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Tumor necrosis factor receptor 2 is required for ischemic preconditioning-mediated neuroprotection in the hippocampus following a subsequent longer transient cerebral ischemia

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ABSTRACT

Tumor Necrosis Factor-α (TNF-α) is a proinflammatory cytokine implicated in neuronal damage in response to cerebral ischemia. Ischemic preconditioning (IPC) provides neuroprotection against a subsequent severer or longer transient ischemia by ischemic tolerance. Here, we focused on the role of TNF-α in IPC-mediated neuroprotection against neuronal death following a subsequent longer transient cerebral ischemia (TCI). Gerbils used in this study were randomly assigned to eight groups; sham group, TCI operated group, IPC plus (+) sham group, IPC + TCI operated group, sham + etanercept (an inhibitor of TNF-a) group, TCI + etanercept group, IPC + sham + etanercept group, and IPC + TCI + etanercept group. IPC was induced by a 2-min sublethal transient ischemia, which was operated 1 day prior to a longer (5-min) TCI. A significant death of neurons was found in the stratum pyramidale (SP) in the CA1 area (CA1) of the hippocampus 5 days after TCI; however, IPC protected SP neurons from TCI. We found that TNF-α immunoreactivity was significantly increased in CA1 pyramidal neurons in the TCI and IPC + TCI groups compared to the sham group. TNF-R1 expression in CA1 pyramidal neurons of the TCI group was also increased 1 and 2 days after TCI; however, in the IPC + TCI group, TNF-R1 expression was significantly lower than that in the TCI group. On the other hand, we did not detect TNF-R2 immunoreactivity in CA1 pyramidal neurons 1 and 2 days after TCI; meanwhile, in the IPC + TCI group, TNF-R2 expression was significantly increased compared to TNF-R2 expression at 1 and 2 days after TCI. In addition, in this group, TNF-R2 was newly expressed in pericytes, which are important cells in the blood brain barrier, from 1 day after TCI. When we treated etanercept to the IPC + TCI group, IPC-induced neuroprotection was significantly weakened. In brief, this study indicates that IPC confers neuroprotection against TCI by TNF-α signaling through TNF-R2 and suggests that the enhancement of TNF-R2 expression by IPC may be a legitimate strategy for a therapeutic intervention of TCI.

1. Introduction

Transient cerebral ischemia (TCI) occurs when the blood flow to the brain is disrupted and causes the degeneration of vulnerable neurons in vulnerable brain regions due to the lack of oxygen and glucose to damaged brain tissues (Kirino, 1982; Kirino and Sano, 1984). Mongolian

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gerbils have been used as an excellent animal model to investigate mechanisms of selective neuronal death following TCI, because most gerbils lack the communicating vessels between the internal carotid and vertebral arteries in the circle of Willis (Mayevsky and Breuer, 1992). Thus, the occlusion of both common carotid arteries eliminates blood flow to the forebrain while the vegetative center of the brain stem is completely intact. In this regard, it has been studied that, in a gerbil model of 5 min of TCI, the hippocampus is sensitive to TCI and pyramidal neurons in the hippocampal CA1 area begin to die about 4 or 5 days after 5 min of TCI (Hwang et al., 2007; Kirino, 1982).

Clinically, brief ischemic episodes induce tolerance by evoking the threshold of brain tissue vulnerability, which is critical for neuroprotection (Wegener et al., 2004). In this regard, many researchers have been studying that TCI-induced neuronal damage is prevented by ischemic preconditioning (IPC), which is able to occur in humans suffering brief transient ischemic attacks (Moncayo et al., 2000; Weih et al., 1999). Also, many studies have demonstrated neuroprotective effects of IPC against a longer or severe subsequent TCI in animal models (Dhodda et al., 2004; Feng et al., 2007; Gidday, 2006; Stenzel-Poore et al., 2007). Recently, we have published papers that show that a brief duration (2 min) of TCI, as IPC, in gerbils does not cause noticeable neuronal damage in the hippocampal CA1 area following a subsequent 5 min of TCI, which causes the neuronal death of pyramidal neurons in the CA1 area 4–5 days after 5-min TCI (Hye Kim et al., 2016; Lee et al., 2014b, 2015, 2017). This phenomenon has been termed “ischemic tolerance,” which is believed to trigger a great many intrinsic neuroprotective mechanisms, but the mechanisms have not been fully defined yet.

Tumor necrosis factor-α (TNF-α), an important inflammatory factor, has been traditionally viewed as a mediator of disease pathogenesis in the central nervous system, including cerebral ischemia, Parkinson's disease, Alzheimer's disease and brain injury (Scherbel et al., 1999; Tchelingerian et al., 1993; Yin et al., 2003). For a long period of time, TNF-α had been believed to induce neuronal cell death, resulting in brain injury (Feuerstein et al., 1998), until Cheng et al. (1994) demonstrated that TNF-α pretreatment protected cultured neurons from glucose deprivation-induced injury and excitatory amino-acid toxicity. The biological action of TNF-α is mediated through two distinct TNF-α receptors on cell surface (Wajant et al., 2003). TNF-R1 is expressed on all cell types and can be activated by both membrane-bound and soluble forms of TNF-α. This is a major signaling receptor for TNF-α (Chen and Goeddel, 2002). TNF-R2 is expressed primarily on hemopoietic and endothelial cells, responds to the membrane-bound form of TNF-α, and mediates limited biological responses (Wicovsky et al., 2009). Interestingly, TNF-Rs can have counteracting functions, at least in neuronal tissues, as recently demonstrated in a retinal ischemic model, where TNF-R1 apparently aggravates tissue damage, whereas TNF-R2 is protective via activation of PKB/Akt (Fontaine et al., 2002). Therefore, its function remains to be defined because both neurotoxic and neuroprotective effects during disease pathogenesis have been described (Kassiotis et al., 1999; Nawashiro et al., 1997; Tsao et al., 2001; Zheng and Yenari, 2004).

To the best of our knowledge, signals in the TNF-α pathway are upregulated after ischemia, yet its role in IPC remains unclear. Also, expression patterns and roles of TNF-α and TNF-Rs proteins in ischemic tolerance by IPC following a subsequent TCI have not been studied. Thus, this study, as a part of ongoing efforts to investigate effects of IPC on a subsequent ischemic insult, examined changes of TNF-α and TNF-Rs immunoreactivities and their protein levels in the hippocampus with IPC after a subsequent longer TCI using gerbils, which are a good animal model of TCI with IPC (Ahn et al., 2016; Lee et al., 2013, 2016a), and tried to identify TNF-α-related function in ischemic tolerance by IPC using etanercept, which is a TNF-α inhibitor competitively inhibits the binding of TNF-α to cell-surface TNF-R2.

2. Material and methods

2.1. Experimental animals

Male Mongolian gerbils (*Meriones unguiculatus,* total number = 140, body weight 65–75 g, 6 months of age) were obtained from the Experimental Animal Center, Kangwon National University, Chuncheon, Republic of Korea. As we described previously (Lee et al., 2014b, 2017), all experimental protocol of this study was approved (approval no. KW-160802-1) by the Institutional Animal Care and Use Committee (IACUC) at Kangwon University and adhered to guidelines that are in compliance with the current international laws and policies (Guide for the Care and Use of Laboratory Animals, The National Academies Press, 8th Ed., 2011).

2.2. Experimental groups

Gerbils were divided into eight groups (n = 14 at each point in time in each group): (1) sham group, which was given sham surgery for TCI (5 min of ischemia); (2) TCI group, which was given a TCI; (3) IPC + sham group, which was subjected to a IPC (2 min of ischemia) and given sham TCI surgery; (4) IPC + TCI group, which was subjected to a IPC and given a TCI; (5) Sham + ENT group, which was subjected to sham TCI surgery and intraperitoneally injected with ENT (etanercept, an inhibitor of TNF-a) for 3 days after TCI; (6) TCI + ENT group, which was subjected to a TCI and treated with ENT for 3 days after TCI; (7) IPC + sham + ENT group, which was subjected to a IPC, given no TCI after IPC, and treated with ENT for 3 days after no TCI; and (8) IPC + TCI + ENT group, which was subjected to a IPC, given TCI after IPC, and treated with ENT for 3 days after TCI. The animals in all the groups were given recovery times of 1 day, 2 days and 5 days after TCI, because pyramidal neurons in the hippocampal CA1 area do not die until 3 days and begin to die 4 days after TCI (Nakamura et al., 2006).

2.3. Induction of IPC and TCI

As previously described (Lee et al., 2015), in brief, the animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. Bilateral common carotid arteries were occluded using non-traumatic aneurysm clips (Yasargil FE 723K, Aesculap, Tuttlingen, Germany). The complete interruption of blood flow was confirmed by observing the central artery in retinae using an ophthalmoscope (HEINE K180®, Heine Optotechnik, Herrsching, Germany). The duration of occlusion (transient ischemia) was 2 min for IPC and 5 min for TCI, respectively, and the aneurysm clips were removed from the common carotid arteries. Normal body (rectal) temperature (37 ± 0.5 °C) was controlled with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA) using thermometric blanket.

2.4. Treatment of ENT

To examine the contribution of TNF-α to IPC-induced neuroprotection, ENT (Enbrel®; Amgen, Thousand Oaks, CA, USA) was reconstituted with normal saline according to the manufacturer's instructions and administered intravenously four times (50 mg/kg of ENT per day) from 1 day to 3 days after TCI. ENT is a commercially available TNF-α inhibitor that functions as a decoy receptor that binds to TNF-α. It was the first fusional monoclonal antibody against TNF-α to be marketed for clinical use and is now commercially available.

2.5. Western blot analyses

Western blot analyses for TNF-α, TNF-R1 and TNF-R2 in hippocampal CA1 areas (n = 7/group) were done according to our published method (Bae et al., 2015). Briefly, the tissues were homogenized, and