Effect of Short Periods of Normobaric Hyperoxia on Local Brain Tissue Oxygenation and Cerebrospinal Fluid Oxidative Stress Markers in Severe Traumatic Brain Injury

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Abstract

Preliminary evidence suggests local brain tissue oxygenation (PbtO₂) values of ≤ 15 mm Hg following severe traumatic brain injury (TBI) represent brain tissue hypoxia. Accordingly, many neurotrauma units attempt to maintain PbtO₂ ≥ 20 mm Hg to avoid hypoxia. This study tested the impact of a short (2 h) trial of normobaric hyperoxia on measures of oxidative stress. We hypothesized this treatment would positively affect cerebral oxygenation but negatively affect the cellular environment via oxidative stress mechanisms. Cerebrospinal fluid (CSF) was serially assessed in 11 adults (9 male, 2 female), aged 26 ± 1.8 years with severe TBI (Glasgow Coma Scale score, 6 ± 1.4) before, during, and after a FiO₂ = 1.0 challenge for markers of oxidative stress, including lipid peroxidation (F₂-isoprostane [ELISA]), protein oxidation (protein sulfhydryl [fluorescence]), and antioxidant defenses (total antioxidant reserve (AOR) [chemiluminescence] and glutathione [fluorescence]). Physiological parameters [PbtO₂, arterial oxygen content (PaO₂), intracranial pressure (ICP), mean arterial pressure (MAP), and cerebral perfusion pressure (CPP)] were assessed at the same time points. Mean ($\pm$ SD) PbtO₂ and PaO₂ levels significantly changed for each time point. Oxidative stress markers, antioxidant reserve defenses, and ICP, MAP, and CPP did not significantly change for any time period. These preliminary findings suggest that brief periods of normobaric hyperoxia do not produce oxidative stress and/or change antioxidant reserves in CSF. Additional studies are required to examine extended periods of normobaric hyperoxia in a larger sample.

Key words: antioxidant reserve; normobaric hyperoxia; oxidative stress; traumatic brain injury

Introduction

THE CURRENT MANAGEMENT of severe traumatic brain injury (TBI) in the acute care setting focuses on prevention, early detection, and treatment of secondary injury through therapy designed to maintain adequate cerebral perfusion and intracranial pressure control ("Guidelines for the management of severe traumatic brain injury," 2007). Recent technological advances allow brain tissue oxygenation (PbtO₂) to be assessed continuously, providing an *in vivo* tool that allows rapid detection of changes in response to the intrinsic evolution of damage, effect of secondary insults, and/or

application of preventive or reactive therapies. In patients with severe TBI, Valadka and colleagues (1998) reported that the longer the time a patient experienced a PbtO₂ of ≤ 15 torr, the greater the likelihood of death. Additionally, any decrease in PbtO₂ to < 6 torr, regardless of its duration, was associated with an increased mortality (Valadka et al., 1998). Subsequently, various treatment modalities to raise the PbtO₂ above these thresholds, such as therapeutic hyperoxia, began to be implemented for patients with severe TBI, albeit with limited empirical evidence. In clinical practice, one approach to managing a low PbtO₂ (< 20 torr) is to increase the delivered fraction of inspired oxygen (FiO₂). Although

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effective in increasing PbtO_2 , and presumably oxygen availability in the brain, the impact of hyperoxia on secondary injury mechanisms, such as oxidative stress, is not well understood.

Diffusion of oxygen into injured brain tissue may be limited in the setting of brain edema and decreased cerebral blood flow (Hlatky, et al., 2008), and thus high levels of dissolved oxygen in arterial blood may facilitate oxygen delivery. In a clinical study comparing patients receiving 24 hours of 100% oxygen to historical controls, normobaric hyperoxic therapy was shown to decrease lactate levels and the lactate-pyruvate ratio, increase glucose levels, decrease intracranial pressure (ICP), and improve outcome (Tolias et al., 2004). This relationship to improved neurological outcome is limited by the historical control design and requires additional examination in a prospectively collected clinical trial. In a prospective clinical study utilizing positron emission tomography (PET), normobaric hyperoxia treatment enhanced cerebral metabolism in "at risk" tissue with a complimentary small reduction in the lactate-pyruvate ratio, but no global changes were seen (Nortje et al., 2008). However, a similar study examining hemispheric cerebral metabolic rate for oxygen (CMRO_2) following a 1-h hyperoxia treatment resulted in no overall CMRO_2 changes, suggesting no improvement in brain oxygen metabolism (Diringer et al., 2007). A study comparing ICP and PbtO_2 monitoring and treatment, including hyperoxia, versus ICP monitoring and treatment alone, reported a favorable and dramatic improvement in neurological outcomes in the group with additional PbtO_2 monitoring (Stiefel et al., 2005).

However, other evidence points to a negative effect of hyperoxia treatment on the injured brain. There exists concern about the use of hyperoxia due to the potential of this therapy to elicit an oxidative stress response (Longhi and Stocchetti, 2004; Zenri et al., 2004). Kaindl and colleagues (2006) reported increased neuronal apoptotic death and protein carbonyls in mice subjected to hyperoxia. Additional evidence in animal models of ischemia and reperfusion suggests a potential powerful, deleterious effect of hyperoxia in early post-ischemic reperfusion of brain (Vereczki et al., 2006), possibly via oxidative post-translational modification and inhibition of the key mitochondrial enzyme, pyruvate dehydrogenase by peroxynitrite (Bogaert et al., 1994). Similarly, studies in children (Bayir et al., 2002; Wagner et al., 2004) and adults (Bayir et al., 2002; Wagner et al., 2004) have shown that, with management strategies, antioxidant reserves are markedly depleted and biomarkers of oxidative stress are increased in cerebrospinal fluid (CSF) in the acute period after TBI. Accordingly, a treatment strategy involving the use of high concentrations of oxygen to achieve a target PbtO_2 could, theoretically, place the already vulnerable brain at risk for secondary injury. It is of clinical interest to determine if any harmful effects occur with the use of therapeutic normobaric hyperoxia to increase a low or normal PbtO_2 value.

The purpose of this study was to examine the effect of a brief, controlled period of normobaric hyperoxia (100% oxygen administered for 2 h) on (1) the key physiological values of PbtO_2 , ICP, global brain perfusion (cerebral perfusion pressure [CPP]), and systemic blood pressure (mean arterial pressure [MAP]); and (2) biochemical markers of oxidative stress (as measured by F_2 -isoprostane, and protein sulphydryl) and antioxidant defenses (as measured by reduced glutathi-

one and antioxidant reserve) in CSF of patients admitted to a Level 1 trauma center who sustained a severe TBI.

Materials and Methods

Subjects

Under the scope of an approved institutional review board protocol, we prospectively studied 11 adult patients (18–45 years of age) with severe TBI who were admitted to our Level 1 trauma center (University of Pittsburgh Medical Center). Severe TBI was determined in the emergency room by the attending neurosurgeon using the following criteria: (1) post-resuscitative Glasgow Coma Scale (GCS) score of ≤ 8 , not following commands and without the influence of pharmacologic agents, alcohol, or paralytics; and (2) positive computer tomography (CT) scan for severe TBI diagnosis. Entry criteria for the study were: (1) $\text{FiO}_2 \leq 0.6$ or $\text{PaO}_2/\text{FiO}_2$ ratio >200 ; (2) external ventricular drain (EVD); (3) PbtO_2 probe (Licor, Integra Neurosciences); (4) informed consent from the legal authorized representative; (5) controlled ICP (≤ 25 mm Hg) and PbtO_2 (≥ 15 mm Hg) parameters at the time of the FiO_2 challenge; and (6) ability to begin data collection within 48 h of injury. Patients who required a $\text{FiO}_2 > 0.6$ or had a $\text{PaO}_2/\text{FiO}_2$ ratio of ≤ 200 were excluded to maximize the ability to detect a difference between standard therapy and normobaric hyperoxia therapy on oxidative measures and to avoid severe impairments in oxygen tension. In addition, patients were excluded if they exhibited prolonged (>30 min) hypotension and hypoxia prior to hospital admission, had an unknown injury time, or an injury time >24 h prior to arrival in the emergency department, or were clinically brain dead. The upper age limit was set at 45 years to avoid enrolling older patients with unreported lung disease.

Critical care management

Intensive care management was provided within a dedicated neurotrauma intensive care unit with care guided by standardized protocols consistent with the "Guidelines for management of severe traumatic brain injury" (2007). All clinical orders are focused on minimization or prevention of secondary injury occurring within this acute recovery period, with an emphasis on cerebral oxygenation and blood flow. MAP endpoint goals were between 90 and 110 mm Hg, PaCO_2 between 33 and 37 mm Hg, central venous pressure (CVP) between 8 and 15 mm Hg; and CPP >60 mm Hg. ICP values of >20 mm Hg were aggressively managed using a stepwise protocol that included pharmacologic paralysis and sedation, continuous CSF drainage, osmotic therapy (bolus mannitol or continuous hypertonic saline infusion), transient escalation of hyperventilation, and barbiturate infusion. The PbtO_2 probe (diameter 0.8 mm, length 460 mm, oxygen sensitive area of 13 mm^2) was placed approximately 35 mm below the dura into the brain parenchyma of the frontal lobe contralateral to the primary site of injury to provide sampling from a relatively normal-appearing brain area, that, consequently, should represent an uninjured brain region at risk for secondary injury. As local microtrauma can occur with probe insertion, and levels of a number of mediators may be increased in CSF during the acute period of sampling (Valadka et al., 1998; van Santbrink et al., 2003), a 24-h interval was allowed between probe insertion and the normobaric hyper-

oxia trial to avoid the overshadowing of a less robust oxidative stress response.

Normobaric hyperoxia trial

Arterial blood gas (ABG) and sterile CSF ventricular samples were obtained 30 min prior to the trial and defined as "before trial"; immediately after the normobaric hyperoxia trial (FiO₂ increased to 1.0 for 2 h), representing the period during the trial and defined as "during trial"; and 2 hours following the return to baseline FiO₂ settings, representing the period following the trial and defined as "after trial." Physiological parameters (ICP, MAP, CPP, PbtO₂) were continuously monitored and data collected every minute via a data acquisition server linked to the patient's monitor (Hewlett Packard/Philips Medical Systems, Bothell, WA). The last 30 min of data collection prior to each CSF sampling was averaged to provide a comparison to the CSF collection interval for each of the three collection points. During the data collection period, nursing activity was minimized and notations were made of any nursing activity that might influence the data obtained, e.g., turning, suctioning, etc. The duration (2 h) of the normobaric hyperoxia trial was chosen to provide sufficient time to produce changes due to oxidative stress to the brain (if present) but not prolonged to cause potential damage if biomarkers were markedly changed by the trial.

CSF sample and marker assays

To obtain CSF, the EVD was placed in the closed position for 15 min and a fresh CSF sample obtained via the proximal access port. All CSF samples were immediately placed on ice, centrifuged, transferred to a cryoprotectant tube, and stored in a -80°C freezer for future batch analysis.

F₂-isoprostane was measured in CSF by a commercially available enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) with a detection limit of 2 pg/mL. Protein sulfhydryls were measured in CSF samples using a maleimide reagent, ThioGlo-1 (Convalent Associates, Woburn, MA), that produces a fluorescent product upon reaction with sulfhydryl groups (Langmuir et al., 1996). Levels of protein sulfhydryls were determined after adding 4 mM SDS to the CSF sample. A Cytofluor 2350 fluorescence plate reader (Millipore, Marlborough, MA) was used to detect fluorescence using an excitation wavelength of 388 nm and an emission wavelength of 500 nm. Glutathione levels (GSH) were measured in CSF utilizing the assay described above. GSH content was estimated by the immediate fluorescent response observed upon addition of ThioGlo-1 to the CSF sample. The response was compared to a standard curve obtained by the addition of known amounts of GSH (0.04–4 μM) to 50 mM disodium phosphate buffer (pH 7.4) containing 10 μM ThioGlo-1 to extrapolate sample values. Total antioxidant reserve (AOR) was measured in CSF samples by a chemiluminescence assay produced in the presence of luminol and a source of peroxyl radicals, previously described by Tyurina and colleagues (1995). For this assay, a water-soluble azo-initiator, AAPH, is used to produce peroxyl radicals at a constant rate. Based on the known rate of peroxyl radical generation by AAPH, the amount of these radicals scavenged by endogenous antioxidants with the sample being assayed, CSF levels were determined. A Microlite ML 1000 microtiter plate luminometer (Dynatech Labs, Chantilly, VA) was used for the final determinations of these amounts.

Statistical analysis

Descriptive statistics and bivariate analyses (parametric and nonparametric based on the distribution) were used. Data were screened for accuracy of input, missing data, and outliers and statistical procedures performed using SPSS (version 12; Chicago, IL), with a statistical significance determined *a priori* at $p < 0.05$. Multivariate analysis of variance (MANOVA) was utilized to detect significant changes in physiological variables (PbtO₂, MAP, ICP, CPP) and biochemical marker levels (F₂-isoprostane, protein sulfhydryls, glutathione, and total antioxidant reserve) over time. If significant results were obtained in the MANOVA procedure, appropriate post-hoc paired student *t*-tests were performed. Significance values were adjusted for multiple comparisons according to Bonferroni, if required.

Results

Subjects (9 male, 2 female) were 26 ± 1.8 years of age, 91% Caucasian, with an initial GCS score of 6 (3,8) (Table 1), and examined 43.7 ± 9.36 (mean $\pm$ SD) from the time of injury. The initial FiO₂ was 0.50 ± 0.09 (range 0.40–0.60). All baseline physiologic parameters were within normal ranges. Compared to baseline, the normobaric hyperoxia challenge increased PaO₂ (173.1 ± 51.4 to 385.5 ± 108.3 mm Hg) and PbtO₂ (27.3 ± 7.4 to 93.9 ± 58.1 mm Hg), as expected. The MANOVA demonstrated a significant overall effect for normobaric hyperoxia on PaO₂ ($F = 38.9$; d.f. = 2, 18; $p < 0.0001$) and PbtO₂ ($F = 15.4$; d.f. = 2, 20; $p < 0.0001$). Normobaric hyperoxia significantly improved PaO₂ ($t = 7.2$; $p < 0.0001$) and PbtO₂ ($t = 4.1$; $p < 0.0001$) from baseline values, with a corresponding significant decrease in PaO₂ ($t = -6.0$; $p < 0.0001$) and PbtO₂ ($t = -3.8$; $p < 0.0001$) when the ventilatory setting was changed back to the baseline FiO₂ setting. No significant resultant changes were seen from before FiO₂ challenge to after FiO₂ challenge for PaO₂ and PbtO₂. No significant changes were seen in ICP, MAP, or CPP (reported in Fig. 1).

Oxidative stress and antioxidant reserve

Markers of oxidative stress (F₂-isoprostane and protein sulfhydryls; Figs. 2 and 3) and antioxidant reserve (glutathione and total AOR; Figs. 4 and 5) did not significantly change at any study time points. Notably, AOR, a sensitive marker of anti-oxidant defenses (Bayir et al., 2002), did not demonstrate even a trend toward reduction, and no substantial trends were seen for any marker versus baseline measures.

TABLE 1. STUDY POPULATION CHARACTERISTICS

Gender, % male	81.8
Race, % Caucasian	91
Age	
Mean $\pm$ SEM	26 ± 1.8
GCS on admission	
Median (range)	6 (3,8)
GCS 3–5	36.4%
GCS 6–8	63.6%

Values are mean $\pm$ SEM or median (range) where appropriate. SEM, standard error of the mean; GCS, Glasgow Coma Scale score.

FIG. 1. Physiological results. Effect of normobaric hyperoxia trial on physiological parameters before, during, and 2 h after normobaric hyperoxia trial. ICP, intracranial pressure; MAP, mean arterial pressure; CPP, cerebral perfusion pressure; PbtO₂, brain tissue oxygenation. Error bars are SEM for each parameter. Note that PaO₂ values are not shown (see text).

FIG. 2. F₂-isoprostane results. Effect of a normobaric hyperoxia trial on F₂ isoprostane levels in CSF. Overall F₂ isoprostane concentration did not significantly change from before, during, or after trial. (A) Individual patients depicted by line graph. (B) Patients grouped and reported in a box plot format (o, outlier value; *extreme outlier value).

FIG. 3. Protein sulfhydryl results. Effect of a normobaric hyperoxia trial on content of protein sulfhydryl concentrations in CSF. Overall concentrations of protein sulfhydryls did not significantly change from before, during, or after trial. **(A)** Individual patients depicted by line graph. **(B)** Patients grouped and reported in a box plot format (*extreme outlier).

Discussion

This interventional study is an initial examination of the effect of a brief (2h) controlled period of normobaric hyperoxia on oxidative stress in an adult severe TBI population, similar to a setting of ischemia/reperfusion damage. The present data demonstrate that raising FiO_2 enhanced PbtO_2 without apparent risk to a short period of normobaric hyperoxia when measuring CSF oxidative stress and antioxidant defense markers. This clinical study is the first to

examine the effect of normobaric hyperoxia on a battery of oxidative stress biomarkers in CSF. The lack of response in the oxidative stress biomarker battery of the present study, in conjunction with the previously reported improvement of cerebral metabolism markers of the lactate-pyruvate ratio (Tolias et al., 2004; Nortje et al., 2008) lends support that short durations of normobaric hyperoxia are potentially safe in this population. However, additional studies have shown no improvement in oxidation of glucose with hyperoxia treatment (Diringer et al., 2007; Magnoni et al., 2003). It is

FIG. 4. Glutathione results. Effect of a normobaric hyperoxia trial on glutathione concentrations in CSF. Overall concentrations of glutathione did not significantly change from before, during, or after trial. (A) Individual patients depicted by line graph. (B) Patients grouped and reported in a box plot format (o, outlier value).

premature to extrapolate these findings to the general severe TBI population without further examination of key variables. Altering various parameters, such as duration of the treatment and timing of the initiation of the hyperoxia, may have differing results.

Although the sample size was small ($n=11$), we chose a homogenous sample in certain aspects to minimize the vari-

ability in secondary injury pathways that might cause a concurrent increase in oxidative stress biomarkers. To control for variations in therapy, all patients were treated with a strict standard protocol for TBI based on the "Guidelines for management of severe traumatic brain injury" (2007). No patients were in crisis situations for ethical considerations, since the use of normobaric hyperoxia is controversial. No patients

FIG. 5. Total antioxidant reserve results. Effect of a normobaric hyperoxia trial on total antioxidant reserve concentrations in CSF. CSF was subjected to free radical attack from peroxyl radicals generated from AAPH in the presence of a reporter molecule, luminol. Overall concentrations did not significantly change from before, during, or after trial. **(A)** Individual patients depicted by line graph. **(B)** Patients grouped and reported in a box plot format.

were experiencing refractory intracranial hypertension, low CPP, or low PbtO₂.

We have previously shown that severe TBI in children and adults results in the compromise of antioxidant defenses and the exacerbation of free radical-mediated lipid peroxidation (Bayir et al., 2002; Wagner et al., 2004). The potential of compromised antioxidant defenses suggests that an intervention, such as normobaric hyperoxia, may place the injured

brain at increased risk for secondary damage in the acute phase of recovery, as a consequence of increased free radical production. Although this study resulted in no significant changes for physiological parameters (CPP, MAP, ICP) or biomarkers for oxidative stress and antioxidant reserves, caution must be taken to extrapolate this to the larger TBI population. The baseline values for PbtO₂ were 27.3 ± 7.4 mm Hg, and ICP were 14.7 ± 4.9 mm Hg, representing normal oxygenation

and pressure values. The resulting non-significant changes in physiology (other than increases in PbtO₂) and biomarker analyses that resulted from the FiO₂ increase to 1.0 may not represent the practice of increasing the FiO₂ when the PbtO₂ is <20 mm Hg, or the ICP is >20 mm Hg. We cannot rule out the possibility that under such conditions normobaric hyperoxia may induce oxidative stress due to the presence of ongoing excitotoxicity, inflammation, activation of xanthine oxidase, or other mechanisms known to mediate oxidative stress. Also, the normobaric hyperoxia trial was performed at least 24 h after PbtO₂ probe insertion to avoid sampling biomarkers resulting from local microtrauma or the initial trauma insult. Normobaric hyperoxia instituted under these early conditions may have an additive effect, with proximity to TBI, ischemia, or other secondary injury pathways such as excitotoxicity. Although it has been theorized that patients experiencing a low PbtO₂ due to low cerebral blood flow in the damaged region may benefit the most from normobaric hyperoxia (Hlatky et al., 2008), additional studies examining patients with low PbtO₂ values and a more extended period of treatment are needed to confirm the absence of adverse events. In addition, because the immature brain is known to be at an increased risk for oxidative damage and there are recognized differences in pathophysiology and outcomes, these findings need to be confirmed in pediatric patients.

The method of CSF sampling chosen was to examine a global representation of the brain under hyperoxia therapy, as opposed to local sampling that microdialysis can offer for specific aims such as pericontusional responses. The injured areas of the brain may respond more or less significantly to this treatment and need to be examined.

The duration of treatment chosen was brief (2h), with the goal of not causing prolonged oxidative stress damage if a dramatic effect with treatment was demonstrated. Some centers use this treatment transiently while others are using this continuously. Although a brief period of normobaric hyperoxia was employed in this current study, an immediate and drastic increase in PbtO₂ occurred, with an average increase of over 200%. In addition, in prior studies, assessment of oxidative stress markers is capable of demonstrable, rapid responses, shown by marked increases in F₂ isoprostane early after TBI (Bayir et al., 2002; Wagner et al., 2004). Because we noted a lack of effect, longer exposure periods need to be examined, as well as long-term neurological outcomes. It has been shown that there is a progressive depletion of antioxidants in CSF after TBI (Bayir et al., 2002), thereby placing the injured brain at increased risk after a TBI. Prolonged exposure of hyperoxia may deplete this reserve even more dramatically. The data herein indicate some individual variability exists across subjects in response to normobaric hyperoxia treatment; however no substantive trends were observed. In patients having an increase in one variable, there was no consistent increase across other measured variables; hence normal physiologic variability is the most likely explanation for our findings. One subject was shown to be an extreme outlier for F₂ isoprostane; however, the values decreased over time and did not significantly increase with the normobaric treatment, suggesting that some individuals inherently have a high basal level of oxidative stress after TBI.

Frequently following TBI, with the development of cerebral edema, diffusion of oxygen to tissue can be compromised and

thus there is a theoretical target for increased oxygen delivery via raising the FiO₂. Subsequently, hyperoxia treatment is being used transiently to increase low PbtO₂ values. It is also believed that the increase in oxygen delivery to compromised regions will produce a compensatory decrease in cerebral blood volume and thus a reduction in intracranial pressure via autoregulation. Although this study did not show an effect of the examined physiological variables, sampling during a crisis situation (i.e., intracranial hypertension, hypoxia, etc.) may have produced different results and additional studies are needed.

Conclusion

This study is the first to examine prospectively the effect of short periods of normobaric hyperoxia on CSF oxidative stress markers. Short periods of normobaric hyperoxia significantly increased PbtO₂ but did not significantly change physiological parameters (ICP, CPP, and MAP) that may influence neurological outcome or CSF markers of oxidative stress and antioxidant reserves in TBI adult patients. This supports the safety of the application of short periods of normobaric hyperoxia after severe TBI in the adult population. However, we cautiously report that extrapolation to the general severe TBI population is premature, and additional studies examining prolonged periods of hyperoxia and various time points of treatment initiation are warranted.

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References

- Bayir, H., Kagan, V.E., Tyurina, Y.Y., Tyurin, V., Ruppel, R.A., Adelson, P.D., Graham, S.H., Janesko, K., Clark, R.S., and Kochanek, P.M. (2002). Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. *Ped. Res.* 51(5), 571–578.
- Bogaert, Y.E., Rosenthal, R.E., and Fiskum, G. (1994). Post-ischemic inhibition of cerebral cortex pyruvate dehydrogenase. *Free Radic. Biol. Med.* 16(6), 811–820.
- Diringer, M.N., Aiyagari, V., Zazulia, A.R., Videen, T.O., and Powers, W.J. (2007). Effect of hyperoxia on cerebral metabolic

- rate for oxygen measured using positron emission tomography in patients with acute severe head injury, *J. Neurosurg.* 106, 526–529.
- Guidelines for the management of severe traumatic brain injury. (2007). *J. Neurotrauma* 24 Suppl 1, S1–106.
- Hlatky, R., Valadka, A.B., Gopinath, S.P., and Robertson, C.S. (2008). Brain tissue oxygen tension response to induced hyperoxia reduced in hypoperfused brain. *J. Neurosurg.* 108(1), 53–58.
- Kaindl, A.M., Siffringer, M., Zabel, C., Nebrich, G., Wacker, M.A., Felderhoff-Mueser, U., Endesfelder, S., von der Hagen, M., Stefovská, V., Klose, J., and Ikonomidou, C. (2006). Acute and long-term proteome changes induced by oxidative stress in the developing brain. *Cell Death Diff.* 13(7), 1097–1109.
- Langmuir, V.K., Laderoute, K.R., Mendonca, H.L., Sutherland, R.M., Hei, T.K., Liu, S.X., Hall, E.J., Naylor, M.A., and Adams, G.E. (1996). Fused pyrazine mono-n-oxides as bioreductive drugs. II. Cytotoxicity in human cells and oncogenicity in a rodent transformation assay. *Int. J. Rad. Oncol. Biol. Phys.* 34(1), 79–84.
- Longhi, L., and Stocchetti, N. (2004). Hyperoxia in head injury: therapeutic tool? *Curr. Opin. Crit. Care*, 10(2), 105–109.
- Magnoni, S., Ghisoni, L., Locatelli, M., Caimi, M., Columbo, A., Valeriani, V., and Stocchetti, N. (2003). Lack of improvement in cerebral metabolism after hyperoxia in severe head injury: a microdialysis study. *J. Neurosurg.* 98(5), 952–958.
- Nortje, J., Coles, J.P., Timofeev, I., Fryer, T.D., Aigbirhio, F.I., Smielewski, P., Outtrim, J.G., Chatfield, D.A., Pickard, J.D., Hutchinson, P.J., Gupta, A.K., and Menon, D.K. (2008). Effect of hyperoxia on regional oxygenation and metabolism after severe traumatic brain injury: preliminary findings. *Crit. Care Med.* 36(1), 273–281.
- Stiefel, M.F., Spiotta, A., Gracias, V.H., Garuffe, A.M., Guillaumondegui, O., Maloney-Wilensky, E., Bloom, S., Grady, M.S., and LeRoux, P.D. (2005). Reduced mortality rate in patients with severe traumatic brain injury treated with brain tissue oxygen monitoring [comment]. *J. Neurosurg.* 103(5), 805–811.
- Tolias, C.M., Reinert, M., Seiler, R., Gilman, C., Scharf, A., and Bullock, M.R. (2004). Normobaric hyperoxia-induced improvement in cerebral metabolism and reduction in intracranial pressure in patients with severe head injury: a prospective historical cohort-matched study [comment]. *J. Neurosurg.* 101(3), 435–444.
- Tyurina, Y.Y., Tyurin, V.A., Yalowich, J.C., Quinn, P.J., Claycamp, H.G., Schor, N.F., Pitt, B.R., and Kagan, V.E. (1995). Phenoxyl radicals of etoposide (VP-16) can directly oxidize intracellular thiols: protective versus damaging effects of phenolic antioxidants. *Toxicol. Appl. Pharmacol.* 131(2), 277–288.
- Valadka, A.B., Gopinath, S.P., Contant, C.F., Uzura, M., and Robertson, C.S. (1998). Relationship of brain tissue PO₂ to outcome after severe head injury. *Crit. Care Med.* 26(9), 1576–1581.
- van Santbrink, H., vd Brink, W.A., Steyerberg, E.W., Carmona Suazo, J.A., Avezaat, C.J., and Maas, A.I. (2003). Brain tissue oxygen response in severe traumatic brain injury. *Acta Neurochir.* 145(6), 429–438; discussion, 438.
- Vereczki, V., Martin, E., Rosenthal, R.E., Hof, P.R., Hoffman, G.E., and Fiskum, G. (2006). Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death. *J. Cereb. Blood Flow Metab.* 26(6), 821–835.
- Wagner, A.K., Bayir, H., Ren, D., Puccio, A., Zafonte, R.D., and Kochanek, P. M. (2004). Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender, age, and hypothermia. *J. Neurotrauma* 21(2), 125–136.
- Zenri, H., Rodriguez-Capote, K., McCaig, L., Yao, L.J., Brackenbury, A., Possmayer, F., Veldhuizen, R., and Lewis, J. (2004). Hyperoxia exposure impairs surfactant function and metabolism. *Crit. Care Med.* 32(5), 1155–1160.

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