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Dimethyl fumarate confers neuroprotection by casein kinase 2 phosphorylation of Nrf2 in murine intracerebral hemorrhage

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ABSTRACT

Background and purpose: Edema formation, inflammation and increased blood–brain barrier permeability contribute to poor outcomes after intracerebral hemorrhage (ICH). This study examined the therapeutic effect of dimethyl fumarate (DMF), a fumaric acid ester that activates nuclear factor erythroid-2 related factor 2 (Nrf2) and Nrf2 heterodimerization effector protein musculo-aponeurotic fibrosarcoma-G (MAFG) in a murine ICH model.

Methods: Male CD-1 mice (n = 176) were subjected to intrastriatal infusion of bacterial collagenase (n = 126), autologous blood (n = 18) or sham surgery (n = 32). Four (4) animals not subjected to ICH (naive) were also included in the study. After ICH, animals either received vehicle, dimethyl fumarate (10 mg or 100 mg/kg) or casein kinase 2 inhibitor (E)-3-(2,3,4,5-tetrabromophenyl)acrylic acid (TBCA). Thirty-two mice also received scrambled siRNA or MAFG siRNA 24 h before ICH. Brain water content and neurological function were evaluated.

Results: Dimethyl fumarate reduced Evans blue dye extravasation, decreased brain water content, and improved neurological deficits at 24 and 72 h after ICH. Casein kinase 2 inhibitor TBCA and MAFG siRNA prevented the effect of dimethyl fumarate on brain edema and neurological function. After ICH, ICAM-1 levels increased and casein kinase 2 levels decreased. Dimethyl fumarate reduced ICAM-1 but enhanced casein kinase 2 levels. Again, casein kinase 2 inhibitor TBCA and MAFG siRNA abolished the effect of dimethyl fumarate on ICAM-1 and casein kinase 2. Dimethyl fumarate preserved pNrf2 and MAFG expression in the nuclear lysate after ICH and the effect of dimethyl fumarate was abolished by casein kinase 2 inhibitor TBCA and MAFG siRNA. Dimethyl fumarate reduced microglia activation in peri-hematoma areas after ICH. The protective effect of dimethyl fumarate on brain edema and neurological function was also observed in a blood injection mouse model.

Conclusion: Dimethyl fumarate ameliorated inflammation, reduced blood-brain barrier permeability, and improved neurological outcomes by casein kinase 2 and Nrf2 signaling pathways after experimental ICH in mice.

1. Introduction

Intracerebral hemorrhage (ICH) which accounts for about 15–20% of all deaths from stroke is the rupturing of small blood vessels in the brain parenchyma. Currently, no effective treatment options are available for this fatal stroke subtype, and, even if patients survive the initial injury, a series of secondary events may lead to cerebral edema, progression of neurobehavioral deficits, and possible death (Chen et al., 2014; Ikram et al., 2012; Keep et al., 2012; Pandey and Xi, 2014).

Evidence from clinical and animal studies suggest that inflammation and oxidative stress which occur after hematoma formation are involved in ICH-induced secondary brain injury and neurological dysfunction (Aronowski and Hall, 2005; Chen et al., 2015). Vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), adhesion molecules expressed in the endothelium that are important in inflammation after injury, are increased upon activation of the nuclear factor-κB (NF-κB)-mediated tumor necrosis factor α (TNFα) signaling pathway. TNFα increases early-onset endothelial adhesion by protein kinase C-dependent up-regulation of ICAM-1 expression, which can worsen outcomes following ICH (Javaid et al., 2003; Wang and Doré, 2007).

Nuclear factor erythroid-2 related factor 2 (Nrf2), a major phase II gene regulator, is a broadly expressed transcription factor that binds

Abbreviations: DMF, dimethyl fumarate; MAF-G, musculo-aponeurotic fibrosarcoma-G; Nrf2, nuclear factor erythroid-2 related factor 2; p-Nrf2, phosphorylated nuclear factor erythroid-2 related factor 2; TBCA, (E)-3-(2,3,4,5 tetrabromophenyl)acrylic acid; CK2, casein kinase 2; blCH, blood induced intracerebral hemorrhage; clCH, collagenase induced intracerebral hemorrhage; ICV, intracerebroventricular injection; ICAM-1, intracellular adhesion molecule-1; DAPI, 4′,6-Diamidino-2-phenylindole.

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to the antioxidant response element (ARE) consensus and regulates expression of phase II detoxifying enzymes (Itoh et al., 1997). In addition to protecting against oxidative and electrophilic stress, recent studies demonstrated that Nrf2 responds to pro-inflammatory stimuli and rescues cells/tissues from inflammatory injuries (Chen et al., 2006; Zhao and Aronowski, 2013). As a transcription factor, it is essential for Nrf2 to translocate into the nucleus in order to stimulate the up-regulation of cytoprotective genes (Zhang et al., 2013). Translocation of Nrf2 from the cytoplasm to the nucleus and export of Nrf2 from the nucleus is regulated by nuclear localization signals, nuclear export sequences and phosphorylation (Jain et al., 2005). Nrf2 is the substrate of several protein kinases, including protein kinase C (Numazawa et al., 2003), phosphatidylinositol 3-kinase (Lee et al., 2001), glycogen synthase kinase-3 (Rada et al., 2012), casein kinase 2 (Apopa et al., 2008), and Fyn (Jain and Jaiswal, 2006). Casein kinase 2, an ubiquitous eukaryotic kinase composed of catalytic (α or α1) and regulatory (β) subunits was elucidated as a major kinase for phosphorylating Nrf2 in in-vitro studies using neuroblastoma cells and human keratinocyte cell lines (Apopa et al., 2008; Pi et al., 2007).

Dimethyl fumarate (DMF), a fumaric acid ester that is effective in the treatment of relapsing/remitting multiple sclerosis, promotes Nrf2 activation and stabilization through direct modification of Keap1 at cysteine residue 151 (Kappos et al., 2008; Linker et al., 2011). Stabilization and phosphorylation of Nrf2 facilitate its nuclear import, forming heterodimers with MAFG, subsequently up-regulating cytoprotective genes and inhibiting NF-κB nuclear translocation, thus decreasing expression of NF-κB-dependent genes, including inflammatory cytokines, chemokines, and adhesion molecules (Jain et al., 2005; Stoof et al., 2001). Although dimethyl fumarate stabilizes Nrf2, the role casein kinase 2 plays in phosphorylating Nrf2 and p-Nrf2 conferred neuroprotection after ICH has not been documented. In the present study, we aimed to test 2 hypotheses, (i) administration of dimethyl fumarate will reduce brain edema and neurological dysfunction in mice after ICH and (ii) casein kinase 2 phosphorylation of Nrf2 will promote Nrf2 nuclear translocation and antioxidant response element activation as well as ameliorate inflammation and blood–brain barrier permeability after ICH. A schema of the study design is presented in Appendix 1.

2. Materials and methods

All procedures were conducted in accordance with the NIH guide for care and use of laboratory animals. Approval was obtained from the Institutional Animal Care and Use Committee of Loma Linda University. CD-1 mice weighing 29–38 g (Charles River, Wilmington, MA) were housed in light and temperature controlled environment with access to food and water ad libitum.

3. Surgical procedures

3.1. ICH

Intracerebral hemorrhage was induced in mice using either the collagenase injection model (cICH) or the autologous blood (bICH) double-injection model, as previously reported (Rosenberg et al., 1990; Wang et al., 2008). Briefly, mice were treated with Atropine (0.22 mg/kg) and anesthetized with co-injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) intraperiteoneally and positioned prone on a stereotactic head frame (Kopf Instruments, Tujunga CA), and eye ointment was applied to keep the eyes moist. A 1 mm burr hole was drilled at 0.2 mm rostral and 2.2 mm right lateral of bregma, and a 27gauge needle (Microliter No. 1701; Hamilton Company, NV) was inserted 3.5 mm below the dura.

For cICH, 0.075 units of bacterial collagenase (Type VII-S, Sigma-Aldrich, St. Louis, MO) dissolved in 0.5 μl saline was infused into the right basal ganglia at a rate of 0.1667 μl/min using a Namonite Syringe Pump (Harvard Apparatus, Holliston, MA). The needle was left for an additional 5 min (to prevent the backflow of bacterial collagenase along the needle tract) and withdrawn slowly at a rate of 1 mm/min. The cranial burr hole was sealed with bone wax, the scalp was sutured, and 0.4 ml of normal saline was injected subcutaneously to avoid post-surgical dehydration. Animals were allowed to recover fully under observation. Mice received DMF (10 or 100 mg/kg) intraperitoneally 1 h after ICH.

For bICH, blood was collected from the tail artery and transferred quickly to a glass syringe. The 27 gauge needle of the syringe was then inserted 3.0 mm below the dura through the burr hole and 5 μl of autologous blood was infused at a rate of 2 μl/min. Next, the needle was advanced an additional 0.5 mm and, after a waiting period of 5 min; 25 μl of blood was infused into the right striatum. The needle was left in place for an additional 10 min after completing the infusion and withdrawn at a rate of 1 mm/min; the burr hole was sealed with bone wax and mice were allowed to recover under observation. Mice received DMF (100 mg/kg) intraperitoneally 1 h after ICH.

Sham operated animals were subjected to needle insertion only.

3.2. ICV

Anesthetized mice were fixed prone onto a stereotactic head apparatus (Kopf Instruments, Tujunga CA), and a 1 mm burr hole was drilled at 0.3 mm posterior and 1 mm left lateral of bregma. A 27 gauge needle of a 10 μl Hamilton syringe (Microliter No. 1701; Hamilton Company, NV) was inserted through a burr hole 2.5 mm below the dura, as previously described (Ma et al., 2011a; Mullier et al., 2010). Following the manufacturer's instructions, and methods described by Ma et al. with slight modifications, MAF-G siRNA or control siRNA, 1.2 nm each in 3 μl siRNA diluted in sterile RNAse free water (Santa Cruz Biotechnology, CA), was injected by a micro infusion pump (Harvard Apparatus, Holliston, MA) at a rate of 0.3750 μl/min 24 h before ICH-induction. The needle was removed over a 5 minute period after waiting for 7 min; the burr hole was sealed with bone wax (Ma et al., 2011a). In order to enhance the gene silence efficiency, MAF-G siRNAs from two different sources were mixed:

1) sense 5′ GGAAGAGAUCAUCCAGCUGtt 3′
Antisense, 5′ CAGCUGGAUGAUCUCUUCCtt 3′
2) sense 5′CAGCGUCAUCACAAUAGUAtt 3′
5′CCUUGAUCAUCUUCGUUGUtt 3′
5′CUGUGGCUGUUGGAGUUUAtt 3′
Antisense, 5′UACUAUUGUGAUGACGCUGtt3′
5′ACAACGAAGAUGAUCAAGGtt 3′
5′UAAACUCCAACAGCCACAGtt 3′.

The sequence for control siRNA is

Sense: 5′UUCUCCGAACGUGUCACGUtt 3′
Antisense: 5′ACGUGACACGUUCGGAGAAtt 3′.

Mice received DMF (100 mg/kg) intraperitoneally 1 h after ICH.

3.3. Experimental groups and pharmacological interventions

One hundred and eighty CD-1 mice were used in this study. Mice were randomly divided into the following groups: naive (n = 4), sham (n = 38); ICH + vehicle (n = 54); ICH + 10 mg/kg DMF (n = 6); ICH + 100 mg/kg DMF (n = 34); ICH + TBCA (100 μm/kg) + 100 mg/kg DMF (n = 12); control siRNA + ICH (n = 4); control siRNA + ICH + 100 mg/kg DMF (n = 12); MAFG siRNA + ICH (n = 4); and MAFG siRNA + ICH + 100 mg/kg DMF (n = 12).

3.4. Assessment of neurological function

Behavioral outcomes were assessed by observers blinded to treatment at 24 and 72 h after ICH. The sensorimotor Garcia test evaluating