Safety and Tolerability of Cyclosporin A in Severe Traumatic Brain Injury Patients: Results from a Prospective Randomized Trial

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Abstract

Cyclosporin A (CsA) has recently been proposed for use in the early phase after traumatic brain injury (TBI), for its ability to preserve mitochondrial integrity in experimental brain injury models, and thereby provide improved behavioral outcomes as well as significant histological protection. The aim of this prospective, randomized, double-blind, dual-center, placebo-controlled trial was to evaluate the safety, tolerability, and pharmacokinetics of a single intravenous infusion of CsA in patients with severe TBI. Fifty adult severe TBI patients were enrolled over a 22-month period. Within 12 h of the injury patients received 5 mg/kg of CsA infused over 24 h, or placebo. Blood urea nitrogen (BUN), creatinine, hemoglobin, platelets, white blood cell count (WBC), and a hepatic panel were monitored on admission, and at 12, 24, 36, and 48 h, and on days 4 and 7. Potential adverse events (AEs) were also recorded. Neurological outcome was recorded at 3 and 6 months after injury. This study revealed only transient differences in BUN levels at 24 and 48 h and for WBC counts at 24 h between the CsA and placebo patients. These modest differences were not clinically significant in that they did not negatively impact on patient course. Both BUN and creatinine values, markers of renal function, remained within their normal limits over the entire monitoring period. There were no significant differences in other mean laboratory values, or in the incidence of AEs at any other measured time point. Also, no significant difference was demonstrated for neurological outcome. Based on these results, we report a good safety profile of CsA infusion when given at the chosen dose of 5 mg/kg, infused over 24 h, during the early phase after severe head injury in humans, with the aim of neuroprotection.

Key words: cyclosporin A; neurological outcome; neuroprotection; safety; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) triggers a complex cascade of pathophysiological events, leading to progressive neuronal and axonal damage (Alessandri et al., 2002; Kroemer and Reed, 2000; Lewen et al., 2001; Okonkwo et al., 1999; Povlishock and Katz, 2005; Signoretti et al., 2004). The traumatically induced influx of calcium is recognized to be a causative factor in triggering early cell death and axonal damage. Elevated intracellular calcium has been linked to an

opening of the mitochondrial permeability transition pore, allowing calcium to flood the mitochondrion and causing mitochondrial swelling, the generation of oxygen free radicals, and the ultimate failure of mitochondrial function (Starkov et al., 2004; Xiong et al., 1997). Such mitochondrial dysfunction plays a significant role in TBI-induced early neuropathological events, causing the loss of ATP and increased production of reactive oxygen species (Fiskum, 2000; Lifshitz et al., 2004; Sullivan et al., 2005; Wang, 2001). These effects, in turn, lead to cell death by either necrotic or

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apoptotic routes, since pro-apoptotic factors such as caspase C are also released from mitochondria (Kroemer and Reed, 2000; Lewen et al., 2001; Li et al., 1997; Li et al., 2000; Sullivan et al., 2002; Uchino et al., 2002; Zou et al., 1997). Similarly, caspase 3 activation has been linked to intra-axonal cytoskeletal damage that leads to axonal failure and disconnection (Büki et al., 2000; Ferrand-Drake et al., 2003).

The complexity of TBI pathophysiology justifies the wide variety of therapeutic approaches that have been tested in experimental animal models and in humans over the last two decades, targeting numerous interrelated aspects of TBI-induced subcellular pathological mechanisms. Such therapeutic screening studies are also complicated by the fact that the patients affected by severe head injury are typically treated in intensive care units, and undergo invasive procedures such as mechanical ventilation, invasive monitoring, and multiple systemic drug therapies, and are thereby subject to multiple secondary risk factors. Accordingly, any proposed drug for use in TBI patients should first be rigorously tested for safety to avoid the possibility of further increasing morbidity and mortality in this already gravely ill patient population.

Recently, the role of cyclosporin A (CsA) as a neuroprotectant has been documented in several animal models of TBI (Alessandri et al., 2002; Okonkwo and Povlishock, 1999; Okonkwo et al., 1999, 2003; Signoretti et al., 2004; Sullivan et al., 1999, 2000b). CsA transiently inhibits the opening of the mitochondrial permeability transition pore by unbinding mitochondrial matrix cyclophilin from the pore (Broekemeier et al., 1989; Broekemeier and Pfeiffer, 1995; Szabó and Zoratti, 1991), and thus protects mitochondria in the face of increased intracellular calcium. In this fashion, CsA prevents mitochondrial destruction and the release of caspase following cellular ischemia or traumatic brain injury (Alessandri et al., 2002; Okonkwo and Povlishock, 1999; Okonkwo et al., 1999; Povlishock and Pettus, 1996; Signoretti et al., 2004; Sullivan et al., 1999, 2000 a,b). Experimental evidence suggests that CsA, via the maintenance of mitochondrial membrane potentials and bioenergetic homeostasis, may attenuate post-traumatic cytoskeletal changes and axonal injury (Okonkwo and Povlishock, 1999; Okonkwo et al., 1999; Sullivan et al., 1999, 2000a), and it may exert beneficial effects on infarct size and global ischemic damage in transient ischemia-reperfusion models (Matsumoto et al., 1999; Shiga et al., 1992; Uchino et al., 1995, 1998; Yoshimoto and Siejo, 1999). It has been proven effective in models of hypoglycemic brain damage (Ferrand-Drake et al., 2003; Friberg et al., 1998), while improving brain tissue oxygen consumption and cognitive performance after TBI (Alessandri et al., 2002). CsA's beneficial effects on infarct size and global ischemic damage have been demonstrated when the drug is given either pre-injury or post-ischemia (Friberg et al., 1998; Li et al., 2000; Yoshimoto and Siejo, 1999).

CsA also belongs to the family of calcineurin inhibitors, that act by impairing the expression of several critical cytokine genes that promote T-cell activation, resulting in a selective inhibition of the killer T-cell-mediated immune response. Its introduction in clinical practice in the 1980s resulted in a marked improvement of the outcome of solid organ transplantation; however, the use of CsA is limited in clinical practice by its adverse-effect profile, especially when the drug is administered chronically.

In the current study we designed a prospective, randomized, double-blind, dual-center, placebo-controlled trial, to evaluate the safety, tolerability, and pharmacokinetics of a single intravenous infusion of CsA in patients with severe TBI. The safety and tolerability of CsA are reported here. Other aspects of drug administration, such as its effects on cell-mediated immunological function, and brain neurochemistry and cerebral and systemic hemodynamics, have been reported previously (Mazzeo et al., 2006, 2008).

Methods

This dual-center National Institutes of Health-National Institute of Neurological Disorders and Stroke (NIH-NINDS)-funded safety study was approved by the Virginia Commonwealth University Institutional Review Board (IRB) for human research, and by the Health Center IRB of the University of Florida. Informed consent was obtained prior to enrollment from the legal representative of each patient using forms and explanations approved by the IRB.

Patients older than 16 years with severe head injury (Glasgow Coma Scale score between 3 and 8) who received a ventriculostomy and a microdialysis catheter were eligible for the study. Exclusion criteria were: both pupils fixed and dilated; blood urea nitrogen (BUN) >20 mg/dL; creatinine >1.3 mg/dL; history of known prior impaired hepatic or renal function; known history of cancer, pregnancy, or the inability to exclude pregnancy; current or prior use of another investigational agent within 30 days; immunosuppression; or deranged coagulation parameters preventing the placement of intracranial pressure (ICP) and microdialysis catheters.

The study was designed to enroll a total of at least 50 patients at both centers. Subjects diagnosed with a severe head injury who received a ventriculostomy and a microdialysis catheter, and for whom consent was obtained, received either an intravenous infusion of CsA or placebo within 12 h of injury. The subjects were assigned to each treatment according to a randomization list in a 3:1 fashion, to allow a greater number of patients in the CsA group. The dose of CsA was 5 mg/kg given over 24 h as a slow continuous infusion, diluted in 250 mL of 5% dextrose. The placebo was 250 mL of 5% dextrose alone. This dose was decided upon based on suggested safe plasma levels from the transplant literature, and also based on neuroprotective effects seen in animal studies. CsA was purchased from Sandoz (Sandimmune[®], Princeton, NJ) and dispensed, along with identical placebo infusions, by the hospital investigational drug pharmacy, who also kept the randomization codes. Blood samples for pharmacokinetic analysis were drawn at baseline, and thereafter every 12 h for 6 days.

Safety monitoring

BUN, creatinine, hemoglobin, platelets, white blood cell (WBC) count, and a hepatic panel were monitored on admission, and at 12, 24, 36, and 48 h, and on days 4 and 7. Potential adverse events (AEs) were also recorded, as were blood levels of CsA. Whole-blood CsA levels were measured using a commercial immunoassay kit by the hospital clinical transplant laboratory (TDx immunoassay).

On admission and before the administration of CsA, blood was collected for the determination of immunologic data: total

lymphocyte count and phenotypic subsets (CD3⁺ mature T cells; CD4⁺ helper/inducer T cells; and CD8⁺ suppressor/cytotoxic T cells). The same analysis was repeated at 48 h after admission and at day 7. Within 7 days post-injury a variety of clinical samples were collected for microbiological analysis. These included blood cultures, suctioned sputum cultures, and urine cultures. Some patients also had respiratory cultures of bronchoalveolar lavage fluid at the discretion of the surgical team. Cultures were also performed as indicated according to the clinical course during the overall period of hospital stay. These immunological data and infection complications have already been reported (Mazzeo et al., 2008).

Outcome measure

Neurological outcome was assessed at 3 and 6 months post-injury using the 5-point Glasgow Outcome Scale (GOS), with a score ranging from 1 (dead) to 5 (good recovery) (Jennett and Bond, 1975).

Statistical analysis

A thorough graphical and exploratory data analysis was carried out to aid in the interpretation of the mean differences, and to perform diagnostic operations. A within-patient trend analysis was performed to diagnose multivariate outliers.

A mean difference test was used to test the differences between the treatment and placebo groups in each indicator. We first performed a diagnostic test for the homogeneity of the variances (Bartlett test), and if the variances were equivalent, a standard *t*-test was used to evaluate the mean difference. If the variances were not equivalent, a corrected *t*-test was employed (Welch test).

A multivariate repeated-measures linear model with covariates was used to test the stability of the measures over time, especially linear trends (increasing or decreasing values). The significance of these results were evaluated with the multivariate *F*-test statistic.

For the analysis of neurological outcome two tests were performed: the Pearson test of independence and the Fisher exact test for the count table.

Results

Fifty adult severe TBI patients (41 males and 9 females) were enrolled in this study over a period of 22 months, starting in January 2003. Thirty-seven patients were randomly assigned to receive 5 mg/kg of CsA within 12 h of injury, which was diluted in 250 mL of 5% dextrose and infused over 24 h. Identical placebo infusions without the CsA were given to 13 patients.

Age, Glasgow Coma Scale score (GCS) on admission, and Injury Severity Score (ISS) of the two groups are presented in Table 1. Of the total of 50 patients, 37 were enrolled at the Neuroscience Intensive Care Unit of the Virginia Commonwealth University Medical Center in Richmond, and 13 patients were enrolled at the Department of Neurosurgery of the University of Florida in Gainesville.

Results from patient 41, randomized to receive CsA, were excluded from further safety analysis due to his multiple severe traumas with hemodynamic instability, and life-

TABLE 1. DESCRIPTIVE STATISTICS OF THE STUDY POPULATION

	Placebo (n = 13)	CsA (n = 37)
Age, mean $\pm$ SD	29 $\pm$ 14	34 $\pm$ 16
Age $\leq$ 20 years	6 (46%)	9 (24%)
Age > 20 and $\leq$ 40 years	4 (31%)	15 (41%)
Age > 40 and $\leq$ 60 years	3 (23%)	10 (27%)
Age > 60 years	0	3 (8%)
GCS score (median)	6	4
GCS 3–4	4 (31%)	23 (62%)
GCS 5–6	4 (31%)	8 (22%)
GCS 7–8	5 (38%)	6 (16%)
ISS category ^a (median)	25	34
Serious (ISS 9–15)	1 (11%)	2 (7%)
Severe (ISS 16–24)	2 (22%)	1 (4%)
Critical (ISS 25–55)	6 (67%)	25 (89%)

^aPlacebo (n = 9); CsA (n = 28).

Note that ISS scores were not available for the 13 patients enrolled at the University of Florida (4 placebo-treated and 9 CsA-treated patients).

The table shows the descriptive statistics of the overall study population (50 patients). Dichotomized groups are expressed as n (%).

Table is modified from Mazzeo et al., 2008.

CsA, cyclosporin A; GCS, Glasgow Coma Scale; ISS = Injury Severity Score.

threatening multiple organ dysfunction that interfered with the analysis of safety of CsA. All his parameters were outliers and therefore he was not similar to the other patients at baseline, so he was excluded from the analysis. Thus in this study we present safety data from 49 patients, 36 receiving CsA and 13 receiving placebo.

Patient 41

This patient was a 52-year-old man admitted after a multiple rollover high-speed sports-car crash requiring prolonged extrication. GCS on admission was 4, Marshall CT classification was 3, and ISS was 45. The head CT scan on admission showed areas of subarachnoid hemorrhage and cortical contusion, a small epidural hemorrhage at the anterior aspect of the right middle fossa, and subdural hemorrhage along the tentorium and the falx, suggestive of diffuse axonal injury. Left frontal, right temporal, and skull base fractures were present, and multiple maxillofacial injuries were also documented.

He also sustained mesenteric artery injury causing ischemic bowel. On the first day of admission the patient therefore underwent exploratory laparotomy, with ligation of bleeding mesenteric artery vessels and resection of ischemic bowel. The intraoperative period was characterized by severe hypotension and he received multiple transfusions during the clinical course (>35 units of red blood, platelets, and plasma). During his hospital stay he underwent cardiocirculatory arrest requiring cardiopulmonary resuscitation, and placement of a chest tube for left pneumothorax. He also underwent placement of an inferior vena cava filter. Several days later, acute renal failure likely secondary to ischemia/hypotension-induced acute tubular necrosis developed, and he underwent continuous veno-venous hemofiltration. On day 19 his family withdrew further care due to poor prognosis.

Safety monitoring results: Effect of CsA on renal function

Blood urea nitrogen. A significant difference was seen for BUN at 24 and 48 h between CsA and placebo patients: 10.1 ± 3.4 versus 7.8 ± 3.5 mg/dL (at 24 h; $p = 0.048$) and 9.8 ± 3.8 versus 7 ± 2.7 mg/dL (at 48 h; $p = 0.017$) in CsA and placebo patients, respectively (Fig. 1). However, mean BUN values remained within their normal range (6–22 mg/dL) in both groups throughout the study period, although a trend of increasing BUN values was observed in both groups at day 7, with mean BUN values of 14.8 ± 4.8 and 17.4 ± 4.5 mg/dL in the CsA and placebo patients, respectively.

Multivariate analysis focusing on BUN values within the CsA group showed there was no significant change over time.

Creatinine. There were no significant differences in creatinine values between CsA- and placebo-treated patients during drug administration and over the monitoring period (Fig. 2). Overall, creatinine remained within the normal range (0.7–1.4 mg/dL) in both groups.

Multivariate analysis of creatinine values in the CsA group showed there were no significant changes over time. The mean baseline creatinine value in the CsA-treated group was 0.9 ± 0.2 mg/dL, and it remained stable at 0.8 ± 0.2 at 48 h, and at 0.75 ± 0.2 and 0.7 ± 0.2 at days 4 and 8, respectively.

Effect of CsA on hepatic function

Aspartate aminotransferase. Mean AST values showed no significant difference between the placebo- and CsA-treated groups, and were lower in the CsA-treated group than in the placebo-treated group throughout the monitoring period. At 48 h, mean AST levels (normal range: 0–75 U/L) were 50.3 ± 38.4 and 63.5 ± 51.5 U/L in CsA-treated and placebo-treated patients, respectively. Toward the end of the first week, all liver function enzyme test values trended upward and exceeded the normal range, but in all patients this later normalized, and no clinical liver abnormalities developed.

Alanine aminotransferase. The same trend described for AST applied for ALT, with mean ALT values showing no significant difference between the placebo-treated and CsA-treated groups, and levels remaining lower in the CsA-treated group than in the placebo-treated group throughout the monitoring period. At 48 h, mean ALT levels were 42.4 ± 25.5

FIG. 1. Mean blood urea nitrogen (BUN) levels (mean $\pm$ standard deviation) in the placebo- and cyclosporin A (CsA)-treated groups, over time. The shaded area indicates normal values of BUN (* $p < 0.05$).

FIG. 2. Mean blood creatinine levels (mean $\pm$ standard deviation) in the placebo- and cyclosporin A (CsA)-treated groups over time. There were no significant differences between the groups. The shaded region indicates normal values of creatinine.

and 49.6 ± 39 U/L, in CsA-treated and placebo-treated patients, respectively. The normal range for ALT is 0–50 U/L.

Total bilirubin. Our study demonstrated no significant difference in total bilirubin levels between the two groups throughout the monitoring period. Total bilirubin remained within the normal range (0.2–1.3 mg/dL) in both study groups.

Alkaline phosphatase. No significant difference in total alkaline phosphatase levels was seen between the two groups throughout the monitoring period. At 48 h, mean alkaline phosphatase levels (normal range: 0–126 U/L) were 78 ± 20 and 77 ± 17 U/L, in CsA-treated and placebo-treated patients, respectively. As with AST and ALT, an increase in alkaline phosphatase was seen at days 4 and 8 in both groups, but was only a little above the normal range, and no overt hepatic clinical changes were seen; values normalized after 2–3 weeks.

Effect of CsA on blood cell parameters

White blood cell count. A significant difference was seen in WBC counts only at 24 hours in CsA-treated versus placebo-treated patients: 15.7 ± 4.8 versus $12.5 \pm 3.2 \times 10^9/L$ ($p = 0.02$), respectively (Fig. 3). Baseline WBC counts prior to CsA or placebo administration were 17.2 ± 6.3 and $15.4 \pm 4.7 \times 10^9/L$ in

FIG. 3. Mean white blood cell count (mean $\pm$ standard deviation) in the placebo- and cyclosporin A (CsA)-treated groups over time. The shaded region indicates normal values of white blood cell count (* $p < 0.05$).

the CsA and placebo patient groups, and at day 7 the values were 12.7 ± 4.1 and $14.5 \pm 5.1 \times 10^9/L$, respectively. Thus the values were above the normal range ($3.2\text{--}11 \times 10^9/L$) throughout the measurement period, consistent with the effect of a severe trauma-induced stress response or infection.

Hemoglobin. Throughout the study period no significant differences were demonstrated between the CsA and placebo groups for hemoglobin. Mean baseline hemoglobin was 12.1 ± 2.3 and 12.8 ± 1.9 g/dL in CsA-treated and placebo-treated groups, respectively. Hemoglobin levels decreased to 10.3 ± 1.8 and 10.4 ± 2.1 g/dL at 24 h, and then remained stable with values of 10.2 ± 1.2 and 9.9 ± 1 at day 7, in CsA-treated and placebo-treated patients, respectively.

Platelets. Our study demonstrated no significant difference in platelet count between the two study groups throughout the monitoring period, with values remaining within the normal range (normal: $165\text{--}350 \times 10^9/L$). Baseline platelet counts were 235 ± 105 and $225 \pm 68 \times 10^9/L$, and at day 7 were 318 ± 136 and $326 \pm 169 \times 10^9/L$, in CsA-treated and placebo-treated patients, respectively.

Occurrence of adverse events

The occurrence of adverse events (AEs) in the CsA- and placebo-treated patients during the first week following drug infusion were recorded, and the analysis demonstrated no significant difference between the two groups. An AE was defined as any clinical event that was treated or found to be clinically significant by the principal investigator. Numbers and percentages of AEs in the CsA-treated and placebo-treated groups, respectively, were as follows.

- **Respiratory:** tachycardia 8 (22%) versus 4 (31%); hypotension 8 (22%) versus 3 (23%); hypertension 7 (19%) versus 6 (46%); bradycardia 1 (3%) versus 1 (8%); positive respiratory culture 13 (35%) versus 3 (23%); pneumonia 9 (24%) versus 3 (23%); hypoxia 9 (24%) versus 1 (8%); pneumothorax 6 (16%) versus 1 (8%); pleural effusion 5 (14%) versus 0; pulmonary edema 2 (5%) versus 1 (8%); pulmonary embolism 1 (3%) versus 0; sinusitis 1 (3%) versus 2 (15%); tachypnea 0 versus 2 (15%); and respiratory failure 0 versus 1 (8%).
- **Cardiac:** cardiac arrest 4 (11%) versus 4 (31%); atrial fibrillation 1 (3%) versus 1 (8%); and myocardial infarction 0 versus 1 (8%).
- **Neurologic:** increased ICP 20 (54%) versus 12 (92%); diabetes insipidus 4 (11%) versus 2 (15%); subdural hematoma 2 (5%) versus 0; seizures 2 (5%) versus 1 (8%); meningitis 2 (5%) versus 0; epidural hematoma 1 (3%) versus 0; enlargement of contusion 1 (3%) versus 1 (8%); cerebrospinal fluid leak 1 (3%) versus 0; uncus herniation 1 (3%) versus 1 (8%); pseudoaneurysm 1 (3%) versus 1 (8%); carotid artery occlusion 1 (3%) versus 0; brain swelling 0 versus 1 (8%); agitation 0 versus 1 (8%); decreased cerebral perfusion pressure 0 versus 1 (8%); and tremor 0 versus 1 (8%).
- **Laboratory:** electrolyte imbalance 34 (92%) versus 13 (100%); anemia 24 (65%) versus 6 (46%); hyperglycemia 21 (57%) versus 7 (54%); increased WBC count 18 (49%) versus 7 (54%); low platelets 10 (27%) versus 3 (23%); elevated liver enzymes 7 (19%) versus 2 (15%); coagu-

lopathy 6 (16%) versus 2 (15%); increased bilirubin 1 (3%) versus 0; and decreased albumin 1 (3%) versus 0.

- **Gastrointestinal:** increased gastric output 2 (5%) versus 0; constipation 2 (5%) versus 0; re-feed syndrome 1 (3%) versus 0; and ileus 1 (3%) versus 0.
- **Renal:** urinary tract infection 2 (5%) versus 0; oliguria 1 (3%) versus 0; polyuria 1 (3%) versus 0; and acute renal failure 0 versus 3 (23%).
- **Other systemic events:** fever 33 (89%) versus 12 (92%); sepsis 2 (5%) versus 0; pancreatitis 2 (5%) versus 0; deep venous thrombosis 2 (5%) versus 0; and hypothermia 1 (3%) versus 0.

Effect on hemodynamics

The effect of CsA infusion on blood pressure after head injury has been previously reported (Mazzeo et al., 2008). After the initiation of the CsA infusion, an increasing mean arterial pressure (MAP) was seen in the drug treated group, with MAP levels reaching a mean value of 105 mm Hg at 56 h after injury (Fig. 4A). MAP values were then maintained at a higher level in the CsA-treated group than the placebo-treated group throughout the monitoring period. The difference in MAP between the two groups was statistically significant at $p < 0.01$ at multiple time points; however, MAP never exceeded the physiologic range. Significantly increased cerebral perfusion pressures (CPPs) became apparent in the CsA-treated patients at 68 h after injury, with mean values of

FIG. 4. Twenty-four-hour values (mean $\pm$ standard deviation) for mean arterial pressure (MAP; $*p < 0.01$) (A) and cerebral perfusion pressure (CPP; $*p < 0.01$ and $*p < 0.05$) (B) in the placebo- and cyclosporin A (CsA)-treated groups over time. The boxes indicate the 24-h period of CsA infusion.

90 mm Hg in the CsA-treated group and 81 mm Hg in the placebo-treated group at this time point (Fig. 4B). The recorded CPP values were subsequently significantly higher in the CsA-treated group than in the placebo-treated group throughout the 7-day monitoring period ($p < 0.01$) (Mazzeo et al., 2008). Nevertheless, no significant difference was recorded in the two groups in the occurrence of clinically significant hypertension, confirming that even if MAP was maintained at higher levels in CsA-treated patients, these values remained within the normal range, and did not require therapeutic intervention to avoid clinical adverse effects.

Infectious complications

No significant difference was demonstrated in the incidence of infections or sepsis between the two study groups. These findings mirror those reported in a previous article, in which we clearly demonstrated that no significant difference exists in the studied immunologic parameters in the placebo-versus CsA-treated groups at any time point (Mazzeo et al., 2006). Furthermore, we could not demonstrate any other significant covariable factors in the incidence of lung infections after admission, other than the early impairment of T-cell activation secondary to injury that was not due to the administration of CsA (Mazzeo et al., 2006).

Results from patients with high blood CsA levels

Four of the 37 CsA-treated patients showed high CsA blood levels (>1000 ng/mL). In Table 2, clinical data for these patients are separately reported. Their CsA blood levels throughout the monitoring period are shown in Figure 5A. No significant effect on any safety parameters was seen in this subgroup with blood levels transiently >1000 ng/mL. Mean blood CsA levels for the entire CsA-treated group are shown in Figure 5B.

Summary of deaths

In Table 3 is presented a summary of the clinical course of the 11 patient deaths that occurred during this clinical trial, along with type and severity of injury, clinical presentation on admission, and cause of death.

Outcome

Neurological outcome was assessed at 3 and 6 months post-injury using the Glasgow Outcome Scale (Jennett and Bond, 1975). We had three missing scores in the CsA group at 6 months due to loss to follow-up, so the analysis for outcome was performed on a total of 47 patients (13 placebo patients and 34 CsA patients; Fig. 6). The distribution of outcome in the 5 GOS categories at 6 months in the placebo and CsA groups, respectively, were: good (4 and 8), moderate (4 and 6), severe (3 and 7), vegetative (0 and 4), and death (2 and 9). For analytical purposes the outcomes were dichotomized as bad outcome (dead, vegetative, or severe disability) and good outcome (moderate disability or good recovery).

Fisher's exact test demonstrated that the difference between the two groups was not significant both at 3 months ($p = 0.7$) and at 6 months ($p = 0.3$). The odds ratios adjusted for age and GOS scores also showed no statistically significant differences.

TABLE 2. CLINICAL DATA FOR THE FOUR PATIENTS WITH CsA BLOOD LEVELS > 1000 NG/ML

Patient no.	Age (y)	ISS	Weight (kg)	CsA dose administered	Mean BUN (mg/dL)	Mean creatinine (mg/dL)	Mean bilirubin (mg/dL)	Mean AST (U/L)	Mean ALT (U/L)	Mean ALP (U/L)	Mean WBC ($\times 10^9$ /L)	Adverse events
5	16	34	80	400 mg	n.v. 6–22 13 $\pm$ 2.9	n.v. 0.7–1.4 0.9 $\pm$ 0.1	n.v. 0.2–1.3 1.5 $\pm$ 0.3	n.v. 0–75 186.7 $\pm$ 47.3	n.v. 0–50 79 $\pm$ 10	n.v. 0–120 80.8 $\pm$ 35	n.v. 3.2–11 17.3 $\pm$ 3.8	Mild leukocytosis, moderate anemia, right subclavian pseudoaneurysm, septicemia
7	19	38	82	410 mg	11.4 $\pm$ 1.6	0.8 $\pm$ 0.1	0.9 $\pm$ 0.3	142.4 $\pm$ 133.8	111.3 $\pm$ 142	145.7 $\pm$ 135.4	10.2 $\pm$ 2.3	Mild leukocytosis, mild anemia, fever, increased ICP, right pneumothorax, deep venous thrombosis
17	49	N.A.	75	375 mg	10.6 $\pm$ 1.6	0.8 $\pm$ 0.2	0.5 $\pm$ 0.3	41.7 $\pm$ 19.5	51.6 $\pm$ 38.2	78.3 $\pm$ 45.9	15.7 $\pm$ 3.7	Hyperglycemia, fever, pulmonary edema
54	74	29	92	460 mg	12.3 $\pm$ 6.7	0.6 $\pm$ 0.1	0.3 $\pm$ 0.04	65.8 $\pm$ 11	30.6 $\pm$ 14.4	96 $\pm$ 15.7	11.1 $\pm$ 1.6	Aspiration pneumonia, mild anemia, fever, hyperglycemia, mild hypertension

For each patient the mean values $\pm$ SD of the studied parameters and the occurrence of adverse events during the 7-day monitoring period are presented, as well as the demographics, the total dose of cyclosporin A administered, and the occurrence of adverse events.
ICP, intracranial pressure; BUN, blood urea nitrogen; CsA, cyclosporin A; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; WBC, white blood cell count; N.A., not available; n.v., normal value; ISS, Injury Severity Score.

FIG. 5. The figure shows the cyclosporin A (CsA) blood level throughout the monitoring period in the 4 patients in whom it exceeded 1000 ng/mL (**A**), and the mean blood levels for the entire CsA-treated group (mean $\pm$ standard deviation) (**B**) (Modified from Mazzeo et al., 2008).

Discussion

This study demonstrated only transient, yet statistically significant, differences for BUN at 24 and 48 h, and for WBC at 24 h, between the CsA-treated and placebo-treated patients. These modest differences were not clinically significant, in that they did not negatively impact patient course. Both BUN and creatinine values, markers of renal function, remained within normal limits over the entire monitoring period in both placebo-treated and CsA-treated patients. There were no

significant differences in any other mean laboratory values, or in the incidence of AEs at any other measured time point. Also, no significant differences were demonstrated for neurological outcome. These results demonstrate the good safety profile of CsA infusion when given at the chosen dose of 5 mg/kg infused over 24 h during the early period after severe head injury in humans.

CsA is a small polypeptide with a molecular weight of 1.203 kDa, that was introduced into clinical practice in 1983 for rejection prophylaxis in transplant patients, and it acts

TABLE 3. SUMMARY OF THE PATIENT DEATHS THAT OCCURRED IN THE STUDY GROUPS

Patient no.	Study drug	Type of injury	Age (y)	GCS	ISS	Craniectomy	Cause of death	Time of death (days from admission)
4	CsA	MVA	18	4	42	No	Family withdrew care	8
18	CsA	MVA	18	4	26	No	Cardiac arrest	8
19	Placebo	MVA	20	3	59	No	Cardiac arrest	9
20	CsA	Fall	65	3	27	Yes	Family withdrew care	21
38	CsA	MVA	17	4	43	Yes	Family withdrew care	10
39	CsA	MVA	20	6	38	No	Brain death	7
40	Placebo	MVA	28	3	43	No	Family withdrew care	18
41	CsA	MVA	52	4	45	No	Family withdrew care	18
49	CsA	Pedestrian in MVA	51	7	36	No	Family withdrew care	20
54	CsA	MVA	74	6	29	No	Discharged to nursing home and then found dead	Discharged at day 21
60	CsA	ATV accident	27	7	41	Yes	Family withdrew care	27

MVA, motor vehicle accident; CsA, cyclosporin A; ATV, all-terrain vehicle; ISS, Injury Severity Score; GCS, Glasgow Coma Scale score.

FIG. 6. Graph showing the percentage of patients with bad outcomes (dead, vegetative, or severe disability) and those with good outcomes (moderate disability or good recovery) in the cyclosporin A (CsA) and placebo groups at 6 months post-injury. The number of patients for each group is shown above each column (GOS, Glasgow Outcome Scale score).

through selective inhibition of the immune response. CsA exerts its immunosuppressant action by binding to an intracellular protein, cyclophilin, and the CsA-cyclophilin complex then inhibits the activity of calcineurin. As a calcineurin inhibitor, CsA selectively targets interleukin-2 (IL-2)-dependent T-cell proliferation, thus blocking the cell-mediated killer T-cell immune response (Danovitch, 2005). Calcineurin inhibitors are the mainstay of prevention of allograft rejection in modern transplant practice, and are approved for this indication by the U.S. Food and Drug Administration (Kapturczak et al., 2004).

With the increased clinical use of CsA in transplantation medicine and to treat autoimmune disease, significant CsA-related side effects have become apparent in the organ transplant population, and the regulation of immunosuppression remains a challenge in the care of these transplant patients (Shaw et al., 1996). Furthermore, CsA is a drug with a narrow therapeutic index, and is characterized by significant pharmacokinetic inter- and intra-individual variability and unpredictability in its pharmacodynamic effects, mandating strict blood level monitoring and careful titration (Danovitch, 2005; Kapturczak et al., 2004; Paul and de Fijter, 2004; Ptachcinski et al., 1986; Schiff et al., 2007).

Several important side effects of CsA have been described in chronically treated transplant patients, in particular nephrotoxicity, acute microvascular disease, electrolyte abnormalities, and hypertension. There are also reports of neurotoxic manifestations, such as tremor, dysesthesia, and seizures, and gastrointestinal symptoms such as dyspepsia, nausea, vomiting, hypertrichosis, hyperlipidemia, glucose intolerance, and hyperuricemia, as well as an increased incidence of thromboembolic events (Danovitch, 2005; Miller, 1996; Min and Monaco, 1991; Remuzzi and Perico, 1995). Among these, CsA-induced acute renal dysfunction is by far the most significant and limiting adverse effect, manifest as reversible increases in BUN and creatinine levels (Al Aly, 2005; Cattaneo et al., 2004; Fellstrom, 2004; Paul and de Fijter, 2004; Rezzani, 2004; Shaw et al., 1996).

The aim of this study was to evaluate the safety profile of cyclosporin A when given in the early phase post-TBI for its presumed neuroprotective effects. Recently, Piot and associates reported no significant adverse effects of CsA when administered in humans at a dose of 2.5 mg/kg for the purpose

of attenuating myocardial reperfusion injury after myocardial infarction (Piot et al., 2008). Furthermore, the results of a randomized, placebo-controlled dose-escalation trial investigating the role of CsA when administered within 8 h of injury to a population with severe TBI have been recently published, and showed no difference in mortality or other adverse events in patients receiving CsA versus those receiving placebo (Hatton et al., 2008).

Factors to be considered in intensive care unit trauma patients

When a drug is introduced into clinical use for a new indication, its proposed benefits must be critically evaluated against its potential adverse effects. This is especially true for those with acute trauma, who are critically ill and often physiologically unstable, and must undergo multiple invasive procedures while receiving multiple drug therapies. These patients are especially prone to develop pneumonia and multiple organ dysfunction, especially if they remain in the ICU for prolonged periods. The incidence is proportional to the severity of the primary injury, and is influenced by the presence of pre-existing disease, such as diabetes and ischemic heart disease. Superimposed infection frequently develops, as well as metabolic derangements, and both contribute to increased morbidity and mortality in these critically ill patients. Therapeutic management in the ICU should focus on preventive measures, with the aim of minimizing organ-specific dysfunction, thus improving outcome.

Possible drug interactions

In TBI patients receiving CsA, the possibility of interactions with other drug therapies should be considered and requires careful vigilance (Danovitch, 2005; Kapturczak et al., 2004). Particularly problematic are drugs that may decrease CsA concentrations by induction of cytochrome P450 activity, such as anticonvulsants (e.g., barbiturates, phenytoin, and carbamazepine), and drugs that may increase CsA concentrations via inhibition of cytochrome P450 or by competition for its pathways, such as calcium channel blockers, antifungal agents (especially ketoconazole), histamine blockers, or macrolide antibiotics. The administration of any additional potentially nephrotoxic drugs such as aminoglycosides, non-steroidal anti-inflammatory drugs, or angiotensin-converting enzyme inhibitors, should be carefully considered and avoided whenever possible (Danovitch, 2005).

In this study, the possible toxic effects of CsA were assessed in detail during the drug infusion period and over the first week after admission in a large number of severely head-injured patients admitted to the ICU, and no clinically significant AEs due to drug interactions were found.

Effect of CsA on renal function

In relation to the potential nephrotoxic effect of CsA in our patient population, we demonstrated a significant difference in mean BUN concentration at 24 and 48 h between CsA- and placebo-treated patients. However, these differences were not clinically relevant since these values remained within their normal limits.

As observed in transplant patients with long-term high-dose CsA treatment, acute and chronic nephrotoxicity are common (Al Aly et al., 2005; Benigni et al., 1999; Paul and de

Fijter, 2004; Shaw et al., 1996). Acute nephrotoxicity is associated with reduced renal blood flow, reduced glomerular filtration rate, and increased renal vascular resistance (Fellstrom, 2004). CsA administration thus produces a highly dose-dependent and rapidly reversible renal vasoconstriction that particularly affects the afferent arteriole and leads to renal hypoperfusion (Cattaneo et al., 2004; Danovitch, 2005; Fellstrom, 2004). This acute nephrotoxicity is thought to be caused by a local imbalance of vasoconstrictor and vasodilator mediators, such as increased endothelin activity, increased levels of angiotensin II, and reduced availability of vasodilator prostaglandins and nitric oxide, as well by activation of the renal sympathetic nerves (Cattaneo et al., 2004; Danovitch, 2005; Fellstrom, 2004; Paul and de Fijter, 2004). Most importantly, this effect is reversible with CsA dose reduction or withdrawal, and is not followed by renal histological changes. It is seldom seen in those with less than 2–3 months of dosing. Conversely, chronic CsA nephrotoxicity represents a more serious concern in patients receiving long-term (lifetime) CsA treatment, and it is associated with progressive and irreversible renal interstitial fibrosis, and clinically significant renal dysfunction eventually leading to end-stage renal failure (Cattaneo et al., 2004; Danovitch, 2005; Fellstrom, 2004; Paul and de Fijter, 2004). Renal exposure to and accumulation of systemically derived cysteinyl leukotrienes, such as leukotriene C₄ and D₄ products, may be underlying factors in CsA nephrotoxicity (Aleo et al., 2008). Our study protocol consisted of brief administration (24 h) of a therapeutic dose of CsA within the recognized dosing range of 5–6 mg/kg, and was associated with none of these effects.

No significant difference in mean serum creatinine level between the CsA and placebo groups was shown in our study, confirming the safe renal profile of CsA when given as a putative neuroprotective drug in the early phase after severe human TBI.

Effects of CsA on hepatic function

The results of this study demonstrated no significant difference between CsA- and placebo-treated patients in the evaluation of parameters of hepatic function, such as AST, ALT, bilirubin, and alkaline phosphatase levels. Increased mean values for AST, ALT, and ALP, above the normal range, were however recorded on days 4 and 8 in both treatment groups, but no clinical signs of liver dysfunction were seen, and in all cases this effect disappeared in a few weeks.

In the literature there are reports of similarly transient gastrointestinal effects of chronic CsA administration, such as subclinical mild and self-limiting dose-dependent elevations in serum aminotransferase levels and mild hyperbilirubinemia in nearly half of the treated patients. This did not seem to be CsA-related, as this was also seen in the placebo group. In the literature, hyperbilirubinemia has been related to disturbed bile secretion, rather than hepatocellular damage, and no hepatic histological lesions have been described in humans after the use of CsA (Aleo et al., 2008; Danovitch, 2005; Min and Monaco, 1991; Rezzani, 2004).

Effects of CsA on blood counts and incidence of infection

In this study we found a transient significant difference in WBC counts at 24 h. No significant differences were detected

in hemoglobin concentrations and platelet counts between the two study groups during the monitoring period. These results support the good hematological safety profile of CsA when given at 5 mg/kg over 24 hours.

Many factors may influence WBC counts, hemoglobin levels, and platelet counts in TBI patients during their ICU stay, such as age, concomitant disease, risk of infection, multiple drug therapies, colloid or crystalloid replacement therapy, and length of hospital stay.

Trauma is a multisystemic disease that interferes with the function of multiple organs and systems, even those not directly related to the site of the initial impact. Infectious complications and sepsis have been reported in up to 50–70% of severe TBI patients, and worsen clinical outcomes, increasing both late morbidity and mortality (Helling et al., 1988; Hoyt et al., 1990; Quattrocchi et al., 1991a,b). Furthermore, severely head-injured patients have other factors known to increase infection rates, such as a prolonged need for ventilatory support, prolonged sedation and paralysis, aspiration of gastric contents, and invasive monitoring.

It is also well-known that immunological function may be impaired in the early phase after TBI, as well in as trauma in general, due to a complex multifactorial effect of suppression of T-cell mediated immune function, and changes in soluble mediators, such as an increase in PGE₂ biosynthesis, and reductions in the levels of the key immunoregulatory cytokines IL-2 and IFN- γ (Boddie et al., 2003; Quattrocchi et al., 1990, 1991a,b, 1992). The administration of any medication that might induce further immunological suppression should therefore be undertaken with caution, in order to avoid any additional or synergistic suppression of immune function after severe TBI. We have previously reported that there were no significant differences between the placebo- and CsA-treated patients in this clinical trial with regard to immunological parameters, such as total lymphocyte count and phenotypic cell subsets (CD3⁺ mature T cells, CD4⁺ helper/inducer T cells, and CD8⁺ suppressor/cytotoxic T cells), or the incidence of infection (Mazzeo et al., 2006). No significant difference was seen in the incidence of infection or sepsis between the two study groups detailed here.

Effects of CsA on hemodynamics

In the literature there are reports of CsA-induced hypertension, which is a potentially serious side effect in patients receiving the drug as an immunosuppressive agent, due to stimulation of the renin-angiotensin system, as well as sympathetic activation, endothelin release, and nitric oxide production, all of which play important roles in the pathogenesis of hypertension (Gardiner et al., 2004; Roullet et al., 1994; Shaltout and Abdel-Rahman, 2003).

The results of our study strongly suggest a significant vasoactive effect of CsA, and we previously reported a significant transient hypertensive effect in the CsA-treated group (Mazzeo et al., 2008). This may be beneficial in the early phase after TBI, especially since maintenance of CPP is an important goal of TBI treatment, and in our trial the need for vasopressors was reduced in the CsA-treated group (Mazzeo et al., 2008).

CsA's other possible toxic effects

A spectrum of neurologic complications has been described in patients receiving CsA as an immunosuppressant. Clinical

manifestations of CsA-associated neurotoxicity include tremors, restlessness, dysesthesias, headaches, and seizures (Miller, 1996; Danovitch, 2005). In our trial neurotoxicity was not evident in the CsA-treated group; the incidence of seizures was 5% in the CsA-treated group and 8% in those receiving placebo.

Cyclosporin blood level pharmacokinetics

The blood CsA levels are shown in Figure 5. As can be seen in Figure 5B, the highest mean blood levels for the entire CsA group was ~ 500 ng/mL, and these levels remained high for about 12 h after the infusion ended. In four patients the blood level exceeded 1000 ng/mL, and this was about 2–3 times higher than the levels seen in the group as a whole. The reasons for these higher blood levels were not clear, but no adverse effects were seen in any of the monitored safety parameters in this small patient group (Table 2), although one of these four patients died.

Outcome measures

Our results demonstrate that there was no significant difference in neurological outcomes at 3 and 6 months in the two study groups. As shown in Table 3, there was a higher mortality rate in the patients receiving CsA. This appears to be due to the severe systemic sequelae suffered by the CsA patient group, rather than any possible drug effect. In fact, as shown in Table 1, the median ISS scores were higher in CsA-treated patients than in those receiving placebo (34 versus 25), as was the percentage of patients with critical ISS scores (89% versus 67%), suggesting that the CsA-treated patients were more severely injured than those receiving placebo. Data analysis also demonstrated that 8 patients in the CsA-treated group showed improvements in GOS scores over time.

Conclusion

This study demonstrates the good safety and tolerability profile of CsA when it is administered early after severe TBI with the goal of neuroprotection. Only brief, transient significant differences were seen for BUN at 24 and 48 h, and for WBC counts at 24 h, between the CsA- and placebo-treated groups; these differences were small and were not clinically significant. There were also no significant differences in the other studied clinical parameters, or in the incidence of adverse events, at any time point. No significant differences were seen with regard to neurological outcomes in the two study groups.

Pharmacokinetic studies have demonstrated that CsA administration is complicated by wide variations in inter- and intra-patient metabolic levels (primarily mediated by changes in cytochrome P450 metabolism), and thus drug levels must be carefully monitored during treatment.

A large Phase III efficacy trial of CsA in severe TBI is currently under peer review by the NIH-NINDS Clinical Trials study section, and if approved, it will be performed in about 50 centers throughout the U.S. to further assess the efficacy of this agent for this indication.

Acknowledgments

This study was funded by the NIH-NINDS as part of project grant no. NS12587 to M.R.B. (the primary investigator),

and by the Lind-Lawrence Foundation and the Reynolds Foundation.

Author Disclosure Statement

No competing financial interests exist.

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