Secondary brain injury in trauma patients: The effects of remote ischemic conditioning

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BACKGROUND: Management of traumatic brain injury (TBI) is focused on preventing secondary brain injury. Remote ischemic conditioning (RIC) is an established treatment modality that has been shown to improve patient outcomes secondary to inflammatory insults. The aim of our study was to assess whether RIC in trauma patients with severe TBI could reduce secondary brain injury.

METHODS: This prospective consented interventional trial included all TBI patients admitted to our Level 1 trauma center with an intracranial hemorrhage and a Glasgow Coma Scale (GCS) score of 8 or lower on admission. In each patient, four cycles of RIC were performed within 1 hour of admission. Each cycle consisted of 5 minutes of controlled upper limb (arm) ischemia followed by 5 minutes of reperfusion using a blood pressure cuff. Serum biomarkers of acute brain injury, S-100B, and neuron-specific enolase (NSE) were measured at 0, 6, and 24 hours. Outcome measure was reduction in the level of serum biomarkers after RIC.

RESULTS: A total of 40 patients (RIC, 20; control, 20) were enrolled. The mean (SD) age was 46.15 (18.64) years, the median GCS score was 8 (interquartile range, 3–8), and the median head Abbreviated Injury Scale (AIS) score was 3 (interquartile range, 3–5), and there was no difference between the RIC and control groups in any of the baseline demographics or injury characteristics including the type and size of intracranial bleed or skull fracture patterns. There was no difference in the 0-hour S-100B (p = 0.9) and NSE (p = 0.72) level between the RIC and the control group. There was a significant reduction in the mean levels of S-100B (p = 0.01) and NSE (p = 0.04) at 6 hours and 24 hours in comparison with the 0-hour level in the RIC group.

CONCLUSION: This study showed that RIC significantly decreased the standard biomarkers of acute brain injury in patients with severe TBI. Our study highlights the novel therapeutic role of RIC for preventing secondary brain insults in TBI patients. (J Trauma Acute Care Surg. 2015;78: 698–705. Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: Therapeutic study, level III.

KEY WORDS: Remote ischemic conditioning; traumatic brain injury; S-100B; neuron-specific enolase.

Traumatic brain injury (TBI) remains one of the leading causes of death and disability in the United States. Secondary brain injury caused by the complex interplay of inflammatory mediators induced by a primary insult is the major contributing factor for morbidity and mortality after TBI. While the primary injury is irreversible, the inflammatory cascade leading to the development of the secondary injury may be preventable. As a result, all the current research in TBI is focused on preventing initiation of this secondary insult.

Remote ischemic conditioning (RIC) is a process where normal tissues are subjected to short cycles of ischemia and reperfusion, which have been shown to reduce the sequelae of an ischemic injury at a remotely injured site. RIC has been shown to improve the outcomes after myocardial infarction, sepsis, transplantation, reimplantation, and elective neurologic surgery. It is thought to work by releasing endogenous systemic anti-inflammatory mediators and humoral factors and by using neural pathways, rendering global protection to the body against subsequent ischemic insults in a remote area. This protection provided by RIC has two phases, an early (short) phase and a late (prolonged) phase, both of which have proven to be effective in reducing ischemic size and improving survival. However, its role in patients with TBI remains unclear. The aim of our study was to assess the effects of RIC in trauma patients with severe TBI. We hypothesized that RIC reduces the serum level of biomarkers of brain injury in trauma patients with severe brain injury.

PATIENTS AND METHODS

After the approval from the institutional review board at the College of Medicine at the University of Arizona, we performed a prospective interventional study to evaluate the effects of RIC in patients with TBI. All patients were evaluated and treated at our Level 1 trauma center from September 2013 to June 2014. A written informed consent was obtained from the patient or patient’s relative within 24 hours of admission for enrollment in the study.
Inclusion and Exclusion

In this study, we included all patients with blunt TBI with Glasgow Coma Scale (GCS) score of 8 or lower and an intracranial hemorrhage on the initial brain computed tomography (CT) scan. Patients who are 18 years or younger, patients on antplatelet or anticoagulation medications, patients transferred from other institutions, patients dead on presentation, patients undergoing emergent neurosurgical intervention (patients taken to the operating room immediately after initial CT scan in whom RIC could not be performed), and patients who did not consent for enrollment were excluded from the study. We also excluded patients with concomitant spinal cord injuries, patients who died before completion of protocol, and patients with an intracranial pressure monitor placement before performance of RIC because of the their potential effect on neuronal markers of injury. These patients were excluded to select a homogenous patient population independent of confounding external factors. The presence of the initial intracranial hemorrhage was based on the evaluation of the attending radiologist, and these findings were confirmed by a single investigator.

Data Points

We prospectively recorded the following data points in each patient: patient demographics, which included age, sex, and mechanism of injury; vital parameters on presentation, which included systolic blood pressure (SBP), heart rate, temperature, and GCS score; neurologic examination on presentation; intoxication (drug or alcohol); details regarding antplatelet and anticoagulation therapy; intubation, loss of consciousness; initial and repeat brain CT scan findings; neurosurgical intervention details; hospital and intensive care unit length of stay (LOS); discharge disposition; GCS score on discharge; and in-hospital mortality. The Injury Severity Score (ISS) and head Abbreviated Injury Scale (h-AIS) score were obtained from the trauma registry. We defined neurosurgical intervention as operative intervention (craniotomy, craniectomy) and/or placement of intracranial pressure monitor. In addition, we also recorded the levels of markers of neuronal injury (neuron-specific enolase [NSE] and S-100B) in the blood samples obtained at 0, 6, and 24 hours. Patients were observed for edema, ecchymosis, thromboembolic disease process, skin breakdown, or development of neurologic complications (loss of sensation or motor function) at the site of application of the cuff during their hospital stay.

Power Analysis

A power analysis was performed to determine the number of patients required to be included in each group (treatment and control group). The sample size was estimated based on previous studies in TBI, which have assessed markers of head injury (NSE and S-100B). The power of the study was calculated to identify differences in the treatment and control groups for a decrease in the level of serum biomarkers on injury. After two-sided power analysis, statistical power of 80%, and an α of 0.05, we calculated a sample size of 20 per group (RIC and control groups).

Study Protocol

1. Patients meeting inclusion criteria were evaluated on admission in the trauma bay of our Level 1 trauma center for enrollment in the study.
2. Based on our power analysis, 20 consecutive patients with brain injury meeting inclusion criteria were enrolled in the RIC group followed by another 20 consecutive TBI patients in the control group.
3. In the RIC group, ischemic conditioning was performed using a standard manual blood pressure cuff. The pressure in the blood pressure cuff was maintained at 30 mm Hg higher than the patient’s SBP. Four cycles of ischemic conditioning were performed within 1 hour of admission. Each cycle consisted of 5 minutes of controlled upper limb ischemia (cuff up) followed by 5 minutes of reperfusion (cuff down). The total treatment cycle was 40 minutes. This study protocol was based on the published literature that has used the concept of RIC in nontrauma patients.16
4. Blood samples were collected in both the groups at 0 hour (before the application of the blood pressure cuff and performance of RIC in treatment group), 6 hours, and 24 hours. Blood samples were assessed for serum biomarkers of acute brain injury, S-100B, and NSE.
5. Patients were followed up through their hospital course to assess for patient outcomes and complications at the site of attachment of cuff.

Outcome Measures

The primary outcome measure was level of markers of neuronal injury (NSE and S-100B) in both the groups (RIC and control). The secondary outcome was local site complications at the site of attachment of cuff. Complications at the site of attachment of cuff were defined as edema, ecchymosis, thromboembolic disease process, skin breakdown, or development of neurologic complications (loss of sensation or motor function).

Neuronal Biomarkers of Injury

In this study, we used S-100B and NSE as biomarkers of neuronal injury. These are established markers of brain injury and are not routinely found in blood. The baseline level of these markers in patients without brain injury is zero. Both NSE (sensitivity, 80%; specificity, 73%) and S-100B (sensitivity, 90%; specificity, 78%) have high sensitivity and specificity for assessing neuronal injury. These markers are routinely elevated after neuronal injury, and the severity of brain injury has been shown to be proportional to the level of these markers in blood stream. In addition, sustained elevation of these markers after brain injury is known to be associated with worse outcomes. The levels of neuronal markers of injury in blood were analyzed by enzyme-linked immunosorbent assay using commercially available kits (NSE, Biotang Inc., Walham, MA; S-100B, Biovendor, Candor, NC).3,17

Statistical Analysis

Data are reported as mean (SD) for continuous descriptive variables, as median (range) for ordinal descriptive variables, and as proportion for categorical variables. We used Mann-Whitney U-test and the Student’s t test to explore for
differences in the two groups (RIC and control) for continuous variables. We used $\chi^2$ test to identify differences in outcomes between the two groups for categorical variables. Analysis of variance was used to assess for difference in the level of biomarkers at different time points. The one-way analysis of variance was used to assess for difference in the level of biomarkers between the two groups for categorical variables. Analysis of variance was used to perform repeated-measures analysis.

**RESULTS**

During the study period, a total of 59 patients were prospectively screened, of whom 40 patients (RIC, 20; control, 20) were included in the study. Of the 19 patients who were screened and excluded, 4 patients underwent emergent craniotomy, 3 patients underwent external ventricular drain placement before performance of RIC, 3 patients died within 24 hours of admission, 5 patients did not consent for enrollment in the study, and the remaining 4 patients were transferred from other hospitals.

Of the patients included in the study ($n = 40$), the mean (SD) age was 46.84 (21.7) years, 72.5% ($n = 29$) were male, the median (IQR) GCS score was 8 (3–8), and the median (IQR) admission SBP was 113.1 (20.6) mm Hg, and the median h-AIS score was 8 (3–8), the mean (SD) age was 46.15 (18.64) years, 72.5% ($n = 29$) were male, the median (IQR) GCS score was 8 (3–8), and the median (IQR) admission SBP was 113.1 (20.6) mm Hg, and the median h-AIS score was 8 (3–8).

Of the patients included in the study ($n = 40$), the ADC was 103.11 (197.96) pg/mL, while the mean (SD) NSE value was 316.22 (291.41) pg/mL for the entire study population. There was no difference in the 0-hour S-100B ($p = 0.9$) and NSE ($p = 0.72$) levels between the RIC and the control group. However, there was a significant reduction in the levels of S-100B ($p = 0.04$) and NSE ($p = 0.01$) at 6 hours and 24 hours in the RIC group when compared with the 0-hour level. However, there was a significant increase in the levels of S-100B ($p = 0.001$) and NSE ($p = 0.004$) at 6 hours and 24 hours in comparison with the 0-hour level in the control group. On performing paired analysis for comparing level of biomarkers, there was a significant reduction in the levels of NSE and S-100B at 6 hours and 24 hours in the RIC group compared with the control group. Figures 1 and 2 demonstrate the comparison of markers between the RIC and the control group at 0, 6, and 24 hours.

On subanalysis of patients without neurosurgical intervention ($n = 31$: RIC, 16; control, 15), all patients in the RIC group had a significant decline in the level of S-100B ($p = 0.01$) and NSE ($p = 0.001$) at 6 hours and 24 hours in comparison with the 0-hour level. Contrastingly, there was a significant increase in the levels of S-100B ($p = 0.001$) and NSE ($p = 0.004$) at 6 hours and 24 hours in comparison with the 0-hour level in the control group. All statistical analyses were performed using the SPSS version 21 (IBM, Inc., Armonk, NY).

**FIGURE 1.** Comparison between the control and the RIC group for the marker S-100B.

<table>
<thead>
<tr>
<th>TABLE 1. Demographics</th>
<th>RIC (n = 20)</th>
<th>No RIC (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>46.84 (21.7)</td>
<td>45.73 (17.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>≥65 y, % (n)</td>
<td>20 (4)</td>
<td>15 (3)</td>
<td>0.67</td>
</tr>
<tr>
<td>Male, % (n)</td>
<td>70 (14)</td>
<td>75 (15)</td>
<td>0.72</td>
</tr>
<tr>
<td>GCS score, median (IQR)</td>
<td>8 (3–8)</td>
<td>8 (3–8)</td>
<td>0.96</td>
</tr>
<tr>
<td>ED SBP, mean (SD)</td>
<td>115.8 (21.5)</td>
<td>109.6 (19.7)</td>
<td>0.72</td>
</tr>
<tr>
<td>ED heart rate, mean (SD)</td>
<td>84.2 (28.4)</td>
<td>87.9 (23.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>ED temperature, mean (SD)</td>
<td>37.3 (1.1)</td>
<td>37.1 (1.6)</td>
<td>0.81</td>
</tr>
<tr>
<td>Platelet count, ×10^12, mean (SD)</td>
<td>196.7 (86.4)</td>
<td>201.4 (72.9)</td>
<td>0.75</td>
</tr>
<tr>
<td>INR, mean (SD)</td>
<td>1.4 (0.3)</td>
<td>1.3 (0.5)</td>
<td>0.68</td>
</tr>
<tr>
<td>Mechanism of injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC, % (n)</td>
<td>60 (12)</td>
<td>70 (14)</td>
<td>0.51</td>
</tr>
<tr>
<td>Fall, % (n)</td>
<td>25 (5)</td>
<td>20 (4)</td>
<td>0.70</td>
</tr>
<tr>
<td>ISS, median (IQR)</td>
<td>18 (9–19)</td>
<td>17 (9–18)</td>
<td>0.68</td>
</tr>
<tr>
<td>Head AIS score, median (IQR)</td>
<td>3 (3–5)</td>
<td>3 (3–5)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

ED, emergency department; INR, international normalized ratio; MVC, motor vehicle collision.

**TABLE 2. Initial Head CT Scan Findings**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RIC (n = 20)</th>
<th>No RIC (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull fracture, % (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondisplaced</td>
<td>30 (6)</td>
<td>35 (7)</td>
<td>0.73</td>
</tr>
<tr>
<td>Displaced</td>
<td>25 (5)</td>
<td>15 (3)</td>
<td>0.42</td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDH, % (n)</td>
<td>45 (9)</td>
<td>50 (10)</td>
<td>0.75</td>
</tr>
<tr>
<td>Size, mean (SD), mm</td>
<td>6.4 (3.8)</td>
<td>5.9 (4.5)</td>
<td>0.38</td>
</tr>
<tr>
<td>EDH, % (n)</td>
<td>20 (4)</td>
<td>30 (6)</td>
<td>0.47</td>
</tr>
<tr>
<td>Size, mean (SD), mm</td>
<td>4.3 (3.7)</td>
<td>4.8 (4.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>SAH, % (n)</td>
<td>55 (11)</td>
<td>45 (9)</td>
<td>0.52</td>
</tr>
<tr>
<td>IPH, % (n)</td>
<td>15 (3)</td>
<td>10 (2)</td>
<td>0.63</td>
</tr>
<tr>
<td>IVH, % (n)</td>
<td>25 (5)</td>
<td>30 (6)</td>
<td>0.72</td>
</tr>
</tbody>
</table>

EDH, epidural hemorrhage; ICH, intracranial hemorrhage; IPH, intraparenchymal hemorrhage; IVH, intraventricular hemorrhage; NC, neurosurgical consultation; SAH, subarachnoid hemorrhage; SDH, subdural hemorrhage.
Nine patients (RIC, 4; control, 5) underwent neurosurgical intervention. Of the four patients (craniotomy, 3; intracranial pressure monitor placement, 1) in the RIC group that had intervention, the level of NSE and S-100B increased in the three patients with craniotomy; however, in the patient with intracranial pressure monitor, the markers remained unchanged at 0, 6, and 24 hours. All the patients in the control group had significant increase in the level of both NSE and S-100 markers at 6 hours and 24 hours in comparison with the 0-hour level. All patients who underwent neurosurgical intervention had an increment in their levels of NSE and S-100B regardless of RIC.

Table 3 demonstrates the outcomes between the RIC and the control group. No patient developed complications at the site of attachment of blood pressure cuff in the study population. Forty-five percent (n = 18) had progression on repeat brain CT scan, and neurosurgical intervention was performed in 22.5% of the patients after performance of the treatment. There was no difference in the rate of progression (p = 0.52) and neurosurgical intervention (p = 0.69) between the RIC and the control group. The median discharge GCS score was 13 (range, 11–15), and 40% (n = 16) of the patients were discharged home. There was no difference in the discharge GCS score (p = 0.76) and discharge disposition (p = 0.52) among the two groups. The overall mortality rate was 22.5% (n = 9). There was no difference in the mortality rate (p = 0.70) between the RIC and the control group.

**DISCUSSION**

The mainstay in the management of blunt TBI patients continues to be focused on the prevention of secondary brain injury. In this study, we have demonstrated a potential therapeutic role of a novel treatment modality, RIC in patients with severe brain injury. In a cohort of severe blunt TBI patients, we found a significant reduction in the markers of neuronal injury in patients who received ischemic conditioning. This was in comparison with the control group that had significant increases in the biomarkers as expected. This study is a proof of concept study, defining the potential role of RIC in TBI.

Several studies have shown an acute increase in the level of both NSE and S-100B after brain injury and a sustained elevation of these makers until 24 hours after neuronal insult. The initial elevation in the level of biomarkers was consistent with the published trends of these markers. In our control group, there was a significant increase in the level of these markers during the 24-hour period. This was consistent with the normal pattern of these markers in patients with neuronal injury. However, we found a significant reduction in the level of both NSE and S-100B after RIC at 6 hours and 24 hours in the RIC group. In addition, there was a significant difference in the level of both the markers (NSE and S-100B) at 6-hour and 24-hours time points between the RIC and the control group. We believe that this difference in the level of markers of neuronal injury may have been contributed by the protective or therapeutic effects of RIC. The activation of multiple inflammatory pathways in the RIC group may have blunted the secondary inflammatory insults and provided a global protection. Although in patients with intervention there was a rise in the levels of serum markers of neuronal injury, we believe that the performance of an intervention caused further damage to the neurons and caused elevation of neuronal markers. However, the level of biomarkers remained unchanged in the patient with intracranial pressure monitor in the RIC group and may demonstrate the protective effect of RIC. We believe that assessing the true clinical outcomes in patients with neurosurgical intervention in the RIC group may actually better help to define the role of RIC in this patient population.

Although there are multiple markers of brain injury, in our study, we used the two (NSE and S-100B) well-established markers of brain injury to assess the effect of RIC in patients with severe TBI. S-100B is a calcium binding protein, which is found in the astrocytes, Schwann cells, and the oligodendrocytes. In contrast, NSE is a cytoplasmic neuronal enzyme. Several studies have shown the association between these markers and severity of brain injury. Stein et al. demonstrated an association between the level of these markers and cerebral

**TABLE 3. Outcomes**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RIC (n = 20)</th>
<th>No RIC (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHCT, % (n)</td>
<td>60 (12)</td>
<td>55 (11)</td>
<td>0.75</td>
</tr>
<tr>
<td>Progression on RHCT, % (n)</td>
<td>40 (8)</td>
<td>50 (10)</td>
<td>0.52</td>
</tr>
<tr>
<td>Neurosurgical intervention, % (n)</td>
<td>20 (4)</td>
<td>25 (5)</td>
<td>0.69</td>
</tr>
<tr>
<td>Cuff site complications, % (n)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.98</td>
</tr>
<tr>
<td>LOS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital LOS, median (IQR)</td>
<td>5 (4–6)</td>
<td>5 (3–6)</td>
<td>0.37</td>
</tr>
<tr>
<td>ICU LOS, median (IQR)</td>
<td>3 (2–3)</td>
<td>3 (2–3)</td>
<td>0.54</td>
</tr>
<tr>
<td>Discharge GCS score</td>
<td>13 (12–15)</td>
<td>13 (11–15)</td>
<td>0.76</td>
</tr>
<tr>
<td>Discharge disposition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home, % (n)</td>
<td>45 (9)</td>
<td>35 (7)</td>
<td>0.52</td>
</tr>
<tr>
<td>Rehabilitation center, % (n)</td>
<td>30 (6)</td>
<td>30 (6)</td>
<td>0.91</td>
</tr>
<tr>
<td>Skilled nursing facility, % (n)</td>
<td>5 (1)</td>
<td>10 (2)</td>
<td>0.54</td>
</tr>
<tr>
<td>Mortality, % (n)</td>
<td>20 (4)</td>
<td>25 (5)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

ED, emergency department; NC, neurosurgical consultation; RHCT, repeat head CT.
hypoperfusion. In addition, in another study, Stein et al. also demonstrated the association of elevated level of these markers and worse outcomes. Although in our study we did not assess long-term brain function, the levels of both NSE and S-100B on admission as well as 6 hours and 24 hours after admission provided evidence to assess the utility of RIC in patients with severe injury.

The development of secondary brain injury is an important determinant of clinical outcomes in patients with TBI. Secondary brain injury is thought to occur hours to days after primary insult. It develops due to the activation of anaerobic glycolysis in the brain leading to initiation of pathophysiologic cascade, which ultimately leads to recruitment of inflammatory cells and release of inflammatory mediators. Cerebral ischemia, cerebral hypoxia, systemic hypotension, cerebral edema, and elevated intracranial pressure are considered to be responsible for the development of secondary injury. Although guidelines have established the management of hypotension, cerebral edema, and intracranial hypertension in patients with TBI, the optimal therapeutic management of ischemic injury in TBI patients remains unclear. Our study demonstrates the novel therapeutic role of RIC that may help in the management of secondary brain injury in patients with TBI. It also proves that RIC can be applied after injury effectively and easily without obvious consequences.

The concept of ischemic preconditioning was initially established by Murry et al., who demonstrated the protective effect of ischemic preconditioning on ischemic myocardium. They demonstrated a 75% reduction in the size of the myocardial infarct in the ischemic conditioning group in comparison with the control group. Since this original study, the protective benefits of ischemic preconditioning have been widely studied in multiple human and animal studies, which have established the safety and protective effects of ischemic conditioning in a number of organs and tissues including the kidneys, lungs, liver, skin flaps, ovaries, intestine, stomach, pancreas, and neurons. However, to our knowledge, there have been no studies assessing the role of RIC in TBI patients. Proposed theoretical mechanism of RIC is through the activation of a basic cellular stress response leading to multiple gene expressions and initiation of humoral, neuronal, and systemic pathways. Activation of these pathways provides protection against subsequent ischemic injury by improving endothelial function and also cerebral blood flow. However, given the similarities in the mechanism of action on RIC and the development of secondary brain injury, we sought out to evaluate its role in patients with TBI. We found that RIC reduced the levels of standard accepted neuronal injury markers compared with the control group (no RIC). RIC may have a protective or therapeutic role in patients with brain injury and may help to prevent secondary brain insults.

There remains a fear of development of local complications when using a blood pressure cuff for providing RIC. However, in our study, we did not identify any complications at the site of application of the cuff. The results of our study are similar to the other studies that demonstrated no local complications while performing RIC. The results of this study should be interpreted in context of its limitations. Although a prospective study, we did not randomize patients and consecutively enrolled patients in both the groups, and the sample size is relatively small to assess the impact of RIC on patient outcomes. We assessed the level of markers in patients’ blood samples for only 24 hours, and the level of markers was not assessed for a longer period. Other known markers of brain injury such as tau protein and glial fibrillary acidic protein were not assessed. We did not assess the time of RIC initiation from the time of injury. We did not assess the impact of RIC on long-term brain function and clinical outcomes of the patients. The lack of clinical differences can be caused by multiple reasons and needs further research. We did not assess the volume of intracranial hemorrhage to compare the severity of injury among the control and RIC groups. The study population may not be ideal as the TBI may have been too severe or not severe enough. RIC could not have been extensive enough or not applied frequently enough or even applied too much. These variables need to be tested in both animal and human subjects. However, despite these limitations, this study is the first study to our knowledge to highlight the beneficial effect of RIC in patients with severe TBI.

CONCLUSION

RIC with a standard blood pressure cuff applied to the arm within 1 hour of patient’s arrival to the trauma center significantly decreased the standard biomarkers of acute brain injury. This study demonstrates safety and feasibility of RIC in TBI patients. RIC may have a novel therapeutic role for potentially preventing secondary brain insults in TBI patients. Further research assessing the impact of RIC in TBI patients to determine the ideal patient population and method for performing RIC could potentially revolutionize the management of TBI.

AUTHORSHIP

B.J., V.P., R.S.F., and P.R. contributed in the study conception and design. B.J., V.P., M.K., N.K., B.Z., A.T., G.V., L.G., R.S.F., and P.R. performed the data analysis and interpretation of data. B.J., V.P., M.K., N.K., B.Z., A.T., L.G., and P.R. performed the analysis and interpretation of data. B.J., V.P., M.K., N.K., B.Z., T.O., L.G., R.S.F., and P.R. drafted the manuscript. All authors participated in the critical revision.

DISCLOSURE

The authors declare no conflicts of interest.

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DISCUSSION

Dr. Deborah M. Stein (Baltimore, Maryland): Dr. Joseph and his colleagues present us a small but well-done study on the use of remote ischemic conditioning in a subset of patients with severe traumatic brain injury.

The primary outcome measure of this study was reduction in serum biomarkers which are known to be associated...
with severity of traumatic brain injury. Dr. Joseph has demonstrated to us in an elegant fashion that when remote ischemic conditioning was applied, levels of these biomarkers were lower at 6 and 24 hours.

Remote ischemic conditioning is not a new concept but these authors are, in fact, applying it to a novel disease state. Why and how remote ischemic conditioning actually works nobody really knows.

But whatever the precise mechanism or combination of mechanisms that provide benefit, there are numerous animal studies and several human studies that suggest benefit in this relatively simple and non-invasive technique most commonly applied in cardiac surgery and after myocardial infarction.

However, despite the fact that remote ischemic conditioning has been studied since 1986, and a few human studies have demonstrated benefit on surrogate proximal endpoints, it seems that the concluding paragraphs of all human studies seem to end with some variation of "our findings merit a larger clinical trial to establish the effects of remote ischemic conditioning on clinical outcomes."

If we can’t demonstrate a real benefit in a disease as common as myocardial infarction over the past 30 years, what type of study would we need to design in order to demonstrate benefit in a disease whose outcome is so largely determined by the primary insult and has as much heterogeneity as traumatic brain injury?

I do have a few comments and questions for the authors. I am curious to know why you chose to look at consecutive patients in each group rather than randomized patients. How might this confound your results with respect to the fact that the intervention and control patients actually were not treated during the same time period?

Second, we know many interventions to improve traumatic brain injury have been tried and have failed. Despite encouraging results in animal studies, a variety of neuroprotective agents have been unsuccessful in demonstrating any clinical benefit in human trials.

Many of these failures may have to do with the application of therapies simply too late after injury to have clinical effects, given that we know that these secondary insults occur in seconds and minutes following the primary insult.

Much of the success of remote ischemic conditioning and other disease states such as cardiac surgery has been with the application of the intervention prior to a clinical insult or very shortly thereafter. I applaud you for applying this therapy so early in your hospital course.

Do you have any data on the time of injury versus the application of remote ischemic conditioning to see if, as we suspect for many other neuroprotective agents, earlier is in fact better?

Third, although this study was powered and designed to look at the effect of remote ischemic conditioning on very proximal outcome-namely serum biomarkers-do you have any data on the clinical proximal endpoints such as intracranial pressure?

And, lastly and most importantly, I am concerned that in this small study, albeit it was not remotely powered to detect clinical outcomes, there was no suggestion of any kind of clinical benefit.

What is your explanation for this and how would you design a study going forward? Just a larger study with more patients? What would the number needed to treat look like? Or are we not looking, perhaps, at the right way of doing remote ischemic conditioning or the right types of patients?

Again, I would like to congratulate the authors on their work. I thank the association for the privilege of discussing this paper. And, Dr. Joseph, I certainly look forward to seeing what you do with this in the future. Thank you.

Dr. Walter Biffl (Denver, Colorado): Bellal, that was an interesting paper. How many of these patients had ICP monitors? And did you see an increase in the ICP with this, which I assume was a painful intervention?

Dr. Aurelio Rodriguez (Wexford, Pennsylvania): Very provocative paper. A few years ago somebody presented a paper, "The effect of praying and outcome of patients in the ICU." It was discussed by Dr. Mattox. You can imagine what he said.

I have a question. You know the way the ACDs, the compressional devices, work is release of fibrinolysis. Basically, you are doing is squeezing with the blood pressure cuff. Have you measured fibrinolysis in these patients, by any chance?

And my second question is, based on your study would you recommend that the nurses in the ICU stop using intraarterial line and start using the blood pressure cuffs? Would that be your recommendation? Thank you.

Dr. Raul Coimbra (San Diego, California): If you have not done repeat imaging, do you think that if you had used advanced imaging modalities such as complex advanced MRI protocols or magnetic encephalography you would see a corresponding decrease in the secondary injury in the brain?

Dr. Bellal Joseph (Tucson, Arizona): Thank you, Dr. Stein, for your comments and questions. So why consecutive patients instead of randomizing them? I will just say that in our ICU we have TBI protocols to manage patients.

Initially, we didn’t know if this would work, to be honest. And we did our first 20 RIC patients and did the control later. But, as you can see, we didn’t change our practice of TBI over the time. As far as the timing of traumatic, or remote ischemic conditioning, you know there is a lot of studies that initially looked at preconditioning and it is referred to. But if you read a lot of the papers you know pre-, post- and peri-conditioning exists.

At our institution we did a septic mice model and looked at those factors to see when it was best, you know. And we saw that two hours post-septic insult was the best time to apply the remote ischemic conditioning. So the real question is, you know, when or how many times. I think those are still the million dollar questions.

We looked at a few patients of how long from injury time to the actual RIC occurred. And we didn’t have the data on all the patients but it was about 89 minutes from time of injury to time of ischemia in this study.

As far as secondary outcomes such as ICP, you know, there were only four patients that had ICP monitors in this study. We did not actually look at any other outcomes.

As far as the clinical outcomes in this study, again, this is a pilot study and you know when you look at outcomes in TBI everyone looks at the GOS as the long-term. It’s kind of like the 30-day mortality. What are the right outcomes?
I think as we move forward we’re going to have to look at both short-term and long-term outcomes, because I think that secondary hit does have some sequelae three and six months down the line. So I think that’s important to look at.

You know, again, to be able to alter two well-known or well-studied biomarkers from a simple blood pressure cuff I think may have some promise.

Dr. Biffl, as far as the ICP and the pain, we did not look specifically. I can go back and look through whatever ICP pressures we have. But you know it’s interesting you brought up the point about pain.

A lot of the patients were intubated, sedated and really couldn’t tell, but in a few of the patients I put it on myself. If you hold a cuff for five minutes at 30 millimeters above what systolic is it doesn’t feel good at five minutes, that’s for sure. So the pain is a question.

As far as the last comments, we did not measure fibrinolysis in the group. I didn’t hear the second part of your second question, something about the blood pressure cuff in the ICU but, you know, I’m not sure.

And then Dr. Coimbra’s points, you know, actually I wish we would have thought of what you just said prior to doing our study. I think as we move forward that will be something that we will do. We will look at advanced MRI protocols or CTA perfusion scans of some sort to kind of better delineate the actual effect in the brain tissue. Thank you.