Association between peripheral oxidative stress and white matter damage in acute traumatic brain injury

Poster No.: C-1489
Congress: ECR 2014
Type: Scientific Exhibit
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Keywords: Trauma, Inflammation, Acute, Laboratory tests, Diagnostic procedure, MR-Diffusion/Perfusion, Neuroradiology brain, Emergency, CNS
DOI: 10.1594/ecr2014/C-1489

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Aims and objectives

Approximately 1.4 million people sustain traumatic brain injury (TBI) in the United States every year [1]. Oxidative stress is believed to be one of the mechanisms involved in the neuronal damage and a close relationship exists between the degree of oxidative stress and the pathogenesis of TBI [2]. Thus, there is compelling clinical need for real-time serum biochemical marker tests to aid in the diagnosis and severity stratification of head injuries.

Lipid peroxidation and inflammatory processes have been shown to increase blood-brain barrier permeability. As an immediate response, vasogenic and cytotoxic edema develop within the first hour after TBI. Thiobarbituric acid reactive species (TBARS) is widely adapted as a sensitive method for measuring lipid peroxidation [3]. In addition, several studies have measured the levels of antioxidants as potential indirect markers of oxidative stress in brain injury. Plasma concentrations of ascorbic acid, α-tocopherol, and protein thiols are likewise associated with the degree of neurological impairment [4]. Biochemical marker testing that provides prognostic information on short-term patient outcome, especially among mild TBI cases, will be immensely valuable for patient management.

Diffuse axonal injury (DAI) and cortical contusions constitute the vast majority of primary intra-axial lesions in cases of TBI and are associated with significant morbidity. During TBI, the sub-cortical white matter, internal capsule, corpus callosum, fornix, and infratentorial white matter (brain stem and cerebellum) are the most common predicted regions of brain injury [5,6]. Conventional computed tomography (CT) and standard magnetic resonance imaging (MRI) often underestimate the extent of white matter damage after TBI [7]. Advances in MRI techniques has allowed for the visualization of changes, particularly after a patient’s earlier exposure to TBI [8]. The MRI diffusion tensor imaging (DTI) is potentially more sensitive for detecting intracranial microstructural change. Among various quantitative parameters such as apparent diffusion coefficient (ADC) and fractional anisotropy (FA), eigenvalues derived from DTI have been recognized as most useful for evaluating the integrity of white matter fibers [9].

To date, the relations of a panel of inflammatory markers and MRI DTI findings in acute TBI patients have not been examined. Under the hypothesis that increased systemic inflammatory biomarkers are associated with loss of anatomic integrity, this study measured DTI indices at the deep brain regions in TBI patients to evaluate their correlation with serum inflammatory biochemical marker levels.
Methods and materials

Patients

Sixty-three patients who sustained TBI between June and December 2012 and admitted at Kaohsiung Chan Gung Memorial Hospital were enrolled. The diagnosis of acute TBI was confirmed by history and brain CT scans. All of the patients underwent brain CT scan shortly after arriving at the emergency room. Repeat brain CT scan or/and MRI were performed for any clinical deterioration (e.g., acute-onset focal neurologic deficits, seizures, status epilepticus, and progressively disturbed consciousness) and as routine post-neurosurgical procedure. After complete neurologic examination and history taking, the patients were under continuous observation and monitored regularly for Glasgow Coma Scale (GCS) Score, electrocardiogram, blood pressure, pulse rate, temperature, fluid balance, and laboratory parameters.

On initial CT study, patients with massive epidural/subdural hemorrhage that could distort the brain tissue or with any parenchymal lesion that might affect diffusion tensor MRI results were excluded. Those with the following were also excluded: 1) age <20 years; 2) under medication with anti-platelet or anti-coagulant drugs before the acute TBI; 3) had evidence of alcoholism or any other addictive disorders, or known affective or other psychiatric diseases than those caused by sedatives or neuroleptics; 4) had known neurologic disorders potentially affecting the central nervous system; and 5) had major systemic diseases like end-stage renal disease, liver cirrhosis, or congestive heart failure.

Among the 63 patients, 25 had at least one of massive EDH, SDH, or SAH that caused anatomical structure distortions, while 11 had minor EDH, SDH, or SAH but combined with parenchymal contusion hematoma. Two patients were excluded due to alcoholism and one for age <20 years. Twenty-four patients with TBI were finally included in this study. For diffusion tensor MRI and biomarker comparison, 24 age- and sex-matched healthy volunteers were also enrolled. The Ethics Committee of the hospital's Institutional Review Board approved the study and all participants provided written informed consent.

Laboratory Measurements for Oxidative Stress Factors

Blood Sampling

Sera were isolated from peripheral blood samples drawn from each subject before and after the expedition. Blood samples were centrifuged at 3000 rpm for 10 minutes. Each serum sample was collected and frozen at -80°C prior to biochemical measurements.

Determination of Serum Thiobarbituric Acid-Reactive Substances

Thiobarbituric acid-reactive substances (TBARS) was measured based on a well-established method for detecting lipid peroxidation (Yagi, 1998). A TBARS Assay
Kit that allowed rapid photometric detection at 532 nm of the thiobarbituric acid-malondialdehyde (TBA-MDA) adduct, as described by the manufacturer (cat. 10009055; Cayman Chemical). Briefly, serum (100 mL) was added in duplicate to sodium dodecyl sulfate (SDS) (100 mL) and color reagent (4 mL). These mixtures were then incubated for 1 hour in boiling water and centrifuged at 1600 g for 10 min at 4°C. After warming for 5 min at 25°C, the samples were read on a micro-plate spectrophotometer (Beckman Coulter). Values for samples were calculated from a linear calibration curve prepared using pure MDA-containing samples (range, 0-50 µmol/L).

**Determination of Serum Free Thiol Content**

The ability of anti-oxidative defense in response to increased oxidative damage was evaluated by measuring the serum level of total reduced thiols since serum thiols were physiologic free radical scavengers. Serum total protein thiols were estimated by directly reacting thiols with 5,5-dithiobis 2-nitrobenzoic acid (DTNB) to form 5-thio-2-nitrobenzoic acid (TNB). The amount of thiols in the sample were calculated from the absorbance determined using the extinction coefficient of TNB (A412 = 13,600 M⁻¹cm⁻¹).

**MRI Acquisition**

The DTI datasets were acquired by using single-shot diffusion spin-echo, echo-planar imaging with a TR/TE of 15 800 millisecond/minimum, a 2.5-mm section, a matrix of 128 X 128, number of excitations of 3, and an FOV of 25.6 X 25.6 cm, yielding an in-plane resolution of 2 mm, with a total acquisition time of 12 min. The DTI encoding entailed 13 non-collinear directions, with b = 1000 s/mm², and a non-diffusion T2-weighted image. Contiguous sections (n = 55) were obtained without an intersection gap to achieve total cerebral coverage.

The DTI sets were transferred to an off-line workstation for further analysis using the FuncTool diffusion tensor protocol (Advanced Workstation 4.2; GE Healthcare), which contained a pre-processing function to remove echo-planar imaging distortions like scaling, shearing, and translation due to eddy current effects from a diffusion gradient. The distortion-corrected data were then interpolated to attain isotropic voxels and decoded to obtain the tensor field for each voxel. The algorithm computed the 6 coefficients of the diffusion tensor for each pixel location. The tensor field data was then used to compute the DTI metrics, including the mean diffusivity (ADC) and FA for each voxel.

**DTI Data Analysis**

Regions of interest, 50-100 mm² depending on the anatomic region, were measured by a radiologist and confirmed by another radiologist to avoid malpositioning (Fig. 1). The measurements were performed according to the method described by Bozzali et al. [10]. Regions of interest were placed on the caudate, putamen, globus pallidum, anterior and posterior limbs of the internal capsule, thalamus, cerebral peduncles, superior and middle
cerebellar peduncles, pontine crossing tract, medial lemniscus, and the genu, body, and splenium of the corpus callosum.

The first level of the region of interest was selected at the foramen of Monro. At this level, regions of interest at the caudate, putamen, globus pallidum, and thalamus were measured on two continuous sections (Figs. 1A and 1B). At the same level, the anterior internal capsule (bounded by the head of the caudate nucleus and the globus pallidus) and the posterior internal capsules (defined by the globus pallidus and thalamus) were measured on two contiguous sections (Fig. 1B).

The second level was selected at the inferior colliculus and the regions of interest at the cerebral peduncle and superior cerebellar peduncle and measured on two continuous sections (Fig. 1C). The third level was selected at the trigeminal nerve origin from the pons and the regions of interest at the middle cerebellar peduncle, pontine crossing tract, and medial lemniscus were measured on two continuous sections (Fig. 1D). Regions of interest at the genu, body, and splenium of the corpus callosum were placed on three consecutive sections of median sagittal section on which their full volume was obtained (Fig. 1E). Cerebral structures were carefully identified on T1- and T2-weighted images to avoid partial volume averaging due to CSF. Regions of interest were drawn on a null image of DTI and were automatically transferred onto FA and ADC maps for each subject. The average FA and ADC of 22 regions of interest were calculated. The raters were blinded to the subjects’ details.

**Statistical Analysis**

The Statistics Package for Social Science, Version 17.0 (SPSS Inc, Chicago; IL USA) software was used to perform all statistical analyses. The Student's t test and the chi-square test were applied to compare the age and sex between groups, respectively. Multivariate analysis of covariance (MANCOVA) model with age and sex as covariates were used to investigate differences in serum oxidative stress factors such as serum thiol and TBARS concentrations between the two groups. Post-hoc tests with Bonferroni’s correction were performed for multiple comparisons. Statistical significance was set at \( p < 0.05 \).

The MANCOVA model with age and sex as covariates was also used to investigate differences in the diffusivity indices of the regions of interest between the two groups. Post-hoc tests with Bonferroni’s correction were performed for multiple comparisons. The DTI indices between the two groups were significance when \( p \) was <0.05.

To access the correlation between serum oxidative stress factors and DTI-related indices for the regions of interest, Pearson’s partial correlation analysis with age and sex as confounding covariates was performed. To further investigate the relationships among TBI severity, serum oxidative stress factors, and DTI-related indices for regions of interest, the patients were graded based on their GCS, with a GCS >12 as grade 1, GCS 9-12 as grade 2, and GCS <9 as grade 3. Pearson’s partial correlation analysis with age
and sex as confounding covariates was again performed. Statistical significance was set at $p<0.05$. 
Fig. 1: Figure 1. Regions of interest were placed on the (A) caudate, putamen, globus pallidum; (B) the anterior and the posterior limbs of the internal capsule, thalamus; (C) cerebral peduncles, superior cerebellar peduncles; (D) middle cerebellar peduncles, pontine crossing tract, medial lemniscus, and (E) the genu, body, and splenium of corpus callosum.

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Results

Patients

The demographic and clinical information were listed in Table 1s. There were no significant differences between two groups in age ($p=0.164$, Student t-test) and sex ($p=1$, chi-square test).

The initial clinical symptoms of the patients with TBI were motor deficit (n=3), post-traumatic amnesia (n=5), and brief unconsciousness. There were no seizure attacks. The imaging findings showed minimal focal epidural hematoma (n=5), subdural hematoma (n=12), subarachnoid hemorrhage (n=12), and pneumocraniun (n=4). There was no depressed skull fracture among these patients. Two received ventriculostomy and craniotomy. With initial GCS of 13.74±3.018, the hospitalization duration reached 11.70±7.945 days, with 3.87±4.506 days in the ICU. One of the patients suffered new-onset neurologic deficit while two had deterioration of consciousness on follow-up.

There were significant differences in oxidative stress factors, including serum thiol and TBARS concentrations, between the two groups. The serum TBARS concentration of patients with TBI was significantly higher than those of the controls ($p=0.014$). There was only borderline difference between two groups in serum thiol concentration ($p=0.050$).

Table 1. Demographic data of patients with traumatic brain injury (TBI) and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients with TBI</th>
<th>Normal controls</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Numbers</td>
<td>24</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>(Male/Female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/13</td>
<td>12/12</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
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<tr>
<td>Age (age)</td>
<td>42.79 ± 15.56</td>
<td>42.67 ± 12.68</td>
<td>2.003</td>
<td>0.164</td>
</tr>
<tr>
<td>Serum Thiol concentration</td>
<td>1.63 ± 0.27</td>
<td>1.45 ± 0.35</td>
<td>4.065</td>
<td>0.050</td>
</tr>
<tr>
<td>Serum TBARS</td>
<td>18.24 ± 13.70</td>
<td>10.66 ± 3.244</td>
<td>6.558</td>
<td>0.014*</td>
</tr>
<tr>
<td>Initial Glasgow Coma Scale</td>
<td>13.74 ± 3.02</td>
<td></td>
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<tr>
<td>Motor deficit</td>
<td>3 (12.5%)</td>
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<td></td>
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</tr>
<tr>
<td>Condition</td>
<td>Count (Percentage)</td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------</td>
<td>--------------------</td>
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<tr>
<td>Posttraumatic amnesia</td>
<td>5 (20.8%)</td>
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<tr>
<td>Seizure</td>
<td>0 (0%)</td>
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<tr>
<td>Brief unconsciousness</td>
<td>7 (29.2%)</td>
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<tr>
<td>Depressed skull fracture</td>
<td>0 (0%)</td>
<td></td>
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<tr>
<td>Pneumocranium</td>
<td>4 (16.7%)</td>
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<tr>
<td>Traumatic subarachnoid hemorrhage</td>
<td>12 (50%)</td>
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<td></td>
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<tr>
<td>Subdural hematoma</td>
<td>12 (50%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidural hematoma</td>
<td>5 (20.8%)</td>
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<tr>
<td>Parenchymal contusion hematoma</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Operation</td>
<td>2 (8.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventriculostomy</td>
<td>2 (8.3%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Craniectomy</td>
<td>0 (0%)</td>
<td></td>
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<tr>
<td>Craniotomy</td>
<td>2 (8.3%)</td>
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<tr>
<td>Days of total hospitalization</td>
<td>11.70 ± 7.95</td>
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<tr>
<td>Days of intensive care unit</td>
<td>3.87 ± 4.51</td>
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<tr>
<td>Newly onset of neurological deficit</td>
<td>1 (4.2%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Deterioration of consciousness</td>
<td>2 (8.3%)</td>
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</tbody>
</table>

Data are demonstrated as means ± standard deviation.

* Significant differences ($p<0.05$)

**FA and ADC Values of DTI**
The FAs were significantly reduced in patients with TBI compared to those of the controls in the anterior limbs of the bilateral internal capsule, bilateral superior cerebellar peduncles, and left cerebral peduncle (Fig. 2). In patients with TBI, the FA was also reduced in most of the regions with abundant WM, including the posterior limb of the internal capsule, whole corpus callosum, right cerebral peduncle, middle cerebellar peduncle, pontine crossing tract, and medial lemniscus, although the differences were not significant (Supp. 1). There was no significant difference between the patients with TBI and normal controls in gray matter (Fig.2A) and in ADC value (Fig. 2B).

**Oxidative Stress Factors in Relation to FA and RD Values of DTI and Disease Severity**

All of the 48 subjects were examined for serum thiol and TBARS concentrations and DTI on the same day (1 day after TBI). There was significant correlation between serum TBARS concentration and the FAs of DTI (Fig. 3). Decreased FA in the left ($r = -0.524$, $p = 0.000$) and right ($r = -0.336$, $p = 0.026$) superior cerebellar peduncles and the right anterior limb of the internal capsule ($r = -0.396$, $p = 0.008$) were associated with higher serum TBARS concentrations (Figs. 3A-3C). The serum oxidative stress factors or FAs of DTI did not correlate with the initial GCS of the patients.
Fig. 2: Figure 2. The (A) FAs and (B) ADC of patients compared to the healthy controls. The al-IC and pl-IC represent the anterior and posterior limbs of the internal capsule, respectively, while the gCC, bCC, and sCC represent the genu, body, and splenium of the corpus callosum. The attached R/L represents the right/left side. *Significant difference between the patients and controls (p<0.05). SCP, superior cerebellar peduncle; CP, cerebral peduncle; MCP, mean middle cerebellar peduncle; PCT, pontine crossing tract; ML, medial lemniscus

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Fig. 3: Figure 3. Increased serum TBARS concentration correlated to the decreased FAs in the (A) left (r= -0.524, p=0.000) and (B) right (r= -0.336, p=0.026) superior cerebellar peduncles, and (C) the right anterior limb of the internal capsule (r= -0.396, p=0.008).

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Conclusion

Increased TBARS level and decreased WM integrity in vulnerable brain areas are found in patients with TBI. Possible interactions between peripheral inflammation and CNS microstructural damage likely represent the acute pathologic processes in TBI.
Fig. 3: Figure 3. Increased serum TBARS concentration correlated to the decreased FAs in the (A) left ($r = -0.524$, $p=0.000$) and (B) right ($r = -0.336$, $p=0.026$) superior cerebellar peduncles, and (C) the right anterior limb of the internal capsule ($r = -0.396$, $p=0.008$).
Personal information

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