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PHARMACOLOGICAL INDUCTION OF THE 70-kDa HEAT SHOCK PROTEIN PROTECTS AGAINST BRAIN INJURY

N. KIM, [†‡] **J. Y. KIM** [†‡] **AND M. A. YENARI** [*]

Department of Neurology, University of California, San Francisco and Veterans Affairs Medical Center, San Francisco, CA 94121, USA

Abstract—The 70-kDa heat shock protein (HSP70) is known to protect the brain from injury through multiple mechanisms. We investigated the effect of pharmacological HSP70 induction in experimental traumatic brain injury (TBI). 3-month-old male C57/B6 mice were given 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) intraperitoneally (IP, 2 mg/kg) or intracerebroventricularly (ICV, 1 μg/kg) to determine whether HSP70 could be induced in the brain. Mice were subjected to TBI via cortical controlled impact, and were treated with 17-AAG (or vehicle) IP according to one of two treatment regimens: (1) 2 mg/kg at the time of injury, (2) a total of three doses (4 mg/kg) at 2 and 1 d prior to TBI and again at the time of injury. Brains were assessed for HSP70 induction, hemorrhage volume at 3 d, and lesion size at 14 d post-injury. Immunohistochemistry showed that both IP and ICV administration of 17-AAG increased HSP70 expression primarily in microglia and in a few neurons by 24 h but not in astrocytes. 17-AAG induced HSP70 in injured brain tissue as early as 6 h, peaking at 48 h and largely subsiding by 72 h after IP injection. Both treatment groups showed decreased hemorrhage volume relative to untreated mice as well as improved neurobehavioral outcomes. These observations indicate that pharmacologic HSP70 induction may prove to be a promising treatment for TBI. Published by Elsevier Ltd. on behalf of IBRO.

Key words: animal studies, traumatic brain injury, therapeutic approaches.

INTRODUCTION

The 70-kDa class of heat shock proteins (HSP70) comprise a highly conserved family of ATP-dependent, cytosolic chaperones that function primarily in facilitating protein folding, degradation, complex assembly, and translocation, consequently preventing harmful protein aggregation (Giffard et al., 2004). They are present in nearly every type of cell in the body, and some are specifically upregulated in response to stress, such as cytotoxic and potentially pathogenetic accumulation of unfolded proteins that arises when normal cellular processes are interrupted by stress (Adachi et al., 2009; Henderson, 2010). The HSP70 family includes an inducible form also known as HSP72, HSP70i, or simply HSP70. HSP70 has also shown to be neuroprotective in animal models of various brain insults, including neurodegenerative disorders, cerebral ischemia, and traumatic brain injury (TBI) (Yenari et al., 2005; Turturici et al., 2011).

Whether by their function as chaperone or by some other yet undetermined mechanism, HSP70 appears to play a role in cytoprotection, reducing inflammation and apoptosis in brain injury models including stroke and TBI (Giffard et al., 2004). Overexpression of HSP70 has been shown to reduce apoptosis, though the exact mechanism remains unclear (Giffard and Yenari, 2004). Thus, strategies to increase intracellular HSPs might be relevant in many neurological conditions such as TBI. Studies have shown that immune response pathways arising after acute neurological insults can exacerbate brain injury, and that suppressing inflammation can reduce cell death and improve recovery. Overexpression of HSP70 in such circumstances appears to be largely anti-inflammatory, as intracellular, innate immune responses appear to be in play (Giffard and Yenari, 2004). Previous studies have also identified a link between inducible HSP70 and matrix metalloprotease regulation in injury conditions (Lee et al., 2004). Recent findings from our lab have shown that HSP70 overexpression suppresses matrix metalloproteinase (MMP) 9, protecting the brain in experimental TBI. Selective knock-down of HSP70 led to more pronounced MMP 2 and MMP 9 activity in the brain and reversed the reduction in hemorrhage and lesion sizes corresponding with HSP70 overexpression (Kim et al., 2013). However, much of the existing research in neuroprotective HSP70 overexpression has been conducted in transgenic models or by gene transfer which may not be practical in clinical settings (Whitesell et al., 1994; Giffard et al., 2008). Pharmaceutical induction of HSP70 may prove to be a viable therapeutic approach for limiting damage due to brain injury.

Under normal, non-stressful conditions HSPs are located intracellularly and are bound to heat shock

[*]Corresponding author. Address: Neurology 127, VAMC 4150 Clement Street, San Francisco, CA 94121, USA. Tel: +1-415-750-2011; fax: +1-415-750-2273.
E-mail addresses: nuri@berkeley.edu (N. Kim), jongyoul74@gmail.com (J. Y. Kim), yenari@alum.mit.edu (M. A. Yenari).
[†] These authors contributed equally to this work.
[‡] Tel: +1-415-221-4810x3691; fax: +1-415-750-2273.

Abbreviations: 17-AAG, 17-N-allylamino-17-demethoxygeldanamycin; ANOVA, analysis of variance; BBB, blood–brain barrier; CCI, controlled cortical impact; DMSO, dimethyl sulfoxide; GA, geldanamycin; GFAP, Glial fibrillary acidic protein; HSEs, heat shock elements; HSFs, heat shock factors; HSP70, 70-kDa heat shock protein; MMP, matrix metalloproteinases; PBS, phosphate-buffered saline; PFA, paraformaldehyde; TBI, traumatic brain injury.

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factors (HSFs) (Kelly and Yenari, 2002). Inducible HSP70 is upregulated following a denaturing stress such as trauma or ischemia. Next, HSFs dissociate from HSPs, leaving HSPs free to bind target proteins. HSFs are then phosphorylated and form activated trimers which bind to highly conserved regulatory sequences on the heat shock gene known as heat shock elements (HSEs). Once bound to HSEs, HSFs control the generation and expression of more HSPs. Newly generated HSPs can then bind denatured proteins and act as a molecular chaperone by contributing to repair, refolding, and trafficking of damaged proteins within the cell. HSP90 can also influence HSP70, since HSP90 is bound to HSF-1. When HSP90 dissociates from HSF-1, HSF-1 leads to HSP70 induction (Kim et al., 2012). HSP90 antagonists can thus function as HSP70 inducers.

At present, there are few published findings evaluating pharmacological HSP70 induction in neurological disorders, and these have exclusively involved ansamycins in acute injury. The research to date has particularly focused on geldanamycin (GA), one of the earliest known HSP90-antagonists first described in a 1994 publication describing HSP90-inhibition in cancer cells (Kwon et al., 2008). The existing research focuses on the application of GA in cerebral ischemia and brain hemorrhage (Lu et al., 2002; Porter et al., 2009; Manaenko et al., 2010). These studies collectively determined that HSP70 induction by GA represses expression of pro-inflammatory markers, reduces infarct size, and downregulates apoptotic pathways in stroke and hemorrhage. Because of toxicity, GA remains a poor candidate for a pharmaceutical treatment, and investigators in the cancer biology field have developed a less toxic GA analog 17-allylamino-17-demethoxygeldanamycin (17-AAG or tanespimycin), which has been shown to be an effective HSP90 antagonist in addition to a more favorable safety profile (Chakraborty et al., 2008; Vaishampayan et al., 2010). 17-AAG has primarily been studied as a pro-apoptotic drug in several cancers, though these studies often use the HSP90 inhibitor in conjunction with other anti-survival conditions (Chatterjee et al., 2007; Tavernier et al., 2012; Zhang et al., 2012). Apart from cancer biology, 17-AAG has been shown to protect neural progenitor cells *in vitro* and reduce inflammation in other non-neurological disease models such as atherosclerosis, uveitis, and acute lung injury (Poulaki et al., 2007; Madrigal-Matute et al., 2010; Wang et al., 2011). There are presently no studies of 17-AAG in brain trauma. Findings from our lab are the first to suggest that 17-AAG may also confer protection in TBI, reducing hemorrhage and improving neurobehavioral outcomes. Further investigation is needed to conclusively determine a potential role in stroke or other neurological insults, although the existing data highlight 17-AAG as a promising therapeutic.

EXPERIMENTAL PROCEDURES

Animals

Male, 3-month-old C57B6 mice weighing 25–30 g each (Simonson Labs, Gilroy, CA, USA) were used for all groups. Mice were housed and handled in compliance with NIH regulations, according to protocols approved by the local Institutional Animal Care & Use Committee.

Controlled cortical impact (CCI)

Mice were anesthetized with isoflurane (5% for induction in a designated chamber, 2% for maintenance by nosecone) and fixed in a stereotaxic frame. CCI was induced using methods previously published by our group (Whitesell et al., 1994). A midline scalp incision was made to expose the skull. A 5-mm-diameter burr hole over the parietal lobe bregma was drilled. The dura was not disturbed during craniectomy. Impactor with 3 mm-diameter convex tip (Pinpoint Precision Cortical impactor, Hatteras Instruments, Cary, NC, USA) was perpendicularly applied at exposed area with 1.5 m/s velocity to a depth of 2 mm and for a 100-ms dwell time. Sham operated mice were studied in parallel. Post-injury, mice were monitored daily and survived for up to 14 d. At the end of the observation periods, mice were euthanized and brains harvested for further study.

17-AAG treatment

For the initial immunohistochemistry panel to determine whether 17-AAG treatment induced HSP70 in the brain, uninjured mice were treated with 1 μl of 17-AAG (Sigma, St. Louis, MO, USA) at a dose of 1 μg/kg via intracerebroventricular (ICV) injection or 100 μl of the same concentration via intraperitoneal (IP) injection. In subsequent uses, sham and injured mice in the treatment groups were given 100 μl of 17-AAG at 100 μg/mL via IP-injection at indicated time points. IP dose of 4 mg/kg was determined based on previous studies and drug safety profile (Sausville et al., 2003). 17-AAG was solubilized in dimethyl sulfoxide (DMSO) and diluted in phosphate-buffered saline (PBS). Single treatment (1X) mice were administered a single injection of 17-AAG concurrent to injury. Triple treatment (3X) mice were given two initial doses, 2 d and 1 d prior to injury, followed by a final injection at the time of injury (Table 1). Untreated groups were given diluted DMSO via IP injection at time of injury as well as at matching pre-injury time points. 1X and 3X DMSO groups did not show significant variation (not shown).

Histology

Frozen tissue sections were prepared from brains collected at 1 d after treatment with 17-AAG or vehicle only (n = 3 per group). Mice were euthanized using (5% isoflurane, dislocation), transcardially reperfused with

Table 1. Treatment paradigms for the CCI experiments. All injections were given intraperitoneally (IP); CCI = controlled cortical impact, 17-AAG = 17-allylamino-17-demethoxygeldanamycin

Group	Treatment
Control	Vehicle injection at the time of CCI
17-AAG (1X)	IP injection at the time of CCI
17-AAG (3X)	IP injections 2 d prior, 1 d prior, and at time of CCI