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PRINCIPAL INVESTIGATOR: Ronald L. Hayes, Ph.D.

CONTRACTING ORGANIZATION: University of Florida
Gainesville, Florida 32611-5500

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INTRODUCTION

In addition to being the leading cause of death for civilians under 45 years of age, recent studies have confirmed that traumatic brain injury (TBI) is also one of the most frequent causes of morbidity and mortality on the modern battlefield. Specifically, 40% of battlefield fatalities in the Viet Nam war were due to head wounds and it has been reported that, patients arriving alive at military field hospitals, 20% with extremely severe brain wounds die before surgery was performed, and 80% received neurosurgical treatment, with a 10% surgical mortality rate. Nearly half of all single and/or multiple wound combat deaths result from injuries to the head, and these injuries are among the most frequent cause of morbidity and mortality on the battlefield. Penetrating head injury alone accounts for 25% of all wartime casualties and approximately 40% of these injuries are fatal.

Cell death following TBI has often been classified into two major biochemical pathways associated with expression of distinct necrotic and apoptotic phenotypes. As recently as five years ago, a relatively simplistic model hypothesized that loss of calcium homeostasis and activation of calcium dependent proteases such as calpain were primarily, and potentially exclusively, associated with necrotic (i.e. oncotic) cell death. Conversely, activation of another cysteine protease, caspase-3, was developed to exclusively mediate apoptotic cell death.

As we pointed out in last year's Annual Report, there has been an explosive increase in our understanding of the complexity of pathways regulating cell death. Enhanced understanding of these pathways and their complexity has required us to modify some of our research strategies. We are pleased and excited by the progress that these approaches have yielded. As outlined in more detail below, we have extended our studies to oligodendrocytes and confirmed that, at least in part, apoptotic-like mechanisms mediated by caspase-3 activation are involved in oligodendrocyte degeneration after experimental TBI in the rat. We have also expanded our efforts to develop biochemical markers of necrotic and apoptotic cell death in TBI by confirming accumulation of calpain and caspase-3 proteolytic fragments of brain-derived α II-spectrin in cerebrospinal fluid after middle cerebral artery occlusion (MCAO) in rats. We have also characterized a novel pathway regulating cell death after TBI by confirming increased expression and activation of caspase-12 after injury. Following up on earlier work in TBI, we have documented increased expression of tissue-type transglutaminase, a potential mediator of apoptotic neuronal injury, following MCAO in rats. We have also developed a new model to study cell death mechanisms. We have characterized the utility of the hippocampal slice model for studies of acute brain injury using diffusion magnetic resonance imaging studies in rats. We have expanded our interest in proteolytic mechanisms by characterizing induction of cathepsin B mRNA and protein expression following contusion spinal cord injury. Finally, we have pioneered application of proteomics technology to the field of Neurotrauma.

BODY

Record Of Research Findings and Approaches

We have investigated oligodendrocyte pathology after TBI, *in vivo*. Animals were killed and examined for proteolipid protein (PLP) and myelin basic protein (MBP), mRNA and protein expression and for immunohistochemical analysis at intervals from 6 hrs to 14 days after injury. We observed transient decreases in mRNA expression in protein levels after injury. However, there was a significant increase of PLP and MBP mRNA and protein at 14 days post trauma.

Moreover, we have provided evidence of apoptotic cell death in at least some oligodendrocytes following TBI (Beer et al., *J Neuropathology & Exp Neurol*, In Press).

We have examined accumulation of calpain and caspase-3 proteolytic fragments of brain-derived α II-spectrin in CSF after 2 hrs of transient focal cerebral ischemia produced by MCAO in rats followed by reperfusion. After MCAO injury, α II-spectrin parent protein was decreased in brain tissue and increased in CSF from 24 to 72 hrs after injury. In addition, injury produced substantial increases in calpain mediated breakdown products of α II-spectrin. Caspase-3 proteolytic products were observed only in CSF of some injured animals. These studies suggest that these markers may be useful diagnostic and prognostic indicators of brain injury following both stroke and traumatic insults to the brain (Pike et al., *JCBFM*, 2003).

We have provided the first evidence of increased expression and activation of caspase-12 after TBI in. The recently characterized caspase-12 is both induced and activated by the unfolded protein response following excess endoplasmic reticulum (ER) stress. In our studies, we confirmed increased mRNA expression in protein levels for up to 5 days following injury. These studies are the first documentation that the caspase-12 mediated ER pathway plays a role in apoptotic cell death following TBI in rats independent of receptor- or mitochondria-mediated pathways of apoptotic cell death rats (Larner et al., *J Neurochem*, In Press).

We have extended previous studies of increased expression of tissue-type transglutaminase in models of TBI, by examining the effects of MCAO on this enzyme. Tissue-type transglutaminase (tTG) has been implicated in neurodegenerative diseases and in protein aggregation associated with these diseases. In this study, we demonstrated induction of tTG in response to ischemic injury. We documented increases in both full length tTG (TG-L) but not in a truncated form, (TG-S). We also showed TG-L and TG-S mRNA transcripts were induced after ischemia. The temporal profile of tTG induction after ischemia was similar to that observed after TBI, suggesting a similar role of tTG in both pathological conditions (Tolentino et al., *J Neurochem*, In Press).

We have expanded our interest in proteolytic mechanisms of cell death by documenting the induction of cathepsin B mRNA and protein expression following contusion spinal cord injury. Spinal cord injury, like TBI, triggers proteolytic attack on neuronal and myelin proteins essential for cellular function and survival. We have provided data that an important member of the lysosomal cathepsin protease family, cathepsin B (Cath B), is upregulated following contusion-SCI. The excessive releases in activation of Cath B have been implicated in several pathologies including tumor metastasis, arthritis and Alzheimer's disease. The induction of Cath B mRNA protein expression following contusion-SCI has not been previously described and suggests that Cath B may be involved in secondary injury cascades not only in SCI but in other acute injuries such as TBI (Ellis et al., *J Neurochem*, In Press).

We have also developed a new model that may have particular relevance to the study of acute brain injury including TBI. We have developed a hippocampal slice model for diffusion magnetic resonance imaging studies in the rat. Employing a chemical toxin and calcium ionifor, A23187 we have confirmed that diffusion-weighted images may be highly sensitive correlates of cell swelling in nervous tissue after acute injury (Shepherd et al, *JCBFM*, 2003).

Finally, our laboratory has begun pioneering applications of proteomics technology to the field of Neurotrauma. We have taken a recognized leadership role in this area which will undoubtedly facilitate accomplishment of SOWs embraced in this funding (Denslow et al., *J Neurotrauma*, 2003).

KEY RESEARCH ACCOMPLISHMENTS

- Provided the first studies characterizing apoptotic mechanisms of oligodendrocyte death following TBI and associated changes in myelin-related proteins.
- Expanded previous work in TBI to confirm accumulation of calpain and caspase-3 proteolytic fragments of brain-derived α II-spectrin in CSF after MCAO in rats.
- Provided the first studies confirming that caspase-12 is a potential mediator of apoptotic cell death following TBI in rats.
- Following previous work in TBI, have confirmed increased expression of tissue-type transglutaminase following MCAO in rats.
- Have expanded our protease studies to confirm that Cath B is upregulated following contusion spinal cord injury in rats.
- Have developed a new hippocampal slice model for acute brain injury useful before diffusion magnetic resonance imaging studies.
- Pioneered application of proteomics technology to the field of Neurotrauma.

REPORTABLE OUTCOMES

Beer R, Franz G, Tauber H, Reindl M, Lassmann H, Pike BR, **Hayes RL**, Krajewski S, Reed JC, Kampfl, A. Oligodendrocyte reaction and changes in myelin protein expression after experimental traumatic brain injury. *J. Neuropathology & Exp. Neurol.* (In Press).

Brian R. Pike, Jeremy Flint, Jitendra R. Dave, X.-C. May. Lu, Kevin K.W. Wang, Frank C. Tortella and **Ronald L. Hayes**. Accumulation of calpain and caspase-3 proteolytic fragments of brain-derived α II-spectrin in CSF after middle cerebral artery occlusion in rats. (*JCBFM*, 2003).

Stephen F. Larner, **Ronald L. Hayes**, Deborah M. McKinsey, Brian R. Pike, Michael Kalai, Peter Vandenabeele and Kevin K.W. Wang. Increased expression and processing of caspase-12 after traumatic brain injury in rats. (In press. *J Neurochem*).

Paul J. Tolentino, Anuradha Waghray, Kevin K.W. Wang and Ronald L. Hayes. Increased expression of tissue-type transglutaminase following middle cerebral artery occlusion in rats. (*J Neurochem*, In press).

Rebecca C. Ellis, J. Nicole Earnhardt, **Ronald L. Hayes**, Kevin K.W. Wang and Douglas K. Anderson. Cathepsin B mRNA and protein expression following contusion spinal cord injury. (In press, *J. Neurochem*).

Timothy M. Shepherd, Peter E. Thelwall, Stephen J. Blackband, Brian R. Pike, **Ronald L. Hayes** and Edward D. Wirth, III. Diffusion MRI study of a rat hippocampal slice model for acute brain injury. (JCBFM, 2003).

N. Denslow, M.E. Michel, M.D. Temple, C.Y. Hsu, K. Saatman and **R.L. Hayes**. Application of proteomics technology to the field of neurotrauma: Report from the human proteome project meeting. J. Neurotrauma 20(5): 401-407, 2003.

CONCLUSIONS

Research during this year has made significant progress in understanding proteolytic mechanisms of TBI. We have provided the first evidence of apoptotic cell death in oligodendrocytes following TBI and have implicated a previously undocumented cell death pathway (caspase-12) following traumatic insults to the CNS. We have expanded previous studies in TBI to document the utility of breakdown products to α II-spectrin in CSF as a potential biomarker of injury. Also following up on previous work in TBI, we have supported the generality of tissue transglutaminase activation following acute brain injury employing studies of MCAO. We have also provided the first evidence of the activation of the protease, cathepsin B, following traumatic injury to the spinal cord, laying the ground work for future studies in TBI. We have also developed a new hippocampal slice model for acute brain injury and pioneered application of proteomics to the field of Neurotrauma.