricle may play an important role in
the development of hearing loss. Mice that exhibited neurological symptoms harbored higher concentrations of *S. suis* in the whole brain than mice that did not show neurological signs. Therefore, high concentrations of residual *S. suis* in the brain white matter strongly correlate with the induction of neurological symptoms in the host.

We also examined AQP4 expression. AQP4 is a membrane protein involved in water transport in many fluid-transporting tissues (Niu et al. 2012; Cruz et al. 2013). Although oedema is highly related to AQP4 expression (Tang et al. 2013), the relationship between neurological damage due to infection and AQP4 is poorly understood. AQP4 expression in the brain was decreased in mice that exhibited neurological symptoms at 2-day post-infection. Although few studies have reported the relationship between AQP4 and prevention of microbial infection, water channel AQP4 partially protects the host from cerebral malaria (Promeneur et al. 2013). Moreover, Shiga toxin released by *E. coli* decreases AQP4 levels throughout the cell, which compromises the integrity of the blood–brain barrier via the activation of astrocytes (Amran et al. 2013).

AQP4 expression in *Streptococcus pneumoniae*, a haemolysin-producing streptococci that can induce pneumococcal meningitis (Nakayama et al. 2014), was investigated using AQP4 null mice and rats (Papadopoulos and Verkman 2005; Du et al. 2015). The authors reported that pneumococcal infection upregulated AQP4 expression. In this study, the downregulation of AQP4 occurred as a result of infection and production of toxins from the *S. suis* strains. Therefore, the results here do not indicate that *S. suis* infection has an opposite effect on AQP4 than observed in *S. pneumoniae*. Further studies are needed to clarify these relationships.

AQP4 upregulation is induced by brain oedema and bacterial meningitis (Huang et al. 2014). In this study, the significant decrease in brain AQP4 expression in mice infected with *S. suis* may be related to the oedema found around neurons of the cerebral cortex and periventricular regions. Oedema formation around vessels may occur as a result of the increase in blood–brain barrier permeability, allowing the passage of fluid into the extracellular space, and a decrease in AQP4 expression that prevents fluid elimination (Verkman et al. 2006; Lucero et al. 2012). In *S. suis* infection, AQP4 expression was decreased more in mice with neurological signs. The AQP4 downregulation detected in astrocytes of mice infected with *S. suis* could reduce the potassium ion clearance from neurons to adjacent astrocytes, resulting in alteration of neuron functionality (Binder et al. 2006). A previous study also showed that AQP4 levels were significantly higher in the cerebral cortex (grey matter) than in other parts of the brain (Han et al. 2004), and immunogold electron microscopy demonstrated that AQP4 is restricted to the glial membrane and ependymal cells (Balladh et al. 2004). Therefore, *S. suis* invaded the brain much better in mice that exhibited neurological symptoms than in symptom-free mice, and *S. suis* broke down the cells of the cerebral cortex, including AQP4. *S. suis* invaded the white matter, at which point the mice began to exhibit neurological symptoms. The present study demonstrated that *S. suis* was present in the white matter of mice that exhibited neurological symptoms. However, we could not clarify the region of white matter that was related to the induction of neurological symptoms due to *S. suis* infection, and this clarification is an important topic for future study.

The downregulation of AQP4 in the brain may also be due to functional mechanisms. Astrocytes are involved in inflammatory processes and are activated in response to brain damage. In this case, they may release inflammatory mediators, which may alter the integrity and permeability of the blood–brain barrier and neuronal survival (Abbott et al. 2000). In addition, astrocytes may release neurotrophic factors, which can be neurotoxic to neurons in the pursuit of brain damage (Pehar et al. 2004; Lucero et al. 2012). It is possible that astrocytes undergo astrogliosis when they come into contact with *S. suis* or extracellular proteins that contain toxins, such as haemolysin released by *S. suis*. Astrogliosis decreases the expression of AQP4 (Hassan-Olive 2019). Therefore, this may have been caused by *S. suis* infection in this study. Based on these findings, in this study, AQP4 might be downregulated by *S. suis* infection.

In conclusion, mice that exhibited neurological symptoms also harboured high *S. suis* levels and downregulated AQP4 levels in the brain. Image analysis demonstrated that *S. suis* progressed to invade the white matter in the brain of infected mice.

**Acknowledgements** I would like to thank the research staff of the Laboratory of Clinical Research on Infectious Disease, RMD, Osaka University (Japan), who helped operate the microscopes. This work was supported by research grants from the Grant-in-Aid for Young Scientists B (24791022) and the Ministry of Education, Science, and Culture of Japan.

**References**

Abbott NJ (2000) Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol 20:131–147. https://doi.org/10.1023/a:1007074420772

Amran MY, Fuji J, Suzuki SO, Kolling GL, Villanueva SY, Kinumwa M, Kobayashi H, Kameyama H, Yoshida S (2013) Investigation of encephalopathy caused by Shiga toxin 2c-producing *Escherichia coli* infection in mice. PLoS ONE 8:e58959. https://doi.org/10.1371/journal.pone.0058959

Balladh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview structure, regulation, and clinical implications. Neurobiol Dis 16:1–13. https://doi.org/10.1016/j.nbd.2003.12.016

Binder DK, Yao X, Verkman AS, Manley GT (2006) Increased seizure duration in mice lacking aquaporin-4 water channels. Acta Neuropsych 96:389–392. https://doi.org/10.1007/3-211-30714-1_80
Cruz NF, Ball KK, Froehner SC, Adams ME, Dienel GA (2013) Regional registration of [6–14 C] glucose metabolism during brain activation of alpha-syntrophin knockout mice. J Neurochem 24:10. https://doi.org/10.1111/jnc.12166

De-Greeff A, Buys H, Verhaar R, Van-Alphen L, Smith HE (2002) Distribution of environmentally regulated genes of Streptococcus suis serotype 2 among S. suis serotypes and other organisms. J Clin Microbiol 40:3261–3268. https://doi.org/10.1128/jcm.40.9.3261-3268.2002

Dominguez-Punaro M, Segura M, Radzioch D, Rivest S, Gottschalk M, Xu J, Calzas C, Segura M (2010) AQP4 expression and MAPK pathway. Neuropharmacology 67:8–41. https://doi.org/10.1016/j.neuropharm.2012.05.005

Verkman AS, Yang B, Song Y, Manley GT, Ma T (2000) Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. Exp Physiol 85:233S–S241. https://doi.org/10.1111/j.1469-445x.2000.tb0028.x

Verkman AS, Binder DK, Bloch O, Auguste K, Papadopoulos MC (2006) Three distinct roles of aquaporin-4 in brain function in infants with congenital cytomegalovirus infection. Brain Dev 36:10–15. https://doi.org/10.1016/j.braindev.2012.12.009

Moinuddin A, McKinstry RC, Martin KA, Neil JJ (2003) Intracranial hemorrhage progressing to porencephaly as a result of congenitally acquired cytomegalovirus infection and illustrative report. Prenat Diagn 23:797–800. https://doi.org/10.1002/pd.688

Morris EB, Li C, Khan RB, Sanford RA, Boop F, Pinlac R, Xiong X, Merchant TE (2009) Evolution of neurological impairment in pediatric infratentorialependymoma patients. J Neurooncol 94(2009):391–398. https://doi.org/10.1007/s11060-009-9866-8

Nakayama T, Eoze H (2014) Heat incubation inactivates streptococcal exotoxins and recombinant cholesterol-dependent cytolysin: sulysin, pneumolysin and streptolysin O. Curr Microbiol 69:690–698. https://doi.org/10.1007/s00284-014-0639-z

Nakayama T, Takeuchi D, Akeda Y, Oishi K (2010) Streptococcus suis infection induces bacterial accumulation in the kidney. Microb Pathog 50:87–93. https://doi.org/10.1016/j.micpath.2010.11.005

Nakayama T, Takeuchi D, Matsumura T, Akeda Y, Fujinaga Y, Oishi K (2013) Alcohol consumption promotes the intestinal translocation of Streptococcus suis infection. Microb Pathog 65:14–20. https://doi.org/10.1016/j.micpath.2013.08.006

Niu D, Kondo T, Nakazawa T, Kawasaki T, Yamane T, Mochizuki K, Yato M, Matsuzaki T, Takata K, Katoh R (2012) Differential expression of aquaporins and its diagnostic utility in thyroid cancer. PLoS ONE 7:e40770. https://doi.org/10.1371/journal.pone.0040770

Olfert ED, Godson DL (2000) Humane endpoints for infectious disease animal models. Inst Lab Anim Res 41:99–104. https://doi.org/10.1093/iilar.41.2.99

Papadopoulos MC, Verkman AS (2005) Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. J Biol Chem 280:13906–13912. https://doi.org/10.1074/jbc.M413627200

Papadopoulos MC, Verkman AS (2007) Aquaporin-4 and brain edema. Pediatr Nephrol 22:778–784. https://doi.org/10.1007/s00467-006-0411-0

Pechar M, Cassina P, Vargas MR, Castellanos R, Viera L, Beckman JS, Estévez AG (2004) Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for amyotrophic lateral sclerosis. J Neurochem 89:46467. https://doi.org/10.1111/j.1471-4159.2004.02357.x

Pommerene D, Lunde LD, Amiry-Moghaddam M, Agre P (2013) Protective role of brain water channel AQP4 in murine cerebral malaria. Proc Natl Acad Sci USA 106:107–112. https://doi.org/10.1073/pnas.122056610

Smith HE, Vecht U, Gielkens AL, Smits MA (1992) Cloning and nucleotide sequence of the gene encoding the 136 kDa surface protein (muramidase-released protein) of Streptococcus suis type 2. Infect Immun 60:2361–2367. https://doi.org/10.1128/IAI.60.6.2361-2367.1992

Smith HE, Reek FH, Vecht U, Gielkens AL, Smits MA (1993) Repeats in an extracellular protein of weakly pathogenic strains of Streptococcus suis type 2 are absent in pathogenic strains. Infect Immun 61:3318–3326. https://doi.org/10.1128/IAI.61.8.3318-3326.1993

Tang Z, Sun X, Huo G, Xie Y, Shi Q, Chen S, Wang X, Liao Z (2013) Protective effects of erythropoietin on astrocytic swelling after oxygen-glucose deprivation and reoxygenation: mediation through AQP4 expression and MAPK pathway. Neuropsychopharmacology 67:8–15. https://doi.org/10.1016/j.neuropharm.2012.10.017

Verkman AS, Yang B, Song Y, Manley GT, Ma T (2000) Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. Exp Physiol 85:233S–S241. https://doi.org/10.1111/j.1469-445x.2000.tb0028.x

Springer
revealed by knockout mice. Biochim Biophys Acta 1758:1085–1093. https://doi.org/10.1016/j.bbamem.2006.02.018

Wellmer A, Gerber J, Ragheb J, Zysk G, Kunst T, Smirnov A, Bruck W, Nau R (2001) Effect of deficiency of tumor necrosis factor alpha or both of its receptors on Streptococcus pneumonia central nervous system infection and peritonitis. Infect Immun 69:6881–6886. https://doi.org/10.1128/IAI.69.11.6881-6886.2001

Wertheim HF, Nguyen HN, Taylor W, Lien TT, Ngo HT, Nguyen TQ, Nguyen BN, Nguyen HH, Nguyen HM, Nguyen CT, Dao TT, Nguyen TV, Fox A, Farrar J, Schultsz C, Nguyen HD, Nguyen KV, Horby P (2009) Streptococcus suis, an important cause of adult bacterial meningitis in northern Vietnam. PLoS ONE 22:e5973. https://doi.org/10.1371/journal.pone.0005973

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.