NEUROSURGERY CLINICS OF NORTH AMERICA

Neurosurg Clin N Am 13 (2002) 169–182

Critical mechanisms of secondary damage after inflicted head injury in infants and children

Randall A. Ruppel, MD[a,b,c], Robert S.B. Clark, MD[a,b,c], Hülya Bayır, MD[a,b,c], Margaret A. Satchell, MD[a,b,c], Patrick M. Kochanek, MD[a,b,c,d,*]

[a]*Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, 3434 Fifth Ave., Pittsburgh, PA 15260, USA*
[b]*Department of Pediatrics, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, 3705 Fifth Ave., Pittsburgh, PA 15213, USA*
[c]*Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, 3434 Fifth Ave., Pittsburgh, PA 15260, USA*
[d]*University of Pittsburgh Center for Injury Control and Research, University of Pittsburgh School of Medicine and Children's Hospital of Pittsburgh, 200 Lothrop St., Pittsburgh, PA 15213, USA*

Inflicted head trauma is a unique entity within the realm of pediatric head trauma. Most of the work on the pathophysiology of inflicted head trauma, or more specifically shaken baby syndrome, has developed as an extension of research in pediatric traumatic brain injury (TBI). This article discusses some of the relevant pathophysiologic mechanisms that have been elucidated in TBI in general, and how these mechanisms apply to child abuse (Fig. 1).

Blood flow and metabolism

Perturbations in cerebral blood flow (CBF) and metabolism are common following severe TBI. Posttraumatic hypoperfusion has been clearly demonstrated in children following TBI. CBF is lowest early after injury and is associated with Glasgow Coma Scale score [1]. In the largest report of CBF in young children ischemic blood flow (defined as <20 mL/100 g/min assessed by stable xenon CT) was found to be most common during the first 24 hours following head injury [2]. In this study, age less than 2 years, early low CBF, and loss of CO_2 vasoreactivity were all associated with poor outcome. Eight of the 13 children less than 2 years of age were victims of child abuse, who are at significant risk for hypoperfusion (Fig. 2). This period of low blood flow occurs during a time when cerebral metabolic rate for oxygen is preserved, suggesting ischemic conditions [3].

The mechanisms involved in the development of reduced CBF are still largely unknown and a number of current efforts are investigating the role of substances that have vasoactive properties. An important peptide that has potent vasoconstrictor properties is endothelin-1 [4]. Endothelin-1 is increased in an experimental model of TBI in newborn and juvenile piglets [5]. Disturbances in cerebral autoregulation are greater in newborn piglets when compared with juveniles, and this abnormality is mitigated by treatment with endothelin-1 antagonists. This implies that endothelin may have an important role in this phenomenon. Increased concentrations of endothelin-1 have been demonstrated in the cerebrospinal fluid (CSF) of infants and children following TBI [6].

Another substance that is of particular interest with regards to inflicted head injury is procalcitonin. Procalcitonin is the propeptide of the hormone calcitonin, belongs to the insulin gene superfamily, and has potent vasodilatory activity.

* Corresponding author.
E-mail address: kochanekpm@anes.upmc.edu (P.M. Kochanek).

PII: S1042-3680(01)00005-5

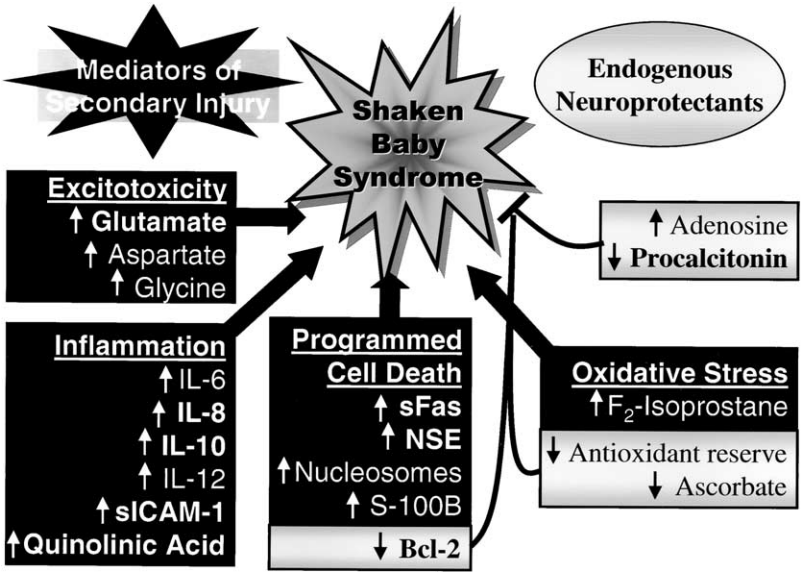

Fig. 1. Critical mechanisms of secondary injury after inflicted head injury in infants and young children. The mechanisms involved can be categorized as mediators of secondary injury (*black*) and endogenous neuroprotectants (*gray*). Mediators of secondary injury include those causing excitotoxicity, inflammation, programmed cell death, and oxidative stress. Endogenous neuroprotectants are substances that protect against programmed cell death, oxidative stress, or work through vasoactive properties. IL = interleukin; sICAM-1 = soluble intercellular adhesion molecule; sFas = soluble Fas; NSE = neuron-specific enolase.

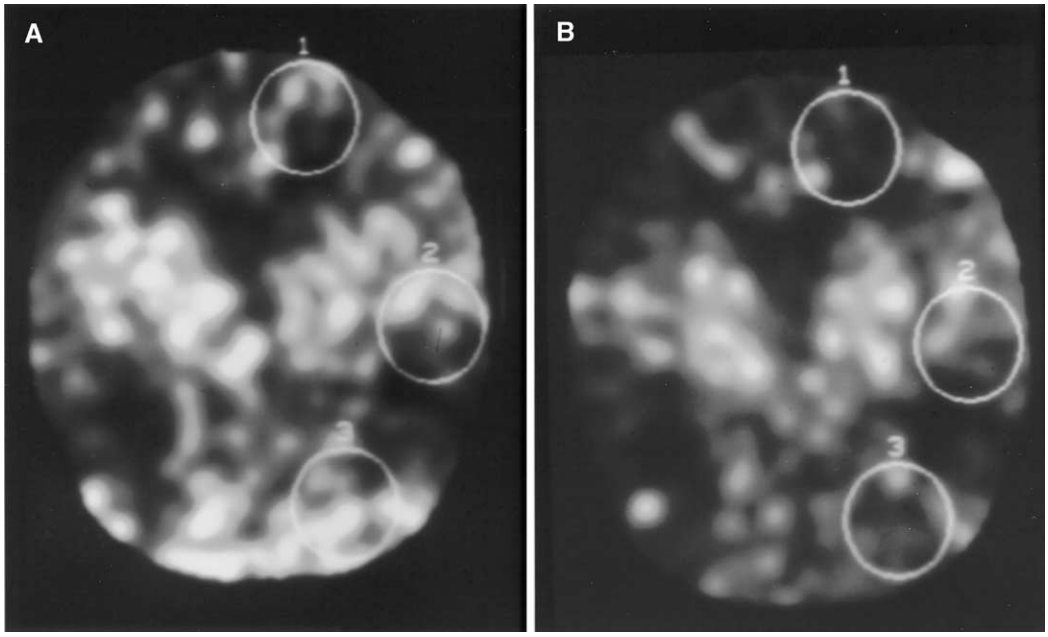

Fig. 2. Cerebral blood flow (CBF) in a child with shaken baby syndrome as measured by stable xenon CT. Low CBF (A), particularly in cortical regions of interest, is further reduced by hyperventilation (B).

It has been hypothesized that this and related substances may provide some neuroprotection during periods of hypoperfusion following TBI. The CSF concentration of procalcitonin is increased in children after head trauma, with the highest levels occurring early after injury, when the risk of hypoperfusion is greatest [7]. Interestingly, an inverse association with CSF procalcitonin level and child abuse as a mechanism of injury has been demonstrated, implying that child-abuse victims may be afforded less protection than victims of accidental injury following injury.

Adenosine is another substance that is neuroprotective that may act, in part, through its vasodilatory properties. Adenosine is produced from the breakdown of ATP and has variable actions depending on the receptor with which it interacts. The A_1 receptor decreases calcium conductance and metabolism in neurons, thereby attenuating excitotoxicity (see later), whereas the A_2a receptor causes vasodilatation. Increased levels of adenosine have been demonstrated after TBI in rats [8], in adults [9], and in children [10] (Fig. 3). The concept that adenosine is neuroprotective is supported by studies showing improved outcome with strategies that augment adenosine levels. Delayed increases in CSF adenosine in adults with severe TBI were associated with increases in CBF, possibly representing pathologic vasodilations [11]. Finally, vasodilatory metabolites, such as nitrosothiols, are formed and present several days after severe head injury in infants and children [12]. It remains to be determined if these substances contribute to the development of cerebral hyperemia and swelling or are retaliatory mediators combating hypoperfusion.

Shaken babies are at great risk for loss of CBF regulation. Loss of blood pressure autoregulation (Fig. 4) occurs in approximately 40% of severely head-injured children [13]. Although a correlation between outcome and loss of autoregulation has never been demonstrated, impaired autoregulation is seen more often in children with abnormal CBF. The occurrence of posttraumatic hyperemia is a debatable problem in children, although malignant cerebral swelling is a well-described phenomenon [14]. Whether hyperemia and edema are linked is not known. It has been theorized that hyperemia is linked to increased metabolic demands as a result of hyperglycolysis [15] or from local production of vasodilatory mediators as described previously. Age-related differences in blood flow are certainly relevant to discussions of shaken baby syndrome. Pericontusional hyperemia has been shown to be much more prominent in the immature versus the adult brain in experimental models [16].

A finding that is somewhat unique to children with abusive head trauma is unilateral or bilateral loss of gray-white differentiation, termed *big black brain* [17]. This radiographic finding of hemispheric hypodensity can develop over the first 48 hours following injury and is associated with parenchymal loss and poor outcome [18]. Because this pattern of damage can be unilateral, it is unlikely a result of a mechanism that should affect the entire brain, but is more likely the result of either vascular compromise or local effects from hemorrhage. The abnormalities seen on CT or MRI develop over a period of days following injury; programmed cell death may play a role in this progression [19].

Excitotoxicity

The potential for excitatory amino acid neurotransmitters to cause neuronal cellular damage has been known for approximately 30 years. *Excitotoxicity*, the term used to describe this phenomenon, plays a key role in the secondary neuronal damage and cell death that occur following TBI. Pathologically high levels of excitatory neurotransmitters result in cell death ultimately by causing a rise in intracellular calcium concentration and triggering a variety of detrimental intracellular mechanisms. Increased intracellular calcium concentration leads to activation of proteases, lipases, endonucleases, and superoxide anion and nitric oxide production, with resultant mitochondrial and DNA damage.

A glutamate concentration of 5 μm can cause neuronal cell death in vitro [20]. Interstitial and ventricular glutamate levels up to 800 μm have been demonstrated in clinical TBI, implicating excitotoxicity as a key mechanism of secondary neuronal cell death in humans [21]. Glutamate is increased in the CSF of infants and children following severe head trauma, the most dramatic levels being in the victims of child abuse (Fig. 5) [22]. Of note, CSF aspartate concentration, which is another intracellular amino acid with excitatory properties, was not increased in this study. Concurrent increases of glutamate and aspartate have been demonstrated in the CSF of adults following head trauma [23] and this finding has been used as an argument for these substances being merely markers of cell disruption rather than causative agents in excitotoxic damage [24]. The unilateral rise in glutamate concentration demonstrated in

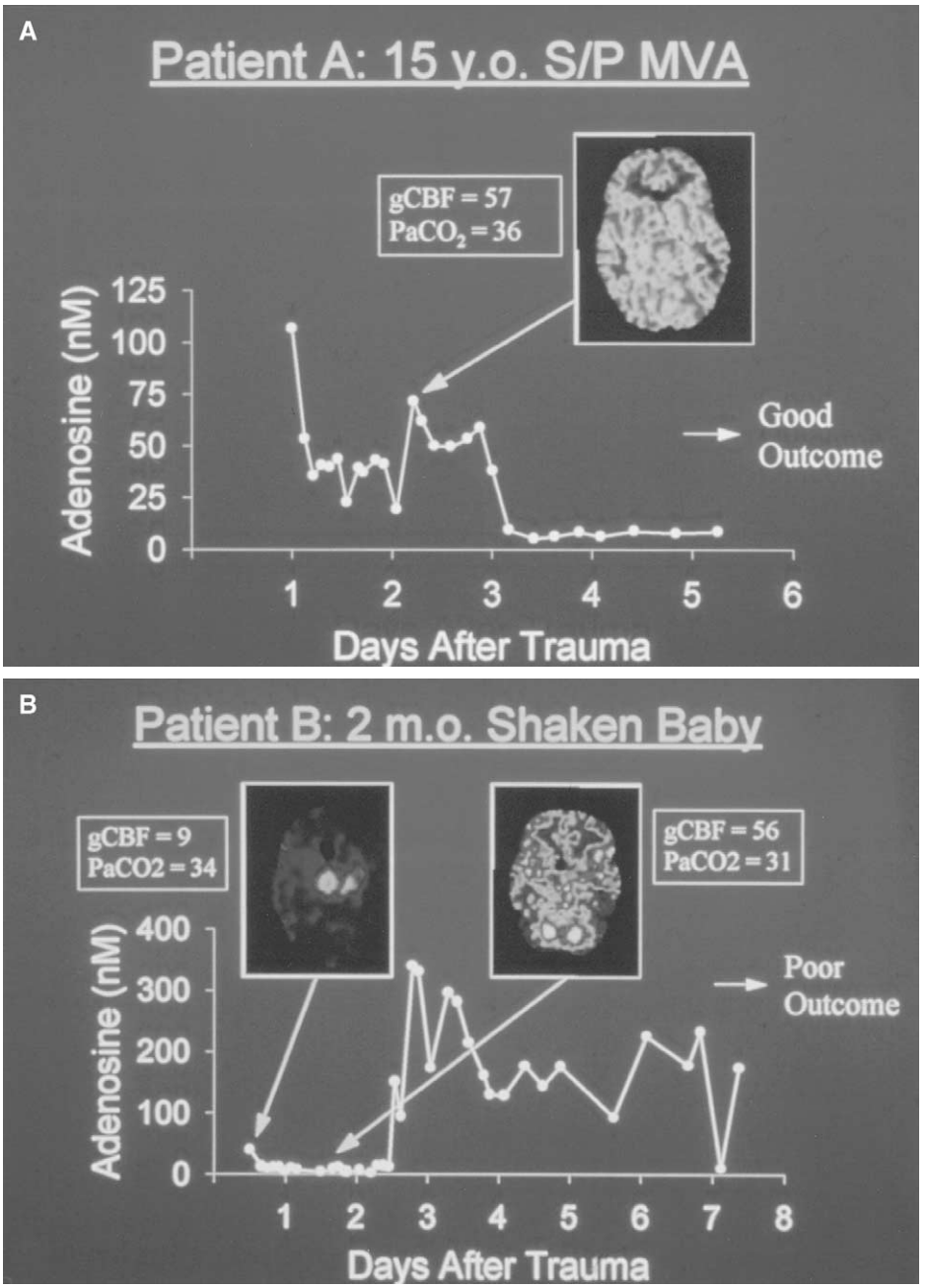

Fig. 3. (A) CBF in a 15-year-old child in a motor vehicle accident (MVA) measured by stable xenon CT. Normal global CBF (gCBF) concurrent with increased cerebrospinal fluid (CSF) adenosine levels was observed. This may reflect an appropriate cerebrovascular response to adenosine release early after injury. (B) CBF in a 2-month-old child with shaken baby syndrome measure by stable xenon CT. Marked early hypoperfusion with a low gCBF was noted concurrent with surprisingly low CSF adenosine levels. Subsequent increases in both gCBF and CSF adenosine levels were seen. This pattern may reflect overwhelming injury with an initial failure of the endogenous adenosine response. The late rise may reflect pathologic vasodilation, CBF dysregulation, or marked CBF demands coupled to increased anaerobic metabolism. (See also Color Plate 1.)

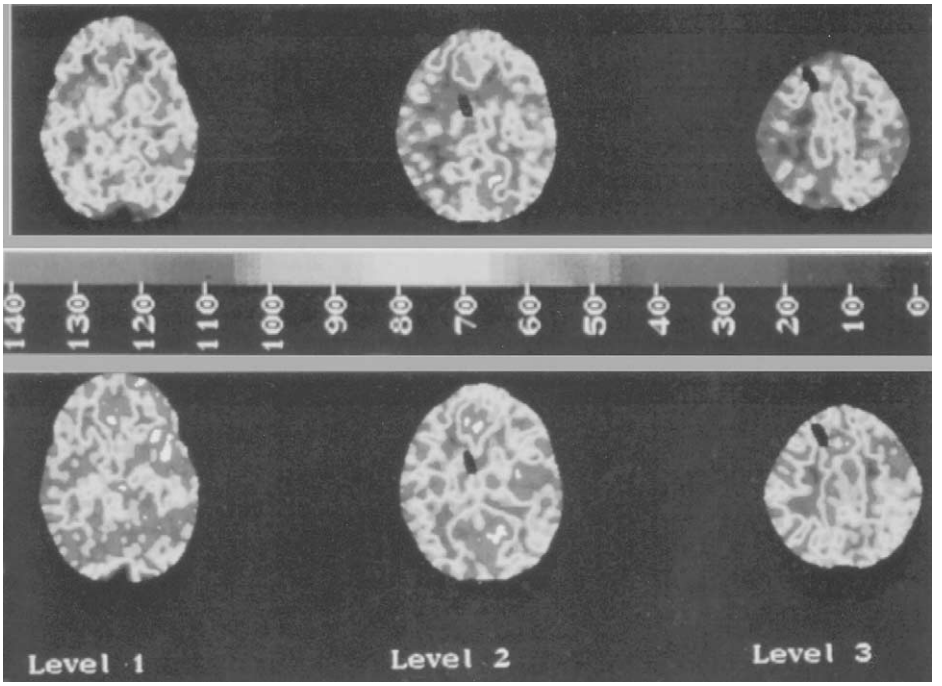

Fig. 4. CBF as measured by stable xenon CT in a shaken baby with refractory intracranial hypertension despite maximal medical management including barbiturate coma. (*Top row*) Normal or increased CBF at a cerebral perfusion pressure (CPP) of approximately 40 mm Hg. (*Bottom row*) Increasing blood pressure by norepinephrine administration (despite CPP within the autoregulation range) resulted in a concomitant increase in CBF and intracranial pressure, demonstrating a loss of autoregulation. (See also Color Plate 2.)

children, however, may support glutamate dysregulation as a primary event rather than representing cell leakage. The pathologic increase in glutamate concentration may be caused by reversed action of the glutamate transporters [25].

Glutamate is the most abundant excitatory neurotransmitter, used in up to 70% of synapses [26], and it is the most important mediator of excitotoxicity. The glutamate receptors fall into two categories: those that have direct actions on ion channels (ionotropic receptors) and those that are linked to second messenger systems (metabotropic receptors [Fig. 6]). The ionotropic receptors are named for the high-affinity analogues that have activity at that receptor, specifically the *N*-methyl-D-aspartate (NMDA), the α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), and the kainate receptor. These receptors can be subdivided further into those that cause receptor-mediated, non–voltage-dependent activation of ion channels with movement primarily of sodium, although there are some subtypes that allow calcium flux. The AMPA and kainate receptors are of this category. The NMDA receptor, on the other hand, requires a voltage-dependent release of magnesium ion from the associated ion channel with subsequent sodium, potassium, or calcium flux [27] and requires glycine as a coagonist [28]. Additionally, there are glutamate transporters on neurons and glial cells that are important for glutamate reuptake.

The NMDA and AMPA receptors are primarily located on the postsynaptic membrane and are probably the most important for mediating glutamate-induced excitotoxic damage. Physiologically, the NMDA receptor is involved in long-term potentiation, which is the process involved in the formation of memory [29]. Until recently the functions of the AMPA and kainate receptors have been difficult to differentiate because of a lack of specific pharmacologic agents. The AMPA receptors, however, are almost exclusively involved in the fast component of synaptic transmission [30]. Kainate receptor activation reduces synaptic inhibition from γ-aminobutyric acid, implying a presynaptic location [31], although it may also have a

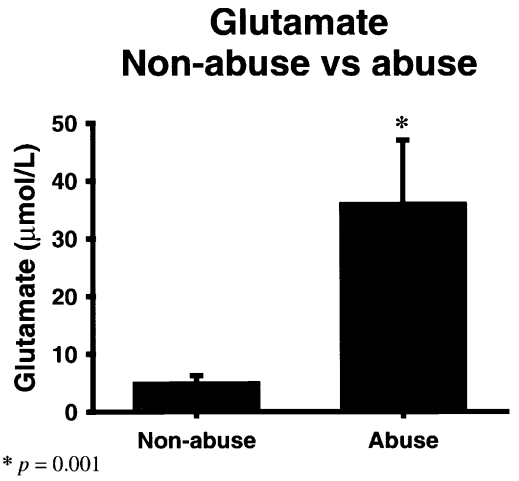

Fig. 5. Mean CSF glutamate concentration is significantly increased in abuse patients versus nonabuse patients. (*From* Ruppel RA, Kochanek PM, Adelson PD, et al. Excitatory amino acid concentrations in ventricular cerebrospinal fluid after severe traumatic brain injury in infants and children: the role of child abuse. J Pediatr 2001;138:18; with permission.)

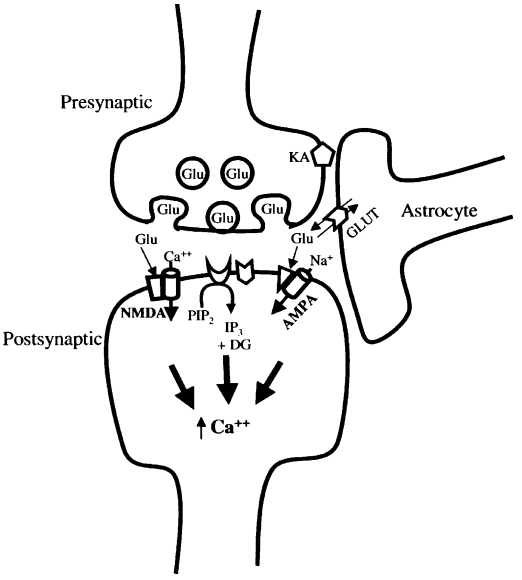

Fig. 6. Mechanisms of excitotoxicity. Glutamate causes an increase in intracellular calcium concentration through receptor-coupled ion channels (α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid [AMPA]); voltage-sensitive channels (*N*-methyl-D-aspartate [NMDA]); or second messenger systems (metabotropic glutamate channels). These channels are found in a postsynaptic location. Glutamate concentration is determined both by presynaptic release, which is mediated by the kainate receptor (KA), and through reverse action of the glutamate transporter (GLUT) found on neurons and astrocytes.

separate, postsynaptic location that causes depolarization of inhibitory neurons.

The subunit composition of the glutamate receptors changes during development, with resulting differences in function and susceptibility to excitotoxicity. The NMDA receptors in the immature rat brain have a weaker magnesium blockade than in adults [32], and are more sensitive to glycine activation [33]. Additionally, the chemical structure of the NMDA receptor channel complex in the immature brain is such that it allows greater amounts of calcium movement [34]. These differences exist because of the role the NMDA receptor plays in activity-dependent synaptic plasticity during development. The most notable example of this concept is the Nobel Prize–winning work that showed reorganization of the visual system in the kitten when input was deprived in one eye, demonstrating the need for tonic neuronal stimulation to preserve function and normal synaptic architecture [35]. These physiologic changes in receptor function become particularly important in pathologic situations because they increase the vulnerability of the immature brain to excitotoxicity. Indeed, an intracerebral injection of NMDA into the immature rat brain shows a dramatically larger area of neuronal destruction than in adults [36].

Developmental factors are particularly important when potential therapies directed at modifying glutamate activity are considered. These concerns have been highlighted by studies in the immature rat. NMDA blockade in 7-day-old rats (developmentally equivalent to third-trimester human fetuses) causes widespread apoptosis in 16 different areas of the brain [37]. Despite the vulnerability of the developing brain to excitotoxicity, this NMDA antagonist-stimulated apoptosis actually outweighs the beneficial reduction in excitotoxic cell death gained from blocking NMDA receptors after experimental TBI in 7-day-old rats [38]. Potentially relevant to this fact, child abuse victims have been shown to have reduced levels of antiapoptotic defense mechanisms, such as Bcl-2 [19]. This is discussed in more detail later in this article.

Although these are important considerations when considering antiexcitotoxic therapies, it is yet to be determined if this risk of inducing apoptosis in the infant brain with NMDA antagonists occurs in injured human brain (Fig. 7). The degree of excitotoxic insult to an infant with shaken baby

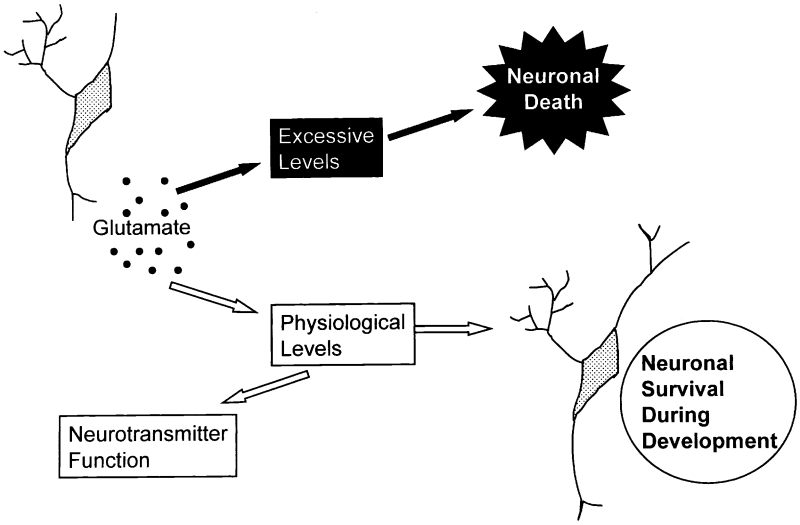

Fig. 7. Illustration of the potential risk in the use of glutamate antagonists in the developing brain after traumatic brain injury (TBI). Physiologic levels of glutamate are integral to neurotransmission; however, excessive levels of glutamate after TBI contribute to excitotoxicity-mediated neuronal death. In postnatal day (PND)-3 or -7 rats, treatment with NMDA antagonists after TBI reduced excitotoxicity-mediated neuronal death in the contusion. Apoptotic neuronal death outside the lesion, however, was exacerbated. This suggests there may be a theoretical risk to antiexcitotoxic treatments during development. The relevance of the PND-3 or -7 rat to infants and children after TBI, however, remains to be determined. (*From* Kochanek PM, Clark RSB, Ruppel RA, et al. Biochemical, cellular and molecular mechanisms in the evolution of secondary damage after severe traumatic brain injury in infants and children: lessons learned from the bedside. Pediatr Crit Care Med 2000;1:4–19; with permission.)

syndrome seems much more dramatic than what has been simulated thus far in experimental models. The fact that excitotoxicity seems to be maximal in abuse victims, most of whom are infants, presents a potential conundrum that will only be addressed with a treatment trial. Relevant to child-abuse victims, it also is important to determine if conventional sedative agents, with antagonistic effects at the NMDA receptor or other excitatory receptors, have detrimental effects in the injured infant brain.

Another important limitation to the use of therapies targeting excitotoxicity is the lack of success of previous clinical trials. Antagonists of the NMDA, AMPA, and metabotropic receptors have all exhibited beneficial effects in a variety of experimental models of TBI. Despite the success of these approaches in experimental brain injury, trials in human head injury have not been successful. The most notable example was a phase III trial of the competitive NMDA antagonist Selfotel. No benefit was demonstrated in this double-blinded, placebo-controlled trial on Glasgow Outcome Score despite enrollment of 693 patients with severe TBI [39]. The lack of benefit of glutamate antagonists in human trials may be a result of problems with study design, because the reported benefits in experimental models have been overwhelming. The central nervous system (CNS) penetration of some of these drugs is uncertain and the patients enrolled were a heterogeneous group. Also, the measures of outcome in clinical head injury trials are inexact at best. All of these factors contribute to unreliable results in treatment trials.

Oxidative stress

Oxidative stress describes the potential for free radical damage that exists in metabolically active tissues. Free radicals are generated during normal metabolism and a number of systems are in place to prevent the potential damage that can occur from oxidation of lipids, proteins, and DNA. Because of the high metabolic rate in the brain, a substantial amount of free radicals are continually generated, which mandates a generous antioxidant reserve. Depletion of these reserves can lead to oxidative stress and tissue damage by free radicals.

TBI initiates a number of processes that result in increased free radical production, including excitotoxicity, inflammation, and electron transport

chain dysfunction. The free radical molecules that are important potential mediators of secondary injury following TBI can be classified as reactive oxygen or reactive nitrogen species. The superoxide radical ($O_2^{\cdot -}$) is converted to hydrogen peroxide through the action of superoxide dismutase (SOD). Both are reactive oxygen species that create highly reactive hydroxyl radicals ($OH^{\cdot}$) in the presence of transitional metals, such as iron and copper. Hydroxyl radicals react with all biomolecules, so their potential for tissue damage is great. Nitric oxide is a reactive nitrogen species that has been linked to excitotoxicity. Nitric oxide reacts with the superoxide radical to form peroxynitrite, which causes lipid peroxidation, DNA damage, and protein oxidation.

Endogenous antioxidants exist to prevent the tissue damage that can result from free radical formation. The antioxidant enzymes that are important in the CNS are SOD, catalase, and glutathione peroxidase. The action of SOD is described previously, converting superoxide to hydrogen peroxide. Catalase then converts hydrogen peroxide to water and oxygen. Glutathione peroxidase can also metabolize hydrogen peroxide, in addition to peroxidated lipids. There are also antioxidant reserves that consist of the low-molecular-weight antioxidants glutathione, ascorbate, and tocopherol. Glutathione either serves as a substrate in the reactions mediated by glutathione peroxidase, or independently reacts with free radicals. Ascorbate and tocopherol can work in conjunction with each other. Tocopherol reacts with lipid radicals to prevent lipid peroxidation, and is then recycled by ascorbate. Ascorbate also scavenges numerous free radicals independently and is present in CSF in concentrations approximately 10 times higher than in plasma [40]. Interestingly, ascorbate also has pro-oxidant properties in the presence of free transitional metals.

A wealth of data exists to support the important role of oxidative stress in secondary injury after head trauma. Hydroxyl radical formation has been demonstrated following experimental closed-head injury in rats, and nitrite and nitrate concentrations (the oxidation products of nitric oxide) are increased in the CSF of humans following TBI [41]. Increased catalase and glutathione peroxidase activities have been found in rats following TBI [42], whereas total antioxidant reserves are decreased and lipid peroxidation products are increased [43].

There is also experimental evidence that supports a role for oxidative stress specifically in shaken baby syndrome [44]. Six-day-old rats were subjected to daily shaking for 3 days using a device that replicates the mechanical acceleration-deceleration injury seen in human infants that are shaken. Hydroxyl radical was increased 1 hour following the third episode of shaking, which was significantly attenuated by antioxidant therapy with tirilazad. Antioxidant treatment also decreased the amount of hemorrhage associated with shaking, but did not impact the loss of cortical tissue measured 1 and 2 weeks after injury. Treatment with tirilazad also improved the effect of antiexcitotoxic therapy in this experimental model [45].

Oxidative stress has been demonstrated in children following TBI [46]. F_2-isoprostane, a marker of lipid peroxidation, is increased fourfold in the CSF of children following head injury compared with control, indicating free radical damage to membrane lipids. Also, the antioxidant reserve of the CSF in these children was decreased, indicating a depletion of the normal antioxidative mechanisms present in the brain. Specifically, ascorbate levels in the CSF following head trauma were much lower than in control samples, with a corresponding increase in ascorbate radical, again supporting a role for ascorbate as a key antioxidant in the brain.

Similar to antiexcitotoxic therapies, clinical trials using antioxidants after TBI in adults have been unsuccessful. The two most prominent clinical trials evaluated the use of polyethylene glycol–SOD and tirilazad [47,48]. Despite the effort to make superoxide dismutase more permeable to the blood-brain barrier and increase its half-life by conjugation with polyethylene glycol, the ability of both of these substances to enter the brain is limited. Also, no measurement of oxidative stress was attempted in the patients enrolled in the study, thereby allowing a potentially heterogeneous group. With the current tools available to quantitate, at least in part, oxidative stress in individual patients, further antioxidant therapies may be more successful. Trials of antioxidant strategies in infants and children with severe TBI are needed.

Inflammation

The brain exhibits a vigorous inflammatory response to TBI. Trauma induces an acute-phase response within the brain, as evidenced by increased levels of tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 in the ventricular CSF of adults with head injury [49–51]. Similarly,

ventricular CSF levels of IL-6 are 60 times higher [52] and IL-1 about 5 times higher [53] in children with TBI than in controls. The acute-phase response is initiated by microglia and neurons and is responsible for fever, neutrophil mobilization, and possibly direct neurotoxicity. These cytokines are also responsible for stimulating inducible nitric oxide synthase, with subsequent free radical formation. A potential pathologic consequence of this inflammatory cascade is vasomotor paralysis, vasodilation, and posttraumatic hyperemia and edema.

There are unique aspects to the inflammatory response that occur after head injury that results from child abuse. IL-8, which is important for neutrophil chemotaxis, is increased in the CSF after head injury and is associated with both child abuse and mortality [54]. The soluble intercellular adhesion molecule-1 is also elevated in the CSF of children after head trauma, and is also associated with child abuse as a mechanism of injury [55]. This inflammatory response is similar in magnitude to that observed in children with meningitis. This suggests a particularly heightened acute inflammatory response in the shaken baby syndrome. This may result from the production of large brain regions with necrosis in severely injured abuse victims [56]. The anti-inflammatory cytokine IL-10 is also increased in the CSF of children after TBI and is associated with young age [52]. In this study, four of the five children less than 4 years old were shaken infants.

Another unique finding in victims of child abuse involves a marker of chronic inflammation, quinolinic acid. Infiltrating macrophages and microglia, in response to cytokine stimulation, produce quinolinic acid as a metabolite of l-tryptophan breakdown by the kynurenine pathway. Although systemically quinolinic acid has no known function, in the CNS it acts as an NMDA agonist, and has been implicated as a mediator of excitotoxicity [57]. Accidental TBI causes a progressive increase in quinolinic acid concentration in the CSF of adults and children, with a peak approximately 3 days after injury [58,59]. This is consistent with the development of a chronic inflammatory response and macrophage infiltration. Quinolinic acid concentration in the initial CSF sample on admission to the ICU, however, was much higher in shaken infants when compared with children with accidental trauma. This likely reflects the chronic nature of shaken baby syndrome or delay in seeking medical care after injury that often occurs.

Therapy directed at modifying the inflammatory process is a complicated proposition. There is evidence that inflammation may contribute to secondary damage following brain injury. Inflammation may be important, however, for initiating recovery and regeneration. In murine models of TBI, deficiency in either TNF-α or iNOS leads to worse long-term outcome [60,61]. This is true despite a short-term benefit to TNF-α deficiency and iNOS inhibition. Similarly, IL-6 and IL-8 have been shown to stimulate nerve growth factor production from cultured astrocytes, further supporting a regenerative role for acute-phase cytokines [62].

Programmed cell death

Cell death is a natural process, particularly in the developing organism when neuronal pruning occurs to refine neuronal connections. Programmed cell death describes an active process that requires energy-dependent activation of a cascade that results in DNA fragmentation and the morphologic appearance of apoptosis [63]. This is in contrast to necrotic cell death, which is typified by energy failure and resultant cellular swelling and lysis, with resulting release of cellular contents into the surrounding tissues. In TBI cellular death may have aspects of both mechanisms, representing an apoptosis-necrosis continuum [64]. As previously discussed in the case of NMDA-receptor activation and NMDA antagonists, this may complicate therapeutic decisions, especially in infants.

Processes that disrupt normal cellular function, such as energy depletion, oxidative stress, or excitotoxicity, can lead to mitochondrial damage. There is also a link between inflammation and programmed cell death in that IL-1–converting enzyme, which is increased in the CSF of children after TBI [53], is also linked to the apoptosis cascade. The release of cytochrome *c* from the mitochondrial membrane is the primary internal trigger for the apoptosis cascade [65]. Recently, studies from the laboratory of Clark and coworkers have demonstrated that cytochrome *c* is increased in the CSF of children with abusive head trauma [53]. Remarkably, CSF cytochrome *c* levels were increased exclusively in victims of child abuse or in infants and children who went on to die from severe accidental injuries. Whether this actually reflects triggering of apoptosis, or results from massive necrosis with release of cell contents, is unclear.

The Bcl-2 family of proteins regulates cytochrome *c* release, with both proapoptotic and

antiapoptotic members. Bcl-2 itself inhibits programmed cell death in vitro [66] and overexpression reduces cortical cell loss in experimental TBI in mice [67]. Bcl-2 is increased in the CSF of infants and children following severe TBI when compared with control and is independently associated with survival [19]. Of considerable relevance to the shaken baby syndrome, the concentration of Bcl-2 in the CSF of child-abuse patients was significantly lower than in children with accidental trauma on univariate analysis, with levels equivalent to control samples. Although mechanism of injury was not independently associated with Bcl-2 levels on multivariate analysis (likely because of the strong association between child abuse and mortality), the failure of child-abuse victims to upregulate this important endogenous defense protein may represent an important finding for understanding the pathophysiology of cell death after inflicted head injury. Whether this is part of an overall failure of specific defense mechanisms in child abuse or a nonspecific failure of protein synthesis remains to be determined.

Programmed cell death can also be initiated by an extracellular pathway that involves cell surface receptor for TNF or Fas [68]. Receptor activation sets in motion an apoptosis cascade that converges with the intracellular pathway at the level of caspase-3. Receptor activity is regulated, in part, by the presence of soluble receptors, which bind soluble ligands and prevent cell-surface activation. Soluble Fas ligand and receptor were measured in the CSF of children after TBI, because this system has been shown to be important in mediating programmed cell death in the CNS [69]. Soluble Fas receptor concentration is increased in the CSF of children after TBI, and is associated with child abuse as a mechanism.

Markers of neuronal injury measured in the CSF of children with TBI support a role for programmed cell death in abusive head trauma. Neuron-specific enolase is a glycolytic enzyme found primarily in the neuronal cytoplasm, and as expected is increased in the CSF of both adults and children after TBI [70,71]. Unlike children with accidental head trauma, who had a peak CSF neuron-specific enolase on the first day after injury, the children with abusive head trauma had a second, delayed rise in neuron-specific enolase concentration [70]. This second peak was higher than the initial peak, and was sustained up to 8 days after injury. This could be consistent with delayed neuronal cell death as a result of apoptosis. If apoptosis represents a particularly important cell death pathway in infants with shaken baby syndrome, it suggests the need to pursue novel therapies directed at this cascade.

Ischemic tolerance

An important component of the overall spectrum of disease present in shaken baby syndrome is the common occurrence of repeated injuries over time. Although there are obvious detrimental effects from these actions, there may actually be some protection afforded to neurons from previous, nonlethal insults. Ischemic tolerance describes the ability of cells to adapt to adverse but nonlethal ischemic conditions, and thereby be more resistant to further ischemic insults. This concept was first described in the context of ischemic heart disease when it was found that myocardial infarct size resulting from coronary occlusion in dogs was reduced if preceded by four brief periods of 5-minute ischemia [72]. This effect has also been demonstrated in the brain [73], showing that 2 minutes of global ischemia in gerbils reduced CA1 hippocampal cell loss after a subsequent, prolonged period of global ischemia. Ischemic preconditioning also reduces contusion volume and hippocampal cell loss following fluid-percussion injury in rats [74]. The developing brain also responds to this tolerance effect. Preconditioning with hypoxia protects the 7-day-old rat from subsequent hypoxic-ischemic brain injury [75]. The hypoxic-ischemic preconditioning event can even occur prenatally [76].

The mechanisms involved in this phenomenon are not clear. Low-level glutamate exposure can create tolerance in cell culture to subsequent excitotoxic challenges [77], and NMDA antagonists block the protective action of ischemic preconditioning in the global ischemia model in the gerbil [78]. Blockade of adenosine A_1 receptors also attenuates the effects of ischemic tolerance [79]. Heat shock proteins, which are important for thermal tolerance, are also induced by brain ischemia [80]. Nitric oxide [76], Bcl-2 [81], and superoxide dismutase [82] have all been implicated as contributing to this process. There is also a specific transcription factor induced called hypoxia-inducible factor–1 that modulates the expression of a number of target genes [83]. Hypoxic preconditioning in neonatal rats alters the enzymes involved in glucose transport and glycolysis, known target genes for hypoxia-inducible factor–1 [84]. Although this mechanism may occur in shaken baby syndrome, there are

important components to the process that limit its potential protective effect. The first limitation is the importance of timing. The protection that occurs from preconditioning occurs during a very specific time after the initial insult, probably beginning at about 12 to 24 hours after the insult, and lasting only 1 or 2 days. The second limitation is the need for the initial insult to be very mild, with no resulting damage. It is very unusual in shaken baby syndrome for a very mild insult to occur at the precise time necessary to afford protection from subsequent injury. In this context, the possible protection that may occur from a preconditioning type of event is vastly overshadowed by the resulting damage, minimizing its effect.

Considerations for the future

Shaken baby syndrome presents a very unique spectrum of disease within the field of pediatric trauma. The combination of trauma and ischemia, the presence of an immature or developing CNS, the unusual biomechanical forces at play, and the preceding social and medical factors with subsequent delays in diagnosis and treatment all conspire to make this entity unlike any other mechanism of head trauma. Because of the notoriously poor outcome of these children and the unique nature of this injury, these children are often excluded from both descriptive studies and therapeutic trials. Although this is appropriate in many situations, it obviates the need for separate trials designed specifically for victims of child abuse. An excellent example is a trial using decompressive craniotomy in shaken infants with intracranial hypertension, which may have shown some benefit to this approach [85]. Given the fortunate lack of large numbers of patients at any given institution, the need for multicenter trials is obvious. This is complicated by the variability of management that occurs with shaken babies, but is absolutely essential if therapeutic breakthroughs are to occur. Further investigation into the critical mechanisms of secondary injury may provide more insight into potential therapeutic targets [86]. Alternatively, it may be discovered that the interaction of these mechanisms is too complex to allow effective modification through targeted therapy with individual agents and that only treatments with widespread effects, such as hypothermia, or surgical decompression will ultimately be efficacious.

Acknowledgments

The authors thank the University of Pittsburgh Center for Injury Research and Control (CIRCL)/ Center for Disease Control, NINDS, NS30318, NICHD T32-NS07485-01, and the Laerdal Foundation for support. The authors also thank Marci Provins and Janice Hasch for assistance preparing the manuscript and Christopher Edwards and Bradley Stezoski for preparation of the figures.

References

[1] Muizelaar JP, Marmarou A, DeSalles AA, et al. Cerebral blood flow and metabolism in severely head-injured children. Part I: Relationship with GCS score, outcome, ICP, and PVI. J Neurosurg 1989;71:63–71.

[2] Adelson PD, Clyde B, Kochanek PM, et al. Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. Pediatr Neurosurg 1997;26: 200–7.

[3] Sharples PM, Stuart AG, Matthews DSF, et al. Cerebral blood flow and metabolism in children with severe head injury. 1. Relation to age, Glasgow Coma Score, outcome, intracranial pressure, and time after injury. J Neurol Neurosurg Psychiatry 1995;58:145–52.

[4] Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–15.

[5] Armstead WM. Role of endothelin-1 in age-dependent cerebrovascular hypotensive responses after brain injury. Am J Physiol 1999;277:H1884–94.

[6] Ruppel RA, Kochanek PM, Adelson PD, et al. Endothelin-1 is increased in cerebrospinal fluid following traumatic brain injury in children. Crit Care Med 1999;27:A76.

[7] Han YY, Carcillo JA, Ruppel RA, et al. Cerebrospinal fluid procalcitonin is increased after traumatic brain injury in children. Crit Care Med 1999;27:A75.

[8] Nilsson P, Hillered L, Ponten U, et al. Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. J Cereb Blood Flow Metab 1990; 10:631–7.

[9] Bell MJ, Robertson CS, Kochanek PM, et al. Interstitial brain adenosine and xanthine increase during jugular venous oxygen desaturations in humans after traumatic brain injury. Crit Care Med 2001;29:399–404.

[10] Robertson CL, Bell MJ, Kochanek PM, et al. Increased adenosine in cerebrospinal fluid after traumatic brain injury in infants and children. J Neurotrauma 1999;16:975 (Abstract).

[11] Clark RS, Carcillo JA, Kochanek PM, et al. Cerebrospinal fluid adenosine concentration and

uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. Neurosurgery 1997;41:1284–93.
[12] Bayır H, Kochanek PM, Janesko KL, et al. S-nitrosothiols increase after traumatic brain injury in humans. Society for Neuroscience Abstracts, 27: Program No. 214.10, 2001.
[13] Muizelaar JP, Ward JD, Marmarou A, et al. Cerebral blood flow and metabolism in severely head-injured children. Part 2. Autoregulation. J Neurosurg 1989;71:72–6.
[14] Bruce DA, Alavi A, Bilaniuk L, et al. Diffuse cerebral swelling following head injuries in children: the syndrome of "malignant brain edema". J Neurosurg 1981;54:170–8.
[15] Bergsneider M, Hovda DA, Shalmon E, et al. Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. J Neurosurg 1997;86:241–51.
[16] Biagas KV, Grundl PD, Kochanek PM, et al. Posttraumatic hyperemia in immature, mature, and aged rats: autoradiographic determination of cerebral blood flow. J Neurotrauma 1996;13: 189–200.
[17] Duhaime AC, Bilaniuk L, Zimmerman R. The "big black brain": radiographic changes after severe inflicted head injury in infancy. J Neurotrauma 1993;10:S59.
[18] Duhaime AC, Christian C, Moss E, et al. Long-term outcome in infants with the shaking-impact syndrome. Pediatr Neurosurg 1996;24:292–8.
[19] Clark RSB, Kochanek PM, Adelson PD, et al. Increases in Bcl-2 protein in cerebrospinal fluid and evidence for programmed-cell death in infants and children following severe traumatic brain injury. J Pediatr 2000;137:197–204.
[20] Rosenberg PA, Aizenman E. Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. Neurosci Lett 1989;103:162–8.
[21] Brown JI, Baker AJ, Konasiewicz SJ, et al. Clinical significance of CSF glutamate concentrations following severe traumatic brain injury in humans. J Neurotrauma 1998;15:253–65.
[22] Ruppel RA, Kochanek PM, Adelson PD, et al. Excitatory amino acid concentrations in ventricular cerebrospinal fluid after severe traumatic brain injury in infants and children: the role of child abuse. J Pediatr 2001;138:18–25.
[23] Palmer AM, Marion DW, Botscheller ML, et al. Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. Neuroreport 1994;6:153–6.
[24] Obrenovitch TP, Urenjak J. Is high extracellular glutamate the key to excitotoxicity in traumatic brain injury? J Neurotrauma 1997;14:677–98.
[25] Rossi DJ, Oshima T, Attwell D. Glutamate release in severe brain ischaemia is mainly by reversed uptake. Nature 2000;403:316–21.
[26] Fonnum F. Glutamate: a neurotransmitter in mammalian brain. J Neurochem 1984;42:1–11.
[27] Nowak L, Bregestovski P, Ascher P, et al. Magnesium gates glutamate-activated channels in mouse central neurons. Nature 1984;307:462–5.
[28] Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987;325:529–31.
[29] Bliss TV, Collingridge GL. A synaptic model of memory: long term potentiation in the hippocampus. Nature 1993;361:31–9.
[30] Trussel LO, Zhang S, Raman IM. Desensitization of AMPA receptors upon multiquantal neurotransmitter release. Neuron 1993;10:1185–96.
[31] Rodriguez-Moreno A, Herreras O, Lerma J. Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. Neuron 1997; 19:893–901.
[32] Kato N, Yoshimura H. Reduced Mg^{2+} block of the *N*-methyl-D-aspartate receptor-mediated synaptic potentials in developing visual cortex. Proc Natl Acad Sci USA 1993;90:7114–8.
[33] Zhong J, Gribkoff VK, Molinoff PB. Use of subunit-specific anti-sense oligodeoxynucleotides to define developmental changes in the properties of *N*-methyl-D-aspartate receptors. Mol Pharmacol 1996;50:631–8.
[34] Barnashav N, Schoepfer R, Monyer H, et al. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. Science 1992;257:1415–19.
[35] Wiesel TN, Hubel DH. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 1965;28: 1029–40.
[36] McDonald JW, Silverstein FS, Johnston MV. Neurotoxicity of *N*-methyl-D-aspartate is markedly enhanced in developing rat central nervous system. Brain Res 1988;459:200–3.
[37] Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283: 70–4.
[38] Pohl D, Bittigau P, Ishimaru MJ, et al. *N*-Methyl-D-aspartate antagonists and apoptotic cell death triggered by head trauma in developing rat brain. Proc Natl Acad Sci U S A 1999;96:2508–13.
[39] Morris GF, Bullock R, Marshall SB, et al. Failure of the competitive *N*-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two phase III clinical trials. J Neurosurg 1999;91:737–43.
[40] Spector R, Eells J. Deoxynucleoside and vitamin transport into the central nervous system. Fed Proc 1984;43:196–200.
[41] Clark RSB, Kochanek PM, Obrist WD, et al. Cerebrospinal fluid and plasma nitrite and nitrate concentrations after head injury in humans. Crit Care Med 1996;24:1243–51.

[42] Goss JR, Taffe KM, Kochanek PM, et al. The antioxidant enzymes glutathione peroxidase and catalase increase following traumatic brain injury in the rat. Exp Neurol 1997;146:291–4.
[43] Tyurin VA, Tyurina YY, Borisenko GG, et al. Oxidative stress following TBI in rats: quantitation of biomarkers and detection of free radical intermediates. J Neurochem 2000;75:2178–89.
[44] Smith SL, Andrus PK, Gleason DD, et al. Infant rat model of the shaken baby syndrome: preliminary characterization and evidence for the role of free radicals in cortical hemorrhaging and progressive neuronal degeneration. J Neurotrauma 1998;15: 693–705.
[45] Smith SL, Hall ED. Tirilazad widens the therapeutic window for riluzole-induced attenuation of progressive cortical degeneration in an infant rat model of the shaken baby syndrome. J Neurotrauma 1998;15:707–19.
[46] Bayır H, Kagan VE, Tyurina YY, et al. Assessment of antioxidant reserve and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. Crit Care Med 2000; 28:A52.
[47] Marshall LF, Maas AIR, Marshall SB, et al. A multicenter trial on the efficacy of using tirilizad mesylate in cases of head injury. J Neurosurg 1998;89: 519–25.
[48] Young B, Runge JW, Waxman KS, et al. Effects of pegorgotein on neurologic outcome of patients with severe head injury: a multicenter, randomized controlled trial. JAMA 1996;276:538–43.
[49] Goodman JC, Robertson CS, Grossman RG, et al. Elevation of tumor necrosis factor in head injury. J Neuroimmunol 1990;30:213–7.
[50] Kossmann T, Hans VHJ, Imhof H-G, et al. Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. Shock 1995;4:311–7.
[51] McClain CF, Cohen D, Phillips R, et al. Increased plasma and ventricular fluid interleukin-6 levels in patients with head injury. J Lab Clin Med 1991; 118:225–31.
[52] Bell MJ, Kochanek PM, Doughty LA, et al. Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. J Neurotrauma 1997;14:451–7.
[53] Janesko K, Satchell M, Kochanek P, et al. IL-1 converting enzyme (ICE), IL-1, and cytochrome *c* in SCF after head injury in infants and children. J Neurotrauma 2000;17:956 (Abstract).
[54] Whalen MJ, Carlos TM, Kochanek PM, et al. Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. Crit Care Med 2000;28:929–34.
[55] Whalen MJ, Carlos TM, Kochanek PM, et al. Soluble adhesion molecules in CSF are increased in children with severe head injury. J Neurotrauma 1998;15:777–87.
[56] Kochanek PM, Clark RSB, Ruppel RA, et al. Pediatric traumatic brain injury: from bench to bedside and back. In: Marwah J, Dixon E, Banik N, editors. Traumatic CNS injury. Scottsdale, AZ: Prominent Press; 2001. p. 251–72.
[57] Heyes MP. Metabolism and neuropathologic significance of quinolinic acid and kynurenic acid. Biochem Soc Trans 1993;21:83–9.
[58] Bell MJ, Kochanek PM, Heyes MP, et al. Quinolinic acid in the cerebrospinal fluid of children after traumatic brain injury. Crit Care Med 1999;27: 493–7.
[59] Sinz EH, Kochanek PM, Heyes MP, et al. Quinolinic acid is increased in CSF and associated with mortality after traumatic brain injury in humans. J Cereb Blood Flow Metab 1998;18:610–15.
[60] Scherbel U, Raghupathi R, Nakamura M, et al. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. Proc Natl Acad Sci U S A 1999;96: 8721–6.
[61] Sinz EH, Kochanek PM, Dixon CE, et al. Inducible nitric oxide syntases an endogenous neuroprotectant after traumatic brain injury in rats and mice. J Clin Invest 1999;104:647–56.
[62] Kossmann T, Hans V, Imhof H-G, et al. Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. Brain Res 1996;713:143–52.
[63] Steller H. Mechanisms and genes of cellular suicide. Science 1995;267:1445–9.
[64] Portera-Cailliau C, Price DL, Martin LJ. Excitotoxic neuronal death in the immature brain is an apoptosis-necrosis morphological continuum. J Comp Neurol 1997;378:70–87.
[65] Cai J, Yang J, Jones DP. Mitochondrial control of apoptosis: the role of cytochrome *c*. Biochim Biophys Acta 1998;1366:139–49.
[66] Hockenbery D, Nunez G, Milliman C, et al. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990;348: 334–6.
[67] Raghupathi R, Fernandez SC, Murai H, et al. Bcl-2 overexpression attenuates cortical cell loss after traumatic brain injury in transgenic mice. J Cereb Blood Flow Metab 1998;18:1259–69.
[68] Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998;281:1305–8.
[69] Cheema ZF, Wade SB, Sata M, et al. Fas/Apo [apoptosis]-1 and associated proteins in the differentiating cerebral cortex: induction of caspase-dependent cell death and activation of NF-kappa B. J Neurosci 1999;19:1754–70.
[70] Berger RP, Janesko KL, Wisniewski SR, et al. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics, 2002; 109(2), URL: http://www.pediatrics.org/cgi/content/full/109/2/e31.

[71] Vazquez M, Rodriquez-Sanchez F, Osuna E, et al. Creatine kinase BB and neuron specific enolase in cerebrospinal fluid in the diagnosis of brain insult. Am J Forensic Med Pathol 1995;16:210–4.
[72] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74: 1124–36.
[73] Kitagawa K, Matsumoto M, Tagaya M, et al. "Ischemic tolerance" phenomenon found in the brain. Brain Res 1990;528:21–4.
[74] Perez-Pinzon MA, Alonso O, Kraydieh S, et al. Induction of tolerance against traumatic brain injury by ischemic preconditioning. Neuroreport 1999;29:2951–4.
[75] Gidday JM, Fitzgibbons JC, Shah AR. Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. Neurosci Lett 1994;168:221–4.
[76] Xiao F, Fratkin JD, Rhodes PG, et al. Reduced nitric oxide is involved in prenatal ischemia-induced tolerance to neonatal hypoxic-ischemic brain injury in rats. Neurosci Lett 2000;285:5–8.
[77] Marini AM, Paul SM. *N*-methyl-D-aspartate receptor mediated neuroprotection in cerebellar granule cells requires new RNA and protein synthesis. Proc Natl Acad Sci U S A 1992;89:6555–9.
[78] Kato H, Liu Y, Araki T, et al. MK-801, but not anisomycin, inhibits the induction of tolerance to ischemia in the gerbil hippocampus. Neurosci Lett 1992;139:118–21.
[79] Heurteaux C, Lauritzen I, Widmann C, et al. Essential role of adenosine, adenosine A_1 receptors, and ATP-sensitive K+ channels in cerebral ischemic preconditioning. Proc Natl Acad Sci U S A 1995;92: 4666–70.
[80] Currie RW, Ellison JA, White RF, et al. Benign focal ischemic preconditioning induces neuronal Hsp70 and prolonged astrogliosis with expression of Hsp27. Brain Res 2000;863:169–81.
[81] Chen J, Simon R. Ischemic tolerance in the brain. Neurology 1997;28:306–11.
[82] Gorgias N, Maidatsi P, Tsolaki M, et al. Hypoxic pretreatment protects against neuronal damage of the rat hippocampus induced by severe hypoxia. Brain Res 1996;714:215–25.
[83] Bergeron M, Gidday JM, Yu AY, et al. Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. Ann Neurol 2000;48:285–96.
[84] Jones NM, Bergeron M. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. J Cereb Blood Flow Metab 2001;21:1105–14.
[85] Cho DY, Wang YC, Chi CS. Decompressive craniotomy for acute shaken/impact baby syndrome. Pediatr Neurosurg 1995;23:192–8.
[86] Kochanek PM, Clark RSB, Ruppel RA, et al. Biochemical, cellular and molecular mechanisms in the evolution of secondary damage after severe traumatic brain injury in infants and children: lessons learned from the bedside. Pediatr Crit Care Med 2000;1:4–19.