In The Name Of God

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The efficacy of Cyclosporine-A on Diffuse Axonal Injury after Traumatic Brain Injury

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Dear Dr. Salman Abbasi Fard

It is my pleasure to inform you as corresponding author and your colleagues ***"Dr. Bahram Aminmansour (first author), Dr. Majid Rezvani Habibabadi, Dr. Payam Moein, Dr. Rasoul Norouzi, Dr. Morteza Naderan"*** that your manuscript entitled ***" The efficacy of Cyclosporine-A on Diffuse Axonal Injury after Traumatic Brain Injury"*** has been accepted for publication in the journal of ***"Advanced Biomedical Research"***, Vol 2; No 2.

Thank you for submitting your article to this journal. We look forward to receiving your next precious articles.

Shaghayegh haghjooy javanmard
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Original Article

The efficacy of Cyclosporine-A on Diffuse Axonal Injury after Traumatic Brain Injury

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Abstract

Background: To evaluate the efficacy and side-effects of cyclosporine-A (CsA) in improvement of consciousness and cognitive dysfunction of patients with diffuse axonal injury (DAI) after traumatic brain injury (TBI).

Materials and Methods: This study is designed as a randomized double-blind placebo-controlled with 100 patients suffered from DAI. CsA was administered to the intervention group ($n = 50$) as 5 mg/kg/24 h via 250 ml dextrose water (DW) 5% solution (DW 5%) during the first 8 h after trauma. The control group ($n = 50$) received only DW 5% in the same course. The presenting Glasgow coma scale in addition to the Glasgow outcome scale-extended (GOS-E) and mini-mental state examination (MMSE) in the 3rd and 6th months after trauma were documented. The serum values for complete blood count (CBC), blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were checked to assess for complications.

Results: Most patients in both groups had type II DAI (46%). There was no significant difference between groups in the GOS-E scores after 3 and 6 months. All participants were in moderate or severe classes of MMSE with no statistically significant difference. Except for the higher BUN level in the cyclosporine treated group, 48 h after admission ($P = 0.012$), the difference in the level of Cr, AST, ALT, and ALP was not significant and all were in the normal range. The CBC results showed only significant difference for White Blood Cell (WBC) count at 12 h ($P = 0.000$).

Conclusion: The administration of CsA is not effective in the improvement of consciousness and cognitive function. However, it brings about no adverse effects.

Key Words: Cyclosporine-a, diffuse axonal injury, neuroprotection, neurorecovery, traumatic brain injury

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INTRODUCTION

The developing countries are facing an increasing burden of closed head injuries mainly as a result of vehicle accidents so that trauma is now a leading cause of mortality in the under 45 years of age.[1] Unfortunately, at least half of these have suffered from severe traumatic brain injury (TBI). It is estimated that the main

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mechanism for TBI in 40-50% of cases is a consequence of diffuse axonal injury (DAI).[2] In fact, the traumatic head injury sets off a train of pathophysiological events culminating in the progressive neuronal and axonal damage.[3-5] A very prominent feature of this process is the rapid influx of Ca^{+2} to the cell, which alters the permeability of mitochondria and induces release of death proteins into the cytoplasm with the resultant apoptosis or necrosis of the neuron.[6]

The role of cyclosporine-A (CsA), as a neuroprotective substance, has been evaluated in some animal studies.[7-11] CsA is a calcineurin inhibitor which temporarily prohibits Ca^{+2} from entering into the mitochondria, the mechanism that is suggested underlying the neuroprotective behavior of the drug. However, the safety profile and efficacy of CsA for use in humans as a neuroprotective drug following TBI has recently been evaluated.[12-14] These studies indicate that the use of CsA may be safe without crucial adverse effects on the immune responses of the patients. Nonetheless, there are randomized double-blind dose-escalation trials which demonstrate neither difference in the mortality rate nor in the associated complications such as infection, renal function impairment, or evidence of liver damage.[15,16]

Despite strives toward elucidating the efficacy of CsA in TBI and some promising findings reported in clinical trials during last decade, current knowledge about the efficacy of CsA in DAI patients is limited and obscured by many unexplained results. The purpose of this study is to evaluate the efficacy of CsA in neural protection after DAI in terms of improvement in early and late level of consciousness and cognitive dysfunction and also to define major side-effects associated with CsA infusion.

MATERIALS AND METHODS

The study is a randomized double-blind placebo-controlled clinical trial performed on patients suffered from TBI with Glasgow coma scale ≤10 with clinical and radiological evidence of DAI. Isfahan University of medical sciences, Isfahan, Iran and the study was conducted from 2010 to 2012.

Inclusion and exclusion criteria complied for selecting participants has been presented in Table 1.

The flow of assessment of patients and enrollment is presented in the study flow diagram [Figure 4]. We divided participants into two groups each containing 50 individuals: Group A, the intervention group, received 5 mg/kg/24 h cyclosporine as a solution in 250 ml dextrose water (DW 5%) started within 8 h after occurrence of the head injury; Group B, the control group, received placebo as only 250 ml DW 5% started at the same time and continued for 24 h. The nursing staff who prepared the drug was different from those who administered it to patients. The allocation of patients was not randomized and we homogenized them with respect to presenting consciousness level, age, and sex.

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age within 16-75	Fixed and dilated pupils
GCS≤10 in the first 24 h next of kin's informed consent about the study	Blood urea nitrogen ≥20 mg/dl at presentation
	Serum creatinine ≥1.3 mg/dl at presentation
negative urine pregnancy test, if female	Accompanying malignancy
	Pregnancy or inability to rule out pregnancy
	Immunodeficiency
	Subjected to clinical research with last 30 days
	Severe and progressing intracranial hematoma and/or extensive brain contusion with surgical indication
	Midline shift above 5 mm; and/or bilateral or extensive brain contusion; and/or subarachnoid hemorrhage apparent in presenting CT scan or MRI
	Acute change in vital signs or laboratory findings compatible with drug's side effect or hypersensitivity
	History of severe traumatic head injury, brain tumor, cerebrovascular lesions such as stroke
	Penetrating head trauma
	Multiple trauma

CT: Computed tomography, GCS: Glasgow coma scale, MRI: Magnetic resonance imaging

The 8-score Glasgow outcome scale-extended (GOS-E) was used to assess for the neural improvement at 3rd and 6th month after trauma. Death of patient scored 1 and complete recovery scored 8. According to GOS-E scores, we further subdivided patients into bad (if GOS = 1-3) and good (if GOS = 5-8) groups considering their outcome.

We also accomplished mini-mental state examination (MMSE) to evaluate the cognitive status of patients 3 and 6 months after trauma. The MMSE scores were classified as normal cognitive function, (MMSE = 25-30), mild impairment (MMSE = 20-24), moderate impairment (MMSE = 10-19), and severe impairment (MMSE = 0-9).

We took blood samples on admission and at 12, 24, 36, 48 h and on days 4 and 7 afterwards to evaluate complete blood count (CBC), blood urea nitrogen (BUN), Creatinine (Cr), and liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline

phosphatase (ALP). We also ordered blood, urine, and sputum culture. Any drug reaction was documented in the patients' medical records.

We calculated the sample size to be 50 patients in each groups.

All information gathered into a data report sheet including anthropometric data, clinical findings, and laboratory tests results, and introduced into SPSS software for windows version 18. We used Chi-square test comparison of qualitative data and *t*-test for comparison of quantitative data and ANOVA test for comparison of the consciousness level at among the follow-up period. We chose *P* value less than 0.05 to be statistically significant.

This study was approved by the Isfahan University of Medical Sciences' ethics committee. All the procedures and course of action were explained clearly to the surrogate decision maker of the patients and appropriate informed consent was acquired.

Table 2: Baseline characteristics and outcome results of the study's population

Variable	Intervention group (*n*=50) (%)	Control group (*n*=50) (%)	*P* value
Gender			
Male	45 (90)	43 (86)	
Female	5 (10)	7 (14)	
Age (years) (mean±SD)	29.9±8.7	31.1±10.7	0.283
Hospitalization (days) (mean±SD)	29.2±5.8	27.8±6.6	0.152
DAI classification			
Type I			0.381
Type II	6 (12)	8 (16)	
Type III	22 (44)	24 (48)	
Type IV	18 (36)	16 (32)	
GOS-E	4 (8)	2 (4)	
After 3 months			
Bad	23 (46)	18 (36)	0.208
Good	27 (54)	32 (64)	
After 6 months			
Bad	29 (58)	22 (44)	0.115
Good	21 (42)	28 (56)	
MMSE			
After 3 months			
Moderate	24 (48)	29 (58)	0.212
Severe	26 (52)	21 (42)	
After 6 months			
Moderate	19 (38)	22 (44)	0.342
Severe	31 (62)	28 (56)	
Infection			
Yes	4 (8)	3 (6)	0.5
No	46 (92)	47 (97)	
Death			
Yes	9 (18)	6 (12)	0.288
No	41 (82)	44 (88)	

DAI: Diffuse axonal injury, GOS-E: Glasgow outcome scale-extended, MMSE: Mini-mental state examination

RESULTS

Our study population included 100 individuals suffered from TBI. The baseline characteristics and outcome results of the participants are outlined in Table 2. Type II DAI was responsible for the greater number of patients in both groups while type IV was found to afflict few patients. We found no significant difference in terms of hospitalization time between those who received cyclosporine and those who received placebo.

We found no significant difference between groups regarding GOS-E scores after 3 months as well as the repeat test after 6 months. All participants at all MMSE examinations were either in the moderate (10-19) or severe (0-9) impairment classes. We found no one with scores high to classify as mild or normal cognitive function. After 3 months, there was no statistical difference between intervention and control groups, which also was the case for the MMSE results after 6 months.

Most patients had no infectious complication and we found no difference between groups in this respect. Seven percent had infectious complications mainly involving the lungs. Although, the death rate reached 15% of the total population, the difference between groups was insignificant.

Assessment of serum biomarkers was done according to a scheduled protocol immediately on admission and at 12, 24, 36, 48 h, and on days 4 and 7 afterwards. Except for the higher BUN level in the cyclosporine treated group at 48 h after admission which came to become statistically significant between groups ($P = 0.012$), the difference in the level of Cr, AST, ALT, and ALP was not significant and all were in the normal range of the laboratory [Figures 1 and 2].

The CBC results showed significantly higher WBCs in the cyclosporine treated group at 12 h after admission ($P = 0.000$); however, the value of hemoglobin and platelets were not different at any of the corresponding measurements [Figure 3].

DISCUSSION

We designed a study in order to evaluate the neuroprotective efficiency of CsA in patients suffering from DAI and we found no significant effect of this drug on accelerating neurorecovery in the study's population in terms of hospitalization time or

long-term cognitive status. The dosing schedule of our study resulted in fairly no significant side-effects and it could be said that CsA is a safe drug in this sense with no actual risk of nephrotoxicity, hepatotoxicity, or bone marrow suppression. However, the significant difference in BUN levels at 48 h after admission may have resulted from CsA but because it was in the normal range and the difference did not last through later measurements, we cannot recognize it as a clinically important occurrence. This is also the case for increased WBC count at 12 h after trauma, which we could not see any clinical relevance into it. The rate of infections was fairly similar in both groups.

Our study is limited by the fact that we could not check the CD4 and CD8 subsets of leukocytes and

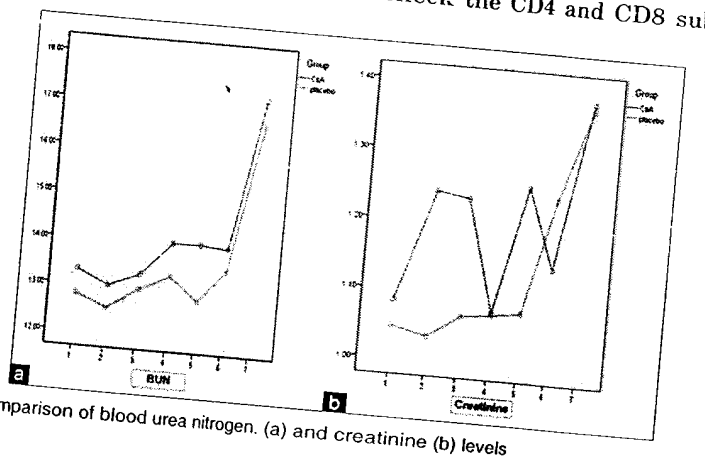

Figure 1: Illustrations for the comparison of blood urea nitrogen. (a) and creatinine (b) levels

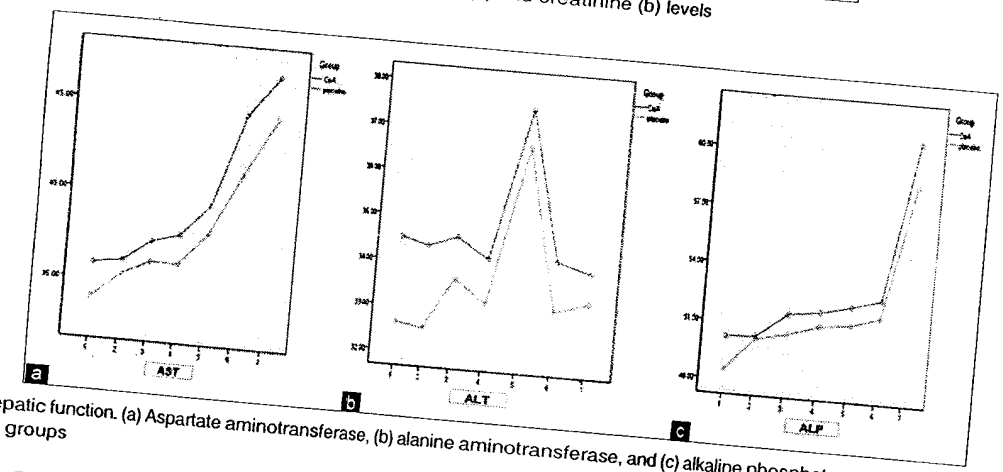

Figure 2: Hepatic function. (a) Aspartate aminotransferase, (b) alanine aminotransferase, and (c) alkaline phosphatase values for the cyclosporine-A and placebo groups

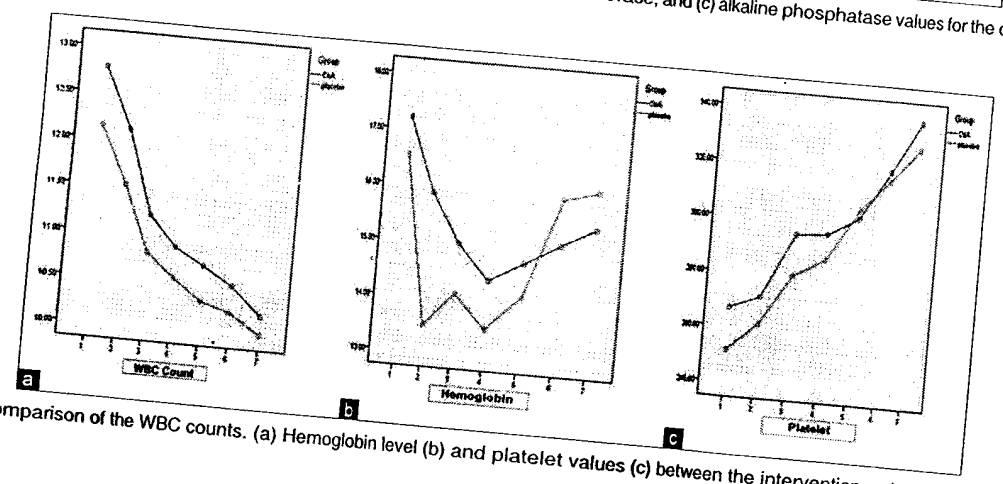

Figure 3: Comparison of the WBC counts. (a) Hemoglobin level (b) and platelet values (c) between the intervention and placebo groups

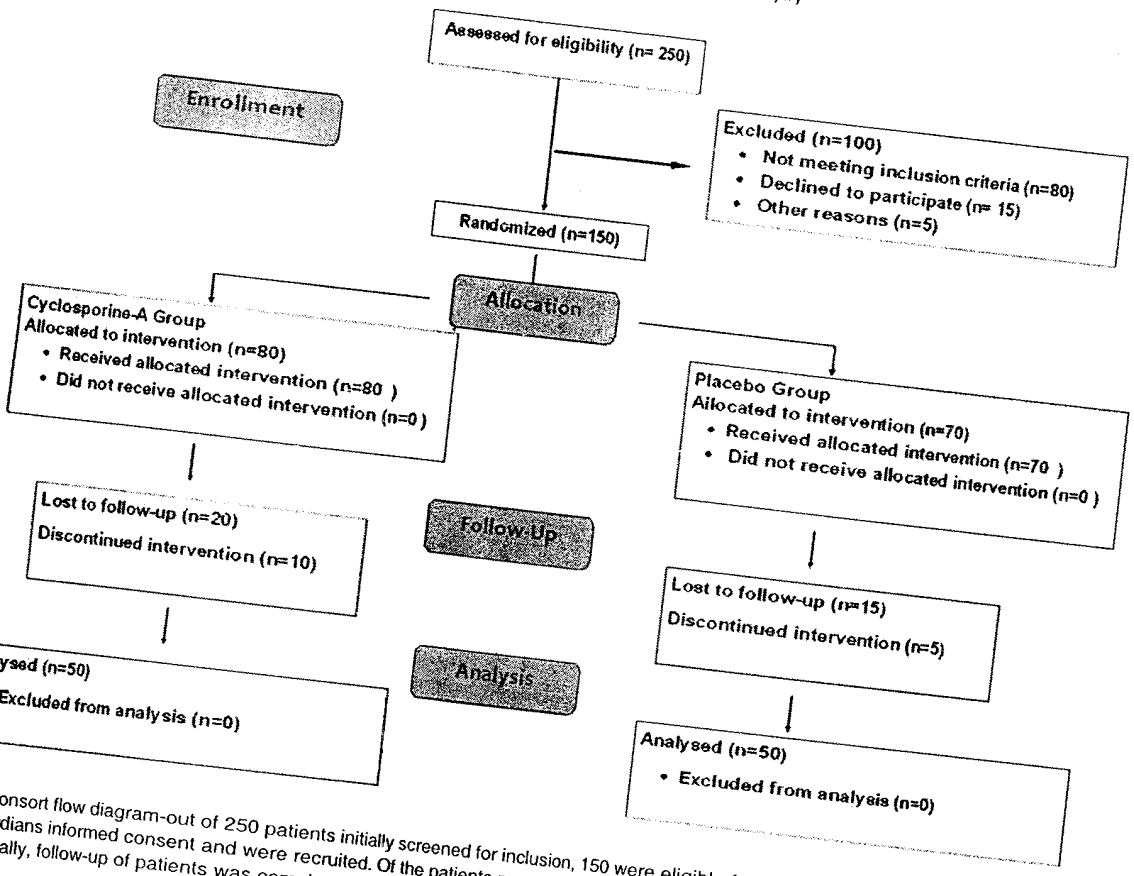

Figure 4: Consort flow diagram-out of 250 patients initially screened for inclusion, 150 were eligible for inclusion, of which 150 gave their written or their guardians informed consent and were recruited. Of the patients recruited, 80 were randomly assigned to Cyclosporine -A arm and 70 to placebo. Finally, follow-up of patients was completed by 100 patients, 50 assigned to Cyclosporine-A and 50 to placebo

immunoglobulin levels as a result of technical obstacles. So difference in the immune regulation of two groups was judged only as a rough deduction from WBC counts or increased numbers of infections. We also had 15% mortality among the study population which reduced the number of participants for final analysis. On the other hand, the infectious complications, mainly pneumonia, lead to inevitable antibiotic administration which we may not be sure about the possible interactions with the effects of CsA or serum findings.

Regarding previous studies related to our work, we mention some famous literature here. In a randomized placebo-controlled trial in 2006, which was conducted as a phase II clinical trial in order to assess the safety of CsA, the method used was escalation schedule and the 30 participants divided as three groups receiving from 0.625 mg/kg (group I) to 1.25 mg/kg (group II) to 2.5 mg/kg (group III) CsA within the first 8 h after trauma. Although the maximum dose they used was half that of ours, but we also found no side effects related to CsA.[12] In a randomized placebo-controlled trial in 2006, researchers evaluated the safety and effects of 5 mg/kg/24 h CsA on 36 patients within 12 h after accident. Sixty percent of total 59 participants experienced decreased lymphocyte counts which we did not check in our study. However, the researcher concluded that respiratory infections were consequence of brain injury itself, not CsA, and it may be used safely in such situations.[13] Our results also agree with this conclusion. In another randomized double-blind study in 2008, 40 patients with severe TBI divided into four groups: Group IV received 2.5 mg/kg CsA as a loading dose no later than 8 h after incident and then placed on 5 mg/kg infusion for 72 h. Their results corresponds those of our study in terms of no difference for death, infectious complications, and end organ failure. These findings are also similar to findings of our study in that the GOS-E scores were not statistically different between groups.[15] In a multi-center double-blind placebo-controlled trial in 2009, the CsA was

administered to 36 patients with severe TBI (while 13 received placebo) at the dose of 5 mg/kg/24 h started within 12 h after trauma. The results were increases, though transient, BUN levels at 24 h and 48 h after treatment and increased WBC count at 24 h after initiation of treatment. No difference in other biochemical factors such as Cr, AST, ALT, ALP, hemoglobin, or platelets were detected. These are fairly similar to the results of our study. In another sense, the results of GOS-E at 3 and 6 months after injury were found not to be different in their study, which we also agree according to our findings. Death rate was slightly higher in their study (20% vs. 15%) but the authors concluded it was not a result of CsA administration.[16]

CONCLUSION

Our results suggest that CsA administration to patients with DAI during first 8 h after damage with the dose of 5 mg/kg for 24 h is safe and no clinically important side-effect may ensue. However, it may not bring about desired effects in terms of neuroprotection and cognitive outcome.

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This clinical trial has been registered in www.irct.ir with the code of IRCT 201311712164N1.

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