

Neurobiology of Perinatal Asphyxia Sept 23rd 2001

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Introduction: Lessons from the Laboratory

Hypoxic-ischemic brain damage is an evolving process that begins during the insult and extends into recovery.[1] We have learnt a lot from neonatal animal models of neonatal hypoxic-ischemic brain injury.[2] This lecture will review the evolution of brain injury from different perspectives to highlight some of the most clinically relevant “lessons from the laboratory”.

Synopsis

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1.0 The Clinical Perspective

Following severe perinatal asphyxia, the newborn infant is affected by multi-organ dysfunction. While other organs may recover, the brain is often permanently injured by a pathophysiologic process that progresses over many days. The clinical encephalopathy peaks in severity after 3-4 days and the neurological sequelae are directly related to the severity of the encephalopathy [3] [4]. What is both disturbing and exciting to the clinician is the fact that the evolving encephalopathy reflects progressive brain injury and that appropriate management can be protective in animal studies even if administered hours after reperfusion. While the therapeutic window has been teased open with novel therapeutic strategies it is critical to elucidate the mechanisms of cell death following perinatal asphyxia. Future therapies will most likely represent a combination of modalities, including rescue hypothermia and various pharmacologic approaches that are appropriate for the phase and mechanism of post asphyxial injury.

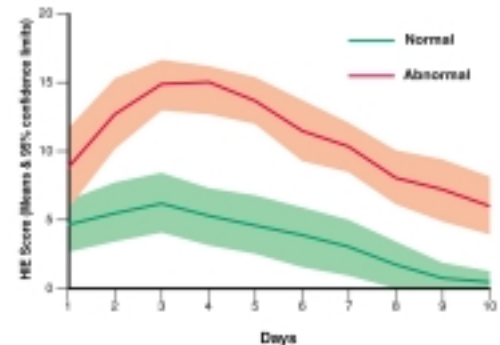


Figure 1 A graphical representation of the “encephalopathy score” which represents the severity of the clinical presentation. It peaks between days 3-4 in infants destined to have an abnormal neurological outcome at 1 year . (from Thompson ref 3.)

2.0 Phases of Cerebral Recovery

2.1 Neurophysiological events

Phases of recovery are characterized by the alterations in cerebral blood flow, EEG intensity, and cortical impedance that occur in the first 5 days after perinatal asphyxia. They have been referred to as:

Fig. 2

Reperfusion phase (\pm 0-4hrs)

Latent phase (0-8hrs)

Secondary energy failure phase(8-72 h)

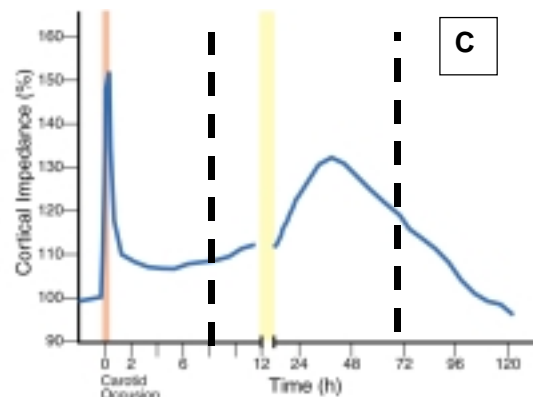
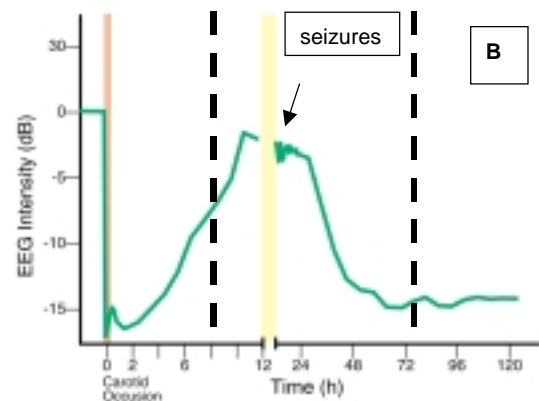
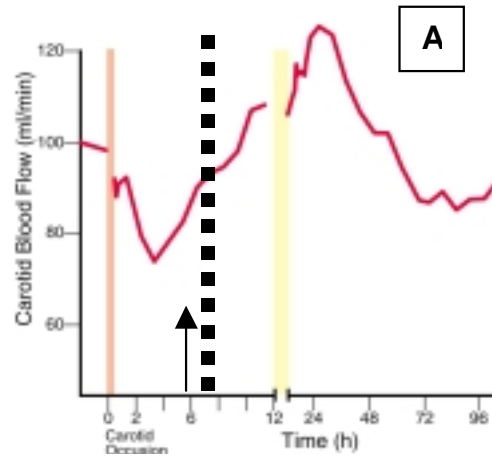
Late phase >72hrs

(The time spans in parentheses are rough estimates)

Figure 2A. Line graph representing the average carotid blood flow following 30 min transient carotid occlusion in fetal sheep (modified from Gunn et al [5]). The interval between the end of carotid occlusion and the dotted line at 8 hours recovery is the *Latent Phase*. The early portion (first few hours) of the *Latent Phase* is the *Reperfusion Phase*. Note: Blood flow falls below normal during this phase. See discussion about “no-reflow” later in text. In the immature rat model of hypoxia ischemia blood flow is not low during this time however between 3-6hrs following HI the cerebral cortex exhibits a pattern of increased regional glucose utilization and evidence of neuronal injury (loss of MAP2 immunostaining). The increased glucose utilization is indicative of impaired mitochondrial function and is a forerunner of overt infarction.[6]

Figure 2B: In the same fetal sheep model, EEG intensity falls precipitously during ischemia and recovers slowly during the *Latent Phase*. During the *Phase of Secondary Energy Failure* which spans the interval between the dotted lines in 2B, the EEG intensity falls for the second time. Seizures are frequent at the recovery interval indicated by the arrow.

Figure 2C. Illustrates changes in cortical impedance which is an indicator of cytotoxic edema. Impedance increases rapidly during carotid occlusion. During the *Latent Phase* it remains constant yet above normal and with the *Phase of Secondary Energy Failure* there is another more gradual rise in impedance. This biphasic increase in cell water can be seen on diffusion weighed MRI of the immature rat brain.[7]



The recovery interval beyond 3 days can be regarded as the *Late Phase* of recovery and it is associated with a reduction in impedance, EEG intensity, and a blood flow reduction to below

normal. As the mechanisms of injury and repair differ during each phase it is important to try and identify the stage of recovery the patient is experiencing. The use of the amplitude integrated EEG, 3 and 6 hours after birth is most useful in helping to identify which infants are likely to benefit from intervention after birth asphyxia[8, 9] Serial diffusion MRI images could also help indicate the changing phases of cytotoxic edema but they are not a practical clinical tool. The ideal cerebral monitor has still to be developed. It is my guess that it will require two or more of the above signals. In each phase the signals move in different directions relative to each other, thus by comparing the relative changes of 2 or more parameters it may be possible to identify the phase of recovery.

2.2 Cellular and Molecular Events

2.2.1 Reperfusion Phase:

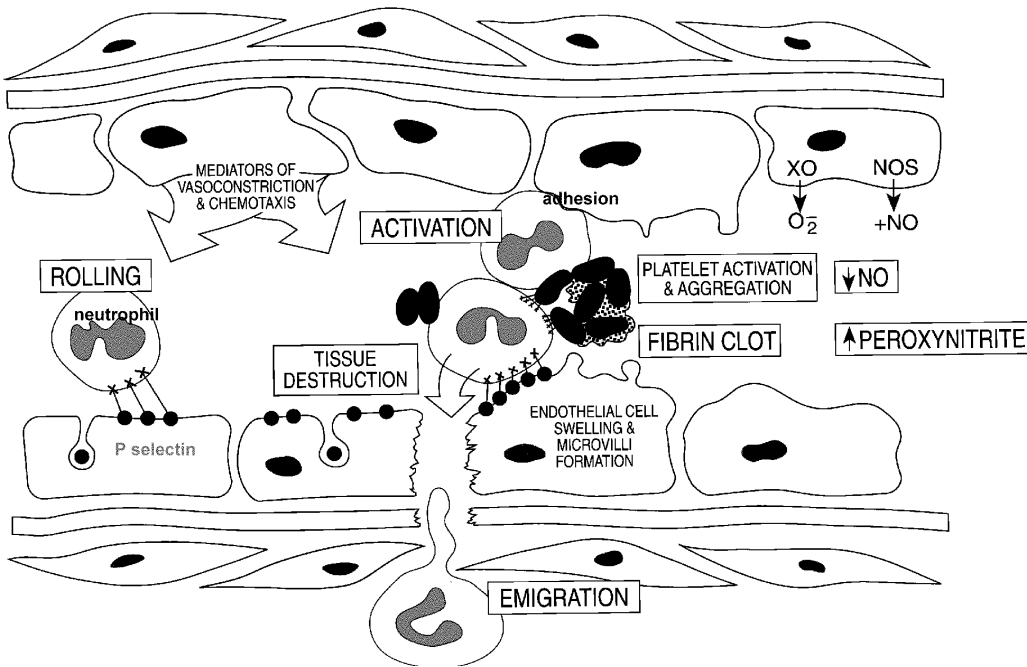
During the *Reperfusion Phase* there is a return of oxygenated blood to previously ischemic brain. The microvasculature bears the brunt of reperfusion injury as free radicals are generated by the return of oxygen to the tissue (See Figure 3). Activated neutrophils adhere to vascular endothelial cells, which are often swollen and the lumens narrowed (see review in ref [10]). If ischemia lasts for more than a few minutes the disturbance in blood flow is not readily reversed when the baby is resuscitated. There is a brief period of hyperemia followed by a few hours of reduced blood flow “no-reflow”. [11] (reviewed in ref [12]) The pathophysiology of no-reflow is multifactorial. It results from mechanical obstruction of the microcirculation, and includes such vascular factors as endothelial blebs, compression by swollen glial cells, blood factors like viscosity changes due to polycythemia, erythrocyte sludging, platelet aggregation, and general cardiovascular factors such as post ischemic hypotension. The treatment of no-reflow consists of maintaining adequate post ischemic blood pressure. In animal models no-reflow can be prevented by raising blood pressure.

Following cerebral ischemia and a brief period of hyperemia, a phenomenon of delayed post ischemic hypoperfusion can also occur.[12] Post ischemic hypoperfusion is a functional disturbance due to increased arteriolar vascular tone. It is important to prevent delayed post ischemic hypoperfusion as it may contribute to secondary delayed tissue injury. The increased vascular tone is due to failure of endothelium mediated vasodilation, possibly an abnormality of the synthesis of nitric oxide. Other contributors are adhesion of polymorphonuclear leukocytes and platelets.[13, 14] Treatment of post ischemic hypoperfusion and the resulting reduction in oxygen and glucose supply can be ameliorated by reducing vascular tone, improving the rheological properties of the blood. Reducing the oxygen and glucose requirements of the blood would also be helpful. For the asphyxiated newborn, maintaining normoglycemia, and normoxia is important. Hyperoxia can produce vasoconstriction[15] and increased lipid peroxidation.[16] Resuscitation with oxygen should be controlled to avoid prolonged hyperoxia. There is growing interest in resuscitating newborns in room air as it is as effective as using 100% oxygen. [17] While this matter is being resolved, clinicians should limit the use of 100% oxygen to the shortest amount of time that ensures adequate resuscitation, then wean to a lower amount as permitted by the other cardiorespiratory factors to ensure adequate oxygenation. Experimental therapies aimed at reperfusion events include free radical scavengers and antineutrophil strategies.

Molecular mechanisms of neutrophil adhesion and microvascular injury during reperfusion.

Inflammatory mediators (for example, C5a, thrombin, histamine, and reactive oxygen species) are released from damaged brain tissue and cause the activation of blood vessel endothelia. The activated endothelium expresses adhesion molecules to attract and then adhere any circulating neutrophils. The first adhesion molecule to be expressed onto the endothelial cell surface is P-selectin. P-selectin, expressed rapidly on the endothelial surface, mediates the tethering of flowing leukocytes to the blood vessel wall by binding to selectin ligands on leukocytes. These interactions coerce leukocytes to "roll" along the vascular endothelium in the direction of blood flow. Rolling is a necessary first step before firm adhesion can occur. All selectins recognize a sialylated carbohydrate component of their ligands (Sialyl Lewis^x)

Figure 3 Brain Microvascular Changes During Reperfusion



The vascular surface is activated by additional inflammatory stimuli (superoxide, the cytokines IL-1 and TNF α) that stimulate the formation of ICAM-1 (intercellular adhesion molecule-1) and E-selectin. These adhesion molecules require mRNA transcription and protein synthesis and usually take 3 to 6 hours to be expressed. The next step in the inflammatory adhesion cascade requires the activation of leukocyte β_2 integrin molecules (CD11/CD18). Chemoattractants (PAF, IL-8, chemokines) induce a conformational change in the leukocyte integrins that allow them to bind to the endothelial cell ligands (ICAM-1). Leukocyte rolling prolongs their exposure and enhances activation by endothelial derived chemoattractants. The appearance of ICAM-1 on the postcapillary venule luminal membrane arrests granulocyte "rolling" and secures adhesion of the neutrophils to the microvascular endothelium.

Cerebral hypoxia-ischemia in the PD7 rat enhances rapid expression of brain inflammatory cytokines (IL6, IL-1B),[18] and the expression of inflammatory cell response to injury that includes neutrophils lymphocytes, and microglia. [19, 20] [21] We, and others, have found that neutrophils are mainly intravascular during the first 12 -24hours of reperfusion.[21, 22] Interestingly, we found that hypoxic-ischemic brain damage in the immature rat could be reduced with neutrophil depletion with antineutrophil serum. If the antineutrophil serum was administered immediately after hypoxia-ischemia (it takes 8 hours to make the rats neutropenic) the protective effect was lost.[23] This suggests that the neutrophils exert their influence in producing ischemic brain injury either during ischemia or shortly afterward in early reperfusion, when one might expect no-reflow or delayed post ischemic hypoperfusion.

Microvascular changes are reviewed in ref[10, 24]

Immediately following reperfusion, cytotoxic edema, which developed during ischemia, rapidly improves in those regions of the brain not permanently injured by the ischemia.[7] The improvement in cytotoxic edema can clearly be visualized by diffusion weighted MRI.[7] Unfortunately, improvement is only transient as cellular energy failure starts the biochemical cascade described below and after a few hours the brain swells from a combination of cytotoxic and vasogenic edema.

2.2.2 Latent Phase

The *Latent Phase* is characterized clinically by absence of seizures (pre seizures) and reduction in early cytotoxic edema. Cerebral blood flow often falls below normal following a transient hyperemia (Figure 2A). Impedance improves rapidly then stabilizes (Figure 2C). EEG activity is suppressed but begins to recover in this phase (Figure 2B).

During this interval of relative neurophysiological suppression, there are a host of biochemical events occurring in the parenchyma and microvessels that contribute to injury. These are summarized below:

Pathways to cell death: See Figure 4, Cell Injury Cycle

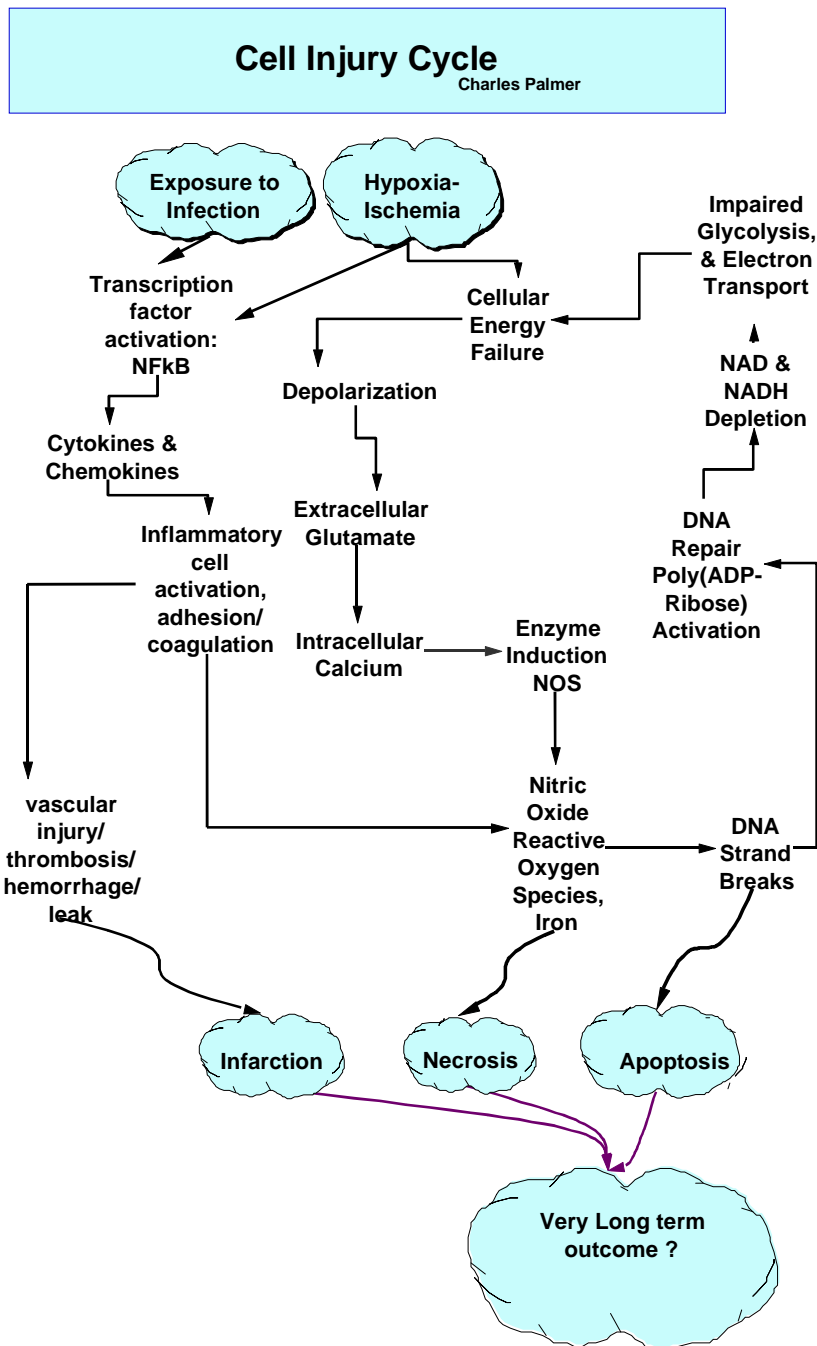
Hypoxia-ischemia results in depletion of ATP and the reduction of resting membrane potentials in neurons and glia (primary energy failure). Potassium leaks out of cells and depolarizes neurons leading to a massive release of glutamate (excitotoxicity). Acting via NMDA receptors, glutamate permits the intracellular influx of calcium, which triggers a number of potentially harmful enzymes including cyclooxygenase, lipoxygenase, proteases and nitric oxide (NO) synthetase.

*Nitric oxide:*NO combines with superoxide anion to form the powerful oxidant peroxynitrite. Peroxynitrite damages proteins, lipids and DNA.[25, 26] Inducible NOS message and protein was expressed from 6 to 24hours after hypoxia ischemia and the activity peaked at 48 hours. The production of 3-nitrotyrosine is a marker for nitrated tyrosine, was elevated with a coincident peak at 48hrs. The clinical relevance of this delayed onset of excess nitric oxide production is that when the rats were treated with a specific inhibitor of inducible NOS infarction was reduced from 31.9 to 10.6%. Studies in my laboratory have confirmed that in the neonatal rat, damage can be reduced by inhibiting NOS (with low dose L-NAME) even when the non specific inhibitor was administered at 15hrs of recovery. [27]

DNA fragments trigger a repair process by the nuclear enzyme Poly (ADP ribose) polymerase (PARP).[28]

Massive activation of PARP leads to ADP ribosylation and depletion of NAD⁺. NAD/NADH are vital cofactors for glycolysis and the electron transport chain. When PARP is overactive, ATP is consumed in an effort to resynthesize NAD⁺. [29] NO may also directly interfere with cellular respiration and deplete cellular ATP via direct inhibition of enzymes in the glycolytic pathway, Krebs cycle, and electron transport chain. [30] The combined NO/peroxynitrite mediated onslaught on cellular energy production leads to cell death by energy depletion (Persistent or Delayed Energy Failure). For surviving neurons, depletion of energy can lead to further loss of membrane potential and amplification of excitotoxicity in another self-perpetuating energy depleting cycle. [Lo, 1998 #96]

Figure 4



2.2.3 Phase of Secondary Energy Failure

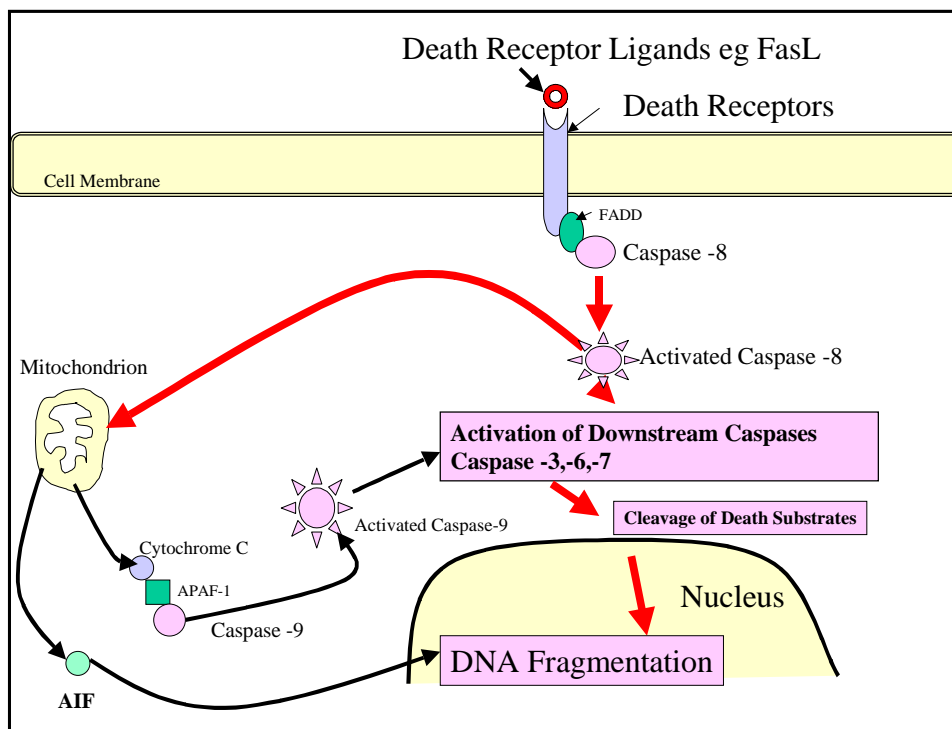
Clinical and experimental studies have determined that following cerebral ischemia, there is a delayed (secondary) phase of injury and energy failure that occurs 8-48h after reperfusion.[31, 32] This has been reported in human infants in whom the severity of the delayed energy failure correlated with poor neuro-development at 1 year of age.[32] In the near-term fetal lamb model (Figure 2) of transient cerebral ischemia, the secondary phase of energy depletion coincides with the onset of cytotoxic edema and seizures. Seizures begin at about 7 hrs after reperfusion and peak at about 28hrs. At the same time there is an accumulation of excitotoxins, increased production of nitric oxide, and a fall in brain electrical activity. [33] [34] [35]

2.2.4 Apoptosis and Delayed Cell Death

In severe hypoxic ischemic injury necrosis is the predominant modality of cell death but in less severe injury apoptosis seems to prevail Figure 5 illustrates how the caspase family of “cell death enzymes” are activated in the initiation and execution of apoptosis .Reviewed by Martin in ref [36]

Caspases (cysteiny aspartate-specific proteinases) are a family of 14 proteases that are activated by regulated proteolysis of proenzymes. Upstream caspases activate downstream “executioner” caspases. Active caspases have numerous target proteins including nuclear proteins, cytoskeletal proteins and cytosolic proteins. Activation of caspase-3 leads to cleavage of the inhibitor of caspase-activated-DNase which triggers the activation of caspase activated DNase and subsequent DNA fragmentation.

Figure 5



Different caspase cascades not necessarily exclusive mediate apoptosis: *The intrinsic pathway* involves cytochrome c release from mitochondria, promoting the activation of caspase -9 through Apaf-1 and then caspase -3 activation. The consequences of mitochondrial injury after cerebral ischemia and reperfusion are numerous.[37] They include metabolic failure , oxidative stress, impaired Ca⁺⁺ buffering and opening of the mitochondrial permeability pore with the release of apoptotic factors such as cytochrome c and apoptosis inducing factor (AIF).In the immature rat mitochondrial dysfunction is characterized by early (3-6hrs of recovery) glucose hyperutilization in areas of the cerebral cortex followed by a secondary phase with low glucose utilization and infarction.[6]When mitochondrial respiration was measured following HI in the neonatal rat it decreased immediately after HI and was followed by a partial recovery between 3-8h. Thereafter a secondary drop in mitochondrial respiration occurred reaching a minimum at 24h of reperfusion. This secondary loss of

respiratory function was accompanied by increased caspase-3 like activity and loss of cell integrity. (MAP2 staining)[38]

The extrinsic pathway to caspase activation leads to activation of cell-surface death receptors, including Fas and tumor necrosis factor receptor, leading to caspase –8 activation that in turn cleaves and activates downstream caspases.[36] Caspase 8 cleaves a cytosolic substrate protein (Bid) that translocates to mitochondria thereby transducing death receptor action at the cell surface to the mitochondrion. Activation of Fas is induced by the binding of Fas ligand, a member of the TNF-cytokine family. Fas is expressed on activated T cells and natural killer cells. Following HI in the 7-day old rat, T cells have been detected in the periinfarct region between 48-96hrs after ischemia.[20] See inflammation 4.0 below.

Recently there have been a number of studies attesting to the role of apoptosis and delayed injury in the 7day old rat model of HI injury: apoptotic cells density is elevated from 12h to 7 days after HI in the cortex and in the basal ganglia. [39] Necrotic cell death can be seen in the striatum and cortex as early as 3hours following hypoxia-ischemia. However a secondary phase of injury occurs after 24-48h in the cortex and not until 6 days in the basal ganglia. The delayed cortical neurodegeneration is a hybrid of necrosis and apoptosis (cell death continuum) while in the thalamus it is apoptosis.[40] Caspase –3 activity peaks at 24hrs of recovery in the injured parietal cortex of the 7day old rat, [41]whereas DNA damage also occurs maximally after 24 hours. [42]

Damage to the forebrain of the neonatal rat is also associated with damage to remote areas that are connected with white matter pathways and that are probably dependent on trophic stimulation from the damaged forebrain. This is consistent with target deprivation mediated injury.[40] Damage to the thalamus is delayed about 24hours. It has structural and molecular features of apoptosis, is mediated by death receptor activation (Fas) and altered mitochondrial function manifested by cytosolic cytochrome C accumulation.[43] Various studies have confirmed that apoptosis has a prolonged role in the neonatal rat.[39]

Strategies for preventing the terminal events in the sequence of events preceding apoptotic cell death may provide a new therapeutic strategy effective many hours after reperfusion. [44, 45]

3.0 Duration of Injury Process

One question that interests the clinical investigator is “For how long does brain injury continue to progress after the primary insult?” Most animal studies are terminated within a few weeks of recovery. There is a need to evaluate even longer recovery timeframes.

Human infants who have a bad neurological outcome following birth asphyxia show increased brain lactate/creatinine for as long as a year later.[46] Preliminary findings obtained from ³¹P NMR studies from the same investigators have shown that the increased lactate /creatinine correlated with an increased pH and a decreased PCr/Pi 9 months after asphyxia. [47] These late changes in high energy phosphates suggest long standing perturbations in the brain energy metabolism. Using the immature rat pup model of unilateral carotid occlusion and

hypoxia we have observed that a microglial and astroglial response to the areas of injury continues for at least a year following the insult.[48] We have also observed plaque-like structures that stain positive for iron reaction product (Perls stain) and calcium as a late recovery finding. These plaques are surrounded by microglia, and the surrounding parenchyma stains positive with a marker for lipid peroxidation. It appears that these plaques may represent foci of chronic inflammation and possibly chronic injury [48].

4.0 Inflammation

Cerebral hypoxia-ischemia in the PD7 rat enhances rapid expression of brain inflammatory cytokines (IL6, IL-1B)[18] and an inflammatory cell response to injury that includes neutrophils, lymphocytes, and microglia. [19, 20] Systemic anticytokine therapy, in the form of receptor antagonists to TNF α , markedly reduces ischemic brain injury[49], as does an IL-1 receptor antagonist.[18] Neutrophil depletion is also neuroprotective in neonatal,[21] and adult stroke models. (see review in [50])

Cytokines and white matter damage in the preterm neonate

Strong epidemiologic evidence indicates that the babies who are at greater risk of developing an adverse outcome are those born to mothers who have chorioamnionitis.[51] [52-55] There are elevated concentrations of the cytokines, tumor necrosis factor (TNF α), and interleukins 1 β (IL-1 β) and IL-6 in the amniotic fluid and umbilical cord plasma of fetuses and prematurely born infants who sustain periventricular leukomalacia (PVL). [56, 57] Both tumor necrosis factor and IL-6 are expressed in areas of PVL in premature infants who expire with such lesions.[58, 59] Thus, infection and ischemia share the cytokine pathway as upstream modulators of brain injury. In a recent report, Nelson et al,[60] showed that children with cerebral palsy had elevated inflammatory cytokines in their cord blood. It is possible that the fetal inflammatory response made the brain more susceptible to asphyxiating insults.[61]

In human infants the neuropathology of PVL is diverse and includes diffuse astrogliosis, characteristic loss of oligodendrocytes, and deficiencies in myelination.[62-65] Cell culture experiments have shown that immature oligodendrocytes are particularly vulnerable to free radical mediated apoptotic cell death. (for review see [66]) In areas of white matter injury, microglia stain positive for inflammatory cytokines TNF- α and IL-6.[59] Activated microglia are capable of secreting a vast array of neurotoxins, including free radicals.[67] Finally, to test the role of intrauterine infection as a cause of fetal brain injury, Yoon et al, [68] developed a model in which fetal rabbits were exposed to intrauterine infection. Some rabbits developed areas of necrosis in the white matter.

There is evidence that blood supply to the white matter is precarious because there are relatively fewer blood vessels to that area, especially in the 28 week premature infant who is most susceptible to PVL.[69] Impaired autoregulation also places the white matter at increased risk of damage. (Reviewed by Perlman in reference[70]) Thus, there are two main contributors to white matter damage in the preterm. The first relates to elevated levels of inflammatory cytokines, and the second to vascular factors leading to ischemia. These two apparently separate mechanisms may have common components. One possible link could be the role of inflammatory cytokines in activating neutrophil adhesion to blood vessels. Under low flow

states, neutrophil plugging might contribute to cerebral ischemia and periventricular leukomalacia.[71-73]

Premature infants born from an infected uterine environment have nearly 3 times as many neutrophils in their blood as infants whose placentas have no evidence of infection. We have found that neutrophils accumulate in cerebral blood vessels during hypoxia-ischemia in the immature rat,[21] and that they contribute to the extent of the brain’s energy failure during hypoxia-ischemia. If neutrophils are depleted prior to subjecting immature rats to hypoxia-ischemia, then subsequent brain injury is reduced.[23] If neutropenia is induced within hours of reperfusion then we lost the neuroprotective effect. Thus it seems likely that in the immature rat, neutrophils contribute to the ischemic insult or early reperfusion phase. Accordingly, the activated neutrophil may be an important link between exposure to infection in-utero and ischemic brain injury.

5.0 Brain Rescue Strategies (see tables)

The Table below is separated into the Phases of Recovery and the common mediators of injury. The middle column contains modifications to current practice that can be made in light of the lessons we have learned from animal experiments. The third column “Future Rescue Therapy” lists the modalities that have a proven neuroprotective effect in animal experiments. The bold type indicates that the treatment is currently available in our pharmacies. Clinical trials are needed before new therapeutic modalities can be applied to clinical practice. Recent reviews of neuroprotective therapies and references for the modalities listed can be found refs [24, 74-76] [77]

Table 1: Neuroprotective Strategies for the Newborn Infant Recovering from a Hypoxic Ischemic Insult

Reperfusion Phase of Recovery (first 4hrs)

Mediators of injury	Current management	Future rescue therapy
Hyperoxemia produces vasoconstriction and increases free radical formation.	Monitor and control oxygen delivery. Avoid prolonged exposure to 100% oxygen. Maintain normoxia.	Free radical scavengers: Allopurinol Metal chelation, antioxidants Antenatal administration of Allopurinol
Free radicals		Allopurinol, ascorbic acid, deferoxamine
Neutrophil adhesion and vascular plugging	Avoid hypotension and poor peripheral perfusion.	Antineutrophil strategy: Prevent adhesion, activation, secretion.(Sialyl Lewis ^x) pentoxifylline
Inflammatory mediators Lipid derived and inflammatory cytokines		PAF antagonists, Thromboxane antagonists, Cyclooxygenase inhibitors, (ibuprofen), Phospholipase A2 antagonists, IL-1 antagonists (zinc protoporphyrin)
No reflow (hypoperfusion)	Maintain adequate blood	ibuprofen

Endothelin (vasoconstrictor)	pressure Avoid hyperviscosity. Avoid hyperoxemia.	Endothelin antagonists Calcium channel blockers (nimodipine)
Endothelial cell alterations/ swelling and villi formation	Avoid hyperviscosity Hct>65.	Hyperosmolar therapy
Pressure passive cerebral circulation.	Maintain adequate blood pressure Monitor and control carbon dioxide levels within normal range.	
Evolving inflammation	Avoid radiant heat to the head.	Rescue Hypothermia
Impaired substrate delivery	Maintain normal serum glucose.	Fructose-1,6-Diphosphate, acetyl-L Carnitine, ketone bodies.
Release of mediators from other organs eg XO, iron, inflammatory mediators and activated coagulation cascade.	Treat multi organ system dysfunction.	Allopurinol , complement antagonists,

Table 2: Latent Phase of Recovery (0- 8hrs of recovery)

Mediator of Injury		Future Rescue Strategy
Ongoing inflammation		Prolonged Rescue Hypothermia
Nitric oxide		Specific inhibitors of neuronal and inducible nitric oxide synthetase. Hypothermia
Intracellular Calcium		Calcium channel blockers (nimodipine) Neuronal calcium channel blockers (Nilvadipine, Conopeptide)
Excitatory amino acids		NMDA receptor antagonists: (Dextromethorphan, Mag sulphate) Glutamate release inhibitors: (Lubeluzole, Lamotrigine) Adenosine transport inhibitors: Hypothermia
Free radicals		Free radical scavengers: Pyrrolopyrimidines Metal chelators: deferoxamine Allopurinol
Proteases		Calpain inhibitors

Phase of Secondary Energy Failure (8-48rs)

Mediator of Injury	Current Management	Future Rescue Strategy
Apoptosis (Programmed cell death)		Caspase Inhibitors. BAF Growth Factors: bFGF, BDNF, IGF-1
Excess Poly (ADP-Ribose) Polymerase activity produces energy failure		PARP antagonists
Nitric oxide /peroxynitrite		See above
Excitatory amino acids		See above
Seizures	Phenobarbitone	Phenobarbitone high dose (40mg/kg before seizures)

Bibliography

1. Vannucci, R.C. and Palmer, C. (1997). Hypoxic-ischemic encephalopathy: pathogenesis and neuropathology. In Diseases of the Fetus and Infant, (ed.A.A. Fanaroff and R.J. Martin) pp856-77, Mosby-Year Book, Inc.,
2. Vannucci, R.C., Connor, J.R., Mauger, D.T., Palmer, C., Smith, M.B., Towfighi, J., et al.(1999). Rat model of perinatal hypoxic-ischemic brain damage. Journal of Neuroscience Research. 55,(2),158-63.
3. Sarnat, H. and Sarnat, M.(1976). Neonatal encephalopathy following fetal distress-a clinical and electroencephalographic study. Arch Neurol. 33,695-706.
4. Thompson, C.M., Puterman, A.S., Linley, L.L., Hann, F.M., Vanderelst, C.W., Molteno, C.D., et al.(1997). The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr. 86,(7),757-61.
5. Gunn, A.J., Gunn, T.R., Gunning, M.I., Williams, C.E. and Gluckman, P.D.(1998). Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. Pediatrics. 102,(5),1098-106.
6. Gilland, E., Bona, E. and Hagberg, H.(1998). Temporal changes of regional glucose use, blood flow, and microtubule-associated protein 2 immunostaining after hypoxia-ischemia in the immature rat brain. Journal of Cerebral Blood Flow and Metabolism. 18,(2),222-8.
7. Nedelcu, J., Klein, M.A., Aguzzi, A., Boesiger, P. and Martin, E.(1999). Biphasic edema after hypoxic-ischemic brain injury in neonatal rats reflects early neuronal and late glial damage. Pediatric Research. 46,(3),297-304.
8. Toet, M.C., Hellstrom-Westas, L., Groenendaal, F., Eken, P. and de Vries, L.S.(1999). Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. Arch Dis Child. 81,(1 Special Issue SI),F19-F23.
9. al Naqeeb, N., Edwards, A.D., Cowan, F.M. and Azzopardi, D.(1999). Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. Pediatrics. 103,(6),1263-71.

10. Palmer, C.(1995). Hypoxic-ischemic encephalopathy: Therapeutic approaches against microvascular injury, and role of neutrophils, PAF, and free radicals. *Clinics in Perinatology*. 22,(2),481-517.
11. Ames, A., Wright, L.W., Kowada, M., Thurston, J.M. and Majno, G.(1968). Cerebral ischemia, II: the no-reflow phenomenon. *American Journal of Pathology*. 52,437-53.
12. Hossmann, K.A.(1997). Reperfusion of the brain after global ischemia - hemodynamic disturbances. *Shock*. 8,(2),95-101.
13. Groggaard, B., Schurer, L., Gerdin, B. and Arfors, K.E.(1989). Delayed hypoperfusion after incomplete forebrain ischemia in the rat. The role of polymorphonuclear leukocytes. *J Cereb Blood Flow & Metab*. 9,(4),500-5.
14. Groggaard, B., Schurer, L., Gerdin, B. and Arfors, K.-E. (1985). The role of polymorphonuclear leukocytes in postischemic delayed hypoperfusion. In *Oxygen Free Radicals in Shock*, (ed.U. Novelli) pp74-78,Karger, Basel, Florence
15. Kennedy, C., Grave, G.D. and Jehle, J.W.(1971). Effect of hyperoxia on the cerebral circulation of the newborn puppy. *Pediatric Research*. 5,659-67.
16. Liu, Y.B., Rosenthal, R.E., Haywood, Y., Miljkovicloic, M., Vanderhoek, J.Y. and Fiskum, G.(1998). Normoxic ventilation after cardiac arrest reduces oxidation of brain lipids and improves neurological outcome. *Stroke*. 29,(8),1679-86.
17. Ramji, S., Ahuja, S., Thirupuram, S., Rootwelt, T., Rooth, G. and Saugstad, O.D.(1993). Resuscitation of asphyxic newborn infants with room air or 100% oxygen. *Pediatric Research*. 34,(6),809-12.
18. Hagberg, H., Gilland, E., Bona, E., Hanson, L.Å., Hahn-Zoric, M., Blennow, M., et al.(1996). Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatric Research*. 40,(4),603-09.
19. Bona, E., Andersson, A.L., Blomgren, K., Gilland, E., Puka-Sundvall, M., Gustafson, K., et al.(1999). Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. *Pediatric Research*. 45,(4 Part 1),500-09.
20. Benjelloun, N., Renolleau, S., Represa, A., Ben-Ari, Y. and Charriaut-Marlangue, C.(1999). Inflammatory responses in the cerebral cortex after ischemia in the P7 neonatal rat. *Stroke*. 30,(9),1916-23.
21. Hudome, S., Palmer, C., Roberts, R.L., Mauger, D., Housman, C. and Towfighi, J.(1997). The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. *Pediatric Research*. 41,(5),607-16.
22. Garcia, J., Liu, K., Yoshida, Y., Lian, J., Chen, S. and del Zoppo, G.(1994). Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). *Am J Pathol*. 144,(1),188-99.
23. Palmer, C., Roberts, R.L. and Young, P.I.(1995). Neutropenia before but not after hypoxia-ischemia reduces brain injury in neonatal rats. *J Cereb Blood Flow Metabol*. 15,(Supp 1),S291.
24. Palmer, C. (1997). Ischemia-reperfusion injury. In *Fetal and Neonatal Brain Injury: Mechanisms, Management, and the Risks of Practice*, (ed.P. Sunshine and D.K. Stevenson) pp38-59,Oxford University Press, New York
25. Tamir, S., Burney, S. and Tannenbaum, S.R.(1996). DNA damage by nitric oxide. *Chem Res Toxicol*. 9,(5),821-27.

26. Dawson, V.L. and Dawson, T.M.(1996). Nitric oxide neurotoxicity. *J Chem Neuroanat.* 10,(3-4),179-90.
27. Palmer, C. and Roberts, R.L.(1997). Delayed inhibition of nitric oxide production reduces post hypoxic-ischemic brain injury in neonatal rats. *Pediatric Research.* 41,294A.
28. Szabó, C., Zingarelli, B., O'Connor, M. and Salzman, A.L.(1996). DNA strand breakage, activation of poly(ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. *Proceedings of the National Academy of Sciences of the United States of America.* 93,(5),1753-58.
29. Endres, M., Wang, Z.Q., Namura, S., Waeber, C. and Moskowitz, M.A.(1997). Ischemic brain injury is mediated by the activation of poly(adp-ribose)polymerase. *J Cereb Blood Flow Metab.* 17,(11),1143-51.
30. Nathan, C.(1992). Nitric oxide as a secretory product of mammalian cells. *Faseb Journal.* 6,3051-64.
31. Thoresen, M., Penrice, J., Lorek, A., Cady, E.B., Wylezinska, M., Kirkbride, V., et al.(1995). Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatric Research.* 37,(5),667-70.
32. Roth, S.C., Edwards, A.D., Cady, E.B., Delpy, D.T., Wyatt, J.S., Azzopardi, D., et al.(1992). Relation between cerebral oxidative metabolism following birth asphyxia and neurodevelopmental outcome and brain growth at one year. *Dev Med Child Neurol.* 34,285-95.
33. Williams, C.E., Gunn, A. and Gluckman, P.D.(1991). Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. *Stroke.* 22,(4),516-21.
34. Marks, K.A., Mallard, E.C., Roberts, I., Williams, C.E., Sirimanne, E.S., Johnston, B., et al.(1996). Delayed vasodilation and altered oxygenation after cerebral ischemia in fetal sheep. *Pediatric Research.* 39,(1),48-54.
35. Gunn, A.J., Gunn, T.R., Dehaan, H.H., Williams, C.E. and Gluckman, P.D.(1997). Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *Journal of Clinical Investigation.* 99,(2),248-56.
36. Martin, L.J.(2001). Neuronal cell death in nervous system development, disease, and injury (Review). *Int J Mol Med.* 7,(5),455-78.
37. Fiskum, G.(1985). Mitochondrial Damage During Cerebral Ischemia. *Ann Emerg Med.* 14,(Aug 8),810-15.
38. Puka-Sundvall, M., Wallin, C., Gilland, E., Hallin, U., Wang, X., Sandberg, M., et al.(2000). Impairment of mitochondrial respiration after cerebral hypoxia-ischemia in immature rats: relationship to activation of caspase-3 and neuronal injury. *Brain Res Dev Brain Res.* 125,(1-2),43-50.
39. Nakajima, W., Ishida, A., Lange, M.S., Gabrielson, K.L., Wilson, M.A., Martin, L.J., et al.(2000). Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. *The Journal of Neuroscience.* 20,(21),7994-8004.
40. Northington, F.J., Ferriero, D.M., Graham, E.M., Traystman, R.J. and Martin, L.J.(2001). Early Neurodegeneration after Hypoxia-Ischemia in Neonatal Rat Is Necrosis while Delayed Neuronal Death Is Apoptosis. *Neurobiol Dis.* 8,(2),207-19.

41. Wang, X., Karlsson, J.O., Zhu, C., Bahr, B.A., Hagberg, H. and Blomgren, K.(2001). Caspase-3 activation after neonatal rat cerebral hypoxia-ischemia. *Biol Neonate*. 79,(3-4),172-9.
42. Zhu, C., Wang, X., Hagberg, H. and Blomgren, K.(2000). Correlation between caspase-3 activation and three different markers of DNA damage in neonatal cerebral hypoxia-ischemia. *J Neurochem*. 75,(2),819-29.
43. Northington, F.J., Ferriero, D.M., Flock, D.L. and Martin, L.J.(2001). Delayed neurodegeneration in neonatal rat thalamus after hypoxia- ischemia is apoptosis. *The Journal of Neuroscience*. 21,(6),1931-8.
44. Cheng, Y., Deshmukh, M., D'Costa, A., Demaro, J.A., Gidday, J.M., Shah, A., et al.(1998). Caspase inhibitor affords neuroprotection with delayed administration in a rat model of neonatal hypoxic-ischemic brain injury. *Journal of Clinical Investigation*. 101,1992-99.
45. Schulz, J.B., Weller, M. and Moskowitz, M.A.(1999). Caspases as treatment targets in stroke and neurodegenerative diseases. *Annals of Neurology*. 45,(4),421-29.
46. Hanrahan, D., Cox, I.J., Edwards, A.D., Cowan, F., Sargentoni, J., Bell, J.D., et al.(1998). Persistent increases in cerebral lactate concentration after birth asphyxia. *Pediatr Res (In press)*.
47. Robertson, N.J., Cox, I.J., Counsell, S., Cowan, F., Azzopardi, D. and Edwards, A.D.(1997). Persistent lactate following perinatal hypoxic-ischemic encephalopathy and its relationship to energy failure studied by magnetic resonance spectroscopy. *Early Hum Devel*.
48. Palmer, C., Menzies, S., Roberts, R.L. and Connor, J.R.(1998). Iron containing "plaques" develop in the brains of rats months after neonatal hypoxic-ischemic brain injury. *Pediatric Research*. 43,322A.
49. Lavine, S.D., Hofman, F.M. and Zlokovic, B.V.(1998). Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury. *Journal of Cerebral Blood Flow and Metabolism*. 18,(1),52-58.
50. Palmer, C.(1995). Hypoxic-ischemic encephalopathy: Therapeutic approaches against microvascular injury, and role of neutrophils, PAF and free radicals. *Clinics in Perinatology*. 22,(2),481-517.
51. Verma, U., Tejani, N., Klein, S., Reale, M.R., Beneck, D., Figueroa, R., et al.(1997). Obstetric antecedents of intraventricular hemorrhage and periventricular leukomalacia in the low-birth-weight neonate. *American Journal of Obstetrics and Gynecology*. 176,(2),275-81.
52. Murphy, D.J., Sellers, S., MacKenzie, I.Z., Yudkin, P.L. and Johnson, A.M.(1995). Case-control study of antenatal and intrapartum risk factors for cerebral palsy in very preterm singleton babies. *The Lancet*. 346,(December 2),1449-51.
53. Bejar, R., Wozniak, P., Allard, M., Benirschke, K., Vaucher, Y., Coen, R., et al.(1988). Antenatal origin of neurologic damage in newborn infants. *American Journal of Obstetrics and Gynecology*. 159,(2),357-63.
54. Bejar, R.F., Vaucher, Y.E., Benirschke, K. and Berry, C.C.(1992). Postnatal white matter necrosis in preterm infants. *J Perinatol*. 12,(1),3-8.
55. Leviton, A., Gilles, F., Neff, R. and Yaney, P.(1976). Multivariate analysis of risk of perinatal telencephalic leucoencephalopathy. *Am J Epidemiol*. 104,(6),621-26.

56. Weatherstone, K.B. and Rich, E.A.(1989). Tumor necrosis factor/cachectin and interleukin-a secretion by cord blood monocytes from premature and term neonates. *Pediatric Research*. 25,(4),342-46.
57. Yoon, B.H., Romero, R., Kim, C.J., Jun, J.K., Gomez, R., Choi, J.H., et al.(1995). Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. *American Journal of Obstetrics and Gynecology*. 172,(3),960-70.
58. Deguchi, K., Mizuguchi, M. and Takashima, S.(1996). Immunohistochemical expression of tumor necrosis factor alpha in neonatal leukomalacia. *Pediatr Neurol*. 14,(1),13-6.
59. Yoon, B.H., Romero, R., Kim, C.J., Koo, J.N., Choe, G., Syn, H.C., et al.(1997). High expression of tumor necrosis factor-alpha and interleukin-6 in periventricular leukomalacia. *American Journal of Obstetrics and Gynecology*. 177,(2),406-11.
60. Nelson, K.B., Dambrosia, J.M., Grether, J.K. and Phillips, T.M.(1998). Neonatal cytokines and coagulation factors in children with cerebral palsy. *Annals of Neurology*. 44,665-75.
61. Nelson, K.B. and Grether, J.K.(1998). Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet & Gynecol*. 179,(2),507-13.
62. Iida, K., Takashima, S. and Ueda, K.(1995). Immunohistochemical study of myelination and oligodendrocyte in infants with periventricular leukomalacia. *Pediatr Neurol*. 13,(4),296-304.
63. Takashima, S., Iida, K. and Deguchi, K.(1995). Periventricular leukomalacia, glial development, and myelination. *Early Hum Dev*. 43,(2),177-84.
64. Leviton, A. and Gilles, F.H.(1984). Acquired perinatal leukoencephalopathy. *Annals of Neurology*. 16,(1 July),1-8.
65. Leviton, A. and Gilles, F.(1996). Ventriculomegaly, delayed myelination, white matter hypoplasia, and "periventricular" leukomalacia: how are they related? *Pediatr Neurol*. 15,(2),127-36.
66. Volpe, J.J.(1997). Brain injury in the premature infant--from pathogenesis to prevention. *Brain and Development*. 19,(8),519-34.
67. Giulian, D., Vaca, K. and Corpuz, M.(1993). Brain glia release factors with opposing actions upon neuronal survival. *The Journal of Neuroscience*. 13,(1),29-37.
68. Yoon, B.H., Kim, C.J., Romero, R., Jun, J.K., Park, K.H., Choi, S.T., et al.(1997). Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. *American Journal of Obstetrics and Gynecology*. 177,(4),797-802.
69. Miyawaki, T., Matsui, K. and Takashima, S.(1998). Developmental characteristics of vessel density in the human fetal and infant brains. *Early Hum Dev*. 53,(1),65-72.
70. Perlman, J.M.(1998). White matter injury in the preterm infant: an important determination of abnormal neurodevelopment outcome [Review]. *Early Hum Dev*. 53,(2),99-120.
71. Yamakawa, T., Yamaguchi, S., Niimi, H. and Sugiyama, I.(1987). White blood cell plugging and blood flow maldistribution in the capillary network of cat cerebral cortex in acute hemorrhagic hypotension: an intravital microscopic study. *Circ Shock*. 22,323-32.
72. Mori, E., del Zoppo, G.J., Chambers, J.D., Copeland, B.R. and Arfors, K.E.(1992). Inhibition of polymorphonuclear leukocyte adherence suppresses no-reflow after focal cerebral ischemia in baboons. *Stroke*. 23,(5),712-8.

73. Del Zoppo, G.J., Schmid-Schonbein, G.W., Mori, E., Copeland, B.R. and Chang, C.M.(1991). Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke*. 22,1276-83.
74. Palmer, C. and Vannucci, R.C.(1993). Potential new therapies for perinatal cerebral hypoxia-ischemia. *Clin Perinatol*. 20,(2),411-32.
75. Palmer, C.(1995). Hypoxic-ischemic encephalopathy: therapeutic approaches against microvascular injury: the role of neutrophils, PAF and free radicals. *Clinics in Perinatology*. 22,(2),481-517.
76. Tan, S. and Parks, D.A.(1999). Preserving brain function during neonatal asphyxia. *Clinics in Perinatology*. 26,(3),733-+.
77. Vannucci, R.C. and Perlman, J.M.(1997). Interventions for perinatal hypoxic-ischemic encephalopathy. *Pediatrics*. 100,(6 Dec),1004-14.