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Antidepressant effects of TBE-31 and MCE-1, the novel Nrf2 activators, in an inflammation model of depression

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ABSTRACT

The Nuclear factor (erythroid 2-derived)-like 2 (Nrf2) plays a key role in inflammation which is implicated in the pathophysiology of depression. The Nrf2 activators have antidepressant effects in animal models of depression. The present study was undertaken to examine whether TBE-31 [(±)-(4bS,8aR,10aS)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10,10a-octahydrophenanthrene-2,6-dicarbonitrile] and MCE-1 [(±)-3-ethynyl-3-methyl-6-oxocyclohexa-1,4-dienecarbonitrile], the novel Nrf2 activators, could show antidepressant effects in inflammation model of depression. We found that TBE-31 and MCE-1 significantly potentiated nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells, in a concentration dependent manner. The *Nrf2* siRNA, but not negative control of siRNA, significantly blocked the potentiating effects of TBE-31 and MCE-1 on neurite outgrowth in PC12 cells. Furthermore, oral administration of TBE-31 or MCE-1 significantly attenuated an increase in serum levels of tumor necrosis factor-α (TNF-α) after administration of lipopolysaccharide (LPS: 0.5 mg/kg). In the tail-suspension test and forced swimming test, oral administration of TBE-31 or MCE-1 significantly attenuated an increase in the immobility time after LPS (0.5 mg/kg) administration. These findings suggest that the novel Nrf2 activators such as TBE-31 and MCE-1 might be potential therapeutic drugs for inflammation-related depression.

1. Introduction

Accumulating evidence suggests that inflammation plays a role in the pathophysiology of depression (Dantzer et al., 2008; Hashimoto, 2009, 2015; Miller et al., 2009; Raison et al., 2010). Systemic administration of lipopolysaccharide (LPS) can induce depression-like behavior in rodents after the induction of inflammation (Dantzer et al., 2008; O'Connor et al., 2009; Zhang et al., 2016). The current antidepressants such as serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) can block alterations in serum pro-inflammatory cytokines and depression-like behaviors induced by LPS (de Paiva et al., 2010; Dong et al., 2016; Ma et al., 2014; Ohgi et al., 2013; Yao et al., 2015). Taken together, it is likely that inflammation plays a role in the depression-like phenotype in rodents, and anti-inflammatory drugs could show antidepressant effect in inflammation model of depression.

Nuclear factor (erythroid 2-derived)-like 2 (Nrf2) is a transcription factor that plays a central role in cellular defense against oxidative and electrophilic insults (Ma and He, 2012; Ma, 2013; Suzuki et al., 2013; Suzuki and Yamamoto, 2015). Nrf2 binds to antioxidant response elements (ARE) located in the promoter region of genes encoding many phase II detoxifying or antioxidant enzymes and related stress-responsive proteins (Ma and He, 2012; Ma, 2013; Suzuki et al., 2013; Suzuki and Yamamoto, 2015). Under normal conditions, Nrf2 is repressed by Keap1 (Kelch-like erythroid cell-derived protein with CNC homology [ECH]-associated protein 1), which is an adaptor protein for the degradation of Nrf2 (Suzuki et al., 2013; Suzuki and Yamamoto, 2015). During oxidative stress, Nrf2 is de-repressed and activates the transcription of cytoprotective genes (Suzuki et al., 2013; Suzuki and Yamamoto, 2015). Interestingly, it is recognized that Keap1-Nrf2 system plays a role in inflammation (Kobayashi et al., 2013; Innamorato et al., 2008; Suzuki et al., 2013; Suzuki and Yamamoto, 2015; O'Connell and Hayes, 2015; Wardyn et al., 2015). Recently, we reported that sulforaphane, a natural compound with Nrf2 activator, shows antidepressant effect in inflammation model of depression (Zhang et al., in press), and that pretreatment with sulforaphane confers resilience to social defeat stress in rodents (Yao et al., 2016). These results suggest that Nrf2 activators would be potential thera-

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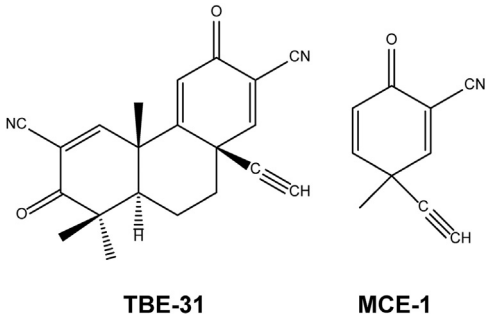

Fig. 1. The chemical structure of TBE-31 and MCE-1.

peutic drugs for depression.

TBE-31 [(±)-(4bS,8aR,10aS)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10,10a-octahydrophenanthrene-2,6-dicarbonitrile] is a novel Nrf2 activator (Fig. 1) (Honda et al., 2007, 2011; Kostov et al., 2015; Dinkova-Kostova et al., 2010; Ma and He, 2012). It retains and even exceeds the potency of CDDO (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid) analogs, which are the most potent compounds in pool of semi-synthetic triterpenoids in various in vitro and in vivo assays, including induction of cytoprotective enzymes. Furthermore, TBE-31 showed anti-inflammation effect in cells, and blocked the formation of aflatoxin-B_1 (AFB_1)-DNA adducts and AFB_1-induced tumorigenesis in vivo (Liby et al., 2008). MCE-1 [(±)-3-ethynyl-3-methyl-6-oxocyclohexa-1,4-dienecarbonitrile] is also a novel Nrf2 activator (Fig. 1) (Dinkova-Kostova et al., 2010; Zheng et al., 2012). A recent study showed that MCE-1 is a highly reactive Michael acceptor leading to reversible adducts with nucleophiles, which displays equal or greater potency than CDDO in inflammation and carcinogenesis related assays (Zheng et al., 2012).

The present study was undertaken to examine whether TBE-31 and MCE-1 show antidepressant effects in inflammation model of depression. First, we examined the effects of these compounds on nerve-growth factor (NGF)-induced neurite outgrowth in PC12 cells. Second, we examined whether these compounds could attenuate alterations in serum pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), after LPS administration. Finally, we examined the effects of TBE-31 and MCE-1 in inflammation-induced model of depression.

2. Materials and methods

2.1. Quantification of neurite outgrowth in PC12 cells

PC12 cells (RIKEN Cell Bank, Tsukuba, Japan) were cultured at 37 ℃, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% heat-inactivated fetal bovine serum (FBS), 10% heat-inactivated horse serum and 1% penicillin. Medium was changed two to three times a week. PC12 cells were plated onto 24-well tissue culture plates coated with poly-D-lysine/laminin. Cells were plated at relatively low density (0.25×104 cells cm-2) in DMEM medium containing 0.5% FBS, 1% penicillin-streptomycin. Medium containing a minimal level of serum (0.5% FBS) was used as previously

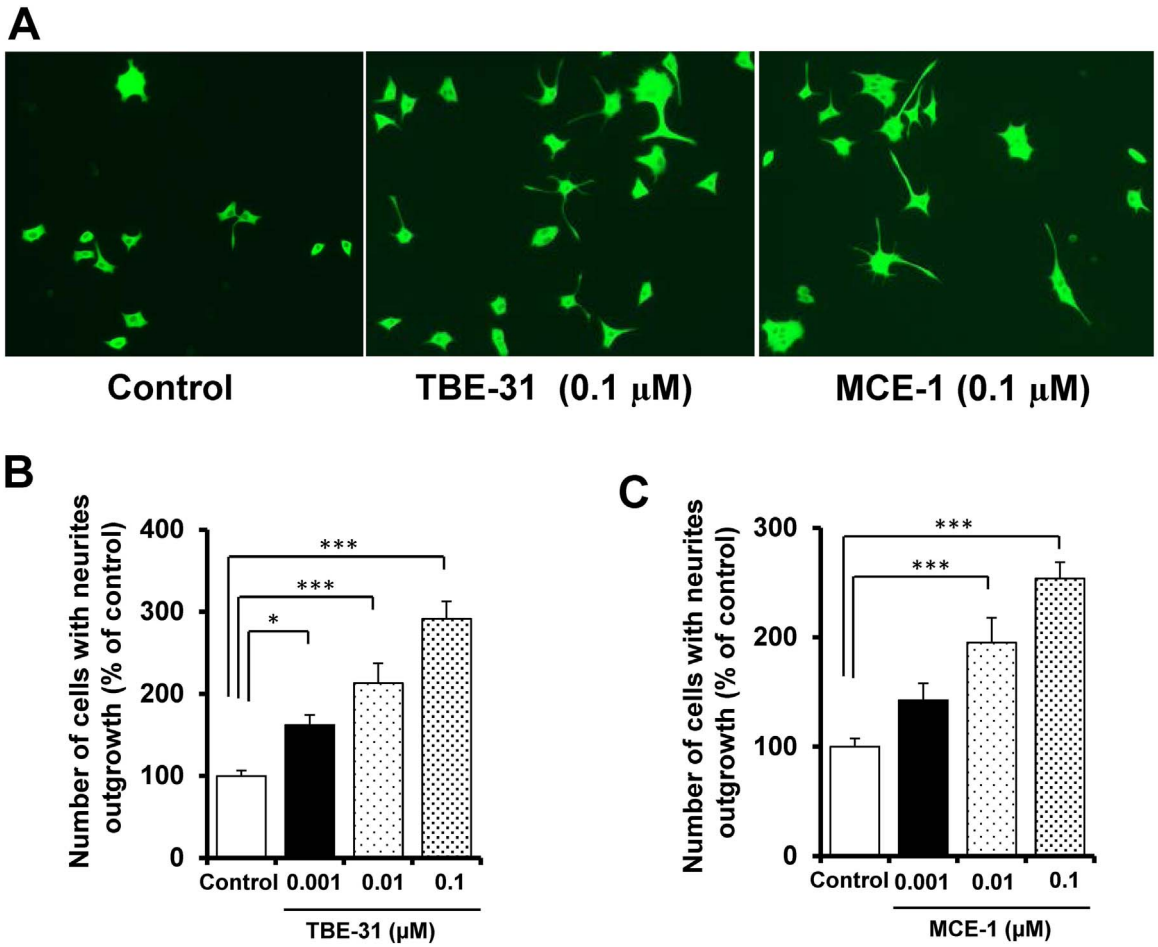

Fig. 2. Potentiation of NGF-induced neurite outgrowth by TBE-31 and MCE-1. (A) Representative photographs of microtubule-associated protein 2 (MAP-2) immunocytochemistry in PC12 cells. Control: NGF (2.5 ng/ml) alone, TBE-31 and MCE-1: NGF (2.5 ng/ml) + TBE-31 (0.1 μM) or MCE-1 (0.1 μM). (B and C) Effects of TBE-31 and MCE-1 on NGF-induced neurite outgrowth in PC12 cells. TBE-31 (0.001, 0.01 and 0.1 μM) and MCE-1 (0.001, 0.01 and 0.1 μM) potentiated NGF-induced neurite outgrowth in PC12 cells, in a concentration-dependent manner. All data represent the mean ± S.E.M. (n =6–14) *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the control group (one-way ANOVA).