BRAIN RESEARCH 1135 (2007) 167–176

available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Pharmacological induction of heat shock protein exerts neuroprotective effects in experimental intracerebral hemorrhage

Dong-In Sinn[a,b,1], *Kon Chu*[a,b,1], *Soon-Tae Lee*[a,b,c], *Eun-Cheol Song*[a,b], *Keun-Hwa Jung*[a,b,d], *Eun-Hee Kim*[a], *Dong-Kyu Park*[a], *Kyung-Mook Kang*[a], *Manho Kim*[a,b], *Jae-Kyu Roh*[a,b,*]

[a]*Stroke and Neural Stem Cell Laboratory in the Clinical Research Institute, Stem Cell Research Center, Department of Neurology, Seoul National University Hospital, Seoul, South Korea*

[b]*Program in Neuroscience, Neuroscience Research Institute of the SNUMRC, College of Medicine, Seoul National University, Seoul, South Korea*

[c]*Center for Alcohol and Drug Addiction Research, Seoul National Hospital, Seoul, South Korea*

[d]*Division of Epidemic Intelligence Service, Korea Center for Disease Control and Prevention, Seoul, South Korea*

ARTICLE INFO

Article history:
Accepted 30 November 2006
Available online 15 December 2006

Keywords:
Intracerebral hemorrhage
Geranylgeranylacetone
Heat shock protein
Cell death
Inflammation
Neuroprotectant

ABSTRACT

Heat shock proteins (HSPs) are reported to reduce inflammation and apoptosis in a variety of brain insults. Geranylgeranylacetone (GGA), developed as an antiulcer in Japan, has been known to induce HSP70 and to exert cytoprotective effects. In this study, we investigated whether GGA, as a specific HSP inducer, exerts therapeutic effects in experimentally induced intracerebral hemorrhage (ICH). ICH was induced with male Sprague–Dawley rats via the collagenase infusion. GGA (800 mg/kg) was administered via oral tube according to various schedules of treatment. The treatment with GGA, beginning before the induction of ICH and continuing until day 3, showed the reduction of brain water content and the increased level of HSP70 protein, as compared to the treatment with vehicle, although GGA started after the induction of ICH or administered as a single dose before ICH failed to up-regulate HSP70 and to reduce brain edema. The rats treated with GGA exhibited better functional recovery than those treated with vehicle. In the pre- and post- treatment group, inflammatory cells and cell death in the perihematomal regions were found to have been decreased. The treatment of GGA inhibited the mRNA expression of MMP-9, uPA, IL-6 and MIP-1, with concomitant increment of eNOS and phosphorylated STAT3 and Akt after ICH. We demonstrated that GGA induced a reduction in the brain edema along with marked inhibitory effects on inflammation and cell death after ICH.

* *Corresponding author*. Department of Neurology, Seoul National University Hospital, 28, Yongeon-Dong, Jongro-Gu, Seoul, 110-744, South Korea. Fax: +822 3672 4949.
E-mail address: rohjk@snu.ac.kr (J.-K. Roh).

Abbreviations: GGA, geranylgeranylacetone; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's modified Eagle's medium; eNOS, endothelial nitric oxide synthase; HSF1, heat shock factor-1; HSP, heat shock protein; ICH, intracerebral hemorrhage; IL-6, interleukin-6; MIP-1, macrophage inflammatory protein-1; MLPT, modified limb placing test; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NF-κB, nuclear factor-kappa B; PAI, plasminogen activator inhibitor; STAT3, signal transducer and activator of transcription-3; tPA, tissue plasminogen activator; uPA, urinary plasminogen activator

[1] These two authors equally contributed to this study.

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.11.098

1. Introduction

Intracerebral hemorrhage (ICH) is an acute and spontaneous bleeding into the parenchyma of the brain, which results in catastrophic disabilities so that more than one-third of patients with ICH die at 1 month after the onset of symptom and only 20% of survivors live independently at 6 months (Broderick et al., 1999; Mendelow et al., 2005). The surgical treatment of ICH is limited to selected patients (Mendelow et al., 2005) and the medical treatment is only supportive in most patients (Broderick et al., 1999). The knowledge about the pathophysiology of ICH has been extended by recent investigations. ICH is now understood as a dynamic and complex process, in which the most important mechanisms are consisted of early hematoma growth and perihematomal injury resulting mainly from inflammation (Mayer and Rincon, 2005). In recent years, the reduction of inflammatory responses and cell death by targeting transcription factors, enzymes, cytokines, oxidative stress or microglial activation has been a hot topic in studies regarding treatment or pathogenesis of ICH (Aronowski and Hall, 2005).

Heat shock proteins (HSPs) are proteins synthesized in eukaryotic organisms and bacteria in response to environmental challenges including hyperthermia, excitotoxic exposure, and other stresses (Welch and Brown, 1996). HSPs play crucial roles in the maintenance of cellular integrity and viability by preventing the protein denaturation and the aggregation of incorrect polypeptides. The 70-kDa heat shock protein (HSP70) may also involve the pathways of apoptosis and inflammation. The overexpression of HSP70 protects neurons from lethal insults (Yenari et al., 2005. HSP70 overexpressed by genetic manipulations or pharmacological inducers is reported to exert neuroprotective effects in cerebral focal ischemia (van der Weerd et al., 2005; Yasuda et al., 2005), polyglutamine-mediated motor neuron disease (Kastuno et al., 2005), severe heat stress (Uney et al., 1993), and other stressful conditions. HSP70 also has the ability to bind to peptides generated during brain inflammation in experimental autoimmune encephalitis and to induce a regulatory natural killer cell population (Galazka et al., 2006). However, the clinical application of HSP70 overexpression has been limited because the genetic manipulations were used for up-regulation of HSP70 in most studies.

Geranylgeranylacetone (GGA), which was originally developed in Japan as an antiulcer drug, is an acyclic isoprenoid compound. GGA induces HSPs and other protective proteins dramatically in a variety of organs or tissues including central nervous system (Fujiki et al., 2003), gastric mucosa (Hirakawa et al., 1996), myocardium (Ooie et al., 2001), liver (Fudaba et al., 2001), cochlea (Mikuriya et al., 2005) and retina (Ishii et al., 2003). Oral administration of GGA up-regulates HSP in central nervous system and exhibits neuroprotection in response to various injuries (Yasuda et al., 2005; Fujiki et al., 2004a,b; Kastuno et al., 2005). Other pharmacological reagents such as ethanol, arsenic, and cadmium also induce heat shock responses. However, in contrast to these agents having toxic or harmful effect clinically, GGA has been commonly used with few side effects reported. In the present study, we aimed to investigate whether oral administration of GGA can induce heat shock proteins and exert antiapoptotic and anti-inflammatory effects in experimental ICH.

2. Results

2.1. The level of HSP70 protein induced in normal PC12 cells by GGA

To investigate the protein level of HSP70 induced by GGA in neural cell line, we treated PC12 cells (pheochromocytoma-derived neuronal cells) with GGA of various concentrations (0–1 μM) for 48–72 h. The changes in the protein level of HSP70 measured by Western blot analysis are not significant. Neither dose-dependent nor time-dependent increment in HSP70 protein was detected (Fig. 1A).

2.2. The induction of experimental ICH

Experimental ICH was induced via stereotaxic intrastriatal administration of type VII bacterial collagenase, using male Sprague–Dawley rats, as described in other studies (Rosenberg et al., 1990; Chu et al., 2004). Rats showing unilateral sensorimotor deficits after the induction of ICH were used for the following experiments. Physiological parameters, including mean arterial blood pressure, blood gases, serum glucose, and body temperatures were not significantly different among all experimental groups, either at 30 min before, during, or 30 min after ICH (data not shown).

2.3. Oral GGA reduced brain edema

All rats were treated with GGA or vehicle. Oral GGA was given to the rats according to three different schedules. To determine the effect of GGA on the brain edema induced by ICH, brain water contents of cerebral hemispheres were measured. Brain water content of the lesioned (left) hemisphere determined 72 h after the induction of ICH was 81.988±0.428% in the ICH-vehicle group. GGA administrated as a single dose before the induction of ICH (the single pre-treatment group) or given after ICH (the post-treatment group) failed to reduce brain water content significantly as compared to the vehicle. The brain water contents of the lesioned hemisphere were 80.822±1.010% in the single pre-treatment group and 81.128±1.056% in the post-treatment group. Treatment with GGA before and after the induction of ICH (the pre- and post-treatment group) resulted in the decreased water content measured as 80.340±0.488%. The difference of water content in this group was significant as compared to the ICH-vehicle group ($p<0.01$, t-test; Table 1). The brain water contents of the non-lesioned (right) hemispheres were not different statistically among all groups (Table 1).

2.4. Oral GGA treatment up-regulated the HSP70 expression

To determine which treatment group up-regulates HSP70 level, we measured HSP70 levels on Western blotting 1 day after the induction of ICH. The induction of HSP70 was