Impaired cerebral mitochondrial function after traumatic brain injury in humans

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Object. Oxygen supply to the brain is often insufficient after traumatic brain injury (TBI), and this results in decreased energy production (adenosine triphosphate [ATP]) with consequent neuronal cell death. It is obviously important to restore oxygen delivery after TBI; however, increasing oxygen delivery alone may not improve ATP production if the patient’s mitochondria (the source of ATP) are impaired. Traumatic brain injury has been shown to impair mitochondrial function in animals; however, no human studies have been previously reported.

Methods. Using tissue fractionation procedures, living mitochondria derived from therapeutically removed brain tissue were analyzed in 16 patients with head injury (Glasgow Coma Scale Scores 3–14) and two patients without head injury. Results revealed that in head-injured patients mitochondrial function was impaired, with subsequent decreased ATP production.

Conclusions. Decreased oxygen metabolism due to mitochondrial dysfunction must be taken into account when clinically defining ischemia and interpreting oxygen measurements such as jugular venous oxygen saturation, arteriovenous difference in oxygen content, direct tissue oxygen tension, and cerebral blood oxygen content determined using near-infrared spectroscopy. Restoring mitochondrial function might be as important as maintaining oxygen delivery.

Key Words • cerebral energy metabolism • cerebral mitochondria • oxidative phosphorylation • cerebral blood flow • traumatic brain injury • cerebral ischemia

the human brain is highly dependent on aerobic metabolism to maintain ion balance and membrane stabilization, neuronal function, and synthesis of structural components.1-9,10,24-26 The major purpose of oxidative phosphorylation in mitochondria is to produce ATP. Adenosine triphosphate is continuously produced almost exclusively by the oxidation of glucose and is the energy carrier for most cellular homeostatic mechanisms. When the supply of ATP is insufficient, homeostatic mechanisms deteriorate, intracellular concentration of calcium increases, and cell death is inevitable.29

In the past, research efforts in patients with TBI have focused on optimizing the delivery of oxygen and glucose to the injured brain in an attempt to maintain the ATP supply and to avoid neuronal compromise. It is possible that limiting factors in synthesizing ATP may be not only inadequate delivery of oxygen and glucose but also impairment of mitochondrial function. It has previously been demonstrated in animal models that TBI results in mitochondrial dysfunction, which is characterized by impairment of ATP production and calcium ion regulation.32 To date there has been no objective evidence of mitochondrial dysfunction after TBI in humans.

Tissue fractionation procedures were used to isolate brain mitochondria in 16 patients with severe TBI and two control patients without TBI. Mitochondrial function was analyzed and characterized in the presence of sufficient substrates and oxygen by using the following parameters.

State 3 Respiratory Rate

The State 3 respiratory rate is the active state during which mitochondria exhibit maximum activity in the presence of sufficient amounts of respiratory substrates (pyruvate, glutamate, malate, and succinate), oxygen, and ADP. High State 3 rates indicate that mitochondria are functioning in a normal fashion.
The P/O Ratio

The P/O ratio is a measure of the efficiency of ATP synthesis. The P/O ratios are determined by dividing the amount of ADP that is phosphorylated during State 3 respiration by the amount of oxygen consumed. High P/O ratios indicate the normal phosphorylating efficiency of the mitochondria.

Mitochondrial Calcium Content

The mitochondrial calcium content is a sensitive indicator of mitochondrial function. Impaired mitochondria are unable to regulate intracellular calcium and, as previously mentioned, when ATP levels are insufficient, intracellular calcium cannot be regulated in a normal fashion. Uncontrolled intracellular calcium content results in cell death.

A portion of the results reported here have been published earlier by us in a preliminary form.30

Clinical Material and Methods

Patient Population and Clinical Management

The Human Investigation Committee of the Wayne State University School of Medicine approved this study, which was conducted at Detroit Receiving Hospital from August 1996 through October 1997. Consent was obtained from immediate family members of all patients, and all TBI cases were clinically managed according to a standard protocol (guidelines set forth by the American Association of Neurological Surgeons for neurotrauma). Eighteen patients who had undergone therapeutic removal of brain tissue were studied. Patient data, tissue characteristics, and clinical parameters are summarized in Table 1. Sixteen patients who suffered from TBI (GCS Scores 3–14), including three patients with GSWs and two control patients were studied. Patient ages ranged from 17 to 58 years, with a mean of 31 years; 75% of patients were men. In the first control patient (Case 17), macroscopically normal brain tissue was obtained when the patient underwent lobectomy for intractable seizures. The second control patient (Case 18) had uncontrollable intracranial hypertension due to a stroke, and a partial temporal lobectomy was performed to decrease the ICP. Noninfarcted tissue resected from the temporal lobe in this case was analyzed.

Before, during, and after surgery, arterial blood pressure and ICP (ventricular drain or intraparenchymal monitor) were continuously recorded using a standard Pentium personal computer and appropriate software.

Isolation of Brain Mitochondria

Mitochondria were isolated according to the procedure described by Lee, et al.,15 with minor modifications.32 Immediately after removal, brain tissue was placed into SEE medium (250 mM sucrose, 10 mM 4-[2-hydroxyethyl]-1-piperazine-ethanesulfonic acid [pH 7.4], 1 mg/ml bovine serum albumin, 0.5 mM ethylenediamine tetraacetic acid, and 0.5 mM EGTA) at 0°C, and tissue analysis was performed within 10 minutes. The tissue was rinsed and minced. The minced tissue was suspended in 5 ml SEE medium containing the proteinase nagarse (2.5 mg/g tissue), homogenized for 2 minutes with the aid of a handheld Teflon–glass homogenizer, and diluted to 10 ml by adding SEE medium. The homogenates were centrifuged at 2000 G for 3 minutes, and the resulting supernatant was decanted and centrifuged at 12,000 G for 8 minutes. The pellets were resuspended in 10 ml SEE medium and re-

<table>
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<tr>
<th>Case No.</th>
<th>Event Main Pathological Findings on CT Scan</th>
<th>Macropscopic Appearance of Tissue</th>
<th>Time to Preop Surgery (hrs)†</th>
<th>Preop GCS Score</th>
<th>Preop ICP (mm Hg)</th>
<th>Preop MABP (mm Hg)</th>
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* GOS = Glasgow Outcome Scale (scores: 1 = death, 2 = persistent vegetative state, 3 = severe disability, 4 = moderate disability, 5 = good recovery); ICH = intracranial hypertension; MABP = mean arterial blood pressure; NA = not applicable; — = unknown or not measured.
† Period between trauma and surgery.
‡ Patient died as a result of aorta rupture.
§ Due to stroke (part of the temporal lobe). After temporal lobectomy, noninfarcted tissue was analyzed.
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centrifuged at 12,000 G for 10 minutes. Finally, the mitochondrial pellets were resuspended in 10 ml of 0.25 M sucrose and recentrifuged at 12,000 G for 10 minutes. The mitochondrial pellets were suspended in 0.25 M sucrose. The entire procedure was completed within 1 hour.

Mitochondrial protein was determined according to the procedure described by Lowry, et al.,16 in which bovine serum albumin is used as the standard.

Assay of Respiratory and Phosphorylating Activities of Mitochondria

Mitochondrial respiratory activities were determined polarographically by using a Clark-type oxygen electrode fitted into a thermostat-regulated chamber with a 1-ml volume. The reaction medium consisted of 150 mM sucrose, 25 mM Tris-HCl, 10 mM phosphate buffer (pH 7.4), and 0.5 to 1.7 mg of mitochondrial protein. The final concentrations of the substrates were 5 mM/2.5 mM for glutamate/malate and pyruvate/malate and 5 mM for succinate (plus 0.25 μM rotenone). State 3 respiration was initiated by the addition of ADP (258–263 nmol for nico- tinamide adenine dinucleotide–linked substrates and 152–175 nmol for succinate).

Fluorescence Assay of Calcium Content and Transport Activities

The mitochondrial inner membrane, which is impermeable to fura-2 K+, was used as a probe for calcium in the medium and placed outside the mitochondrial permeability barrier. A downward deflection of the fluorescence ratio signified the disappearance of calcium from the fura-2 K+ accessible pool into the inaccessible one. Ionomycin, the calcium ionophore, released the intramitochondrial free calcium. Sodium dodecyl sulfate did not significantly alter the fluorescence ratio, indicating that there was no appreciable amount of intramitochondrial membrane-bound calcium.

Mitochondrial calcium content and uptake activity were assayed fluorometrically by using a spectrofluorometer. The assay system included the calcium probe, fura-2 K+ with 340 nm or 380 nm excitation and 510 nm emission, following the program provided by the manufacturer. The assay medium consisted of 100 mM potassium chloride, 10 mM 4-morpholinepropanesulfonic acid (pH 7.2), 0.4 to 1.8 μM calcium, 2.5 μM rotenone, and 2 μM fura-2 K+, with a total volume of 3 ml. The temperature was maintained at 30°C. The calcium uptake was induced by the addition of 5 mM succinate. Increases in the calcium ionophore (ionomycin)– and sodium dodecyl sulfate–induced fluorescence ratios represent the intramitochondrial levels of free and membrane-bound calcium, respectively. The calcium concentration was calculated according to the equation proposed by Grynkiewicz, et al.,13 [Ca++] = [Kd (R – Rmin)/(Rmax – R) × Ss/Sr], in which Kd is equal to 120 nM at 30°C, R represents the ratio of signals obtained with excitation at 340 nm and 380 nm and emission at 510 nm, Rmin is the minimum fluorescence ratio at a concentration of zero calcium (achieved with excess of EGTA), Rmax is the maximum fluorescence ratio at a saturating calcium concentration, Ss is the fluorescence signal of free fura-2 K+ at 380 nm, and Sr is the fluorescence signal of the fura-2 calcium complex at 380 nm.

Inhibitory and Uncoupling Effects Induced by TBI on the Respiratory and Phosphorylating Activities of Mitochondria

The State 3 respiratory rates of brain mitochondria derived from patients with TBI varied widely. However, these rates were markedly impaired when compared with those of the control patients (Cases 17 and 18). The P/O ratios were also decreased and showed great variability in patients with TBI (Table 2).

Effects of TBI on Calcium Transport

In patients in whom a k value could be determined (in most cases there was insufficient tissue for analysis), there appeared to be a decrease in calcium uptake by the mitochondria. This was also seen in the patient in Case 18. The amount of calcium associated with mitochondria in TBI patients appeared to be increased in the Cases 7 to 11, 15, and 18 (Table 2).

Discussion

Analysis of the findings of our study demonstrates that severe human TBI produces impaired mitochondrial function; this is similar to data documented in animal studies.32 The State 3 respiratory rate was slowed and there was impairment of ATP production. In addition, there was in-

Sources of Supplies and Equipment

The intraparenchymal monitor was purchased from Camino–Neurocare (San Diego, CA) and the accompanying computer software was developed in cooperation with Neurocare. The nagarse was purchased from Teikoku Chemical Co. (Osaka, Japan). The Clark-type oxygen electrode was acquired from Yellow Springs Instruments Co. (Yellow Springs, OH).

For fluorescence assays, we used a spectrofluorometer (model 8000C) purchased from SLM-AMINCO (Urbana, IL) and a fura-2 K+ obtained from Molecular Probes, Inc. (Eugene, OR).
increased intramitochondrial calcium. These results are indicative of impaired mitochondrial function.

The present study was clearly preliminary. In addition, normal healthy human values are not known; it is obviously not possible to obtain absolutely healthy control patients. However, our control patients had similar State 3 values and P/O ratios, and the differences between these findings in patients with TBI and control patients were very large. Finally, there was no way to control for the TBI mechanism of injury and disease, time from trauma to analysis, and factors such as ICP. Nonetheless, these are important results because they are the first studies in humans to be reported. There does appear to be evidence of mitochondrial dysfunction in traumatized injured human brain tissue.

In five of 13 patients tested, mitochondrial efficacy (P/O ratio) improved by at least 20% when calcium was decreased out of the mitochondrial membrane by adding the calcium chelator EGTA to the medium, again providing findings similar to those in our experimental study of brain injury.22 Of these five patients, the two demonstrating the most improvements (Cases 6 and 12) also made the best outcomes of all the patients. This is an important finding because it indicates that, at least initially, the mitochondrial dysfunction is not structural but only functional and related to an excess of intracellular calcium, which can possibly be reversed by pharmacological treatment.11,23

Results of previously reported studies have indirectly demonstrated that mitochondrial dysfunction does in fact occur in humans with TBI. When aerobic metabolism is insufficient, far less efficient anaerobic metabolism occurs (lactate is produced as a result) in an effort to avert energy failure. In clinical TBI studies cerebrospinal fluid lactate was found to be increased, despite the presence of sufficient oxygen and glucose.8 This indicates that, despite an abundance of substrates, aerobic metabolism was depressed. In addition, the increased demand on the glycolytic pathway results not only in lactic acid formation, but also in hyperglycolysis, which is an abnormal cellular state characterized by increased glucose metabolism relative to oxygen utilization. Hyperglycolysis has been demonstrated in 56% of patients who were studied by using positron emission tomography scanning within 1 week after severe TBI, even when sufficient oxygen was present.2 Finally, when CBF remains sufficient throughout the post-traumatic period, the AVDO2 decreases to lower than normal levels after 12 to 48 hours, whereas the SjvO2 and PiO2 increase.4,6,12,14,19,21,23,28,31 This delayed, decreased utilization of oxygen by the brain may indicate development of mitochondrial dysfunction over time. This phenomenon has been observed in animal models of TBI.2 In those experiments rats sustained a cortical impact injury, mitochondria gradually became dysfunctional, and maximum impairment occurred 12 to 24 hours after injury. This impairment persisted for 14 days. Depression of the CMRO2 has previously been demonstrated in humans with TBI.16,17,19,20,22,27 however, it has not yet been established whether this was due to diminished metabolic requirements of the neurons or to an impaired functional state of the mitochondria. Continuous measurement of mitochondrial function would be necessary to verify this phenomenon in patients; however, this is technically impossible at present. Figure 1 shows schematically the possible role of mitochondrial dysfunction in the pathophysiology of head injury.

Clinical Implications

The definitions of ischemia and hyperemia and the balance between delivery and use of oxygen and substrates have been subjects of heated debate.7,18 Considering the findings of this preliminary study, it may be necessary to rethink the clinical definitions of hyperemia and ischemia. If mitochondrial dysfunction does occur in humans with TBI, oxygen demand will be decreased, lactate levels will increase, and CBF will decrease. However, if hyperemia and ischemia are defined solely on the basis of measurements of CBF, oxygen, and lactate levels, the causes of these changes will be misinterpreted. For example, if mitochondrial function is impaired, maintenance of adequate perfusion (cerebral perfusion pressure > 70 mm Hg) may not result in sufficient ATP synthesis and, therefore, may not improve clinical outcome.

Van Santbrink and colleagues28 found increasing PiO2 values and Cormio and colleagues29 found increasing SjvO2 values over time following human head injury. This increase in PiO2 and SjvO2 may be due to decreased utilization of oxygen caused by mitochondrial dysfunction, which evolved over time, a phenomenon we observed in our animal experiments.32 This might have important clinical implications because it has recently been suggested...
that an increase in SjvO\textsubscript{2} due to a decrease in CMRO\textsubscript{2} has been linked to an unfavorable outcome\textsuperscript{7}.

Preventing mitochondrial dysfunction or restoring mitochondrial function might be as important as maintaining oxygen delivery. In animal models of TBI it has been shown that pharmacological intervention effectively maintains mitochondrial function.\textsuperscript{29,30,33,34} In another study of TBI in animals, neurobehavioral outcome improved in response to pharmacotherapy that had previously been shown to maintain mitochondrial function.\textsuperscript{3} The similarity between human and experimental TBI mitochondrial studies is encouraging. It may be possible to use data obtained in TBI animal studies to clarify the pathophysiological processes in TBI in humans and to evaluate the effectiveness of pharmacotherapies.

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**References**


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