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Intracellular and extracellular expression of the major inducible 70kDa heat shock protein in experimental ischemia-reperfusion injury of the spinal cord

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ABSTRACT

Inflammatory responses exacerbate ischemia-reperfusion (IR) injury of spinal cord, although understanding of mediators is incomplete. The major inducible 70kDa heat shock protein (hsp70) is induced by ischemia and extracellular hsp70 (e-hsp70) can modulate inflammatory responses, but there is no published information regarding e-hsp70 levels in the cerebrospinal fluid (CSF) or serum as part of any neurological disease state save trauma. The present work addresses this deficiency by examining e-hsp70 in serum and CSF of dogs in an experimental model of spinal cord IR injury. IR injury of spinal cord caused hind limb paraplegia within 2–3 h that was correlated to lumbosacral poliomalacia with T cell infiltrates at 3 d post-ischemia. In this context, we showed a 5.2-fold elevation of e-hsp70 in CSF that was induced by ischemia and was sustained for the following 3 d observation interval. Plasma e-hsp70 levels were unaffected by IR injury, indicating e-hsp70 release from within the central nervous system. A putative source of this e-hsp70 was ependymal cells in the ischemic penumbra, based upon elevated i-hsp70 levels detected within these cells. Results warrant further investigation of e-hsp70's potential to modulate spinal cord IR injury.

Introduction

Surgical repair of aneurisms of the thoracic and thoracoabdomenal aorta can cause ischemia-reperfusion (IR) injury of the spinal cord that results in paraplegia or paraparesis in 4–40% of patients, even when using newer endovascular approaches (Peppelenbosch et al., 2005). The proximate mechanism of IR injury in the central nervous system (CNS) involves inflammatory responses initiated in the microvasculature. Platelet and neutrophil adhesion are key components of this response, giving rise to vasoactive compounds and reactive oxygen species that are responsible for tissue injury (Ishikawa et al., 2004). In the brain, this early event has been shown to be dependent upon CD4+ and CD8+ T cells and interferon-γ, with the volume of tissue damage and leukocyte adhesion being significantly reduced in T cell deficient or interferon-γ knockout mice (Yilmaz et al., 2006). Experimental ischemic injury of the brain is accompanied by a rapid activation of the peripheral immune system, suggesting that T cell involvement may be part of a more generalized immune response (Offner et al., 2006). Lacking however is the identification of an endogenous ligand induced in the injured tissue that is capable of stimulating this response.

Cellular heat shock proteins represent attractive candidate ligands based upon their induction by ischemia and their ability to stimulate both innate and adaptive immune responses when released into the extracellular environment (Boros and Bromberg, 2006). Classified by molecular mass, the 70kDa family includes both constitutively expressed and highly inducible cytosolic isoforms. The major inducible isoform is designated hsp70, also known as hsp72. Intracellular hsp70 (i-hsp70) serves a cytoprotective function and confers ischemic tolerance in the brain when overexpressed by a transgene or viral vector (Rajdev et al., 2000; Yenari et al., 1998; Kelly et al., 2001). Sublethal cellular stress, such as that mediated by hyperthermia or mild transient ischemia, can also elevate i-hsp70 levels and confer ischemic tolerance in the spinal cords of rats, rabbits, mice, and dogs (Cizkova et al., 2004; Sakurai et al., 1998; Matsuyama et al., 1997).

It is extracellular hsp70 (e-hsp70) that takes on the immune modulatory role of "chaperokine" (Asea, 2005). e-hsp70 interacts through Toll-like receptors and CD14 to stimulate a proinflammatory state, consistent with the view that i-hsp70 released from necrotic cells may serve as a "danger signal" activating the innate immune system (Vabulas et al., 2002; Asea et al., 2000). Correlation between IR injury, induction of hsp70 and

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stimulation of innate immunity has been established in the kidney, where injury resulted in the induction of TLR-2, TLR-4 and hsp70 within affected tubular epithelium and was associated with maturation of dendritic cells (Kim et al., 2005). But e-hsp70 can also suppress chronic inflammatory responses by stimulating hsp70-specific T cells to produce IL-10 (van Eden et al., 2005). Determinants of e-hsp70's role as a proinflammatory versus anti-inflammatory mediator are poorly understood, such that e-hsp70 should be viewed in more general terms — an immunoregulatory protein whose precise function may be context dependent. Unknown is whether e-hsp70 is released into the cerebrospinal fluid (CSF) and/or serum following IR injury of the CNS, and this must be established before an immunoregulatory role can be considered. Currently, the only published reports of e-hsp70 in association with CNS disease describes elevations in CSF (Lai et al., 2004) or serum (da Rocha et al., 2005) associated with traumatic brain injury.

The current work is the first step in addressing this deficiency by characterizing expression of i-hsp70 and e-hsp70 in a canine model of spinal cord IR injury (Berguer et al., 1992). The e-hsp70 levels were evaluated in the context of established CSF markers/mediators of acute spinal cord IR injury: oxidative insult mediated by myeloperoxidase and reflected in the release of lipoperoxides; release of vasoactive compounds, reflected in elevation of thromboxane B_2. Post mortem analyses confirmed an inflammatory component of the IR injury. In this context, we evaluated e-hsp70 levels in the CSF, measuring serum e-hsp70 levels and i-hsp72 in the spinal cord to identify a putative source of the e-hsp70.

Materials and methods

The Institutional Laboratory Animal Care and Use Committee (ILACUC) for The Ohio State University provided supervision of animal care for all aspects of the study, including review and approval of the experimental protocol and animal care facilities at The Ohio State University which are AAALAC accredited.

Treatment and control groups

Anesthesia was administered with 25 mg/kg of pentobarbital and maintained with 1–2% isofluorane for all surgical procedures. An intrathecal catheter was inserted through a mini laminectomy at L4 in nine adult cross-bred dogs weighing 19.6 to 30.2 kg. The catheter was advanced to the level of T13/L1 and connected to a one-way subcutaneous value allowing repeated sterile sampling of the CSF. Animals were allowed a one week recovery interval, at which point they were re-anesthetized for a left thoracotomy. Animals were randomly assigned to one of two groups. The experimental group was subjected to a 60 min aortic cross-clamp distal to the subclavian artery, in addition to ligation of the four pairs of intercostal arteries penetrating the 4th, 5th, 6th and 7th intercostal spaces. Thoracotomy without cross-clamp or ligations was performed in the control group. Animal core body temperature was maintained at 37 °C during the operative procedure by using warmed intravenous fluids and by using a solid-state surgical warming blanket and heater.

Intraoperative assessment of the following parameters was performed every 15 min: urine output through a Foley catheter; electrocardiographic tracing using a telemetry monitor (model 78304A, Hewlett Packard Medical Products Corp, Waltham MA); proximal arterial perfusion pressure through an arterial line with pressure monitor in the common carotid artery (model 78205D, Hewlett Packard Medical Products Corp, Waltham MA); distal artery perfusion pressure (DAAPP) through an arterial line with pressure monitor in the common femoral artery; blood temperature and rectal temperature using a rectal temperature probe and recorder (model 43TA, YSI Telethermometer, YSI Co Inc., Yellow Springs OH).

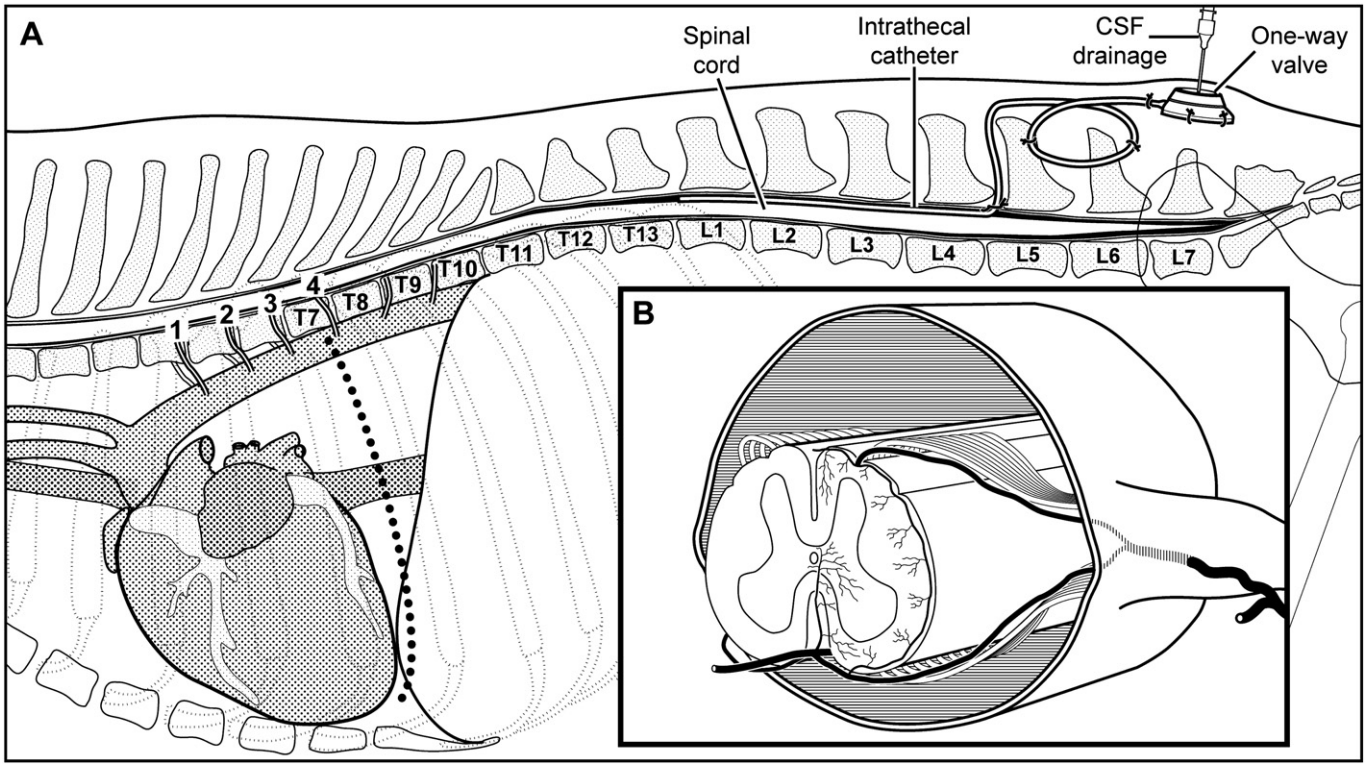

Fig. 1. A) Relationship between ligated intercostals arteries, intrathecal catheter placement, and regions of the caudal thoracic, lumbar and sacral spinal cord segments that were affected by IR treatment in the dog. The intrathecal catheter was introduced between the 4th and 5th lumbar vertebrae and advanced to the level of the 13th thoracic vertebra (corresponding to the 13th thoracic cord segment) and connected to a one-way value placed under the skin. This catheter was used for repeated sampling of cerebrospinal fluid (CSF). Intercostal arteries 1–4 were ligated in the IR treatment group and the aorta cross-clamped caudal to the subclavian artery for 1 h, resulting in grey matter degenerative changes that extended caudally from spinal cord segment L3 (corresponding to vertebral body L3). B) Contribution of the spinal branches of the intercostal artery to the ventral spinal artery (which corresponds to the anterior spinal artery of humans), branches of which are the source of blood supply to the grey matter.