Eric M. Deshaies, MSIV
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Selective Paper

Neuroprotection In A Model Of Acute Global Ischemia/Reperfusion Of The Rodent Brain

ABSTRACT

Neurologic dysfunction due to ischemic injury represents a common and costly modern medical problem. Disease states such as cerebrovascular disease and neurologic complications associated with major cardiovascular surgical procedures all result from irreversible, ischemic neuronal cell death. The aim of this study is to apply techniques of neuronal cytoprotection that have been successful in ischemic protection of the spinal cord with the aim to protect the ischemic brain. The goal is to demonstrate a clinically relevant model of cerebral protection which could be used preoperatively during surgical procedures that risk the complications of cerebral ischemia. We employed a rodent model of global cerebral ischemia to test whether induction of the cellular stress response prior to the ischemic event will prevent loss of neurologic function. Our results showed that we have firmly established a 4-vessel occlusion model of global cerebral ischemia affirmed by our 100% fatality rate in our untreated group. We are currently awaiting data on the effects of Heat Shock and Stannous Chloride administration on neuroprotection, Heat Shock Protein Expression, and Xanthine Oxidase Activity in this model.

INTRODUCTION

Focal and global cerebral ischemia is the cause of significant medical morbidity and mortality. The clinical syndromes associated with this pathologic entity range from primary diseases such as cerebrovascular disease (TIA's and CVA's) to diseases secondary to global hypoxia or hypotension (shock and cardiac arrest). The outcomes of many major surgical procedures, such as coronary artery bypass grafting and carotid endarterectomy, are negatively impacted by iatrogenic, ischemic CNS insults. The recently published practice guidelines for coronary bypass surgery from the American College of Cardiology and the American Heart Association listed the top 10 target areas for perioperative cardiac surgery management.[1] First and second on that list are reduction of Types I (focal) and II (global) brain injury which occur in 3.0% and 3.1% respectively, of this patient population. The North American Symptomatic Carotid Endarterectomy Trial has determined that the benefit of carotid endarterectomy (CEA) is dependent upon the complication rate associate with surgery and should be less than 6% (combined rates of stroke and death).[2] The combined mortality and stroke rates were estimated to range between 5-11% for all Medicare patients in 1991 and does not reflect less severe degrees of neurologic dysfuntion experienced by this patient population.[3] A similar situation exists for patients undergoing coronary artery bypass grafting (CABG) which involves the use of cardiopulmonary bypass (CPB).[1] Neurologic abnormalities after CABG are a dreaded complication. The reported incidence ranges form 0.4-80%, depending on how the deficit is defined. Postoperative stroke is the second most common cause of operative mortality, after low cardiac output states and accounts for

30% of the post-coronary bypass mortality rate. Currently, stroke is the third leading cause of death in the U.S. today and the leading cause of debilitation. There are approximately 2 million stroke victims alive in the U.S. alone, with 550,000 new strokes/year, and 150,000 stroke-related deaths/year.[4,5] Billions of dollars are expended annually for the acute and chronic care of these patients. The neurologic injury associated with operative procedures represents and ideal opportunity to employ preventative neuroprotection protocols.

The fundamental pathophysiologic process for all of these diverse clinical syndromes is neuronal cell death secondary to acute ischemia/reperfusion injury (IRI). A well documented cascade of biochemical events leading to the point of irreversible injury has been described, see Fig 1 for summary. In brief, loss of molecular oxygen as a terminal acceptor within the mitochondrial electron transport chain results in a rapid decline in the cellular energy charge. Reduced metabolic energy leads to the dysfunction of ionic pumps within the neuronal cell membrane and cell swelling results. Associated with this cellular swelling is a rapid elevation in the intracellular concentration of free ionized calcium that leads to uncontrolled activation of cellular proteases. The cellular proteases then indiscriminately act to denature enzymes critical to neuronal cell function. Irreversible neuronal cell death results through pathologic and physiologic death pathways. From a clinical standpoint, IRI of the central nervous system results in severe neurological deficits in a large number of patients annually.

Figure 1 Pathophysiology of Acute Ischemia/Reperfusion Injury*

INADEQUATE OXYGEN DELIVERY
↓
REDUCED ENERGY CHARGE
↓
FAILURE OF IONIC PUMPS
↓
CELLULAR SWELLING
↓
IONIZED CALCIUM INFLUX

PROTEASE ACTIVATION
↓
REDUCED ANTIOXIDANTS
↓
INCREASED XANTHINE OXIDASE
↓
REACTIVE OXYGEN SPECIES
↓
UNIVERSAL BIOCHEMICAL DAMAGE
↓
CELL DEATH

*** The events depicted occur in a time related and dynamic fashion. Oxygen may be reintroduced at any point along the course of these events. Oxygen delivery must be restored at the cellular level for survival to occur, however depending upon the extent of the above changes, a variable degree of reperfusion injury my result, secondary to enhanced generation**

of reactive oxygen species and the acute reduction in antioxidant capacity typical of ischemic tissues. The second wave of cellular insult and injury has been previously referred to as the "oxygen paradox."

Investigators have consistently taken a very specific approach when trying to induce protection against cerebral IRI. For example, multiple reports exist on the use of NMDA antagonists as neuroprotective agents.[6,7,8] While these compounds are active at a cellular and biochemical level, in general they have failed to provide consistently significant neuronal protection *in vivo*. Clearly, the mechanisms involved in neuronal cell death during IRI are legion. Thus, a global technique of neuronal cytoprotection may prove to be a more fruitful approach, compared to these single agent therapies. Therefore, we have chosen to pursue a *general* approach to the problem of neuronal cytoprotection during acute cerebral IRI through the activation of the cellular stress response genes.

A generalized and universal response to cellular stress has been recently identified at the molecular level and designated the "heat-shock response" (HSR).[9] The HSR is a universal cellular response to stress and is defined by the rapid synthesis of heat-shock proteins (HSPs) in response to diverse cellular insults including--hypoxia, ischemia, hypoglycemia, and direct cellular toxins. There are approximately 12 genes controlling the cellular stress response--all have been highly conserved by nature, Table 1. Expression of the heat shock genes appears to be part of an early, adaptive cellular response to threatening conditions. This response has been associated with the transient acquisition of protection or tolerance to subsequent *lethal* injury. Thus, cells that would normally die from hyperthermic, hypoxic, ischemic, or nutritional stress, are now able to survive after initiation of the HSR. This protective phenomenon has been observed in a wide variety of species from bacteria to humans--and at all levels of development from single cells *in vitro* to whole organisms *in vivo*. Initial work using rodent cardiac and renal models demonstrated that protection against IRI could be achieved by prior induction of the HSR.[10,11] Since that time, reports have consistently confirmed that induction of the HSR can provide a wide variety of tissues with protection from IR and oxidant injuries, reviewed elsewhere.[12] In a neuronal model, the photoreceptor layer in the rodent retina was successfully protected from bright light damage by prior induction of the heat-shock gene expression.[13] Taken together, these studies suggest that neuronal tissue might be protected from IRI by prior induction of the heat-shock response. We previously reported protection in a model of acute spinal cord ischemia in the rabbit at the American College of Surgeons, Surgical Forum in October 1996.[14] Briefly, control animals subjected to 20 minutes of acute spinal cord ischemia followed by 24 hours of reperfusion uniformly developed paralysis (7/8), while animals that had been pretreated with whole-body hyperthermia consistently demonstrated normal neurologic function, see Table 1. This acquired state of cytoprotection is transient (lasting approximately 24 hrs) and is consistently correlated with enhanced HSP expression within the protected tissues.

TABLE 1- PRELIMINARY DATA

The Heat-Shock Response Protects the Rabbit Spinal Cord From Ischemia/Reperfusion Injury.

Group (n)	Reperfusion (24hrs)			Paralysis (%)
	normal	paretic	paralyzed	
1. Control (8)	0	1	7	88
2. Heat Shock (9)	8	1	0	0*
3. Sham (7)	1	5	1	14
4. Stannous Chloride (4)	4	0	0	0**

From Lena [14]

*Heat Shock vs Control $p<0.05$
Heat Shock vs Sham $p<0.05$

We wish to test the hypothesis that pretreatment of the whole animal with systemic hyperthermia or stannous chloride administration will protect the rodent brain from acute global ischemia/reperfusion injury. Preservation of neurologic function will be associated with the enhanced production of stress proteins and a reduction in xanthine oxidase activity within pretreated brain tissues.

MATERIALS AND METHODS

A. Whole Body Hyperthermia (Heat Shock)

Animals undergoing heat shock or sham heating will be anesthetized with sodium pentobarbital (65 mg/kg, ip) and rectal temperature probes placed for constant monitoring of core body temperature. Animals are submersed to the neck line in a water bath pre-incubated to 45°C, and heated until the target rectal temperature is reached and maintained for 15 minutes (heat shock 42.5±0.2°C or sham heat shock 37±0.2°C for sham heat shock). Following heat shock, the animals are removed from the water bath, dried, and then allowed to recover at room temperature for 8 hours prior to four-vessel occlusion.

B. Anesthesia and Monitoring:

Wistar rats (250-300 grams, 10 week, male) will be fasted overnight and anesthetized with ketamine (75-90mg/kg, IP) and Xylozine (10mg/kg IP) with additional ketamine as needed (30mg/kg, IP, prn). A rectal temperature probe is placed for continuous monitoring of core body temperature. Core body temperature is maintained at 37±0.5°C throughout the procedure by the intermittent use of a heating lamp. Arterial blood pressure and blood gases are monitored via a left femoral arterial line (P.E.50 tubing). A left femoral venous line is placed for fluid and heparin (40U) administration.

C. Surgical Procedure: Four-Vessel Occlusion

Permanent vertebral artery (VA) occlusion and 20 min common carotid artery (CCA) occlusion will be induced as described elsewhere.[15] Briefly, a 1-cm dorsal midline incision will be made in a sterile fashion at the occiput-C2 level and the paraspinal muscles will be separated from the midline. The alar foramina of C1 will be isolated with the aid of an operating microscope, the VA's cauterized with a 0.5mm electrocaurtery needle through the alar foramina, and the incision closed with 4-0 nylon. 100U heparin given via femoral a-line and allowed to circulate for 2 minutes before bilateral CCA cessel occlusion. A second 1-cm midline incision will be made in a sterile fashion over the ventral aspect of the neck, the CCA's isolated, and atraumatic vascular occlusion clips placed onto each CCA. CCA occlusion will be induced for 20 min after which the clips will be removed, the incision closed with 4-0 nylon, and the animal allowed to reperfuse and recover.

D. Neurologic Evaluation

Clinical neurologic assessments are blindly performed by designee at 12, 24, 48, and 72 hours after reperfusion according to the methods reported by LeMay, et al, see Table 2.[16]

TABLE 2- Neurologic Examination

Score	Degree of Neurologic Deficit
Consciousness (maximum score 10)	
0	normal (no deficit)
2	conscious continuously
4	conscious intermittently
6	stuporous
8	light coma
10	deep coma
Motor Function (maximum score 17)	
0	no deficit
1	minimal ataxia
2	ataxia
3	rights to ventral from dorsal recumbency
4	rights to side from dorsal recumbency
5	remains in dorsal recumbency
6	reflex, spastic, or convulsive movement only
7	no movement
Rope platform (upper extremities placed on platform edge)	
0	climbs onto platform
1	hangs on 5 seconds and brings hind limbs up without climbing onto platform
2	hangs on for 5 seconds without bringing hindlimbs up
3	hangs on for <5 seconds
4	no attempt to grasp
Rotating Screen (rat placed on horizontal screen [0^o] rotated through vertical position [270^o] to inverted position [180^o]	
0	grasps screen to 180^o for > 5 seconds
1	grasps screen to 180^o for <5 seconds
2	grasps screen past 270^o but not to 180^o
3	falls from vertical screen
Wooden Bar 1" diameter at 45^o (best of three trials)	
0	grasps bar for >10 seconds
1	grasps bar for 5-10 seconds
2	grasps bar for <5 seconds
3	no attempt to grasp bar
Cranial Nerves (maximum score 8)	
2	corneal reflex absent

2	pinna reflex absent
2	gag reflex absent
2	swallowing reflex absent
Spinal Nerves (maximum score 6)	
limb tone	
0	normal
1	spastic
2	flaccid
pain reflex	
0	normal
2	hyperactive
4	absent
total= 41	

From: LeMay[16]

E. Cerebral Infarct Size Determination

Determination of infarct size in both the whole-body hyperthermia-treated group, stannous chloride-treated group, and in the control and sham groups by staining with tetrazolium red solution 72 hrs after reperfusion.[15]

F. Euthanasia

All animals will be euthanized with sodium pentobarbital/KCl euthanasia solution according to guidelines established and overseen by Theresa DiGuilio, DVM, at Hartford Hospital, 72 hours following reperfusion.

G. Heat-Shock Proteins

A one-dimensional Western Blot analysis will be performed according to the methods described elsewhere.[17] Briefly, tissues are obtained from representative animals from each group at the conclusion of the recovery period or at 30 minutes of reperfusion and snap frozen in liquid nitrogen (-80°C), see Figure 4. Tissues are homogenized and the proteins separated by 1-dimensional polyacrylamide gel electrophoresis. The separated proteins are transferred to a nitrocellulose membrane and probed with commercially available antibodies (StressGen, Victoria, BC, Canada) specific for HSP's 70, 60, 45, 32, and 27. Bound antibody is detected by a conjugated horse radish peroxidase secondary antibody and visualized by a commercial chemiluminesence system. Data is quantitated using scanning densitometry (StrataGene Eagle Eye II, LaJolla, CA).

H. Xanthine oxidase

XO/XDH tissue will be quantified by measuring uric acid (ƛ360nm) with the Automated ILAB^{TM} 1800 Chemical Systems Spectrophotometer via modifications of a technique reported elsewhere.[18] Briefly, a frozen tissue sample (0.100g) is homogenized and divided into 0.100 mL aliquots and incubated with 75µM xanthine as substrate (37°C x 30 min). The enzymatic reaction is stopped by a 1:2 dilution of o-phosphoric acid. The samples are centrifuged (15,000g x 10 min, 4^{O}C) and this supernatant is removed and centrifuged again (10,000g x 20 min, 4^{O}C). This final supernatant is removed and frozen at -70°C until assayed for uric acid spectrophotometry by measuring the formation of the chromagen, quinoneiomine dye at a wavelength of 510nm according to the procedure developed by Wu *et al* in the Hartford Hospital Clinical Chemistry Lab.[19] Briefly the reaction is outlined in Eq. 4a-b.

Eq.4a: Uric acid + $2H_2O$ + O_2 + uricase --> allantoin + CO_2 + H_2O_2

Eq.4b: H_2O_2 + 4-aminoantipyrine + 3-hydroxy-2,4,6 triiodobenzoic acid + peroxidase ---> quinoneiomine dye + $2H_2O$

I. Statistics

Groups will be analyzed for differences in infarct size, xanthine oxidase, and xanthine dyhydrogenase levels by analysis of variance (ANOVA). Neurological assessment at 12, 24 and 48 hours will be analyzed by repeated measures ANOVA. Both ANOVA procedures will be analyzed for multiple comparison using the Scheffe's procedure. HSP expression will be graded nominally and analyzed by chi-square. All significance will be set at $p \leq 0.05$.

RESULTS

There are currently a variety of models in the literature which are used to look at cerebral ischemia/reperfusion injury. We chose to use the 4-vessel occlusion model described by Pulsinelli *et al.* because it has been well established as a method for inducing severe global cerebral ischemia and reperfusion injury. Our results confirmed this finding and showed that 20 minutes of unilateral (1 VO) and bilateral (2 VO) common carotid artery (CCA) occlusion and 72hrs of warm reperfusion caused minimal neurological deficit. The neurological deficit scores using the neurological exam described by LeMay *et al.* were 1/41 after unilateral CCA occlusion and 5/41 (0=best score, 41=worst score) after bilateral CCA occlusion and 72hrs reperfusion, see Table 3. Our data also show that the rats do not survive 30 minutes of global cerebral ischemia (4VO).

Table 3. Average Neurological Deficit Score (0-41) after 20min CCA Vessel Occlusion and 72hrs Reperfusion

	1 VO (N=1)	**2VO** (N=2)	***4VO** (30 ischemia)
baseline	*0*	*0*	*0*
12	*0*	*2*	---
24	*1*	*3*	---
48	*1*	*5*	---
72	*1*	*5*	---

*4VO, 30 min warm global cerebral ischemia (N=1) died at 30 min bilateral CCA occlusion.

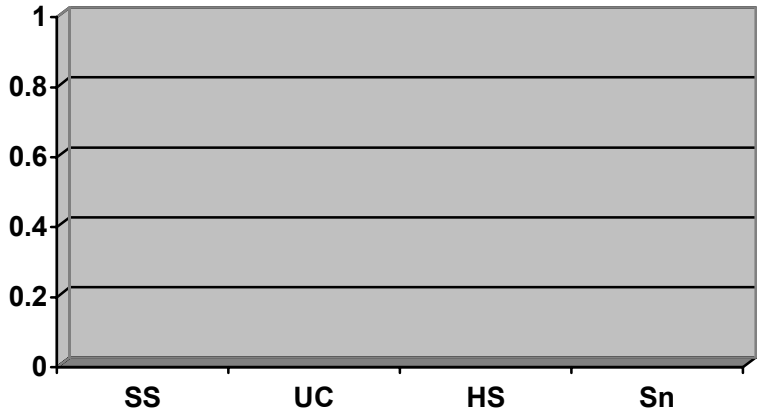
We demonstrated that our surgical shams, those animals which underwent the surgical procedure without actually caurterizing the vertebral arteries or occluding the CCA's, scored perfectly on the neurological exam throughout the 72hrs of reperfusion and had a 0% fatality rate. However, the unstressed control group, those animals receiving the 4VO without stress conditioning, had no animals survive 24hrs of reperfusion. Of the five unstressed controls, 1 animal died of tonic-clonic seizures after 23hrs of reperfusion, 2 died within 3hrs of reperfusion, and 2 died intraoperatively after 3 minutes of bilateral CCA occlusion (2 had tonic-clonic seizures), see Table 4. The data for our Heat Shock and Stannous Chloride group are currently pending.

Table 4. Average Neurological Deficit Score (0-41) after 20min global Ischemia and Reperfusion

	Surgical Sham (N=5)	Unstressed Control (N=5)	Heat Shock	Stannous Chloride
baseline	0	0		
12	0	22*		
24	0	---		
48	0	---		
72	0	---		
****% fatality**	0	100		

Our data on xanthine oxidase is not currently available, see Figure-2.

Figure 2. Xanthine Oxidase Activity in Rat Brain after 20 minutes of Warm Global Ischemia and Reperfusion (mg Uric Acid/mg Whole Brain).



Data on HSP expression in not currently available but when it is, it will consist of photographs of Western Blot gels and placed below, see Figure-3.

Figure 3. Western Blots of Heat Shock Protein Expression (HSP70 & HSP32)

DISCUSSION

Our study has shown that the fatality rate in those animals who are not stress conditioned is 100% and the incidence of tonic-clonic seizures is 60% at 20 minutes of global cerebral ischemia and reperfusion. These statistics differ from those observed by Pulsinelli *et al.* who had a lower fatality rate and only a 10% incidence of seizures. We believe that the reason for our higher incidence of seizures and a higher fatality rate is because we perform 4VO on the same day whereas Pulsinelli's group caurterized the vertebral arteries the day prior to bilateral common carotid artery occlusion. The vertebral artery occlusion may have *stress conditioned* the animals against subsequent injury during the 4VO the next day and indeed this may be the reason for their better neurological outcomes. In our model, we have a truly unstressed control group who's Heat Shock Protein Expression has not been induced and hence are not protected from the subsequent lethal injury that occurs during 20 minutes of global cerebral ischemia/reperfusion. We would like to demonstrate that stress conditioning with heat shock or stannous chloride administration will lower the fatality rate, decrease the incidence of seizures, and improve neurological outcome andrelate this to Heat Shock Protein (HSP) expression.

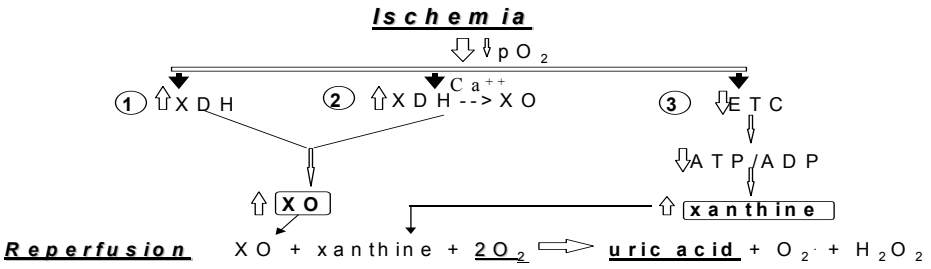
HSP gene expression has been shown to dramatically increase in cerebral tissues following whole-body hyperthermia and I/R injury. However, investigators did not test for cerebral protection in these reports.[20,21] We are aware of only two reports which provide direct evidence that the heat-shock response will provide protection agents against IRI in the rat brain. Transient hyperthermia (41.5°C x 15') followed by 24 hours of recovery conferred protection against subsequent forebrain ischemic cell damage in an in vivo rat model.[22] This study used brain histology as the major measure of IRI and did not report functional data. More recently, it has been demonstrated that mild thermal stress (42°C x 40') followed by 24 hours of recovery conferred protection against subsequent simulated ischemia in primary cultures of dorsal root ganglion neurons.[23] It is clear from these studies that a degree of histologic and biochemical cytoprotection within the thermally conditioned tissues, the functional significance remains unknown.

The mechanism behind the hyperthermia-associated cytoprotection remains a mystery. Studies that employ transgenic cell lines and animals that overexpress inducible HSP70 demonstrate that HSP70 expressed alone, outside of the context of an acute cellular stress response, has the ability to increase the ischemic tolerance of cardiac cells and tissues.[24] Clearly HSP70 expression is a major component of the cytoprotection observed to follow hyperthermic pretreatments but does not account for all of the protective effects. In an effort to better understand the nature of the cytoprotected state, we have focused our attention on two additional potential molecular mechanisms; the inducible isoform of the heme oxygenase enzyme (HO-1) and xanthine oxidase (XO) activity.

Heme-oxygenase-1 is a stress-inducible protein recently classified as a heat-shock protein (HSP32) and functions as the rate limiting enzyme in heme pigment metabolism.[25] The constitutively expressed isoenzyme of heme oxygenase, heme-oxygenase-2, is found in very high levels in the rodent brain tissues.[26] We have unpublished data demonstrating the same degree of ischemic spinal cord protection can be achieved by pretreatment of rabbits with stannous chloride ($SnCl_2$) sixteen hours prior to the ischemic insult, see Table 2. The latter observation is in keeping with the fact that $SnCl_2$ is a potent inducer of HO-1. This preliminary data supports the hypothesis that pretreatment of the rat brain with $SnCl_2$ will provide neuronal protection from IRI. The cytoprotective effect associated with acute induction of HO-1 gene expression may in part be attributed to its classification as a member of a class of molecules called "molecular chaperones" and/or because this molecule possesses potent anti-inflammatory effects in vivo.[27,28] In our current model, we expect to see the induction of HSP's in the Heat Shock and Stannous Chloride pretreated groups and that this expression will be correlated with improved neurological outcome and decreased xanthine oxidase activity.

The second approach we have taken is to ask the question, given that hyperthermia-associated ischemic cytoprotection exists, what effect does hyperthermia have on the expression of biochemical species held to be central to the conventional pathways of IRI. Central to the current dogma of IRI is the rapid rise in the production of reactive oxygen species (ROS) at the time that molecular oxygen is reintroduced to ischemic tissue. This phenomenon has been recognized for 25 years and has been labeled the "oxygen paradox".[29] Two major biochemical changes within ischemic tissues are felt to be responsible for the this rapid and excessive generation of ROS, depicted in Figure 4. During ischemia, the low partial pressuure of oxygen inhibits the electron transport chain (ETC) and subsequently there is a rapid degradation of ATP to AMP and finally to xanthine while at the same time there is a protease-dependent conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO). Once molecular oxygen is reintroduced, XO rapidly degrades xanthine to uric acid and converts molecular oxygen into ROS. We hypothesize that hyperthermia pretreatments will decrease protease-dependent conversion of XD to XO during acute ischemia and thereby limit the generation of ROS and associated IRI.

Figure-4.



During the past four years we have established the assay for measuring XO in rat brain tissue. Our preliminary data showed that rodent brain tissue exposed to ninety minutes of global warm (37C) ischemia demonstrates an 11% increase in XO activity compared to nonischemic brain tissue. Hyperthermia pretreatment prior to ninety minutes of ischemia demonstrates a 14% reduction in XO compared to the nonischemic controls and a 25% reduction compared to the nonpretreated ischemic brain tissue. In our current model of global ischemia, we expect to see xanthine oxidase levels increase after ischemia and reperfusion injury, but to be low-normal in the Heat Shock and Stannous Chloride pretreated groups. We speculate that such findings would be the result of elevated levels HSP's which serve as chaperones, bind to Xanthine Dehydrogenase (precursor enzyme of xanthine oxidase) during ischemia, and prevent calcium-dependent proteases from converting xanthine dehydrogenase to xanthine oxidase. At the time of reperfusion, xanthine oxidase levels will be too low to cause oxidative tissue injury and thereby protect neurons from injury.

CONCLUSION

From the basic science perspective, this research will contribute to the biochemical understanding of the mechanisms underlying cerebral I/R injury, the heat shock response, and neuronal cytoprotection. This will allow us to make connections between the "classical" biochemical pathways known to be important in acute IRI and the less well understood fields of heat-shock biology and molecular chaperones. From the clinical perspective, a reduction in the degree of neurological debilitation would have a significant impact on the costs of cure and the quality of life for patients at risk for cerebral IRI. While whole-body hyperthermia may not have a practical clinical use, the prior administration of $SnCl_2$ to patients who are at high risk for cerebral injury--such as those undergoing carotid endarterectomy or for procedures requiring cardiopulmonary bypass, may prove economical, convenient, and very effective.

ADDENDUM

Selective Paper: Part 2, requested by Judy Lewis.

Research always presents the unexpected to a willing subject.

During the past two months I have been working on my Selective entitled, "Neuroprotection in a Rodent Model of Cerebral Ischemia/Reperfusion" in the

Departments of Neurosurgery and Emergency Medicine/Trauma at Hartford Hospital. Currently, we are beginning to collect data from our animal model but the statistical power is too small to present any meaningful data at this time.

The goals I had set for my two selective months are as follows: 1) refine my grant-writing skills which will be critical in my future career as an academic Neurosurgeon, 2) learn and refine my microsurgical skills which will be necessary and critical during my residency training and career in Neurosurgery, 3) pursue my academic interest in "Stress Conditioning as a mode of Neuroprotection". 4) continue to investigate the role of Xanthine Oxidase in CNS damage.

1) During the past two months, I have worked intensively in collaboration with Drs. Perdrizet and Onyiuke on a grant proposal entitled "Neuroprotection in a Model of Acute Global Ischemia/Reperfusion of the Rodent Brain". We submitted this grant to Hartford Hospital's *Small Grant Initiative* and received the maximum award of $10,000 for this project. I am the primary investigator on this grant and since then a Neurosurgical Fellow from China and a second year UConn Medical Student will be joining this project in our lab.
2) Our data collection has just begun over the past two weeks. I am currently refining my microsurgical dissection techniques to ensure that the model works. This entails using the operating microscope to perform tedious neurovascular dissections, placement of arterial lines, and managing preoperative, operative, and postoperative anesthesia, analgesia, and intensive neurological, and cardiovascular care to these animals. Each of these techniques is very similar to that of hospitalized patients in the surgical intensive care unit.
3) My interest in neuroprotection is of utmost importance to my future career as an academic and clinical Neurosurgeon. Acute injury to the central nervous system commonly presents to the Neurosurgeon in the form of Traumatic Brain Injury, acute and chronic intracranial hemorrhages, and operative ischemia/reperfusion injury during Neurosurgical and cardiovascular procedures. The microscopic neuronal damage and the resulting clinical manifestations namely stroke and death, are all too common and much research is needed in these areas to prevent the neurological damage incurred to this group of patients. Our lab has shown that "Stress Conditioning" in the form of whole-body-hyperthermia or the administration of Stannous Chloride consistently protects against ischemia/reperfusion injury in a variety of tissues including heart, pancreas, kidney, musculocutaneous flaps, and spinal cord in rodent, rabbit, and porcine models. We have also shown that this protection is associated with Heat Shock Protein expression and decreased Xanthine Oxidase levels. Our preliminary data which we presented at Uconn Health Center's Medical Student Research Day has been awarded the Abbott-Kline Laboratory Award for best research presentation in 1998. Since then we have been awarded the *Small Grant Initiative* from Hartford Hospital to continue this research. At Albany Medical Center I will begin working closely with the Neurosurgical Research Department who has a strong interest in neuroprotection where I will continue my laboratory and clinical research in neuroprotection.
4) During the past two months, there have been a group of Orthopedic Surgeons who are working with Dr. Perdrizet who have been looking at the effects of fat emboli

syndrome on Adult Respiratory Distress Syndrome and cerebral injury in a rabbit oleic acid model. I have joined the team to retrieve the rabbit brains and examine them for xanthine oxidase activity via the protocol I have developed in the past for our lab. Again, this is a model of brain injury and the surgical protocol they use also makes this a left hemispheric model of cerebral ischemia since they ligate the left common carotid artery for 20 minutes. This model compliments my interest in cerebral ischemia/reperfusion injury and oxidant injury.

In summary, the four goals I had set at the beginning of my selective months have been accomplished and very successfully at that. We are currently funded to pursue my research project on Neuroprotection and we are on the road to understanding the roles of Xanthine Oxidase in the pathophysiology of acute brain injury and *Stress Conditioning* in neuroprotection. This selective served as a good beginning for refining my skills and developing a model for examining neuroprotection which I will use frequently in my future career as a Neurosurgeon.

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