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Title

Minocycline prevents the impairment of hippocampal long-term potentiation in the septic mouse

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Running head

MINO prevents LTP impairment during sepsis

Abstract

Sepsis-associated encephalopathy is a major complication during sepsis, and an effective treatment remains unknown. Although minocycline (MINO) has neuroprotective effects and is an attractive candidate for treating sepsis-associated encephalopathy, the effect of MINO on synaptic plasticity during sepsis is still unclear. In the present study, we investigated the effects of MINO on long-term potentiation (LTP) in the hippocampus in a cecal ligation and puncture (CLP) mouse model. We divided mice into 4 groups; (1) sham + vehicle, (2) sham + MINO (60mg/kg, i.p. for 3 consecutive days before slice preparation), (3) CLP + vehicle, and (4) CLP + MINO. We tested LTP in the CA1 region of the hippocampus, using slices taken 24 h after surgery. Because MINO is also anti-inflammatory, LTP was analyzed following 30 min of IL-1 receptor antagonist (IL-1ra) perfusion. The endotoxin level in the blood was increased at 24 h after CLP operations regardless of MINO administrations, and LTP in the CLP + vehicle group mice was severely impaired ($P < 0.05$). High doses of MINO prevented the LTP impairment during sepsis in the CLP + MINO group. Interleukin (IL) -1ra administration ameliorated LTP impairment only in the CLP + vehicle group ($P < 0.05$); it had no additional effects on LTP in the CLP + MINO group. In conclusion, we have provided the first evidence that MINO prevents impaired LTP related to sepsis-induced

encephalopathy in the mouse hippocampus, and that mechanisms associated with IL-1 receptor activity may be involved.

Keywords

sepsis-associated encephalopathy; microglia; synaptic plasticity; interleukin-1 beta; neuroinflammation

Introduction

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis-associated encephalopathy (SAE), which is a rapid decline in cognitive function, especially memory, is a major complication of sepsis. SAE occurs in over half of patients with sepsis (1), which is itself an independent predictor of death (2). Furthermore, functional deficits caused by SAE often never recovers (3, 4). Although early neuroprotective intervention is thought to be important, effective therapies have not yet been established.

Microglia activation and subsequent release of pro-inflammatory cytokines, including tumor necrosis factor- α and interleukin (IL)-1 β , are hypothesized to play a pivotal role in the development of SAE (5, 6). Minocycline (MINO), a second-generation semisynthetic tetracycline derivative, not only has anti-microbial activity, but also anti-inflammatory and anti-apoptotic activities (7). These characteristics derive from the ability of MINO to inhibit microglia activation, and to attenuate the expression of inflammatory genes, including IL-1 β (7). Although some clinical and animal studies have shown neuroprotective effects of MINO in ischemic brain injury and SAE, with behavioral tests and immunohistochemistry (8-10), it is still unclear whether MINO affects SAE-induced deficits in synaptic plasticity. Long-term potentiation (LTP) is a

form of synaptic plasticity identified by Bliss et al. in 1973 (11). They found that brief, high frequency synaptic stimulation resulted in a long-lasting increase in synaptic strength. Whether SAE affects LTP is an important question to address, given that LTP is considered the cellular basis of learning and memory (12). The hippocampus is known to be associated with learning and memory, and notably, is also the region most vulnerable to experimental sepsis (13). Therefore, we investigated the effects of MINO on hippocampal LTP in the standard mouse model of sepsis, the cecal ligation and puncture (CLP) mouse (14).

Materials and Methods

Ethical approval

All experiments were performed according to the guidelines for the care and use of laboratory animals of Hokkaido University, as well as the ARRIVE guidelines. The study protocol was approved by the Animal Care and Use Committee of the Hokkaido University Medical School (No. 15-0069).

Cecal ligation and puncture (CLP)

Male C57BL/6J mice (6 weeks old) were purchased from SLC (SLC Japan Inc., Shizuoka, Japan). Mice were kept at a controlled temperature (22–26°C) with a 12:12 light-dark cycle, with free access to water and food. Mice were deeply anesthetized by pentobarbital (40 mg/kg, i.p.) and butorphanol (5 mg/kg, i.p.). The CLP model was developed by Chaudry et al. (14) as a standard procedure in sepsis research due to the reproducibility of its hemodynamic and metabolic changes. In brief, after a midline abdominal incision, the cecum was carefully mobilized and then ligated with 3-0 silk below the ileocecal valve, approximately 1.2 cm from the tip of the cecum. The cecum was then perforated once with a 21-gauge needle and was squeezed to extrude some feces into the peritoneal cavity. Based on the descriptions in a previous report (15), we

selected the “medium” level of severity because we expected that the prolonged hypoperfusion generated in the highly severe model would make it inadequate for evaluating electrophysiological changes. The abdomen was closed in two layers with 4-0 PROLENE® (ETHICON Inc. NJ, USA) sutures, and the mouse resuscitated with a subcutaneous lactated Ringer’s (30 mL/kg) injection immediately after surgery. The entire procedure was completed within 10 min. Sham-operated mice received the same surgical procedures without ligation and puncture of the cecum.

Endotoxin measurement

Endotoxin concentration in the blood was examined in order to confirm the validity of the model mice 24 h after surgery. After mice were deeply anesthetized with pentobarbital and butorphanol, as described above, an approximately 500 µL blood sample was extracted through cardiac puncture with a heparinized syringe. Endotoxin concentration was measured by a toxinometer (MT-6500, Wako, Osaka, Japan).

Electrophysiology

Transverse hippocampal slices (300 µm thick) were prepared with a Pro 7 microslicer (DOSAKA-EM, Kyoto, Japan). Mice were deeply anesthetized with isoflurane, and the

brain was dissected in an ice-cold sucrose solution composed of the following (in mM): 40 NaCl, 25 NaHCO₃, 10 glucose, 150 sucrose, 4 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, and 7 MgCl₂. Next, the sucrose-containing solution was replaced with artificial cerebrospinal fluid (ACSF) containing the following (in mM): 127 NaCl, 1.5 KCl, 1.2 KH₂PO₄, 26 NaHCO₃, 10 glucose, 2.4 CaCl₂, and 1.3 MgCl₂; the slices were incubated in ACSF for 30 min at 30°C, and subsequently for approximately 30 min at room temperature in an interface-type chamber with saturated 95% O₂ and 5% CO₂. Slices were transferred into an observation chamber and continuously superfused at 2 mL/min with ACSF that was equilibrated with 95% O₂ and 5% CO₂. Electrical stimuli (200 μ s duration, < 400 μ A intensity) were delivered every 10 s through a tungsten concentric bipolar electrode inserted into the stratum radiatum of the CA1 region of the hippocampus, and the resultant excitatory postsynaptic potentials (EPSPs) were recorded from the same region via glass microelectrodes with 10- μ m diameter tips filled with ACSF. Stimulation intensity was increased up to the threshold at which action potentials would be produced. We confirmed the stability of the EPSPs amplitude before LTP induction or drug application for at least 20 min. Theta-burst stimulation (TBS), which consists of 3 trains of stimuli delivered 20 s apart, robustly induced LTP. Each train is composed of 10 bursts of 4 pulses at 100 Hz with an interval of 200 ms. We

compared the EPSP amplitudes expressed 60 min after TBS to the baseline, as described in a previous report (16). All recordings were made at room temperature using a MEG-5200[®] amplifier (NIHON KOHDEN, Tokyo, Japan) and LabChart[®] 7 software (AD Instruments, CO, USA).

Drug administration

MINO was obtained from Wako (Osaka, Japan). To evaluate the effect of MINO on LTP, we randomly divided mice into the following 4 groups: sham + vehicle group, sham + MINO group, CLP + vehicle group, and CLP + MINO group. Mice received an intraperitoneal (i.p.) injection of either MINO (60 mg/kg) or the vehicle (equivalent dose of normal saline) for 3 consecutive days. The last injection was performed just after the CLP or sham operation was completed. In the experiments to investigate the dose-response effects of MINO, we tested three doses (60 mg/kg, 30 mg/kg, and 5 mg/kg, all i.p.); all mice were injected as described above. We selected the dose of 60 mg/kg i.p. because high doses of MINO (≥ 40 mg/kg) has previously been used in many studies on neuroprotection using MINO (9, 17). The dose of 5 mg/kg i.p. is similar to the clinical dose in humans for treating infection (18), and the dose of 30 mg/kg is an intermediate value between them. A repeated-injection regimen was utilized since this

was shown to be necessary to attenuate neuroinflammation in a number of previous reports (9, 17). Mouse recombinant IL-1 receptor antagonist (IL-1ra; 97% pure) was purchased from R&D systems (MN, USA). Bovine serum albumin (BSA, 1 µg/mL) was used as a carrier protein to prevent nonspecific adsorption of IL-1ra to the recording chamber, tubing, etc. During drug delivery to the slices, a solution containing IL-1ra (50 ng/mL) was kept in a polypropylene container, also to prevent nonspecific adsorption. For control group recordings, BSA (1 µg/mL) alone was applied. We deemed that the concentration of IL-1ra, 50 ng/mL, was enough to block the IL-1 receptor based upon previously published work (19).

Statistical analysis

Data are presented as the mean $\pm$ standard deviation (SD) and analyzed with JMP[®] Pro 12.0 (SAS Institute Inc., NC, USA). We used Student's t-tests for pair-wise group comparisons. The difference among multiple groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's test when appropriate. The survival rate was estimated by the Kaplan-Meier method and compared by the log-rank test and Bonferroni's post hoc tests. P values < 0.05 were regarded as significant.

Results

CLP induces endotoxemia accompanied with high mortality rate

We measured the endotoxin concentration in blood samples to evaluate the validity of CLP mice. Endotoxin values in the CLP group were much higher than those in the sham group (1773 ± 596 pg/ml vs. 61 ± 102 pg/ml, $n = 5$ respectively; $P < 0.05$) (Fig. 1A). MINO treatment did not decrease the endotoxin values (1439 ± 778 pg/ml, $n = 5$) (Fig. 1A). Furthermore, CLP was accompanied by a high mortality rate through 7 days against sham group (survival rate in 7 days: 43.8% vs. 100%, $n = 16$, respectively, $P < 0.05$) (Fig. 1B). MINO treatment did not improve the survival rate (survival rate in 7 days: 40.0%, $n = 15$) (Fig. 1B). These results suggest that the antibiotic effect of MINO was not enough to treat the induced peritonitis.

MINO administration at higher doses prevent LTP impairment in CLP mice

We divided animals into 4 groups (see “Drug administration” above) and examined LTP in the CA1 region of the hippocampus (Fig.2A). EPSPs in the sham + vehicle group immediately increased after TBS, resulting in robust LTP. However, LTP was notably impaired in the CLP + vehicle group ($197.5 \pm 29.3\%$ vs. $149.1 \pm 6.2\%$ of EPSP amplitude to the baseline, $n = 5, 6$ respectively; $P < 0.05$) (Fig. 2B–D). Three consecutive days of 60 mg/kg MINO administration (CLP + MINO group) prevented

the LTP impairment observed in the CLP + vehicle group, rescuing LTP to approximately the same values as the sham-operated groups ($196.7 \pm 24.2\%$ vs. $149.1 \pm 6.2\%$, $n = 5, 6$ respectively; $P < 0.05$) (Fig. 2C and D). In the sham-operated groups, MINO administration had no effect on LTP ($197.5 \pm 29.3\%$ vs. $196.7 \pm 24.2\%$, $n = 5$ respectively; $P > 0.05$) (Fig. 2B and D).

We conducted the same experiment with the 30 mg/kg and 5 mg/kg doses as well. Although 30 mg/kg of MINO significantly blocked LTP impairment, to about the same degree as 60 mg/kg ($208.8 \pm 32.5\%$, $n = 5$; $P > 0.05$ compared with CLP + vehicle group), LTP was still impaired at 5 mg/kg of MINO ($151.8 \pm 29.2\%$, $n = 6$, $P > 0.05$ compared with CLP + vehicle group) (Fig. 3A and B).

IL-1ra rescues LTP impairment in CLP mice

To investigate the relationship between MINO and IL-1 β inhibition, we applied IL-1ra (50 ng/mL) to slices for 30 min before LTP induction. First, IL-1ra did not affect LTP in the sham-operated group ($194.4 \pm 54.1\%$ vs. $185.4 \pm 35.8\%$, $n = 5$ respectively; $P > 0.05$) (Fig. 4A and D). Conversely, IL-1ra administration increased the LTP value in the CLP + vehicle group to values similar to the sham-operated group ($206.7 \pm 27.7\%$ vs. $157.8 \pm 18.2\%$, $n = 5$ respectively; $P < 0.05$) (Fig. 4B and D); the effect of IL-1ra on

LTP among MINO-treated mice was limited ($199.8 \pm 35.3\%$ vs. $196.4 \pm 38.3\%$, $n = 5$ respectively; $P > 0.05$) (Fig. 4C and D).

Discussion

To our knowledge, the present results provide the first evidence that high doses of MINO prevents LTP impairment related to SAE in the mouse hippocampus. These effects were most likely due to its anti-inflammatory activity, given that blocking IL-1 receptor signaling rescued LTP in the CLP mice. The effectiveness of IL-1 antagonism in rescuing LTP suggests that MINO acts to restore LTP through an IL-1 receptor-associated mechanism.

SAE is characterized by disorientation, delirium, impaired attention, and coma; additionally, it causes long-term functional disability (3), and greatly increases mortality due to sepsis (2). Pathophysiology indicative of SAE includes systemic inflammation, which leads to cerebral endothelial activation and transport of pro-inflammatory mediators such as cytokines and nitric oxide through the impaired blood-brain barrier (BBB). This neuroinflammatory state results in modulation of neurotransmission and neuronal apoptosis. In this sequence of events, activated microglia are hypothesized to play a key role (5). Early activation of microglia in lipopolysaccharide (LPS)-induced

septic mice was reported by Terrando et al. within 4 hours following LPS injection (20), suggesting that microglia in our CLP mice (24 h after surgery) could have been activated. A similar activation lasted for a month after sepsis (21), and has been found in humans that died of sepsis (22). Although resting microglia usually monitor pathogens in their microenvironment, activated microglia undergo a morphological change, and function to induce neuroinflammation by producing cytokines, immune molecules, and gliotransmitters (5).

It is well known that neuroinflammation modulates cognitive dysfunction and synaptic plasticity (23), and IL-1 β in particular degrades LTP (24). Several previous studies have reported that IL-1 β impairs LTP in the various regions of the hippocampus in slices (25, 26). IL-1 β is considered to be a key molecule in the mechanism of LTP impairment in sepsis (15); it acts through type I IL-1 receptors, which are highly expressed in the hippocampus and antagonized by IL-1ra (27). Moraes et al. reported that CLP mice led to increased activated microglia-derived IL-1 β in the hippocampus 24 h after the surgery (6). Therefore, we feel our data are the result of neuroinflammatory activity, which would consistent with previous reports (15, 16).

MINO is a highly lipophilic molecule that can easily pass thorough the BBB, and exerts additional effects beyond its antibiotic activity. MINO administration decreases

the level of IL-1 β in the hippocampus; notably, its anti-inflammatory effect is reported to act only within the brain (9). Several previous reports have shown that MINO improved sickness behavior in septic animals (9, 28); our data showed no improvement in mortality, but are the first to demonstrated the positive effect of MINO on synaptic plasticity related to sepsis. Several mechanisms by which MINO acts as a neuroprotective agent have been suggested. One popular theory is that MINO inhibits nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAP kinase) dependent signaling pathways in microglia (29). MAP kinase activation is critical for the expression of many inflammatory mediators, and is associated with the impairment of hippocampal LTP by IL-1 β (19). Our data showed IL-1ra reversed the degradation of LTP only in vehicle-treated CLP mice. Therefore, IL-1 receptor signaling appears to be involved in the pathogenesis of SAE, and its inhibition appears to be associated with the ability of MINO to rescue LTP in the septic mouse.

According to our results, and previous reports describing the ability of MINO to improve sickness behavior in septic animals (9, 28), MINO has the potential to successfully treat SAE. Although mortality was not improved with MINO administration, it would not be expected to improve because the effect of MINO on LTP improvement was not derived from the antibiotic effect, but from the anti-inflammatory

effect; furthermore, the gold standard therapy for CLP is not antibiotic therapy, but surgical drainage and cecal resection. Even if mortality is not improved using MINO after radical surgery, SAE could be reduced in those who survive CLP, which would be a clinically meaningful advance. The doses used for neuroprotection in most experimental models are much higher than those for the treatment of infection (9, 17). Fagan et al. described that high doses of MINO were optimally delivered to the brain even with intraperitoneal injection (18). They indicated that the clinical dose in humans would be similar to the 5 mg/kg dose given to rats. Our results have shown that the ability of MINO to prevent LTP impairment during sepsis was limited to the higher (≥ 30 mg/kg) doses. Therefore, it remains to be determined if the concentration required to achieve LTP improvement seen in our experiment can be translated to a safe human dose.

Mina et al. reported that transcisternal IL-1ra administration immediately after the CLP operation improved cognitive impairment, and rescued the increase of IL-1 β levels in the hippocampus (30). This suggests that IL-1ra could be also an attractive candidate for treating SAE; however, it comes with substantial problems, because physiological concentrations of IL-1 β are required for normal memory function (31) and the maintenance of LTP (32).

It is important to consider several limitations when interpreting our results. First, since SAE is a multifactorial event, it is difficult to treat clinically with 1 agent. In addition to MINO, resveratrol and edaravone should also be mentioned as possible therapeutic agents because of their anti-inflammatory and anti-oxidative properties (33, 34). Furthermore, other cytokines besides IL-1 β are also associated with the etiology of SAE (30), so other cytokine antagonists will need to be considered and tested in future work. Secondly, we only tested hippocampal synaptic plasticity at 24 h after surgery; therefore, we could not speak of the long-term effects of MINO. Thirdly, we used 6-week-old mice, because microglial activation can occur without any manipulations in aged animals and lead to impaired LTP (35). Thus, different results might be found in slices from aged mice. Finally, LTP is only one aspect of learning and memory, and our results might not reflect the full clinical situation; in other words, this level of LTP change might not be meaningful for cognition in human cases, although a previous report demonstrated that minocycline did improve cognitive dysfunction in CLP animal models (28).

In conclusion, we have shown the first evidence that MINO prevented LTP impairment in the mouse hippocampus induced by sepsis, and that these effects may be

associated with the inhibition of IL-1 receptor-mediated signaling. Further study will be necessary to fully elucidate these mechanisms.

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References

1. Gofton TE, Young GB: Sepsis-associated encephalopathy. *Nat Rev Neurol* 8:557-566, 2012.
2. Sprung CL, Peduzzi PN, Shatney CH, Schein RM, Wilson MF, Sheagren JN, Hinshaw LB: Impact of encephalopathy on mortality in the sepsis syndrome. The Veterans Administration Systemic Sepsis Cooperative Study Group. *Crit Care Med* 18:801-806, 1990.
3. Iwashyna TJ, Ely EW, Smith DM, Langa KM: Long-term cognitive impairment and functional disability among survivors of severe sepsis. *JAMA* 304:1787-1794, 2010.
4. Widmann CN, Heneka MT: Long-term cerebral consequences of sepsis. *Lancet*

Neurol 13:630-636, 2014.

5. Michels M, Danielski LG, Dal-Pizzol F, Petronilho F: Neuroinflammation: microglial activation during sepsis. *Curr Neurovasc Res* 11:262-270, 2014.
6. Moraes CA, Santos G, Spohr TC, D'Avila JC, Lima FR, Benjamim CF, Bozza FA, Gomes FC: Activated Microglia-Induced Deficits in Excitatory Synapses Through IL-1 β : Implications for Cognitive Impairment in Sepsis. *Mol Neurobiol* 52:653-663, 2015.
7. Garrido-Mesa N, Zarzuelo A, Galvez J: Minocycline: far beyond an antibiotic. *Br J Pharmacol* 169:337-352, 2013.
8. Amiri-Nikpour MR, Nazarbaghi S, Hamdi-Holasou M, Rezaei Y: An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. *Acta Neurol Scand* 131:45-50, 2015.
9. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, Sheridan JF, Godbout JP: Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *J Neuroinflammation* 5:15, 2008.
10. Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J: Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting

- activation and proliferation of microglia. *J Neurosci* 21:2580-2588, 2001.
11. Bliss TV, Lomo T: Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232:331-356, 1973.
 12. Bliss TV, Collingridge GL: A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31-39, 1993.
 13. Semmler A, Okulla T, Sastre M, Dumitrescu-Ozimek L, Heneka MT: Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. *J Chem Neuroanat* 30:144-157, 2005.
 14. Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LWI, Bland KI, Chaudry IH: CECAL LIGATION AND PUNCTURE. *Shock* 24:52-57, 2005.
 15. Imamura Y, Wang H, Matsumoto N, Muroya T, Shimazaki J, Ogura H, Shimazu T: Interleukin-1beta causes long-term potentiation deficiency in a mouse model of septic encephalopathy. *Neuroscience* 187:63-69, 2011.
 16. Zivkovic AR, Sedlacek O, von Haken R, Schmidt K, Brenner T, Weigand MA, Bading H, Bengtson CP, Hofer S: Muscarinic M1 receptors modulate endotoxemia-induced loss of synaptic plasticity. *Acta Neuropathol Commun* 3:67, 2015.

17. Tomas-Camardiel M, Rite I, Herrera AJ, de Pablos RM, Cano J, Machado A, Venero JL: Minocycline reduces the lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption of the blood-brain barrier, and damage in the nigral dopaminergic system. *Neurobiol Dis* 16:190-201, 2004.
18. Fagan SC, Edwards DJ, Borlongan CV, Xu L, Arora A, Feuerstein G, Hess DC: Optimal delivery of minocycline to the brain: implication for human studies of acute neuroprotection. *Exp Neurol* 186:248-251, 2004.
19. Coogan AN, O'Neill LA, O'Connor JJ: The P38 mitogen-activated protein kinase inhibitor SB203580 antagonizes the inhibitory effects of interleukin-1beta on long-term potentiation in the rat dentate gyrus in vitro. *Neuroscience* 93:57-69, 1999.
20. Terrando N, Rei Fidalgo A, Vizcaychipi M, Cibelli M, Ma D, Monaco C, Feldmann M, Maze M: The impact of IL-1 modulation on the development of lipopolysaccharide-induced cognitive dysfunction. *Crit Care* 14:R88, 2010.
21. Anderson ST, Commins S, Moynagh PN, Coogan AN: Lipopolysaccharide-induced sepsis induces long-lasting affective changes in the mouse. *Brain Behav Immun* 43:98-109, 2015.

22. Lemstra AW, Groen in't Woud JC, Hoozemans JJ, van Haastert ES, Rozemuller AJ, Eikelenboom P, van Gool WA: Microglia activation in sepsis: a case-control study. *J Neuroinflammation* 4:4, 2007.
23. Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D: Neuroinflammation: the role and consequences. *Neurosci Res* 79:1-12, 2014.
24. Lynch MA: Neuroinflammatory changes negatively impact on LTP: A focus on IL-1beta. *Brain Res* 1621:197-204, 2015.
25. Bellinger FP, Madamba S, Siggins GR: Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res* 628:227-234, 1993.
26. Cunningham AJ, Murray CA, O'Neill LA, Lynch MA, O'Connor JJ: Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. *Neurosci Lett* 203:17-20, 1996.
27. Cunningham ET, Jr., Wada E, Carter DB, Tracey DE, Battey JF, De Souza EB: In situ histochemical localization of type I interleukin-1 receptor messenger RNA in the central nervous system, pituitary, and adrenal gland of the mouse. *J Neurosci* 12:1101-1114, 1992.

28. Michels M, Vieira AS, Vuolo F, Zapelini HG, Mendonca B, Mina F, Domingui D, Steckert A, Schuck PF, Quevedo J, Petronilho F, Dal-Pizzol F: The role of microglia activation in the development of sepsis-induced long-term cognitive impairment. *Brain Behav Immun* 43:54-59, 2015.
29. Nikodemova M, Duncan ID, Watters JJ: Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and IkappaBalpha degradation in a stimulus-specific manner in microglia. *J Neurochem* 96:314-323, 2006.
30. Mina F, Comim CM, Domingui D, Cassol-Jr OJ, Dall Igna DM, Ferreira GK, Silva MC, Galant LS, Streck EL, Quevedo J, Dal-Pizzol F: Il1-beta involvement in cognitive impairment after sepsis. *Mol Neurobiol* 49:1069-1076, 2014.
31. McAfoose J, Baune BT: Evidence for a cytokine model of cognitive function. *Neurosci Biobehav Rev* 33:355-366, 2009.
32. Ross FM, Allan SM, Rothwell NJ, Verkhratsky A: A dual role for interleukin-1 in LTP in mouse hippocampal slices. *J Neuroimmunol* 144:61-67, 2003.
33. Sui DM, Xie Q, Yi WJ, Gupta S, Yu XY, Li JB, Wang J, Wang JF, Deng XM: Resveratrol Protects against Sepsis-Associated Encephalopathy and Inhibits the NLRP3/IL-1beta Axis in Microglia. *Mediators Inflamm* 2016:1045657, 2016.
34. Hara N, Chijiwa M, Yara M, Ishida Y, Ogiwara Y, Inazu M, Kuroda M,

Karlsson M, Sjövall F, Elmér E, Uchino H: Metabolomic Analyses of Brain Tissue in Sepsis Induced by Cecal Ligation Reveal Specific Redox Alterations—Protective Effects of the Oxygen Radical Scavenger Edaravone. *Shock* 44:578-584, 2015.

35. Griffin R, Nally R, Nolan Y, McCartney Y, Linden J, Lynch MA: The age-related attenuation in long-term potentiation is associated with microglial activation. *J Neurochem* 99:1263-1272, 2006.

Figure legends

Fig. 1 Cecal ligation and puncture (CLP)-induced endotoxemia and survival rate within 7 days after CLP. A, The CLP operation led to the successful induction of endotoxemia, and minocycline did not decrease the level of endotoxin. $n = 5$ per group. Data are expressed as the mean $\pm$ SD. B, A high mortality rate was found in CLP mice, and minocycline did not improve the 7-day survival after CLP. sham: $n = 16$, CLP: $n = 16$, CLP + MINO: $n = 15$.

Fig. 2 Minocycline prevents impaired long-term potentiation (LTP) related to sepsis. A, Schematic representation of measurements for field excitatory postsynaptic potentials

(EPSPs). Stim: stimulating electrode, Rec: recording electrode. B, Minocycline (MINO) did not have additional effects on hippocampal LTP in the sham-operated groups. The graph shows the time course of EPSP amplitudes, and representative example in B were sampled at the time points labeled. TBS: theta-burst stimulation. C, Minocycline prevented the LTP impairment in the cecal ligation and puncture (CLP) groups. Robust LTP was induced in the CLP + MINO group to approximately the same values as sham-operated groups. Representative examples in C were sampled at the time points labeled. D, Scatter plots of the percentage of mean EPSP amplitude 60 min after LTP induction relative to baseline in the 4 groups. Data are expressed as the mean $\pm$ SD. CLP + vehicle: n = 6, the other three groups: n = 5, respectively

Fig. 3 LTP impairment during sepsis is prevented only with high doses of minocycline administration. Note that MINO was effective only at doses of 60 mg/kg and 30 mg/kg. A, Time course of excitatory post-synaptic potential (EPSP) amplitudes in the CLP +vehicle group, CLP + MINO (60 mg/kg) group, CLP + MINO (30 mg/kg) group, and CLP + MINO (5 mg/kg) group. TBS: theta-burst stimulation B, Scatter plots of the percentage of the mean EPSP amplitude 60 min after LTP induction relative to the baseline in each group. Data are expressed as the mean $\pm$ SD. CLP + vehicle and CLP

+MINO (5 mg/kg): n = 6, the other two groups: n = 5, respectively

Fig. 4 IL-1receptor antagonist (IL-1ra) administration reverses long-term potentiation (LTP) impairment in the cecal ligation and puncture (CLP) mouse. Note that IL-1ra was effective in the CLP + vehicle group, but had no effect in addition to minocycline (MINO) in the CLP + MINO group. A–C, Time course of excitatory post-synaptic potential (EPSP) amplitudes in the (A) sham + vehicle group, (B) CLP + vehicle group, and (C) CLP +MINO group. Representative examples in A–C were sampled at the time points labeled. TBS: theta-burst stimulation D, Scatter plots of the percentage of the mean EPSP amplitude 60 min after LTP induction relative to the baseline in each group. Data are expressed as the mean $\pm$ SD. n = 5 per group.

Fig.1

Fig.2

Fig.3

Fig.4