Therapeutic Window Analysis of the Neuroprotective Effects of Cyclosporine A after Traumatic Brain Injury

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Abstract

Mitochondrial dysfunction plays a pivotal role in secondary cell death mechanisms following traumatic brain injury (TBI). Several reports have demonstrated that inhibition of the mitochondrial permeability transition pore with the immunosuppressant drug cyclosporine A (CsA) is efficacious. Accordingly, CsA is being moved forward into late-stage clinical trials for the treatment of moderate and severe TBI. However, several unknowns exist concerning the optimal therapeutic window for administering CsA at the proposed dosages to be used in human studies. The present study utilized a moderate (1.75 mm) unilateral controlled cortical impact model of TBI to determine the most efficacious therapeutic window for initiating CsA therapy. Rats were administered an IP dose of CsA (20 mg/kg) or vehicle at 1, 3, 4, 5, 6, and 8 h post-injury. Immediately following the initial IP dose, osmotic mini-pumps were implanted at these time points to deliver 10 mg/kg/d of CsA or vehicle. Seventy-two hours following the initiation of treatment the pumps were removed to stop CsA administration. Quantitative analysis of cortical tissue sparing 7 days post-injury revealed that CsA treatment initiated at any of the post-injury initiation times out to 8 h resulted in significantly less cortical damage compared to animals receiving vehicle treatment. However, earlier treatment begun in the first 3 h was significantly more protective than that begun at 4 and 8 h. Treatment initiated at 1 h post-injury (~68% decrease) was not significantly different than that seen at 3 h (~46% decrease), but resulted in significantly greater cortical tissue sparing compared to CsA treatment initiated at least 4 h post-injury (28% decrease). Together these results illustrate the importance of initiating therapeutic interventions such as CsA as soon as possible following TBI, preferably within 4 h post-injury, to achieve the best possible neuroprotective effect. However, the drug appears to retain some protective efficacy even when initiated as late as 8 h post-injury.

Key words: brain injury; cortical contusion; cyclosporine; cyclosporine A; mitochondrial permeability transition; neuroprotection

Introduction

MUCH OF THE BRAIN DAMAGE that occurs following acute traumatic brain injury (TBI) is due to a complex secondary injury cascade that is triggered by the primary mechanical trauma. Several of the mechanisms believed to be involved in cell death following TBI are excitotoxicity, inflammation, metabolic failure, oxidative stress/damage, calpain activation, and caspase activation (Deng-Bryant et al., 2008; Deng et al., 2007; Hall et al., 2004; Kelley et al., 2007; Lifshitz et al., 2003, 2004; McGinn et al., 2009; Morganti-Kossmann et al., 2007; Saatman et al., 2010; Singh et al., 2006). Several studies in recent years have indicated that mitochondria play a pivotal role in neuronal cell survival, and mitochondrial dysfunction is considered an early event in

central nervous system (CNS) injury that can cause neuronal cell death (Fiskum, 2000; Fiskum et al., 1999; Sullivan, 2005; Sullivan et al., 2004b; Zipfel et al., 2000). Experimental data also indicate that excitotoxicity may be the initial mechanism that leads to TBI-induced neuronal cell death due to accumulation of high levels of intracellular calcium (Choi et al., 1987; Faden et al., 1989; Zipfel et al., 2000).

Increases in cytosolic Ca^{2+} result in the rapid uptake of Ca^{2+} into the mitochondria, which act as cellular Ca^{2+} sinks, allowing mitochondria to respond to dynamic changes in cytosolic Ca^{2+} levels (Budd and Nicholls, 1996; Ichas and Mazat, 1998; Rizzuto et al., 2000; Sullivan et al., 2005). However, excessive increases in mitochondrial Ca^{2+} have been shown to have a detrimental effect on mitochondrial homeostasis by inhibiting ATP production, and increasing the

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production of reactive oxygen species (ROS), leading to the induction of mitochondrial permeability transition (mPT), which ultimately results in neuronal cell death (Ankarcrona et al., 1995; Dugan et al., 1995; Dykens, 1994; Reynolds and Hastings, 1995; Starkov and Fiskum, 2003; Sullivan et al., 2005, 1999b). Studies from our group have demonstrated that changes in mitochondrial Ca^{2+} levels/cycling are coupled with increases in oxidative damage and significant mitochondrial dysfunction, which occurs acutely and is progressive for up to 48 h post-injury (Maragos and Korde, 2004; Mbye et al., 2008; Pandya et al., 2009; Sullivan et al., 2004a, 2004b). The opening of the mitochondrial permeability transition pore (mPTP) is suggested to be a key mediator in this process.

It has now been clearly demonstrated that targeting the mPTP following TBI is a viable neuroprotective approach (Cook et al., 2009; Vink et al., 2001). Consistent with this effect, post-TBI administration of cyclosporine A (CsA), which is known to bind to cyclophilin D, an essential component of the mPTP, interferes with its construction and inhibits subsequent mPT, which has been shown to significantly attenuate post-traumatic mitochondrial dysfunction, and to subsequently decrease neuronal damage in multiple models of TBI (Alessandri et al., 2002; Buki et al., 1999; Okonkwo and Povlishock, 1999; Scheff and Sullivan, 1999; Sullivan et al., 2000a, 2000b, 1999b).

As a result of these neuroprotective findings across multiple laboratories, CsA has been studied in two Phase II clinical trials, which have shown it to be safe and well tolerated when doses as high as 5 mg/kg IV every 24 h were administered for 72 h in severe TBI patients (Hatton et al., 2008; Mazzeo et al., 2009b). In the first of those studies, which was a dose-escalation study involving treatment initiation within 8 h post-injury, there was a statistically significant dose-related improvement in favorable outcomes as assessed by the extended Glasgow Outcome Scale (GOS) score compared to placebo-treated patients. In contrast, the second Phase II study, which involved a broader 12-h post-injury treatment initiation window, did not show any evidence of efficacy (Mazzeo et al., 2009b). A comparison of these two studies appears to preliminarily suggest that the therapeutic efficacy of CsA, at least in severe TBI patients, may last as long as 8 h, but does not extend to 12 h.

Based on the multi-laboratory preclinical efficacy of CsA in rodent TBI models, and its safety, tolerability, and preliminary evidence of efficacy in severe TBI patients, CsA is being proposed for a Phase III multi-center clinical trial of its efficacy in severe TBI patients. However, several blind spots still exist concerning the therapeutic window of opportunity for CsA following TBI, especially regarding early time points for administration. Secondly, the proposed dosing paradigm for humans has not yet been tested in any experimental TBI models. Therefore, in the present study we decided to re-evaluate the therapeutic window for CsA in a controlled cortical impact model of TBI in order to get a more complete and accurate efficacy profile as a function of an increasing delay in post-injury administration. To accomplish this goal, we employed a dosage and dosing paradigm equivalent in terms of total drug exposure to that being proposed for the Phase III trial (Cook et al., 2009; Hatton et al., 2008). The results are generally consistent with those reported in severely injured TBI patients, which preliminarily suggest an 8-h

therapeutic window. However, we also observed that in order to obtain optimal neuroprotection using the proposed human dosing paradigm, CsA treatment should be initiated as soon as possible following TBI, preferably within 4 h post-injury.

Methods

Animals

Young adult male Sprague-Dawley rats (300–350 g) were used for these experiments. The animals ($n = 56$) were housed in group cages (3/group) with a 12-h light/dark cycle with free access to water and food. The core body temperature of all animals was maintained at 37°C throughout the surgical procedures and recovery period. All of the procedures and protocols were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Cortical contusion injury

The animals ($n = 56$) were subjected to a unilateral cortical contusion (1.75 mm) using an electronically-controlled pneumatic impact device (TBI 0310; Precision Systems & Instrumentation, Fairfax Station, VA) as previously described (Scheff and Sullivan, 1999; Sullivan et al., 1999a). The cortical contusion impact (CCI) injury used in these experiments results in significant loss of cortical tissue, blood-brain barrier disruption, and a loss of hippocampal neurons, as previously described (Baldwin et al., 1996, 1997; Scheff et al., 1997; Scheff and Sullivan, 1999; Sullivan et al., 1999a), thus mimicking the pathology of human TBI. All animals were anesthetized with isoflurane (2%) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) prior to CCI injury. At this point the animals were randomly assigned to one of seven treatment groups as described in the experimental design section. Following group designation, the skin was retracted and a 6-mm unilateral craniotomy was performed that was centered between the bregma and the lambda. The skull cap was removed without disruption of the underlying dura. The exposed brain was injured using a 5-mm beveled tip that compressed the cortex at 3.5 m/sec to a depth of 1.75 mm. Following injury surgical foam was laid upon the dura, the skull cap was replaced, and a thin coat of dental acrylic was spread over the craniotomy site and allowed to dry before the wound was stapled closed.

Experimental design

The animals ($n = 8$ /group) were randomly assigned to one of seven groups that received an intraperitoneal (IP) injection of CsA (20 mg/kg) in a solution of polyethylene glycol/sterile saline/cremophor oil solution at 1, 3, 4, 5, 6, or 8 h post-injury, or an equivalent volume of vehicle (300 μL) at 1 h post-injury. We have previously shown that this dose is optimal in rodent TBI studies, since lower and higher doses do not produce as great a neuroprotective effect in terms of an increase in cortical tissue sparing after CCI TBI (Sullivan et al., 2000a). Moreover, we estimated that the initial 20 mg/kg IP dose, which would achieve plasma levels in the rats roughly equivalent to that produced by the initial 2-h 2.5-mg/kg IV bolus dose proposed for patient studies (Cook et al., 2009; Hatton et al., 2008). Depending on group designation, the animals were anesthetized with isoflurane (2%) at the time point indicated, the surgical wound was unstapled and an osmotic mini-pump

was placed subcutaneously at the nape of the neck, and the wound was restapled (Sullivan et al., 2000b). The pumps were loaded prior to insertion with either CsA in a solution of polyethylene glycol/sterile saline/cremophor oil solution at concentrations measured to deliver 10 mg/kg/d (240 μ L/d total volume) for a period of 3 d, or an equivalent volume of vehicle. At 72 h post-pump implantation, all animals were anesthetized with isoflurane (2%) and the pumps were removed in all groups. As with the choice of the initial IP CsA dose, we estimated that the continuous subcutaneous infusion at 10 mg/kg/d for 72 h via osmotic mini-pump would pharmacokinetically mimic the planned 72-h human 5-mg/kg IV infusion (Cook et al., 2009).

Tissue processing and measurements of cortical tissue sparing

After a survival period of 7 days, the animals were anesthetized by an overdose of pentobarbital (95 mg/kg body weight), and transcardially perfused with physiological saline, followed by 10% buffered formalin (w/v) at pH 7.4. One animal (1-h group) was discarded from the analysis due to an overt infection at the injury. The brains were removed, post-fixed for 24 h, and subsequently placed in formalin-sucrose (15%) for an additional 24 h. Coronal sections 50- μ m thick were cut with a freezing microtome throughout the rostral-caudal extent of the damaged hemisphere, extending from the septal area (intra-aural level 10.7), to the most posterior extent of the hippocampus (intra-aural level -0.3). The sections were stained with cresyl violet and subjected to image analysis (BIOQUANT Image Analysis; R&M Biometrics, Nashville, TN). The extent of tissue sparing following TBI was assessed blindly with respect to group designation. The assessment employed an unbiased stereological protocol, the Cavalieri method (Michel and Cruz-Orive, 1988), as previously described (Davis et al., 2008; Pandya et al., 2007). Briefly, a systematic random subset of sections (minimum of 12), separated by a known distance (d) is selected for analysis from the region of interest. The distance d is known because it is the mean measured section thickness multiplied by the number of sections between the sampled sections. For each section, the total cortical area, defined as the dorsal aspect of lamina I to the dorsal aspect of the corpus callosum, was determined for each hemisphere. Each area was multiplied by d to calculate a subvolume, and the subvolumes were then summed to yield the total volume. The volume of the ipsilateral hemisphere is then compared with the volume of tissue measured in the contralateral hemisphere, and the results are expressed as percentage of tissue spared (ipsilateral/contralateral $\times$ 100). These methods obviate the need to adjust values due to possible differential shrinkage resulting from fixation and tissue processing. Analysis of histological data was performed utilizing an analysis of variance (ANOVA). When warranted ($p < 0.05$) *post-hoc* comparisons employed the Student-Newman-Keuls (SNK) multiple comparison test. Significance was set at $p < 0.05$.

Results

Therapeutic window of cyclosporine A treatment

To determine the most efficacious therapeutic window for initiating CsA therapy, the rats were administered an IP dose

of CsA (20 mg/kg) or vehicle at 1, 3, 4, 5, 6, and 8 h post-injury. At these same time points osmotic mini-pumps were implanted to deliver 10 mg/kg/d of CsA or vehicle (1-h time point only). Seventy-two hours after the initiation of treatment and pump placement, the pumps were removed from all animals. At 7 d post-injury, qualitative assessment of tissue damage revealed that all animals sustained conspicuous injury to the cortex immediately below the site of impact (Fig. 1). Rats treated with CsA appeared differentially affected, depending upon the time point CsA was initiated. Quantitative analysis of cortical tissue sparing using ANOVA revealed a significant group-dependent difference [$F(6,48) = 6.569$, $p < 0.0001$; Fig. 2]. *Post-hoc* comparisons (SNK) revealed that animals in which CsA treatment was initiated at any time point post-injury had significantly less cortical damage ($p < 0.05$) than animals receiving vehicle treatment. Treatment initiated at 1 h post-injury (~68% decrease) was not significantly different from 3 h treatment (~46% decrease), but resulted in a significant ($p < 0.05$) increase in cortical tissue sparing compared to CsA treatment initiated at 4, 5, 6, or 8 h post-injury (28% decrease).

Discussion

The present results are the first to demonstrate the efficacy of CsA when employing the proposed human dosing paradigm (Hatton et al., 2008), using a well-established rodent model of TBI. They also support and extend our previous reports by more clearly defining the optimal therapeutic window for administering CsA following TBI. The most effective CsA therapy was achieved when it was initiated at the earliest time point (1 h) post-injury, but all the groups receiving CsA therapy between 1 and 8 h post-injury demonstrated a significant increase in tissue sparing compared to vehicle-treated animals. These results illustrate that the mechanisms responsible for the neuropathology associated with TBI are modifiable by neuroprotective therapy for at least 8 h post-injury.

In previous experiments, we demonstrated a dose-response effect in which higher doses of CsA were as effective as lower doses (Scheff and Sullivan, 1999). In those experiments, animals received a single bolus IP injection of CsA immediately prior to or after (15 min post-injury) TBI, followed by an additional treatment 24 h later. Importantly, the post-injury therapy was almost as effective as pre-injury administration. Based on these findings, we determined the dose-response curve and the neuroprotective therapeutic window using bolus administrations (Sullivan et al., 2000a). However, in those studies we only assessed initiating treatment at 15 min and 1, 6, and 24 h post-injury, followed by a second bolus injection given 24 h later. We then addressed the important question concerning the need for a multiple post-injury dosing regimen, in part based on the findings that delaying treatment until 6 h post-injury did not result in significant neuroprotection, whereas initiating treatment at 1 h or 24 h post-injury did afford significant neuroprotection (Sullivan et al., 2000a).

It was demonstrated in those studies that all animals treated with CsA had significantly less cortical tissue damage than vehicle-treated controls (Sullivan et al., 2000b). Injured animals receiving only a single bolus injection were afforded the least amount of protection, and animals receiving a second

FIG. 1. Coronal sections through the damaged hemisphere in adult rats following a moderate controlled cortical impact (1.75 mm). The injury creates an obvious cavitation in the cortex immediately below the impact site that is apparent 7 days post-injury. Animals treated with cyclosporine (CsA) appeared differentially affected, depending upon the time point CsA treatment was initiated. The optimal sparing of cortical tissue was seen in animals that received CsA at 1 h post-injury (scale bar = 1 mm).

bolus injection 24 h post-injury were afforded even greater neuroprotection. Both groups receiving the continuous infusion of CsA for 7 days demonstrated the greatest sparing of tissue (Sullivan et al., 2000b). These results suggest that the first 24 h is a very critical window, given the fact that animals that received a bolus followed immediately by pump implantation were afforded significantly greater neuroprotection than when the initial bolus injection was coupled with a second bolus injection at 24 h post-injury (Sullivan et al., 2000b). Those results posed the question of whether or not the therapeutic efficacy window could be altered if a continuous infusion of CsA was employed. It also remained unclear if continuous pharmacological therapy would be necessary for

the entire post-injury recovery period. Previous studies have clearly demonstrated that continuous infusion of CsA for prolonged periods can have significant neurological side effects, ranging from seizures to overt cell death (Famiglio et al., 1989; Kahan, 1989; Patchell, 1994; Walker and Brochstein, 1988; Wijdicks et al., 1995). Not surprisingly, the bulk of the neurological complications have been found to be dose-dependent (Berden et al., 1985; de Groen et al., 1987; Hughes, 1990; Reece et al., 1991).

Additionally, when considering the treatment duration with therapeutic intervention, it is important to consider the time course of the target mechanism in order to design an appropriate dosing regimen. The proposed mechanism of the

FIG. 2. Cyclosporine (CsA) increases cortical tissue sparing across a wide therapeutic window that extends out to 8 h following traumatic brain injury (TBI). Adult Sprague-Dawley rats were administered either vehicle or a bolus of CsA (20 mg/kg IP) at 1, 3, 4, 5, 6, or 8 h post-injury. At these same time points mini-osmotic pumps loaded with vehicle or CsA were implanted subcutaneously and set to deliver 10 mg/kg/d. The pumps were then removed after 72 h, and tissue sparing was assessed at 7 days post-injury. All animals that received CsA had significantly more spared cortical tissue than the vehicle-treated animals. Initiating CsA treatment at 1 h post-injury was not significantly better than 3 h, but was significantly better than any time point >4 h post-injury (* $p < 0.05$ compared to vehicle treatment; # $p < 0.05$ compared to the 1-h treatment group).

neuroprotective action of CsA has been demonstrated to involve inhibition of mitochondrial failure in injured brain tissue by binding to cyclophilin D (cypD; Sullivan et al., 2005, 1999b). The binding of CsA to cypD prevents the formation of the mPTP, a catastrophic event with regard to neuronal cell survival. mPTP causes an immediate collapse in the mitochondrial membrane potential, which uncouples the electron transport system from ATP production (Sullivan et al., 2005). The release of pro-apoptotic molecules (e.g., cytochrome C, Smac/Diablo, and apoptosis-inducing factor) from the mitochondria is in part orchestrated by mPTP formation, and leads to the activation of cell death pathways (Jordan et al., 2003). An additional consequence of mPTP formation is the production of ROS, which contribute to cellular damage by oxidizing cellular proteins and lipids (Mazzeo et al., 2009a).

While several studies have demonstrated mitochondrial failure in rodent TBI models over the past 15 years, only recently have careful time course studies been carried out that demonstrate its temporal profile. First of all, in the mouse CCI-TBI model, we have shown that mitochondrial failure is significant by 3 h within the cortical tissue surrounding the injury site, and that this progressive failure peaks at 12 h (Singh et al., 2006). Secondly, several other studies have also determined that the onset of mitochondrial dysfunction is even more rapid in the tissue that makes up the core and penumbra following CCI. In these studies, significant loss of mitochondrial bioenergetics begins as early as 1 h post-injury, and continues for up to 48 h post-injury (Gilmer et al., 2009; Pandya et al., 2007, 2009). Furthermore, mitochondrial Ca^{2+} overload, which directly initiates mPTP formation, was found to occur in the same time frame as the loss of bioenergetics. However, both mitochondrial bioenergetics and Ca^{2+} loading were most amenable to treatment with a mitochondrial uncoupler administered within a 6-h post-injury window (Pandya et al., 2009). Thus, these data sets show that a critical

time for intervention occurs <6 h post-injury. In fact, early administration of CsA within a 3-h post-injury time window would be needed in order to maximally rescue mitochondria at the epicenter of the injury, and within the first 6 h to prevent mitochondrial failure in the cortical tissue surrounding the epicenter.

With all these factors in mind, the goal of the current study was to assess the optimal therapeutic window of CsA utilizing continuous dosing at the concentration proposed to be used in humans during the first 8 h post-injury. Our study design also included cessation of CsA treatment after 3 days (72 h), as currently planned for the proposed Phase III clinical trial in moderate and severe TBI patients. The 72-h CsA treatment duration has been suggested to improve outcome in a small Phase IIa study in severe TBI patients (Hatton et al., 2008). The current results demonstrate several things, including the fact that this proposed dosing paradigm for CsA affords significant neuroprotection, even if treatment is delayed for up to 8 h post-injury. However, it is apparent that the earlier treatment is initiated, the more neuroprotection is afforded, which is evident by the fact that beginning treatment at 1 h post-injury significantly increases tissue sparing compared to delaying the onset of treatment to 4 h or later. This becomes even more evident when one considers that the 1-h treatment group had ~68% less cortical damage than the vehicle-treatment group. Delaying treatment to 3 h post-injury resulted in a reduction of ~46% in cortical tissue damage, and delaying treatment to >4 h reduced cortical damage by ~28% compared to vehicle treatment.

These data clearly illustrate the importance of initiating treatment as soon as possible following TBI, and at a time point before the target mechanism, mitochondrial mPTP, has crested. In fact, these results were predicted based on the known time course of mitochondrial dysfunction that occurs in the tissue where sparing was being assessed in this study

(Gilmer et al., 2009; Singh et al., 2006). In contrast, in our previous findings we did not see any change in tissue sparing when treatment was initiated at 6 h post-injury (Sullivan et al., 2000a). However, this was not entirely unexpected, considering that the present experimental design employed a somewhat less severe injury paradigm than our previous study. Additionally, the current study employed a bolus injection coupled with a continuous CsA dosing strategy given over 72 h, whereas our previous study used two bolus injections separated by 24 h. Finally, in the current study CsA treatment was terminated at 72 h, compared to 7 days in our previous studies, yet the neuroprotection afforded was comparable (74% versus 68% reduction in cortical damage), even though treatment was delayed from 15 min to 1 h post-injury in the current study. These data would seem to indicate that after 72 h our target mechanism may be out of reach and may no longer be amenable to therapeutic intervention. Furthermore, the data also support recent findings demonstrating the post-traumatic evolution of neurodegeneration that appears to peak between 24 and 72 h post-injury (Hall et al., 2008).

The present study clearly demonstrates the utility of CsA to reduce the significant necrosis and secondary injury that occur following TBI. The dosing parameters employed in this study are comparable to the proposed human experimental design, and demonstrate that treatment should be initiated before 4 h post-injury to achieve optimal cortical tissue preservation, although neuroprotective efficacy appears to extend to as long as 8 h post-injury.

In closing, three issues should be addressed that are relevant to the extrapolation of the current study and results in the rat CCI-TBI model to future clinical trial design. The first concerns the question of whether an improvement in cortical tissue sparing (i.e., a reduction in overt cortical tissue loss) at 72 h is an adequate assessment of neuroprotection. For instance, the reduction in lesion volume in cresyl violet-stained brain sections does not reveal more subtle neuropathology, which can be seen in the tissue surrounding the lesion with other techniques such as de Olmos silver staining of axonal injury (Hall et al., 2008), or immunoblotting of axonal cytoskeletal degradation (Saatman et al., 1996; Thompson et al., 2006). However, recent studies from our laboratories have shown that a reduction is cortical lesion volume by CsA in the mouse CCI-TBI model is paralleled by at least a roughly proportional reduction in axonal neuropathology (e.g., silver staining and α -spectrin degradation) in the surrounding spared cortical tissue (Mbye et al., 2009).

A second issue has to do with the fact that the current study did not include any behavioral measures of motor functional recovery. While this is a legitimate limitation, we have recently shown that a 40% reduction in cortical lesion volume in the mouse CCI-TBI paradigm by CsA is associated with complete preservation of motor function at 7 days post-injury (Mbye et al., 2009). Thus it is reasonable to presume that the current 7-day post-injury cortical tissue preservation achieved by CsA, even when begun as much as 8 h post-injury, should be paralleled by an improvement in motor functional recovery.

A third legitimate concern regarding the current study is whether the CsA neuroprotective therapeutic window in a rodent TBI model will be similar to that in humans. While it is not possible to definitively state that the windows will be the same, recent studies in which the time course of biomarkers of

proteolytic degradation of neuronal cytoskeletal proteins (α -spectrin) were measured by immunoassay techniques in brain tissue and cerebrospinal fluid (CSF) from rodents after TBI, and in CSF of TBI patients, suggest that the pathophysiological time course of secondary injury may be similar for rodents and humans. After rat CCI-TBI, CSF α -spectrin degradation products are significantly elevated between 24 and 72 h (Pike et al., 2001), and the levels correlate with cortical lesion volume (Ringger et al., 2004). In the mouse CCI-TBI model, an increase in α -spectrin degradation products in injured brain tissue begins within the first hour, and does not peak until 24 h post-injury (Thompson et al., 2006), following the time of maximal mitochondrial failure at 12 h (Singh et al., 2006). Similarly, in human severe TBI patients, cortical tissue levels of α -spectrin degradation products peak between 24 and 48 h. Furthermore, CSF levels of α -spectrin degradation products are significantly increased after severe TBI in humans during the first 6 h post-injury (Brophy et al., 2009), and remain significantly elevated for the first 72 h (Brophy et al., 2009; Pineda et al., 2007). These results strongly suggest that the secondary injury cascade may proceed at a similar rate after rodent and human TBI, which suggests that the neuroprotective therapeutic window for CsA may also be similar. Thus, in the absence of evidence to the contrary, we believe that it is prudent to take the current rodent TBI data seriously when planning for future CsA clinical trials in TBI patients.

Acknowledgments

This research was supported by grants from the National Institutes of Health, U.S. Public Health Service grants R01 NS48191 and R01 NS062993 (to P.G.S.), and P30 NS051220 (to E.D.H.), and funding from the Kentucky Spinal Cord and Head Injury Research Trust.

Author Disclosure Statement

No competing financial interests exist.

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