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Single administration of soluble epoxide hydrolase inhibitor suppresses neuroinflammation and improves neuronal damage after cardiac arrest in mice

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ABSTRACT

Cardiac arrest (CA) causes ischemia-reperfusion injury in the whole body among victims. Especially in the brain, inflammation and neuronal cell death can lead to irreversible dysfunction. Our goal was to determine whether a single administration of soluble epoxide hydrolase inhibitor (AS2586144-CL) has a neuroprotective effect and decreases the inflammatory response after CA and cardiopulmonary resuscitation (CPR). Global cerebral ischemia was induced in male C57BL/6 mice with 8 min of CA. Thirty minutes after recovery of spontaneous circulation, the mice were randomly assigned to three groups and administered AS2586144-CL: 1 mg/kg ($n = 25$), 10 mg/kg ($n = 25$), or 0 mg/kg (vehicle, $n = 25$). At 6 and 7 days after CA/CPR, behavioral tests were conducted and brains were removed for histological evaluation. Analysis of histological damage 7 days after CA/CPR revealed that 10 mg/kg of AS2586144-CL protected neurons, and suppressed cytokine production and microglial migration into the hippocampus. Two hours after CA/CPR, 10 mg/kg of AS2586144-CL suppressed serum tumor necrosis factor-α and hippocampal nuclear factor κB expression. Our data show that 10 mg/kg of AS2586144-CL administered following CA/CPR suppresses inflammation and decreases neuronal damage.

1. Introduction

Sudden cardiac arrest (CA) is a major cause of death. The degree of cerebral damage, cardiac dysfunction, and systemic ischemic/reperfusion determine the prognosis and mortality among patients who achieve recovery of spontaneous circulation (ROSC). Targeted temperature management lowers the mortality rate; however, the majority of survivors show some degree of brain damage as evidenced by impairment of memory or cognitive function (Laver et al., 2004; Stub et al., 2011). Recent studies on the use of therapeutic drugs, such as minocycline and edaravone for neuroprotection have shown promising experimental results, but the clinical efficacy of these drugs has been controversial (Kubo et al., 2009; Keilhoff et al., 2011; Wu et al., 2014).

In addition to brain damage, whole-body ischemia/reperfusion injury occurs after CA and cardiopulmonary resuscitation (CPR). Brief periods of global brain ischemia induce neuronal inflammation and selective and delayed neuronal death, mostly in the hippocampus and basal ganglia. At the same time, various inflammatory cytokines are released throughout the entire body, including the brain (Callaway et al., 2008; Niemann et al., 2008; Qureshi, 2008). Recent data suggest that these changes exacerbate the neuroinflammation in the hippocampus after brain ischemia through the spread of proinflammatory cytokines and damage-associated molecular pattern molecules, such as tumor necrosis factor-α (TNF-α) and high-mobility group box protein-1 (HMGB1) (Degos et al., 2013). The whole-body reaction to the changes in the brain after CA/CPR is important to consider in the clinical setting (An et al., 2014).

Epoxyeicosatrienoic acids (EETs) have recently emerged as potential therapeutic targets for the treatment of brain ischemia (Li et al., 2012). They exhibit various beneficial actions including neuroprotection, vasodilation, furtherance of angiogenesis, and suppression of platelet aggregation, inflammation and oxidative stress (Iliff and Alkayed, 2009). Soluble epoxide hydrolase (sEH)

Abbreviations: CA, cardiac arrest; CPR, cardiopulmonary resuscitation; EETs, epoxyeicosatrienoic acids; HMGB1, high-mobility group box protein-1; IBA1, ionized calcium-binding adaptor molecule-1; Neu-N, neuronal nuclei; NFκB, nuclear factor κB; sEH, soluble epoxide hydrolase; TNF-α, tumor necrosis factor-α; ROSC, recovery of spontaneous circulation.

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is a key enzyme involved in the metabolic conversion and degradation of EETs. Studies have shown that a single administration of a low dose of sEH inhibitor reduces the infarct volume after middle cerebral artery occlusion in rats, and repeated administration of this drug had a neuroprotective effect after CA/CPR in mice (Shaik et al., 2013; Wang et al., 2013). Therefore, the aim of this study was to determine whether a single administration of sEH inhibitor (AS2586144-CL) at the early phase of reperfusion decreases the inflammatory response, and has a neuroprotective effect 7 days after CA/CPR.

2. Experimental procedures

2.1. Experimental animals

Wild-type male mice (C57BL/6, 7 weeks old, 20–27 g, Charles River, Japan) were housed in a temperature-controlled room (23.5 °C) with 12 h of light and 12 h of dark exposure. All animal experiments were carried out in accordance with the Regulations for Animal Experiments of the University of Tsukuba and Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology of Japan and with approval from the Institutional Animal Experiment Committee of our university. The animals ($n = 75$) were randomized into three groups to determine the neuroprotective effects of AS2586114-CL. An additional 30 mice were included for the measurement of proinflammatory cytokines at two time points (2 h or 7 days after CA/CPR).

To evaluate the physiological variables (blood gas analysis and mean arterial blood pressure) in our CA/CPR protocol, we carried out a preliminary study with a femoral artery catheter in 10 mice that were not included the main study.

2.2. Materials

AS2586114-CL, 3-cyclopropyl-4-{4-[2-({[(1S,2R,5S)-6,6-dimethylbicyclo[3,1,1]heptan-2-yl]methyl}amino)-2-oxoethyl]piperidin-1-yl}benzoic acid monohydrochloride (hydrochloride salt of compound Ex33 in the reference (Miura et al., 2011)) was obtained from Astellas Pharma Inc. (Tsukuba, Japan). This drug is a potent sEH inhibitor (IC50 = 5.9 nM for rat sEH) and has a favorably prolonged action and ability to cross the blood–brain barrier (in-house data of Astellas Pharma Inc.). This drug is a highly potent inhibitor of sEH such that a single oral dose inhibits more than 99% of the sEH activity in the blood (Ma et al., 2013). AS2586144-CL was dissolved in 5% PEG400, 5% Tween 80, and 0.5 M $NaCO_3$ to 1 mg/ml.

2.3. Cardiac arrest procedures

The experimental procedures were performed with modification of previously described studies (Taguchi et al., 2012). Anesthesia was induced with intraperitoneal injection of 40 mg/kg of pentobarbital and 80 μg/kg of fentanyl. After tracheal intubation with a 22-gauge catheter, the mice were mechanically ventilated with 10% oxygen and 90% air (model 845; Harvard Apparatus, Holliston, MA, USA). Temperature probes were inserted into the left temporalis muscle and the rectum (Mon-a-therm, model 4070; Covidien-Nellcor and Puritan Bennett, Boulder, CO, USA). The rectal temperature was controlled at 37 °C with a warming pad and a heating lamp. An AP-10 catheter was inserted into the right jugular vein. The electrocardiogram was monitored with the surgical monitoring system (Mouse Monitor S; Indus Instruments, Houston, TX, USA). CA was induced by injection of 0.5 mol/l KCl (0.05 ml) via the jugular venous catheter and confirmed by electrocardiography. The endotracheal tube was disconnected, and the oxygen and air were stopped. During CA, the rectal temperature was lowered to 28 °C and the pericranial temperature was maintained at 37 °C. CPR was initiated 8 min after the induction of CA by injection of 0.5 ml of epinephrine (8 μg) from the jugular vein, finger chest compressions (approximately 300/min), and ventilation with 100% oxygen. When ROSC was confirmed by electrocardiographic activity and femoral artery pulsation, CPR was stopped. If CA persisted for 2 min, an additional 0.1 ml dose of epinephrine was administered every 60 s. The body temperature was controlled with a mat and a lamp during the recovery period. Thirty minutes after ROSC, AS2586144-CL or vehicle was administrated intraperitoneally and the catheters and temperature probes were removed. After sufficient spontaneous breathing was confirmed, the endotracheal tube was removed.

2.4. Neurologic evaluation

Passive avoidance tests were performed as previously described, with modification (RIKEN GSC, http://www.brc.riken.jp/lab/bpmp/SOPs/Classification; Johns Hopkins University, Department of psychological and brain sciences, http://nbc.jhu.edu/behavioraltasks/tasks/passiveavoidance protocol.html). The test was performed 6 days after CA/CPR. All mice were individually placed on the floor of a lighted compartment, and they generally entered an adjacent dark compartment within a short time. During the conditioning procedure, the mice placed in the bright compartment received a foot shock (0.5 A, 2 s) when they entered the dark compartment (MPB-M 010; Merquest, Tokyo, Japan). Twenty-four hours later, the retention test was performed using the same behavioral instrument as that used in the conditioning procedure. Mice avoided entering the dark compartment because of memory of fear in the dark compartment. The latency of entering the dark compartment was recorded as the avoidance latency. The cut-off point of latency was up to a maximum of 300 s. If the mouse did not enter within this period, the experiment was terminated and the mouse was returned to the cage. Seven days after CA/CPR, the neurologic deficit score of each animal was determined 1 h after the passive avoidance test by an investigator unaware of the group identities as previously described. Five parameters were assessed and scored: level of consciousness, corneal reflex, respiration, coordination, and movement/activity. The total score was reported as the neurological function score (total possible score = 10) (Minamishima et al., 2009).

2.5. Brain tissue harvest and immunohistological analysis

Seven days after CA/CPR, the mice were deeply anesthetized with intraperitoneal administration of pentobarbital for transcardial perfusion with 0.9% saline and were fixed with 4% paraformaldehyde. The brains were removed and embedded in paraffin, and 8 μm coronal sections were serially cut and stained with hematoxylin and eosin. The primary antibodies used were goat anti-ionized calcium-binding adaptor molecule-1 (IBA1) (Abcam, Cambridge, UK) and rabbit anti-neuronal nuclei (Neu-N) (Millipore, Billerica, MA, USA). An investigator blinded to the treatment groups counted all Neu-N-positive cells by light microscopy (100×). Regions vulnerable to ischemia were also evaluated, namely the caudate putamen (bregma 0.5 mm) and the dorsal region of the hippocampus (bregma −2.0 mm) (Fig. 3D). For analysis of microglia, Image J software (National Institutes of Health) was used to manually quantify the pixel area above the background IBA1 staining (threshold area) in the region of interest.