



# Preclinical evidence of sphingosine kinase 1 inhibition in alleviation of intestinal epithelial injury in polymicrobial sepsis

Felix Nnaemeka Ugwu<sup>1</sup> · Jeffery Ho<sup>2</sup>

Received: 22 April 2019 / Revised: 27 May 2019 / Accepted: 28 May 2019 / Published online: 1 June 2019  
© Springer Nature Switzerland AG 2019

## Abstract

**Background** Intestinal epithelial injury in septic patients predicts subsequent development of multiple organ failure, but its regulation by host factors remains unclear. Sphingosine kinase 1 is an enzyme-regulating inflammatory response.

**Methods** Cecal ligation and puncture was used to induce sepsis in C57BL/6 mice with and without *N,N*-dimethylsphingosine, a SphK1 inhibitor. Symptom severity was monitored by murine sepsis severity score. The intestinal barrier function was determined using 4KDa fluorescein-dextran. Bacterial load in the bloodstream was determined by 16S rRNA gene amplification.

**Results and conclusions** Our preliminary experimental data showed that expression of sphingosine kinase 1 in ileum was increased by sixfold in septic mice. Pharmacological blockade of sphingosine kinase 1 alleviated septic symptoms. The intestinal permeability and bacterial load in the bloodstream were also reduced in these animals. We hypothesized that inhibition of sphingosine kinase 1 may reduce pro-inflammatory cytokine production, and alleviate intestinal epithelial injury during sepsis. Further mechanistic studies and clinical specimen analyses are warranted.

**Keywords** Sepsis · Sphingosine kinase 1 · Intestinal epithelium · Caecal ligation and puncture

## Introduction

Sepsis is a state of organ dysfunction as a result of altered host response to an infection which involves multiple cellular pathways [1]. Accumulating evidence suggests that the intestinal epithelium segregating the intestinal lumen from the blood circulation could be altered. This manifested as increased apoptosis, disrupted tight junctions, and heightened permeability [2, 3]. In clinical settings, abnormal intestinal permeability has been reported in critically ill patients whose clinical outcomes were associated with the degree of its permeability change [3]. In this connection, attempts

were sought to sustain the gut barrier function in systemic inflammation as in sepsis.

Sphingosine kinase 1 (SphK1) is an enzyme catalyzing the formation of sphingosine-1-phosphate (S1P), which is responsible for the downstream nuclear factor kappa B (NFkB)-mediated pro-inflammatory cytokine production. It was anticipated that SphK1 inhibition could control inflammation and its associated tissue injury. In SphK1-depleted (SphK1<sup>-/-</sup>) mice treated with dextran sulfate sodium (DSS), they exhibited less colonic damage, were protected against the development of systemic inflammation, and reduced production of pro-inflammatory cytokines [4]. In endothelial cells exposed to activated neutrophils, the endothelial permeability increased but was significantly alleviated by pharmacological blockade of SphK1 using *N,N*-dimethylsphingosine, a potent SphK1 inhibitor [5]. In a rat endotoxemia model, suppression of SphK1 reduced myocardial apoptosis and inflammation [6]. Importantly, ongoing clinical trials have highlighted the therapeutic potential of targeting SphK1–S1P axis in inflammatory diseases [7]. Nevertheless, the role of SphK1 in sepsis-associated intestinal barrier dysfunction has not yet been reported.

---

Responsible Editor: John Di Battista.

✉ Jeffery Ho  
jeffho@cuhk.edu.hk

<sup>1</sup> Department of Anaesthesia and Intensive Care, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, China

<sup>2</sup> Department of Microbiology, Faculty of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China

## Materials and methods

### Mouse strain and experimental sepsis

C57BL/6 mice were used in this study. Cecal ligation and puncture (CLP) was used to induce sepsis in these animals [8]. Briefly, mice that were 8–10 weeks old were anesthetized with 3% isoflurane during which a 1-cm midline incision was made on the anterior abdomen. The cecum was exposed and ligated, and the distance from the distal end of the cecum to the ligation point was approximately 1 cm. A double puncture was made with a 22-gauge needle to induce sepsis. The cecum was squeezed to allow the cecum contents to flow through the punctures. The cecum was then placed back in the abdominal cavity, and the peritoneal wall, and skin incision was closed. Sham-operated animals underwent identical laparotomy, but the cecum was neither ligated nor punctured. The survival rates and severity of the septic animals were recorded daily until 7 days after the surgery with reference to murine sepsis severity (MSS) score as previously described [9]. At any given time point of observation, mice with a MSS score more than 21 or had an increment of more than 3 points between two consecutive observations was euthanized. To ensure consistency of the performance of the experiments, the number of puncture, proportion of cecal ligation, and animal demographics including age, sex, and strains were kept the same. All mice were fasted overnight before CLP to minimize the variation of fecal content between animals.

### Intestinal permeability, tissue apoptosis and SphK1 activity

The animals were gavaged with 4 kD fluorescein isothiocyanate (FITC)-dextran (500 mg/kg body weight in a 50 mg/mL concentration) after CLP using a 4-cm long, curved needle with a plastic ball at a tip. After the animals were sacrificed, the blood was collected and the intensity of FITC was determined by a fluorometer using an excitation wavelength of 488 nm and detecting the emission at 520 nm. Total proteins were extracted from ileal tissues using standard methods followed by immunoblotting with antibodies against cleaved poly (ADP-ribose) polymerase (PARP) and beta-tubulin. The expression of gene encoding SphK1 and its activity level were measured by real-time polymerase chain reaction and enzyme-linked immunosorbent assay (Mouse SphK1 ELISA, R&D Systems), respectively, according to manufacturer's instructions.

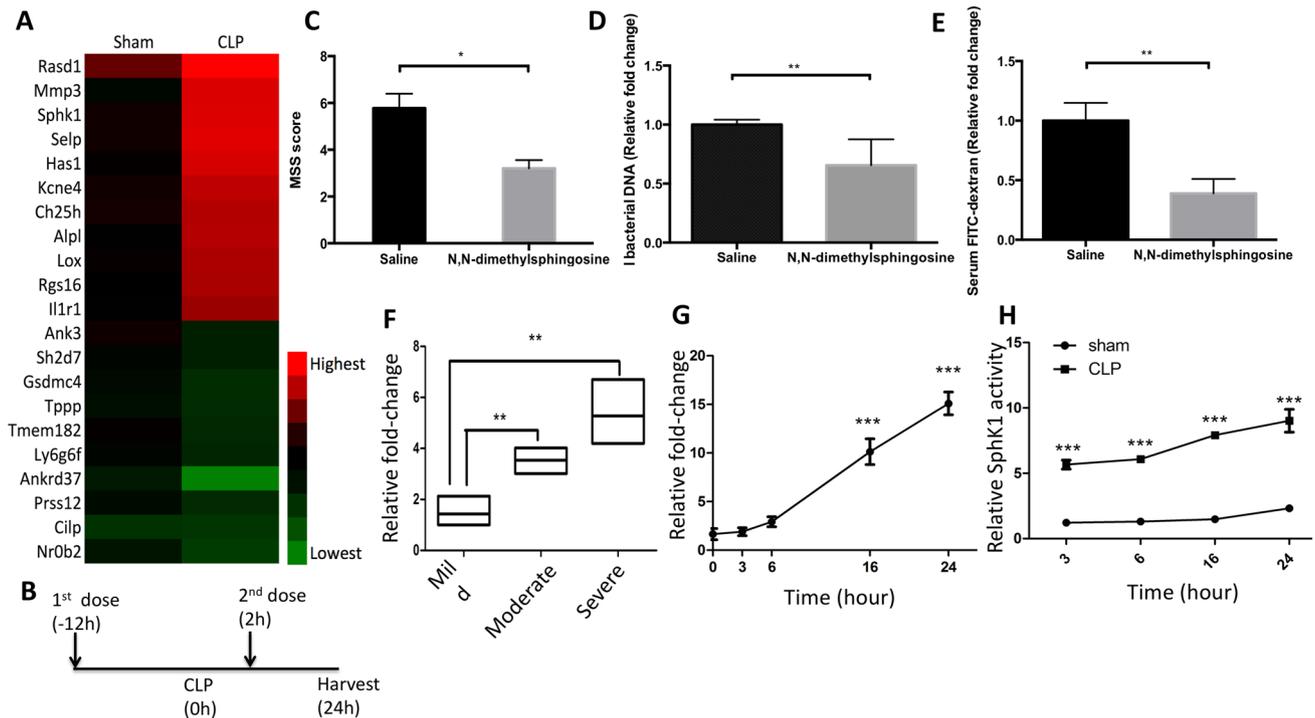
## Ethical statement

The conduct of the above animal experiments was approved by the animal experimentation ethics committee of the Chinese University of Hong Kong.

## Results and discussion

In the animal group that underwent CLP, the level of SphK1 increased considerably up to sixfold as compared to the sham-operated group (Fig. 1). To confirm the protective function of SphK1 inhibition in sepsis, we performed CLP in mice with and without *N,N*-dimethylsphingosine, a SphK1 inhibitor. In this regard, mice that received *N,N*-dimethylsphingosine not only showed milder symptoms as revealed by the MSS score, but also had a reduced level of circulating bacteria of potential origin from the gut. Importantly, the gut permeability, as evidenced by the measurement of FITC dextran, reduced in the animal group treated with *N,N*-dimethylsphingosine. It was demonstrated that the increase of mRNA level of SphK1 was dependent on the severity of sepsis symptoms as varied by the point of ligation at the distal ileum. Similarly, the expression of SphK1 and enzyme activity increased significantly over time after CLP. More importantly, the SphK1 activity was significantly correlated with the level of cleaved PARP, an effector protein of active apoptosis, indicating that sepsis-induced epithelial injury was associated with SphK1 expression. Cecal ligation and puncture is regarded as the gold standard for inducing experimental sepsis, in which the animals develop an initial hyperdynamic cardiovascular response similar to human sepsis [8]. In our experiments, we compared animals treated with SphK1 inhibitor to sham-operated animals for their outcomes following CLP. Our results suggested that inhibition of SphK1 may prevent intestinal epithelial apoptosis and reduce the gut permeability under systemic inflammation such as sepsis. SphK1 is known to activate NF $\kappa$ B in mediating inflammatory response [10]. In this regard, characterization of activity pertinent to the NF $\kappa$ B pathway will be beneficial. The activation status of NF- $\kappa$ B signaling can be determined by measuring the levels of IKK $\alpha$ , a catalytic subunit of IKK complex that degrades I $\kappa$ B $\alpha$ , total and phosphorylated I $\kappa$ B $\alpha$  and nuclear NF- $\kappa$ B2 (p52/p100), NF- $\kappa$ B1 (p50/p105), cRel, RelA/p65, and RelB, using immunoblotting. The activities of NF- $\kappa$ B in the intestinal tissues subject to induction of sepsis could be determined by Cignal dual luciferase assay (Qiagen), which measures the activity of pathway-specific transcription activities.

In the future, more evidence would be required to confirm the therapeutic potential of SphK1 inhibitor in



**Fig. 1** **a** Genome-wide transcriptome of ileal tissues from mice at 24 h after cecal ligation and puncture as compared to sham surgery. Sphingosine kinase 1 is highly upregulated, **b** *N,N*-dimethylsphingosine or saline was administered at 12 h before and 2 h after CLP. **c** Murine sepsis severity score on day one after CLP, **d** bacterial DNA load determined by real-time PCR, **e** oral gavage of FITC-dextran was performed at 21 h after CLP and its serum level determined after 3 h, **f** relative fold change (CLP vs Sham) in SphK1 gene expression level in mice with mild, moderate, and severe sepsis at 24 h ( $n=3$

per group) after CLP as compared to sham surgery, **g** relative fold change (CLP vs Sham) in SphK1 gene expression level in mice over 24 h ( $n=3$  per group) after CLP as compared to those that underwent sham surgery. **h** Dynamic change in relative SphK1 enzyme activity over 24 h after cecal ligation and puncture or sham surgery ( $n=3$  per group). The Sphk1 enzyme activity was significantly higher in mice that underwent CLP as compared to those that received sham surgery at all time points ( $P<0.001$ ). The level of activity increased by more than 50% as compared to that 3 h after CLP

protecting against intestinal epithelial injury in sepsis. Demonstrating such effects would have significant therapeutic implications. Clinical sample analysis is warranted to determine the positive association, if any, between SphK1 activity and intestinal mucosal damage in septic patients.

**Acknowledgements** Part of this study was presented in International Sepsis Forum, Bangkok, Thailand, October 2018. The authors would like to thank Health and Medical Research Fund, Food and Health Bureau, The Government of Hong Kong Special Administrative Region, China (05160746) for supporting this study.

### Compliance with ethical standards

**Conflict of interest** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### References

1. Ho J, Zhang L, Liu X, Wong SH, Wang MHT, Lau BWM, et al. Pathological role and diagnostic value of endogenous host defense peptides in adult and neonatal sepsis: a systematic review. *Shock*. 2017;47:673–9.
2. Doig CJ, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. *Am J Respir Crit Care Med*. 1998;158(2):444–51.
3. Assimakopoulos SF, Triantos C, Thomopoulos K, Fligou F, Maroulis I, Marangos M, et al. Gut-origin sepsis in the critically ill patient: pathophysiology and treatment. *Infection*. 2018;46:751–60.
4. Snider AJ, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Hannun YA, et al. A role for sphingosine kinase 1 in dextran sulphate sodium-induced colitis. *The FASEB J*. 2009;23(1):143–52.

5. Itagaki K, Zhang Q, Hauser CJ. Sphingosine kinase inhibition alleviates endothelial permeability induced by thrombin and activated neutrophils. *Shock*. 2010;33(4):381–6.
6. Zhang T, Yan T, Du J, Wang S, Yang H. Apigenin attenuates heart injury in lipopolysaccharide-induced endotoxemic model by suppressing sphingosine kinase 1/sphingosine-1-phosphate signalling pathway. *Chem Biol Interact*. 2015;233:46–55.
7. Kunkei GT, Maceyka M, Milstien S, Spiegel S. Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nat Rev Drug Dis*. 2013;12(9):688–702.
8. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009;302(21):2323–9.
9. Shrum B, Anantha RV, Xu SX, Donnelly M, Haeryfar SM, McCormick JK, et al. A robust scoring system to evaluate sepsis severity in an animal model. *BMC Res Notes*. 2014;7:233.
10. Pyne NJ, Pyne S. Spingosine-1-phosphate is a missing link between chronic inflammation and colon cancer. *Cancer Cell*. 2013;23:5–7.

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