available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Expression of tissue-type transglutaminase (tTG) and the effect of tTG inhibitor on the hippocampal CA1 region after transient ischemia in gerbils

In Koo Hwang[a], *Ki-Yeon Yoo*[b], *Sun Shin Yi*[a], *Il Yong Kim*[a], *Hye Sook Hwang*[a], *Kyung-Yul Lee*[c], *Sun Mi Choi*[d], *In Se Lee*[a], *Yeo Sung Yoon*[a], *Soo Youl Kim*[e], *Moo Ho Won*[b,f,*], *Je Kyung Seong*[a,g,h,*]

[a]*Department of Anatomy and Cell Biology, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul 151-742, South Korea*
[b]*Department of Anatomy and Neurobiology, and Institute of Neurodegeneration and Neuroregeneration, College of Medicine, Hallym University, Chuncheon 200-702, South Korea*
[c]*Department of Neurology, Yonsei University College of Medicine, Seoul 120-749, South Korea*
[d]*Korea Institute of Oriental Medicine, Daejeon 305-811, South Korea*
[e]*Molecular Oncology Branch, Division of Basic Sciences, Research Institute and Cancer Biostatistics Branch, Center for Clinical Trials, National Cancer Center, Goyang 410-769, South Korea*
[f]*MRC Research Institute, Hallym University, Chuncheon 200-702, South Korea*
[g]*Cancer Research Institute, Seoul National University, Seoul 151-742, South Korea*
[h]*Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul 151-742, South Korea*

ARTICLE INFO

Article history:
Accepted 21 January 2009
Available online 31 January 2009

Keywords:
Transglutaminase 2
Ischemia
Iba-1
Microglia
PECAM-1
Blood vessel
Cystamine

ABSTRACT

Chronological changes of tissue-type transglutaminase (tTG) were observed in the hippocampal CA1 region after transient forebrain ischemia in gerbils. In the sham-operated group, tTG immunoreactivity was weakly detected in blood vessels which were immunostained with platelet endothelial cell adhesion molecule-1 (PECAM-1), and tTG immunoreactivity in blood vessels was highest 5 days after ischemia/reperfusion. In addition, tTG immunoreaction was expressed in microglia which were immunostained with Iba-1 at 4 days post-ischemia, and tTG immunoreactivity in the microglia was also highest at 5 days post-ischemia. In Western blot analysis, tTG protein levels in the CA1 region after ischemia/reperfusion began to increase 3 days after ischemia/reperfusion and peaked 5 days after ischemia/reperfusion. The expression of tTG in PECAM-1-immunoreactive blood vessels may be associated with integrin regulation or transendothelial migration of leukocytes in the ischemic CA1 region. In this study, we also observed the effect of cystamine, a tTG inhibitor, against ischemic damage. Administration of cystamine protected in certain degree neuronal damage from ischemic damage in the CA1 region. These results suggest that tTG may be associated with neuronal death in the hippocampal CA1 region induced by ischemia/reperfusion.

* *Corresponding authors*. M.H. Won is to be contacted at Department of Anatomy and Neurobiology, College of Medicine, Hallym University, Chuncheon 200-702, South Korea. Fax: +82 33 256 1614. J.K. Seong, Department of Anatomy and Cell Biology, College of Veterinary Medicine and BK21 Program for Veterinary Science, Seoul National University, Seoul 151-742, South Korea. Fax: +82 2 875 8395.
E-mail addresses: mhwon@hallym.ac.kr (M.H. Won), snumouse@snu.ac.kr (J.K. Seong).

0006-8993/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.01.038

1. Introduction

Transient occlusion of the common carotid artery deprives the brain of blood flow and significantly reduces glucose and oxygen levels in the blood. These cause insidious delayed degeneration of specific vulnerable neurons within the hippocampus, neocortex, and thalamus (Kirino, 1982; Petito and Pulsinelli, 1984). Among these susceptible regions, pyramidal cells of the hippocampal CA1 region are most vulnerable to cerebral ischemia (Kirino, 1982; Petito and Pulsinelli, 1984), and extensive neuronal death occurs in this region. One of the possible mechanisms for this neuronal death is glutamate-mediated toxicity. The occlusion-mediated elevation of extracellular glutamate leads to a massive increase in intracellular calcium, which triggers the neuronal degeneration in the hippocampal CA1 region (Rothman and Olney, 1986; Choi and Rothman, 1990; Hota et al., 2008; Jung et al., 2008).

Transglutaminase (TG, EC 2.3.2.13) is a member of a family of Ca^{2+}-activated enzymes that catalyze the formation of a covalent bond between a free amine group (*e.g.*, protein- or peptide-bound lysine) and the γ-carboxamide group of protein- or peptide-bound glutamine via an acyl transfer reaction (Greenberg et al., 1992). Among TGs, tissue-type TG (tTG) is the major TG in the mammalian nervous system, localizing predominantly in neurons. There are some contradictory reports that tTG has been implicated in axonal regeneration, cellular differentiation, neurite outgrowth, apoptosis and neurodegeneration (Piredda et al., 1999; Citron et al., 2001; Tucholski et al., 2001; Fesus and Piacentini, 2002; Griffin et al., 2002).

tTG has been studied in neurodegenerative diseases because cross-linking reactions by tTG directly or indirectly exacerbate the formation of protein aggregates in affected brain regions (Kim et al., 1999; Lesort et al., 1999; Cooper et al., 2002; Ientile et al., 2004). Furthermore, this enzyme has conventionally been used as a marker of tissue degeneration (Fesus et al., 1988; Walter et al., 2005; Wang et al., 2005; Vig et al., 2007).

Several researchers have reported ischemia-related increases of tTG in the hippocampus after ischemia/reperfusion. However, such studies only measured the protein levels and tTG activity in tissue homogenates using biochemical methods (Caccamo et al., 2004; Ientile et al., 2004; Tolentino et al., 2004). Understanding the localization of tTG in the hippocampal CA1 region after transient ischemia is very important in assessing the mechanisms of tTG activity after transient forebrain ischemia. In the present study, therefore, we investigated the chronological changes of tTG immunoreactivity and protein levels in the hippocampal CA1 region after 5 min of transient forebrain ischemia in gerbils. In addition, we observed the neuroprotective effects of cystamine, a tTG inhibitor, against ischemic damage.

2. Results

2.1. Change in tTG immunoreactivity

In the sham-operated group, tTG immunoreactivity was weakly detected in the CA1 region (Fig. 1A). In this group, tTG immunoreaction was found in blood vessel-like structures. Between 30 min and 2 days after ischemia/reperfusion, tTG immunoreaction was similar to that in the sham-operated group (data not shown). Three days after ischemia/reperfusion, tTG immunoreactivity was increased in blood vessel-like structures in the CA1 region (Fig. 1B). Four days after ischemia/reperfusion, tTG immunoreaction was detected in some glia as well as blood vessel-like structures (Fig. 1C). Five days after ischemia/reperfusion, tTG immunoreactivity was increased in the CA1 region: Strong tTG immunoreactivity was detected in many glia and blood vessel-like structures (Fig. 1D). Thereafter, tTG immunoreactivity was decreased in the CA1 region (Fig. 1G). Seven to ten days after ischemia/reperfusion, tTG immunoreactivity in those was weaker than that in the 5 days post-ischemia group. However, at these time points, tTG immunoreaction was concentrated in the stratum pyramidale (Figs. 1E and F).

2.2. Expression of tTG in Iba-1-immunoreactive microglia

To verify the glial type of tTG-immunoreactive cells, we performed double immunofluorescence staining using tTG and Iba-1 in the CA1 region 5 days after ischemia/reperfusion; microglia were numerous in the stratum pyramidale of the CA1 region at this time point after ischemia/reperfusion (Hwang et al., 2006). We found that tTG immunoreactive glial cells in the CA1 region were identified as Iba-1-immunoreactive microglia (Figs. 2A–C).

2.3. Expression of tTG in PECAM-1-immunoreactive blood vessels

To confirm the expression of tTG in blood vessels, we performed double immunofluorescence staining using tTG and PECAM-1 in the CA1 region 5 days after ischemia/reperfusion. We found that tTG immunoreaction was in blood vessels which were immunostained with PECAM in the CA1 region after ischemia/reperfusion (Figs. 2D–F).

2.4. Change in tTG protein level

Western blot analysis revealed that the pattern of changes in tTG levels in the CA1 region after ischemia/reperfusion was similar to that observed in the immunohistochemical data (Fig. 3). tTG protein level began to increase 3 days after ischemia/reperfusion, peaked 5 days after ischemia/reperfusion, and thereafter decreased in the CA1 homogenates (Fig. 3).

2.5. Effects of cystamine against ischemic damage

2.5.1. Spontaneous motor activity

In the sham-operated group, locomotor activity was similar in the vehicle- and cystamine-treated groups (Fig. 4). One day after ischemia/reperfusion, locomotor activity increased significantly in the vehicle-treated group by 317% compared to that in the sham-operated group and decreased thereafter in a time-dependent manner. In the 7 mg/kg cystamine-treated group, locomotor activity increased significantly by 213% 1 day after ischemia/reperfusion, but not in the 35 mg/kg cystamine-treated group (127%). Thereafter, locomotor activity decreased