

Synthes Award for Resident Research on Craniofacial and Brain Injury: Effect of Cyclosporin A, Topiramate, or 100% Oxygen as Proposed “Neuroprotective” Therapies on the Neurochemical Analytes in Patients with Severe Traumatic Brain Injury

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INTRODUCTION

Three separate phase II, mechanistic, clinical trials have recently evaluated the biological and “neuroprotective” effects of Cyclosporin A,^{14,15} topiramate,³ and 100% oxygen¹⁷ in patients with severe traumatic brain injury (TBI). The current study is a combined analysis of these three trials, and a direct comparison of the effect of these three drugs on the local brain neurochemistry, as assessed by microdialysis.

Cyclosporin A has received attention for its ability to block the mitochondrial transition pore (MTP).^{1,2} This pore forms on the surface of mitochondria because of the large cytoplasmic calcium influx after injury.¹³ Through this pore, the mitochondrial homeostasis and the potential for energy production are lost. The rationale for the use of cyclosporin is to protect the mitochondria, preserve ATP production, and help stop apoptosis after head injury.^{1,2,16} In a double-blinded, placebo-controlled, randomized clinical trial, 37 patients received the drug cyclosporin and 13 patients received placebo. Findings from this study show changes in the microdialysis parameters as well as improvements in the mean arterial pressure and cerebral perfusion pressure.^{14,15}

Topiramate is an anticonvulsant and neuroprotectant. It has previously been studied in trials for epilepsy and stroke.²⁰ Among its various cellular effects, topiramate functions as a presynaptic glutamate release inhibitor.³ Glutamate and other excitatory amino acids are released in massive amounts from the neurons after the most severe forms of TBI.⁶ This creates an environment of excitotoxicity on neighboring neurons.⁷ The rationale for using topiramate is to help block this prominent pathway to cell damage and cell death after inju-

ry.¹⁰ In an open label trial, 20 patients received the drug topiramate in a dose escalation protocol.³

Brain oxygen therapy, or “normobaric hyperoxia,” consisted of 100% oxygen given through the ventilator for the first 24 hours after injury.¹⁷ The rationale is to help combat the known negative consequences and increased mortality associated with low brain tissue oxygen tension.¹⁸ In this prospective, multicenter, open-label trial, 42 patients received both brain oxygen therapy and microdialysis catheters. These patients were compared with 109 matched patients from a severe head injury microdialysis data base. Findings from this oxygen study showed changes in microdialysis parameters as well as improvements in intracranial pressure and brain tissue oxygen tension.¹⁷

Microdialysis is a technique for determining the concentration of small molecular weight substances in the extracellular space of the brain, in small aliquots (60 μ l) of dialysate fluid. Measurements are typically made every hour. Commonly, glutamate, glucose, pyruvate, and lactate are measured by enzyme-linked immunosorbent assay (ELISA) or high-performance liquid chromatography (HPLC). Three patterns of neurochemical substrate changes are commonly seen after injury. First, glutamate levels after injury are always elevated.¹⁹ Significant secondary increases, up to 50 to 100 times normal levels, can be seen if the head injury is complicated by other insults, such as ischemia.¹⁹ Second, anaerobic cerebral metabolism has the characteristic signature of elevations in lactate and significant reductions in glucose¹⁸—sometimes approaching zero. The lactate/pyruvate ratio (normally less than 25) and the lactate/glucose ratio (normally less than 2) are also sensitive markers for ischemia.

MATERIALS AND METHODS

An academic teaching hospital in the United States performed three separate clinical trials. The trial of oxygen

therapy was performed in cooperation with a European teaching hospital. Results from these three studies are currently available in the literature.^{3,14,15,17} The studies focussed on adult patients with severe TBI. Each trial had individualized inclusion and exclusion criteria, as well as varied but specific details for therapy administration.^{3,15,17} All trials were approved by the institutional review board, and appropriate family consents were obtained for each patient.

To permit direct comparison between these three trials, the current secondary analysis was performed. The raw microdialysis data, which had been prospectively gathered for each earlier trial, was collected. Data analysis was standardized by “hours after injury.”

Four groups were analyzed. Thirty-seven patients received cyclosporin (Sandimmune, Novartis, East Hanover, NJ). Twenty patients received topiramate (Topamax; Ortho-McNeil Neurologics, Inc., Titusville, NJ). Forty-two patients received oxygen therapy and were equipped for microdialysis monitoring.¹⁷ A fourth group of 109 patients from the Richmond Microdialysis in TBI Data Bank had previously been matched to the oxygen-treatment group. These severe TBI patients all received microdialysis monitoring at a time before any of the three trials. Data from these 109 patients was used without further selection as a historic, matched, control cohort with similar TBI severity.

Microdialysis (CMA Microdialysis AB, Solna, Sweden) data included concentrations of glutamate, glucose, pyruvate, and lactate obtained every 60 to 90 minutes. Monitoring was begun as soon as possible after injury and continued for up to 5 full days. The lactate/pyruvate and lactate/glucose ratios were calculated. *Figures 35.1 to 35.7* show the variation in extracellular analytes for all four groups over time.

Raw microdialysate values were reported (2 $\mu\text{l}/\text{min}$ perfusion with normal saline dialysate). No corrections were

made because the “tortuosity fraction” of the extracellular fluid space could not be reliably estimated. In *in vitro* experiments, the CMA 70 probes (10 mm active length, 20 kD cutoff MW) yielded 40 to 45% recovery for all analytes reported.⁶

A Microsoft Access (Microsoft Corporation, Redmond, WA) database was used for collection of microdialysis and other patient data. SPSS 11.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. The mean hourly values for each group were calculated. To smooth the data, combined data from each group, averaged over 12-hour intervals was used for analysis of variance (ANOVA), and a post hoc analysis with the Tukey honestly significantly different (HSD) statistic was used to evaluate the between-group differences. A total of approximately 90,000 measurements were analyzed.

For simplicity, only the data-regression lines are shown on *Figs. 35.2 to 35.7*. From a Microsoft Excel spreadsheet, the closest fitting equation was selected for each regression line. The graphs use mostly high-order polynomial equations. For each, the highest-order of polynomial equation, which best fit the data, and provided the highest R^2 value, was chosen. If a polynomial equation mapped a curve that crossed the x axis, it was excluded, and the next order lower equation was chosen. The trend line for glutamate concentration in the control group best fits a power function suggestive of a typical “clearance pattern.” The trend line for lactate/glucose ratio in the oxygen group was also best fit by a power function, and the trend line for glucose concentration in the topiramate group was best fit by an exponential function.

In each graph, the bars at the top of the graph represent periods of time for which there is a statistically significant difference. The P value between named groups is noted beside each bar. Two asterisks represent a difference from the control population and three asterisks represent the difference from all other treatment groups.

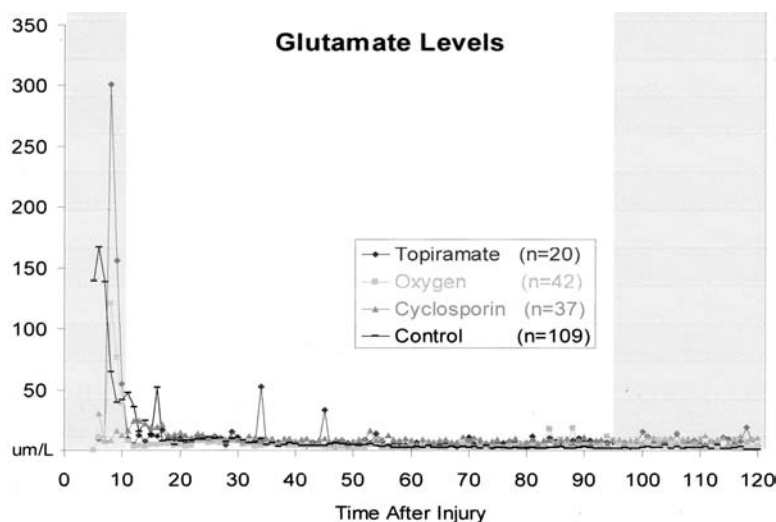


FIGURE 35.1 Average glutamate concentrations for each of four study groups versus time (hours after injury). Glutamate levels for all groups rise high early in response to injury, and then rapidly decline. “Normal” glutamate concentration is approximately 2 $\mu\text{mol}/\text{L}$.

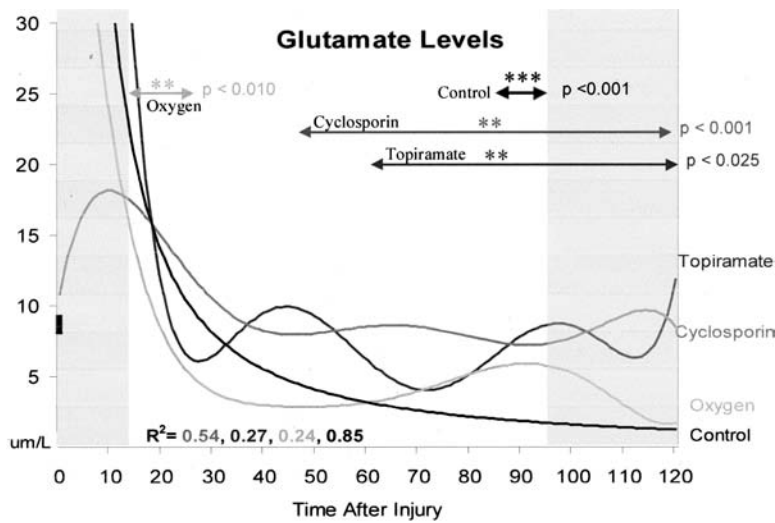


FIGURE 35.2 Glutamate concentrations from 0 to 30 $\mu\text{mol/L}$. The oxygen group declines more rapidly and is significantly lower than the control population from 12 to 24 hours after injury. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

RESULTS

The first microdialysis data graph (Fig. 35.1) shows the overall concentration of glutamate versus time. Characteristic of TBI, glutamate levels rise high early in response to injury, and then rapidly decline to very low levels (normal values near 2 $\mu\text{mol/L}$) for the remainder of the 5-day monitoring period.

Figure 35.2 focuses on the glutamate concentrations from 0 to 30 $\mu\text{mol/L}$. The oxygen group declines more rapidly—ahead of the control population. From 12 to 24 hours after injury, the oxygen group has achieved the very low levels associated with tissue recovery, and is statistically significantly lower than the control population.

The graph of glucose concentrations (Fig. 35.3) shows that the cyclosporin group was associated with significant elevation in extracellular fluid glucose over all other groups

for almost the entire monitoring period. Topiramate has a similar, but less marked effect, and it is elevated above the control population during the second, third, and fourth days.

The graph of pyruvate concentrations (Fig. 35.4) shows that the cyclosporin group was associated with values that are significantly elevated over the other groups. Pyruvate, in the oxygen group, was significantly lower than all of the other groups from 12 to 24 hours after injury.

Lactate concentrations (Fig. 35.5) again show that the cyclosporin group was associated with significantly elevated lactate over all other groups throughout most of the monitoring period. The oxygen group was associated with significantly lower values for lactate than the control population for the first 48 hours after injury.

As expected in TBI, the lactate/pyruvate ratio (Fig. 35.6) demonstrates significant elevations in all groups. An

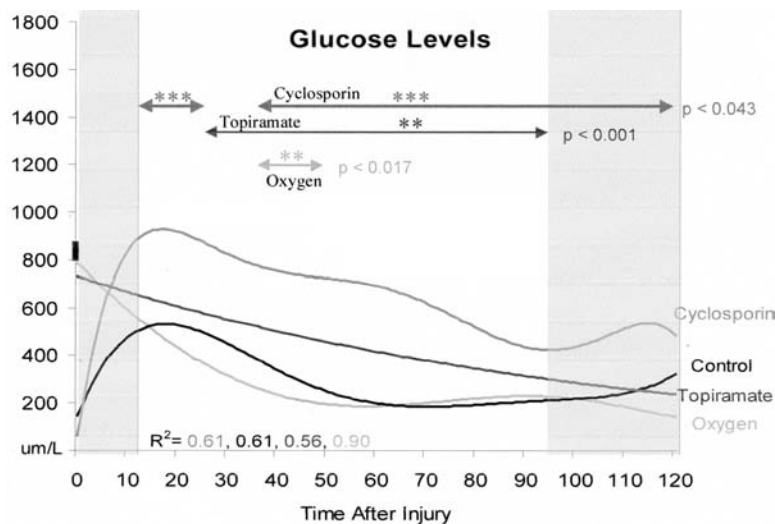


FIGURE 35.3 Glucose concentrations versus time. The cyclosporin group is significantly elevated though much of the monitoring period. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

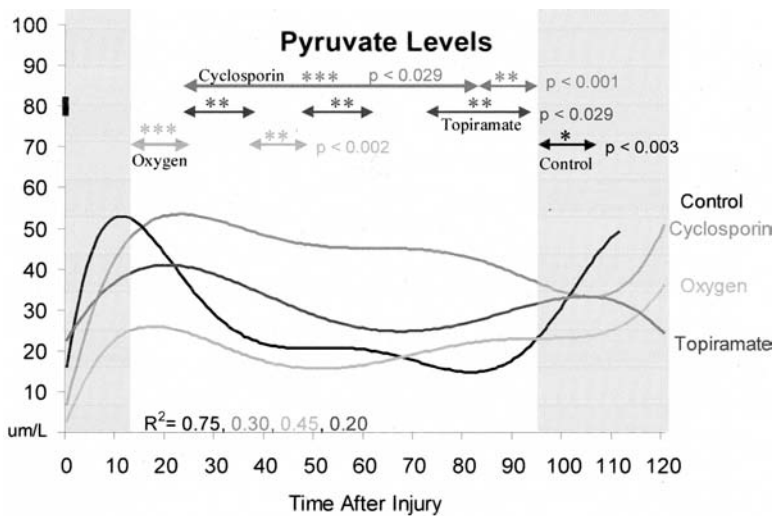


FIGURE 35.4 Pyruvate concentrations versus time. The cyclosporin group is significantly elevated from the 2nd through the 4th day. The oxygen group is lower from 12 to 24 hours after injury. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

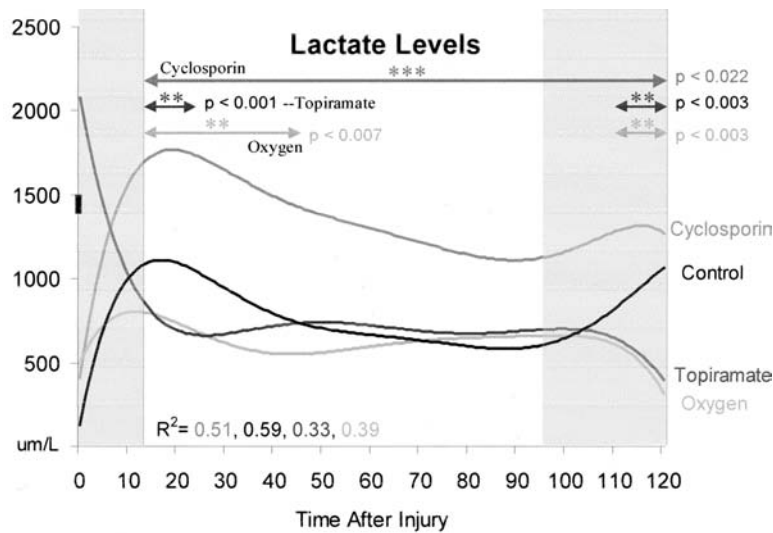


FIGURE 35.5 Lactate concentrations versus time. The cyclosporin group is significantly elevated over all other groups. The oxygen group is lower than control from 12 to 48 hours after injury. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

lactate/pyruvate ratio greater than 25 is evidence for ischemia. This data, thus, supports the high frequency of ischemia, or disturbed substrate metabolism, in the majority of the study patients. Only the topiramate group came below the threshold value of 25, from 12 to 24 and 72 to 96 hours after injury.

Although lactate values for the cyclosporin group were significantly elevated, the lactate/pyruvate ratio was the same as, or less than, the control population. The same pattern was seen for the cyclosporin group in terms of the lactate/glucose ratio (Fig. 35.7). However, it is the topiramate group that has the longest duration of this effect.

DISCUSSION

This study evaluated the neurochemical consequences of three potentially “neuroprotective” drugs. Each

of these “drugs” was evaluated in a phase II clinical trial. Therefore, their effect on long-term outcome has not been established. The cyclosporin group was notable, however, for significant elevations in both energy substrates. If cyclosporin is later shown to be “neuroprotective,” it may be because of this effect on cellular energy metabolism. In this study, this effect seems clear and robust over more than 20,000 measurements.

Cyclosporin treatment was also associated with delayed glutamate clearance and elevation in lactate concentrations. However, elevated lactate levels were not accompanied by corresponding changes in the glucose concentration or lactate/pyruvate and lactate/glucose ratios. Without these secondary changes, there is not clear evidence to suggest anaerobic metabolism.⁸ Alternatively, lactate is known to transfer from astrocyte to neuron as an energy substrate during in-

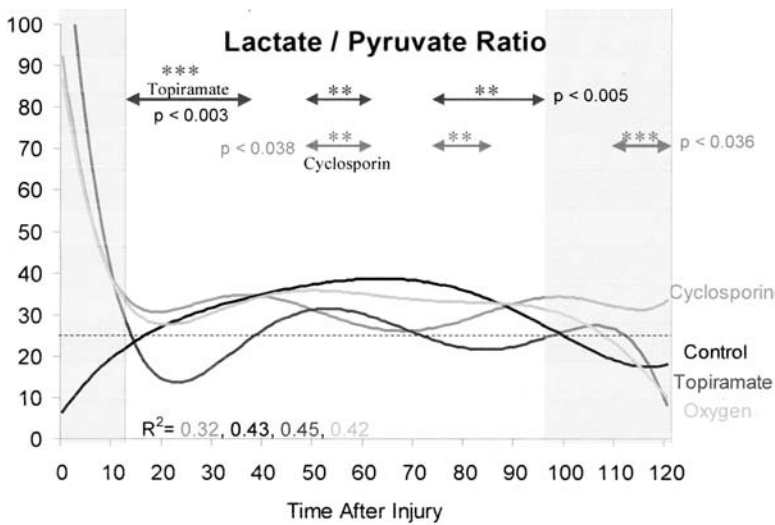


FIGURE 35.6 Lactate/pyruvate ratio versus time. Only the topiramate group comes below an ischemia threshold value of 25. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

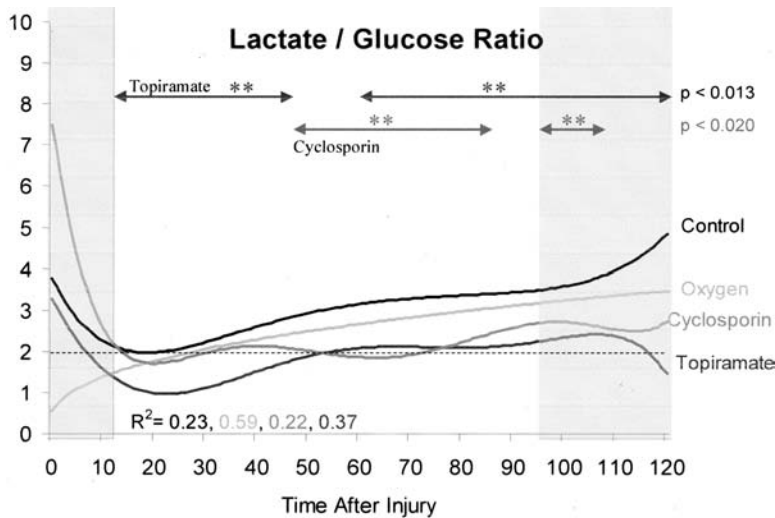


FIGURE 35.7 Lactate/glucose ratio versus time. The topiramate and cyclosporin groups are the same as, or lower than control. Only data-regression lines are shown (see text). Bars show periods of time with statistical difference. **Difference from the control population. ***Difference from all other groups.

creased synaptic activity.^{4,9,12} Whether a similar mechanism occurs within the injured brain in response to this “mitochondrially active agent” remains unknown.

Topiramate showed a similar effect to cyclosporin, with elevations in energy substrates and improvements in the lactate/pyruvate and lactate/glucose ratios.

This study also shows that oxygen therapy affects the injured brain differently. Oxygen therapy was associated with a more rapid glutamate clearance and profoundly lowered lactate values during the first 3 days after injury. There was less glucose and pyruvate sparing effect with oxygen than with cyclosporin. Therefore, one possible conclusion from this study suggests that oxygen therapy may offset and perhaps augment either cyclosporin or topiramate. Future research is needed to investigate the potential benefits of such dual and potentially synergistic therapy.⁵

FINANCIAL SPONSORS

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