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CHAPTER 3

Linking impact to cellular and molecular sequelae of CNS injury: Modeling in vivo complexity with in vitro simplicity

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Abstract: Traumatic brain injury (TBI) represents one of most common disorders to the central nervous system (CNS). Despite significant efforts, though, an effective clinical treatment for TBI is not yet available. The complexity of human TBI is modeled with a broad group of experimental models, with each model matching some aspect of the human condition. In the past 15 years, these in vivo models were complemented with a group of in vitro models, with these in vitro models allowing investigators to more precisely identify the mechanism(s) of TBI, the different intracellular events that occur in acute period following injury, and the possible treatment of this injury in vitro. In this paper, we review the available in vitro models to study TBI, discuss their biomechanical basis for human TBI, and review the findings from these in vitro models. Finally, we synthesize the current knowledge and point out possible future directions for this group of models, especially in the effort toward developing new therapies for the traumatically brain injured patient.

Keywords: traumatic brain injury; biomechanics; in vitro models

Introduction

The enormous consequences of traumatic brain injury (TBI) continue in society, despite the rapid advance of technology to reduce the severity of injuries and new approaches in trauma patient care. Even with these changes, traumatic brain injuries remain the leading cause of death in people less than 45 years old (Thurman et al., 1999). The incidence rate is equally startling — the number of people hospitalized each year for traumatic brain injuries exceed those diagnosed with multiple sclerosis, breast cancer, and spinal cord injury combined (Langlois and Sattin, 2005). In the elderly, TBI is only eclipsed by cardiovascular disease and cancer as a major cause of death.

Clinically, brain injuries are categorized broadly as focal or diffuse (Gennarelli et al., 1982a). Focal injuries are readily observable lesions that appear on standard CT or MRI scans, and include injuries to the vasculature (epidural, subdural hematoma), the microvasculature (cerebral contusions), and visible tears in the brain parenchyma (intracerebral hemorrhage). Diffuse injuries are diagnosed when no visible lesions are present using conventional imaging, yet the patient has clear neurological impairment. A major substrate of diffuse brain injuries is diffuse axonal injury (DAI), but this category of injury also includes diffuse brain swelling

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and brain edema (Graham et al., 1995; Povlishock and Katz, 2005).

Generations of investigators directed their efforts toward understanding the mechanisms of TBI. From these efforts, researchers and clinicians recognize that TBI for a given patient is not captured well with only a 'diffuse' or 'focal' description. Rather, many different injuries are grouped with the broad clinical subtypes and leads to a tremendous diversity of injuries in the patient population. Injury mechanisms share a similar diversity — the mechanisms of injury for one specific traumatic injury may not apply universally to other injury types, and vice versa. As a field, we often use a reductionist approach to understand individual components of clinical TBI.

Models to study the in vivo complexity of TBI exist in different species, along different length scales that span from the single cell to the whole organism. The purpose of this review is to provide a current synthesis of the findings from models intended to study TBI in vitro, with an emphasis on discussing how these models relate to experimental efforts using animal models of TBI. In turn, we will summarize the findings from these models and point out new areas of opportunity where these models may prove invaluable in developing new treatments for TBI.

Biomechanical mechanisms that cause TBI in vivo

A discussion of the linkages between in vitro and in vivo experimental TBI studies would be incomplete without a brief review of the underlying biomechanical mechanisms that cause TBI. One major aim of in vitro models is to replicate the underlying physical forces that the tissue experiences during traumatic injury. However, the forces experienced by the tissue during even a single head impact can vary greatly with impact direction, force, and the impacting surface. A person designing an in vitro model must ask — which of these forces to the tissue are important for causing injury? In addition, one asks — how does one control the inherent variability of these forces?

Pressures developed within the brain during injury were considered one of the primary mechanisms for causing immediate impairment after brain injury, with the first modern studies dating back over six decades (Denny-Brown and Russel, 1941; Denny-Brown, 1945). These studies were soon followed by a number of in vivo experimental models that simulated these pressures to cause a concussive insult in animals (Stalhammar and Olsson, 1975; Sullivan et al., 1976). The intracranial pressure patterns that are generated in the human brain during impact is known (Nahum et al., 1977) and is predicted accurately with computational models (e.g., Zhang et al., 2004). Therefore, one can draw a clear relationship between the pressures generated in the in vivo models and within the brain during TBI. The effect of transient pressure changes on tissue function, though, is less clear. Of the possible mechanisms, there is only consensus that pressure cause damages is when the pressure drops below the cavitation threshold for tissue, thereby causing immediate (primary) tissue damage (Nusholtz et al., 1995). Much less agreement exists on whether high positive pressures will cause impairment, or an additional physical injury mechanism is needed.

Tissue deformation during impact is the second major physical mechanism explaining the primary patterns of injury in the brain. Although the brain shape under pressure loading largely does not change, the compliant properties of the tissue make the brain susceptible to shearing deformations during impact. Until recently, we knew little about the properties of the brain during situations causing injury. Recent information suggests that the brain softens as it deforms (Prange and Margulies, 2002; Coats and Margulies, 2006), an intriguing material property that may lead to unexpected patterns of tissue damage. Although there is wide recognition that brain deformation/stress is a major mechanism leading to cellular damage, exactly how the stresses are transferred from the tissue to the cellular structures within the material are not well known. Estimates of the tissue strain needed to cause injury are now available (Shreiber et al., 1997; Bain and Meaney, 2000; Zhu et al., 2006), but there remains considerable discussion about how these strains should be modeled in either in vivo or in vitro models, and if these

models can truly capture the complete simulation of injury.

Reproducing injury mechanisms with in vivo TBI models

Much like other diseases and disorders, an investigator faces several challenges when modeling human TBI in an animal model. The clinical mix of focal and diffuse injuries conflicts with the need to develop a consistent, reliable, and repeatable model in the laboratory. Inevitably, the investigator chooses which components of clinical TBI to study in the laboratory. Here, we briefly categorize the different experimental models and describe their uses. We use this description as background material for the in vitro models discussed in the next section.

For nonpenetrating injuries, an early area of study was to model the effects of pressure loading on the brain, as the concussive effects of pressure were reported in the early literature (Denny-Brown, 1945). The effect of pressure led to the so called 'percussion concussion' models (Gennarelli, 1994) that used a pulse of either air or fluid on the exposed cortex to cause neurological impairment. The most common model in current use is the fluid percussion model, available in many species and used in different configurations (Stalhammar and Olsson, 1975; Sullivan et al., 1976; Dixon et al., 1988; McIntosh et al., 1989a). Early work with fluid percussion showed how the pressure applied to the cortex was dissipated throughout the brain and the spinal canal (Stalhammar and Olsson, 1975). Later work showed that this pressure also caused a movement of intracranial tissue, leading to pressure and strain throughout the brain (Thibault et al., 1992). Possibly due to the complex mechanics of this model, different variations of the percussion model can lead to forms of injury that resemble components of human TBI.

A more recent technique to study brain injury in vivo is to directly deform the brain with a solid indentor, often referred to as the cortical impact model (Lighthall, 1988). The advantages of the model include a highly quantified impact condition, an ability to easily scale the impact condition across species (Smith et al., 1995), and modifying the model to injure different areas of the cortex. Recent modifications of the model used a direct skull impact, leading to a closed head impact model that could be considered closer to the clinical condition. With the precise control of the model, the cortical impact model is frequently the choice when studying TBI in transgenic animals. From an injury mechanism standpoint, the cortical model is qualitatively similar to the fluid percussion technique — apply a mechanical input locally to the cortex and study the progressive pattern of injury throughout the brain.

A final method for TBI models is using acceleration to injure the brain, reproducing a common mechanical loading that causes TBI in humans. Studies show the necessary acceleration to cause injury increases quickly with decreasing brain mass (Ommaya et al., 1967); therefore, the acceleration method is most readily used in large animal species that include the nonhuman primate and the miniature pig (Gennarelli et al., 1982b; Meaney et al., 1995). Although models using acceleration input in small animals (e.g., rat, ferret) appear in the literature (Marmarou et al., 1994; Xiao-Sheng et al., 2000; Gutierrez et al., 2001), it is difficult to separate the effects of the acceleration from the possible shape changes in the skull caused by the relatively large force needed to create these accelerations. A combination of the acceleration input and the higher proportion of white matter in the gyrencephalic brains of the nonhuman primate and pig makes these models ideal for studying injury to the white matter.

Broad categorization of in vivo models — what do they reproduce?

The different in vivo models described above are generally designed to simulate the mechanisms of injury that occur in humans. Results from in vivo models are sometimes difficult to interpret, though, as measurements of intracellular signaling, single cell function, and the role of different cell types are difficult. Moreover, the effect of mechanical and hypoxic injury are not easily separable with in vivo models, but are easily divided with in vitro

approaches. The use of in vitro models to simulate TBI began several decades ago and has significantly evolved in complexity (Morrison et al., 1998b). In this section, we describe the in vitro models that are currently in use to study TBI, and provide an in vivo correlate for these in vitro methods.

Scratch model/laceration

The most direct method for mechanical injury is to tear or lacerate cultures with a stylus or punch. The direct mechanical disruption of cultures is one of the earliest methods to study the progression of injury, beginning with tissue chunks (Epstein, 1971) but soon moving to mixed cultures of neurons and glia (Tecoma et al., 1989; Regan and Choi, 1994; Regan and Panter, 1995). The scratch method is well suited for high throughput drug screening, and has been used by Faden and colleagues to examine both inhibitors and antisense oligonucleotide treatment (Faden et al., 1997; Mukhin et al., 1997, 1998). This technology also scales easily to slice culture tissue (Sieg et al., 1999), and has suggested factors that influence the vulnerability or survivability of neurons in different regions of the brain. The direct mechanical injury remains in use, now with an emphasis that includes the release of molecules that could be considered biomarkers of the injury (Yang et al., 2006) and the potential factors that can regulate the migratory activity of glial cells to the injury site (Barral-Moran et al., 2003). The technique best correlates to the primary tissue tearing that can occur in different brain regions following severe head injury, a penetrating ballistic injury, or the local tissue damage that occurs with depressed skull fractures.

An interesting variation of the tearing model is using a laser to focally disrupt or transect the processes of neurons in culture (Gross et al., 1983). The relative position of the laser cut can be controlled and can be close to or distant from the neuronal soma, yielding distinct differences in cell fate (Lucas, 1987). The distance from the lesion to the soma also changes the subsequent ultrastructure response, as well as the electrophysiological properties of the cell (Lucas et al., 1985). Moreover, the cuts can lead to gene expression changes over time (Raghupathi et al., 1998). This remains probably the most precise model to study distal and proximal effects of transection. The model best corresponds to the physical disruption that can occur to some neuronal processes following injury (Maxwell et al., 1997), especially at the severe levels. The most important utility of the model, though, may be the ability to distinguish the role of local and remote signaling on cell survival/death following injury (Singleton et al., 2002).

Weight drop/compression

An additional and straightforward technique to use on cultures is to mechanically compress the cultures with a weight, akin to the weight drop method developed initially by Allen (1911) to study spinal cord injury in vivo. Indeed, one of the first in vitro models for central nervous system (CNS) injury used this technique of spinal cord cultures (Balentine et al., 1988). The technique is well suited to organotypic cultures that have a defined thickness and more realistic 3D architecture, and can be used to study the effects of both mechanical injury and a superimposed hypoxic injury (Adamchik et al., 2000). The order of the injuries can be changed, so that the mechanical injury can be considered the secondary injury, or vice versa. The technique to compress the tissue construct can also change; a dropped weight can be replaced by a rolling stainless steel bar, or a composite foam indentor over a region of the culture (Adamchik et al., 2000). Recently, this type of model showed the potential spreading depression that can occur from mechanical injury (Church and Andrew, 2005). One primary disadvantage of this technique, though, is drawing the direct in vivo correlate for this method. Crush injuries to the brain parenchyma are rare, and are complicated by overlying skull fracture.

Cell/substrate stretch model

Based on the number of completed studies, the most commonly used in vitro technique to study the consequences of TBI in vitro is the cell stretch

(or substrate deformation) model. These models replicate the magnitude and rate of tissue deformation that occurs in vivo during injury (Meaney et al., 1995), but often simplify the multiple oscillations of tissue deformation into a single, transient stretch insult. One early feature of the model was using a design where the cultured cells, plated to an elastic substrate, were simultaneously deformed in two directions. Although first used in astrocytes, this technique was rapidly tested in many different cell types of the CNS including neuron and neuron-like cell lines, endothelial cells, and, more recently, microglia (Cargill and Thibault, 1996; Ellis et al., 1995; McKinney et al., 1996; Rzigalinski et al., 1997). A variation of the initial stretch models is now available, where the cultures are stretched only in one direction (Lusardi et al., 2004). The stretch can be confined to just cultured axons to model diffuse axonal injury (Smith et al., 1999). Methods to stretch dissociated cultures transfer easily to studying the effects of stretch on tissue slice cultures (Morrison et al., 1998a), where the transfer between the substrate stretch and the resulting tissue stretch are defined (Cater et al., 2006). More recent work shows the cell stretch technique can scale to study the effect of more complex, 3D strain patterns on the morphology and viability of cells in tissue constructs (LaPlaca et al., 2005). With the increasing number of controlled mechanical manipulations available on cultures and tissue constructs, it is now possible to identify if CNS cells are uniquely vulnerable to a certain type of mechanical deformation (Geddes-Klein et al., 2006a), if the effects of injury are cumulative (Slemmer et al., 2002), or if the mechanism(s) of injury will change if the cells experience stretch, compression, or fluid shear deformation (LaPlaca et al., 1997).

Mechanoactivation — the first therapeutic target

The increasing diversity of models used to study TBI in vitro leads one to a common question — what processes do these forces activate rapidly following injury, and how do these 'mechanoactivated' signals lead to the pathological changes observed hours to days following the initial injury? Identifying these early changes in CNS cells provides initial therapeutic targets. Knowing how the mechanoactivated targets lead to subsequent intracellular events and/or consequences will naturally generate new therapeutic targets, is perhaps as important. In this section, we review the current knowledge on the early changes that occur following mechanical injury and their relative utility as a target for reversing the effects of the injury.

NMDAR are the most commonly studied mechanoactivated target

Alterations in ionic homeostasis have been observed across nearly all in vitro and in vivo models of TBI and therefore provide a natural starting point for identifying mechanoactivated receptors. Due to the role of glutamate receptors in both physiologic (learning and memory) and pathologic (stroke and epilepsy) conditions, *N*-methyl D-aspartate receptors (NMDAR) are among the most widely studied receptors responsible for the cytosolic calcium overload (Gagliardi, 2000; Arundine and Tymianski, 2004). Multiple models of TBI have shown that the bulk of the loss of ionic homeostasis can be attributed to activation of NMDARs. The connectivity of NMDARs to the actin cytoskeleton may provide a potential mechanism as to how these receptors are activated by stretch (Geddes-Klein et al., 2006b). One unique feature of mechanical injury to neurons is the mechanically induced reduction in the normal magnesium block of the NMDAR (Zhang et al., 1996). Altering the magnesium block of NMDARs changes the relative influence of NMDAR even under normal neurotransmission signaling and provides a therapeutic target that has been found to be somewhat effective in vivo (McIntosh et al., 1989a, b). It is worth noting that mechanically induced changes in the NMDAR properties are not observed across all models of TBI, including mixed cultures (Faden et al., 2001), or when the cultures are subject to a sublethal stretch (Arundine et al., 2003, 2004). Additionally, the mechanically induced activation or modulation of NMDARs leads to the propagation of intracellular calcium into adjacent, uninjured cells (Lusardi et al., 2004). While

NMDAR antagonists have not been effective in the clinic, recent data suggests that targeting the location of NMDAR (synaptic vs. extrasynaptic) may differentiate between the protective and excitotoxic processes of NMDARs (DeRidder et al., 2006).

Are other glutamate receptors involved?

The roles of other glutamate receptors are also beginning to generate serious attention in treating traumatic brain injuries. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) undergo a unique transformation, losing their rapid desensitization property following mechanical injury (Goforth et al., 1999). The loss in desensitization is persistent for at least 24 h following injury, and can be avoided if the NMDARs are inhibited prior to mechanical injury (Goforth et al., 2004). The impact of this loss in desensitization of the AMPARs on neuronal function is not yet well described, though. As AMPARs are centrally involved in fundamental processes such as synaptic plasticity and metaplasticity, even a transient alteration in the normal desensitizing properties of the AMPAR can lead to immediate and long term changes in neuronal network behavior.

Although there is evidence that inhibition of metabotropic glutamate receptors (mGluRs) will provide some protection against the effects of mechanical injury, it is not known if these changes are directly caused by mechanical modulation of the mGluRs, or if these protective effects are from simply inhibiting the activation of these receptors from enhanced glutamate levels following injury (Faden et al., 2001; Movsesyan et al., 2001; Movsesyan and Faden, 2006). One interesting recent theory is the role of potential physical coupling between the group I mGluR receptors, IP3, and phospholipase C (PLC) (Floyd et al., 2004). The role of different mGluR subtypes also reveals a complex interplay between mGluRs after injury, where the inhibition of one subtype (type III) can eliminate the protection offered by an antagonist directed against a second subtype (type II) (Movsesyan and Faden, 2006). Given the role of mGluRs in either enhancing or modulating synaptic efficacy (Gubellini et al., 2004; Simonyi et al., 2005), these mechanically initiated changes may have long lasting effects on synaptic physiology following injury.

Voltage-gated sodium channels can contribute to regional changes in neuronal axons

Voltage-gated sodium channels have been shown to indirectly contribute to shifts in intracellular calcium following mechanical injury. The relative role of the voltage-gated sodium channel only appears when the axonal segment of the neuron is deformed, potentially because the NMDAR and AMPAR mediated changes dominate for dendritic processes and the neuronal soma. Moreover, it is worth noting, that little in vitro work exists for myelinated axons due to the difficulties of creating myelinated cultures in vitro, even though it has been shown that myelinated axons respond differently to stretch than myelinated axon (Reeves et al., 2005). Studies show the sodium channel activation following mechanical injury leads to a dramatic and sudden rise in axoplasmic calcium, but not from calcium entering directly through the sodium channel (Wolf et al., 2001). Rather, the source of increased axoplasmic calcium is through voltage-gated calcium channels and through reversal of the sodium calcium exchanger. These changes in the sodium channel occur simultaneously with a larger, but reversible change in the morphology of axons subjected to mechanical injury (Smith et al., 1999). Over time, these sodium channels are the targets of proteolysis, which can lead to a sustained change in neuronal activity (Iwata et al., 2004).

Changes in mechanical permeability

One final consequence of mechanical force is an immediate, but transient, change in the plasma membrane permeability termed ‘mechanoporation’. In a series of studies, both the relative size and duration of these transient pores in the membrane were estimated following neuronal stretch (Geddes and Cargill, 2001; Geddes et al., 2003). These effects are also measured following traumatic injury in vivo, although these changes appear to occur over a

longer time period than in vitro preparations (Pettus et al., 1994; Stone et al., 2004; Farkas et al., 2006). It is not clear, though, how these changes in permeability can change for different neuronal regions (axon, dendrite, soma), or if this process is limited to one or more cell types. The effect of these transient pores can be minimized by resealing the membrane with surfactants (Serbest et al., 2006). Even though these changes may be brief, recent evidence suggests this mechanism may be capable of specifically stimulating different components of the mitogen-activated protein kinase (MAPK) cascade, and can therefore play a role in the ensuing cell death that occurs after injury (Serbest et al., 2006). However, the role of permeability increases in viability seems to be inconsistent across models, as some studies reveal no change in membrane resistance or permeability to a fluorescent dye (Tavalin et al., 1995; Zhang et al., 1996; Smith et al., 1999).

Mechanoactivation cascade — the next target

Even though the early events of mechanical injury in vitro are becoming clear, these efforts only begin to establish the complex cascade of events that will influence neuronal or glial survival after TBI. The initial mechanoactivated receptors and ensuing loss of ionic homeostasis activates a cascade of cellular processes that ultimately result in loss of function or cell death. Understanding these cascades will be essential to developing novel therapeutic targets that are admissible in a clinically relevant therapeutic window. We term these next events as the 'mechanoactivation cascade'. These cascades can occur rapidly following the initial mechanoactivation step, or can progress more slowly over time. In this section, we group these cascades according to the cell types studied to date in the CNS.

Neuronal cascades

The changes in neurons following mechanical injury include alterations in the electrophysiological properties, organelle function, receptor profile, and intracellular calcium buffering. Some of these changes lead to neuronal vulnerability, while others will transiently impair function. Early after injury, there is a delayed, but persistent depolarization that is linked to the alteration in the electrogenic sodium/potassium exchanger (Tavalin et al., 1995, 1997). The depolarization is triggered with a mild glutamate stimulation, and can be attenuated by restoring intracellular ATP levels. Findings from several groups show that mechanical injury leads to an enhanced response to glutamate up to 24 h following injury (Weber et al., 1999; Arundine et al., 2004; Geddes-Klein et al., 2006a), in turn leading to an enhanced vulnerability to glutamate excitotoxicity (Arundine et al., 2003). Although most reports focus on the role of the NMDAR as the underlying factor for this enhanced glutamate response, this is not the only ligand-gated receptor that changes following injury. The loss in desensitization of the AMPAR appears in parallel with an enhancement of AMPA currents after injury, as does the enhancement of γ-aminobutyric acid (GABA) currents. Both the AMPA and GABA current changes are linked to the NMDAR, as inhibiting the NMDA prior to injury eliminates the enhancement of both currents (Goforth et al., 2004; Kao et al., 2004). These changes may also contribute to the changes in neural network activity that appears following mechanical injury (Prado et al., 2005), although these changes remain to be explored in detail. These changes are likely among the initiating factors that contribute to failure to induce LTP in in vivo models and ultimately result in learning and memory deficits.

Neuronal mitochondria are also affected, with a reduction in the mitochondria membrane potential that can persist and which is dependent on the presence of surrounding glia (Ahmed et al., 2000, 2002). A series of reports show that the normal regulation of intracellular calcium stores are altered soon after mechanical injury, and this change in capacitive calcium influx can alter the homeostatic mechanisms for calcium induced calcium release (Weber et al., 2001; Chen et al., 2004; Weber, 2004).

Cysteine protease activation is dependent on the severity of the injury, and can be affected by NMDAR activation inhibition/activation (Pike et al., 2000; DeRidder et al., 2006). Inhibiting the activation of one cysteine protease, caspase-3, will have a short therapeutic window but also reveals

some crosstalk among the classic apoptotic and necrotic pathways (Knoblach et al., 2004). Gene expression changes correlate with the mechanical input applied to the culture (Morrison et al., 2000); some of these changes reflect changes observed in single neurons following TBI in vivo (O'Dell et al., 2000). The role of MAPKs in vitro are similar to the role observed in vivo — extracellular signal regulated kinase (ERK) inhibition may be a potential target (Mori et al., 2002), but the timing of the therapy needs to be better defined (Dash et al., 2002).

Astrocytic cascades

The role of astrocyte-based changes can influence not only glial reactivity in the injured brain, but also neuronal activity through the coupling of astrocytes and neurons at the synapse. Intracellular calcium signaling in astrocytes after injury is coupled to the presence of extracellular calcium, and the absence of extracellular calcium will influence astrocytic death (Rzigalinski et al., 1997). The complex intracellular regulation of calcium also changes, as stimulation of mGluRs is less coupled to IP3 mediated calcium release and is regulated, in part, by PLC (Rzigalinski et al., 1998; Floyd et al., 2001). Changes in intracellular sodium occur in parallel with these calcium changes and are linked with glutamate uptake in astrocytes, but may also be loosely coupled to intracellular calcium changes through changes in the sodium calcium exchanger (Floyd et al., 2005). These changes in ion homeostasis lead to alterations in MAPK signaling (Neary et al., 2003), some of which can be linked to the eventual glial reactivity phase that forms an important part of the neuropathology found in human TBI.

Perhaps more than studies using cultured neurons, there are now several reports focusing on the 'crossover' effect of mechanically injured astrocytes on other CNS cell types. For example, injured astrocytes generate isoprostanes that can influence vasoconstriction and reduce blood flow after trauma (Hoffman et al., 2000). Generation of reactive oxygen species from mechanically injured astrocytes can affect not only metabolic changes within astrocytes, but also mediate damage in adjacent neurons (Lamb et al., 1997). Changes in mitochondria membrane potential and ATP levels occur only transiently in astrocytes after injury, but the presence of the astrocytes in cultures change the time course of neuronal mitochondria changes (Ahmed et al., 2000). The presence of astrocytes also appears to influence the release of matrix metalloproteinases following injury in vitro, a factor that may significantly affect the regeneration phase after injury (Wang et al., 2002). Gene expression changes can be altered in the presence or absence of glia (Katano et al., 1999), indicating that the crossover effect extends to the genomic level.

What therapies have emerged?

A primary advantage of in vitro models is the ability to quickly test the efficacy of new or existing compounds in reducing the effects of mechanical injury. Most work to date, though, has focused on the mechanisms that contribute to either early changes in intracellular signaling or cell function. Surprisingly few studies have moved this work toward studying therapies that may be used for in vivo TBI treatment. Early work showed the effectiveness of NMDA antagonists (Regan and Panter, 1995), free radical scavengers (Lamb et al., 1997; Shah et al., 1997), and mGluR activation or inhibition (Allen et al., 1999; Movsesyan et al., 2001). More recent work includes the development of new peptides to reduce cell death after injury, even if the compound is delivered hours following injury (Faden et al., 2003, 2004, 2005). These peptides offer a strategic advantage over receptor antagonists by offering more selective inhibition of intracellular signaling triggered by a target receptor. Customized peptide designs can interfere with critical regulatory points in the mechanoactivation cascade, such as the point where NMDAR activation can lead to the formation of reactive oxygen species. Using a peptide to inhibit the linkage between the NMDAR and PSD-95, a prominent postsynaptic density protein, leads to an interruption in the ROS cascade and will lead to neuronal protection (Arundine et al., 2004). Delayed efficacy is also possible by using subunit specific

antagonists for the NMDAR (DeRidder et al., 2006), perhaps due to the recent data showing the dual role for NMDAR in cell survival and apoptosis (Hardingham. and Bading, 2003).

In vitro models are also useful in determining more specific biomarkers for both TBI diagnosis and treatment. A group of released factors have been found in the media following mechanical injury, with some contributing to the ensuing neuronal death. Injury using a stylus transection method is inhibited with NMDAR antagonists and aminosteroids (Regan and Panter, 1995), and the surrounding media can contain free radicals that, if applied to uninjured cultures, would cause apoptotic cell death within hours (Shah et al., 1997). A physical scratching injury also activates inflammatory mediated injury in neuronal cultures, as well as astrocytic migration (Fitch et al., 1999), MAPK activation, and the release of matrix metalloproteinases (MMPs) (Wang et al., 2002). Mechanical stretch injury will cause the synaptic release of zinc (Cho et al., 2003), an increase in s100β in the media (Willoughby et al., 2004). It is interesting to note that at least some of these molecules are being investigated as potential biomarkers for in vivo injury (Pineda et al., 2004).

Pointing toward the future

Collectively, this work shows the extensive efforts completed in understanding how mechanical forces are transmitted to cells of the CNS, what processes are activated by these mechanical forces, and how this information reveals different intervention points for treating the effects of mechanical injury in the CNS. Motivated by an understanding of the physical forces that occur within the tissue during injury, there are now models to study the effect of both simple and more complex mechanical loading to cultures. These models are well controlled, can be easily extended to understand more realistic loading conditions, and are even approaching the very complex question of how these forces are transferred to cells in 3D tissue. One needs to consider if additional in vitro models are needed, or if the complex mechanical environment is suitably modeled with existing technology. Certainly, the current work does not comprehensively address the initial mechanoactivation process for all cell types in the brain; notable exceptions are the oligodendrocytes, brain endothelial cells, and microglia. Even within a given cell type such as neurons, there are differences that appear among neurons from different brain regions. Moreover, there is little consensus information on how the culture platform — nearly pure or mixed cultures, tissue constructs, or organotypic slice cultures — contribute to the measured response; information to date show the potential synergistic interactions that appear between different cell types. These areas, as well as many others, can provide more insight into how these models will move toward representing the in vivo environment following injury.

In closing, it is worth noting that the natural division between in vivo and in vitro models of injury is slowly disappearing. The culturing techniques are becoming more sophisticated, allowing one to construct functional tissues with highly precise and more 'in vivo like' architectures. The methods to injure these cultures is now developed enough to mechanically load these constructs with any desired loading profile, mimicking the loading profiles occurring in vivo following injury. In comparison, rapid advances with in vivo imaging technology now allows one to use fluorescent indicator dyes in vivo to monitor shifts ion homeostasis, and technology is already emerging to monitor the electrical activity in living, awake animals over time. As a result, technologies and measurement normally restricted to in vitro systems are now available for testing in the in vivo animal. The increasing convergence of these different approaches will likely make comparative approaches in the future more common, and will lead to a more rapid translation of in vitro findings to the in vivo setting. Ultimately, this will translate into the more rapid testing of therapeutics in vivo that originated from in vitro systems, and lead to more therapeutic options for the traumatically brain injured patient.

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