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Research report

# Leptomycin B attenuates neuronal death via PKA- and PP2B-mediated ERK1/2 activation in the rat hippocampus following status epilepticus

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ABSTRACT

Leptomycin B (LMB), originally developed as an anti-fungal agent, has potent neuroprotective properties against status epilepticus (SE, a prolonged seizure activity). However, the pharmacological profiles and mechanisms of LMB for neuroprotection remain elusive. In the present study, we found that LMB increased phosphorylation levels of protein kinase A (PKA) catalytic subunits, protein phosphatase 2B (PP2B, calcineurin) and extracellular signal–regulated kinase 1/2 (ERK1/2) under normal condition, and abolished SE-induced neuronal death. Co-treatment of H-89 (a PKA inhibitor) with LMB could not affect the seizure latency and its severity in response to pilocarpine. However, H-89 co-treatment abrogated the protective effect of LMB on SE-induced neuronal damage. Cyclosporin A (CsA, a PP2B inhibitor) co-treatment effectively prevented SE-induced neuronal death without altered seizure susceptibility in response to pilocarpine more than LMB alone. H-89 co-treatment inhibited LMB-mediated ERK1/2 phosphorylation, but CsA enhanced it. U0126 (an ERK1/2 inhibitor) co-treatment abolished the protective effect of LMB on SE-induced neuronal death without alterations in PKA and PP2B phosphorylations. To the best of our knowledge, the present data demonstrate a previously unreported potential neuroprotective role of LMB against SE via PKA- and PP2B-mediated ERK1/2 activation.



## 1. Introduction

Leptomycin B (LMB), originally developed as an anti-fungal agent, has potent anti-inflammatory and anti-tumor properties. In addition, LMB inhibits chromosome region maintenance 1 (CRM1)/exportin 1 nuclear export carrier protein (Loewe et al., 2002; Lu et al., 2012). Recently, we have reported that LMB attenuates neuronal death induced by status epilepticus (SE, prolonged seizure activity, Hyun et al., 2016). SE-induced neuronal death is closely related to dysfunction of mitochondrial dynamics (fusion and fission). Indeed, impaired dynamin-related protein-1 (DRP1)-mediated mitochondrial fission results in programmed necrosis in CA1 neurons following SE (Kim et al., 2014; Ko et al., 2015; Hyun et al., 2016). In this process, translocation of high mobility group box 1 (HMGB1) into mitochondria plays an important role in the facilitation of SE-induced programmed necrosis, which is abrogated by LMB (Hyun et al., 2016).

HMGB1 is a nuclear protein to regulate gene transcription, and is transported to the cytoplasm by CRM1/exportin 1 during necrosis (Scaffidi et al., 2002; Faraco et al., 2007). When HMGB1 is released into the extracellular space from damaged or dying cells during necrosis (Qiu et al., 2008), it induces potentially inflammatory responses via toll-like receptor 4 (TLR4) or receptor for advanced glycan endproducts (RAGE) (Abraham et al., 2000; Scaffidi et al., 2002; Maroso et al., 2010). Interestingly, exogenous HMGB1 enters the mitochondria and leads to the formation of giant mitochondria independently of HMGB1 receptors (Gdynia et al., 2010). However, we have reported that inhibition of nuclear HMGB1 export by LMB cannot ameliorate the impaired mitochondrial dynamics in CA1 neurons following SE, although LMB abolishes SE-induced neuronal death (Hyun et al., 2016). This discrepancy suggests the additional signaling pathways of neuronal damage affected by LMB, which remain elusive, independent of mitochondrial elongation. Thus, these findings raise the question concerning the pharmacological properties of LMB against SE-induced neuronal death.

On the other hand, LMB inhibits the nuclear export of cAMP-dependent protein kinase inhibitor-α (PKI), which inhibits the activity of protein kinase A (PKA) catalytic subunits (Chen et al., 2005). Furthermore, PKA inhibits CRM1/exportin-dependent nuclear export (Nishiyama et al., 2007). Therefore, it is likely that LMB may affect PKA activity. With regard to these connections, it

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is worthy of note that LMB would affect PKA-mediated signaling pathway and neuronal viability in response to SE. We report herein that LMB enhanced phosphorylations of PKA, protein phosphatase 2B (PP2B, calcineurin) and extracellular signal-regulated kinase 1/2 (ERK1/2) under normal condition, and ameliorated SE-induced neuronal death. Furthermore, this effect of LMB was reversed by H-89 (a PKA inhibitor) and U0126 (an ERK1/2 inhibitor). Cyclosporin A (CsA, a PP2B inhibitor) enhanced the LMB-mediated ERK1/2 phosphorylation without changed PKA phosphorylation, and increased the neuroprotective effect of LMB on SE-induced neuronal death. These findings suggest that LMB may protect neurons from SE via PKA- and PP2B-mediated ERK1/2 activations.

## 2. Results

### 2.1. LMB attenuates SE-induced neuronal death via PKA activation

Fig. 1 shows that LMB could not affect the seizure latency and its severity induced by pilocarpine (Fig. 1A–C). Consistent with our previous study (Hyun et al., 2016), LMB alleviated SE-induced neuronal death as compared to vehicle ($p < 0.05$; Fig. 1D–E). These findings indicate that LMB may attenuate SE-induced neuronal death without changed seizure activity.

Since LMB inhibits the nuclear export of cAMP-dependent protein kinase inhibitor-α (PKI), which inhibits the activity of PKA catalytic subunits (Chen et al., 2005), we investigated whether LMB affects PKA activity under physiological condition and post-SE condition. LMB increased the phospho (p)-PKA catalytic subunit (T197) level to 2.78-fold of vehicle-treated control animals ($p < 0.05$; Fig. 2A-B). Following SE, pPKA catalytic subunit level in LMB-treated animals was 2.64-fold of vehicle-treated control animals ($p < 0.05$; Fig. 2A-B). However, vehicle did not affect pPKA level (Fig. 2A-B). Consistent with a previous study (Chen et al., 2005), these findings indicate that LMB may increase PKA activity in control and post-SE animals. Next, we co-applied H-89 with LMB to investigate the role of LMB-mediated PKA activation in SE-induced neuronal death. Co-treatment of H-89 with LMB could not affect the seizure latency and its severity in response to pilocarpine (Fig. 2C–E). However, H-89 co-treatment abolished the protective effect of LMB on SE-induced neuronal damage (Fig. 2F–G). These findings indicate that LMB-mediated PKA activation may protect neurons from SE insult without changed seizure activity.

### 2.2. LMB increases PP2B serine-197 phosphorylation independent of PKA activity

It is well known that SE-induced neuronal death is regulated by various protein phosphatase activities (Zeng et al., 2007; Shin et al., 2012). Furthermore, PKA-mediated phosphorylation affects the

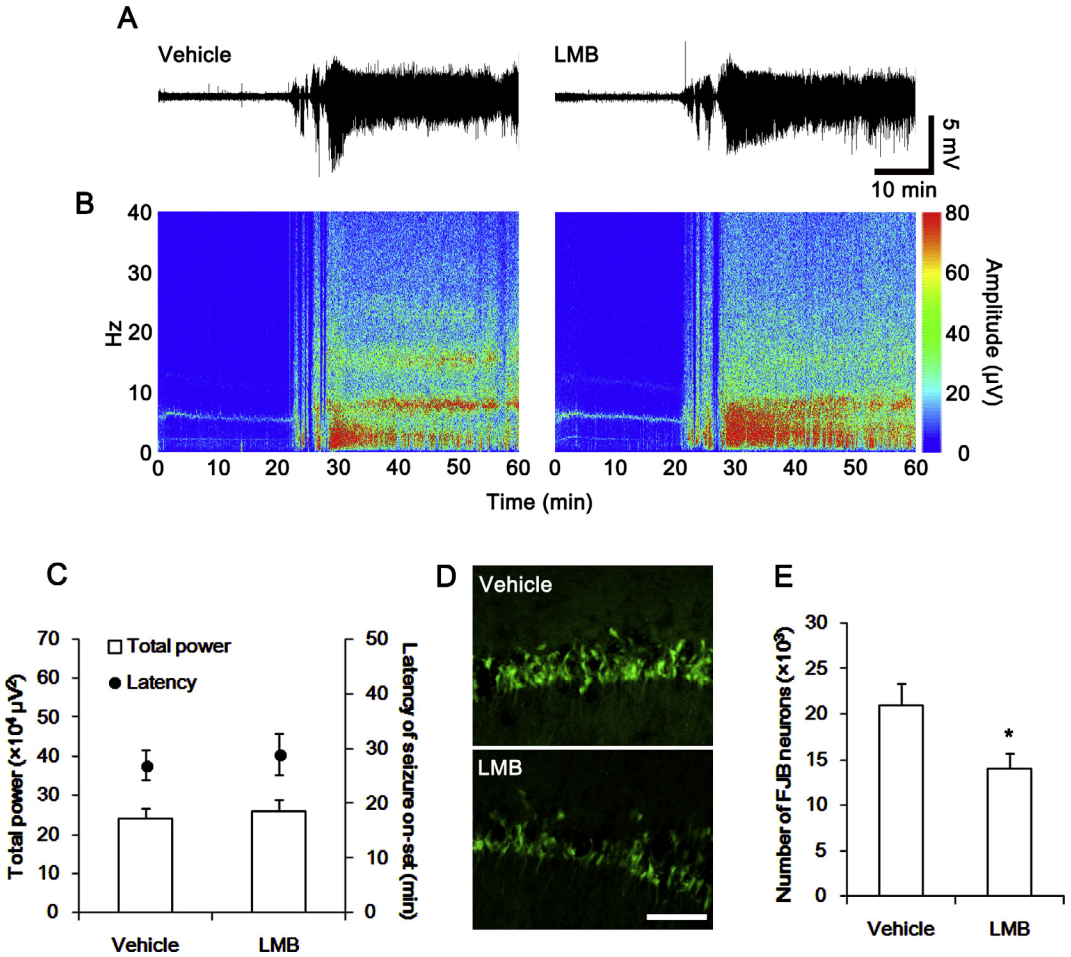


**Fig. 1.** The effect of LMB on neuronal death and seizure activity induced by pilocarpine. (A–C) The effect of LMB on seizure susceptibility induced by pilocarpine. LMB does not affect the seizure susceptibility and its severity in response to pilocarpine. (A) Representative EEG traces in response to pilocarpine. (B) Representative frequency-power spectral temporal maps in response to pilocarpine. (C) Quantification of effect of LMB on SE induction, latency and total EEG power (mean ± S.E.M.; n = 7, respectively). (D–E) The effect of LMB on SE-induced neuronal death. LMB effectively attenuates SE-induced neuronal death. (D) Representative FJB staining in the CA1 region 3 days after SE. Bar = 50 μm. (E) Quantification of effect of LMB on SE-induced neuronal death (mean ± S.E.M.; $^{*}p < 0.05$ vs. vehicle; n = 7, respectively).