Effect of Hyperoxia on Resuscitation of Experimental Combined Traumatic Brain Injury and Hemorrhagic Shock in Mice

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ABSTRACT

Background: Hypotension and hypoxemia worsen traumatic brain injury outcomes. Hyperoxic resuscitation is controversial. The authors proposed that hyperoxia would improve hemodynamics and neuronal survival by augmenting oxygen delivery despite increased oxidative stress and neuroinflammation in experimental combined controlled cortical impact plus hemorrhagic shock in mice.

Methods: Adult C57BL6 mice received controlled cortical impact followed by 35 min of hemorrhagic shock (mean arterial pressure, 25–27 mmHg). The resuscitation phase consisted of lactated Ringer's boluses titrated to mean arterial pressure greater than 70 mmHg. Definitive care included returning shed blood. Either oxygen or room air was administered during the resuscitation phases. Brain tissue levels of oxidative stress and inflammatory markers were measured at 24 h and hippocampal neuronal survival was quantified at 7 days.

Results: Hyperoxia markedly increased brain tissue oxygen tension approximately four- to fivefold (n = 8) and reduced resuscitation fluid requirements approximately 15% (n = 53; both $P < 0.05$). Systemic and cerebral physiologic variables were not significantly affected by hyperoxia. Hippocampal neuron survival was approximately 40% greater with oxygen *versus* room air (n = 18, $P = 0.03$). However, ascorbate depletion doubled with oxygen *versus* room air (n = 11, $P < 0.05$). Brain tissue cytokines and chemokines were increased approximately 2- to 20-fold (n = 10) after combined controlled cortical impact injury plus hemorrhagic shock, whereas hyperoxia shifted cytokines toward a proinflammatory profile.

Conclusions: Hyperoxic resuscitation of cortical impact plus hemorrhagic shock reduced fluid requirements and increased brain tissue oxygen tension and hippocampal neuronal survival but exacerbated ascorbate depletion and neuroinflammation. The benefits of enhanced oxygen delivery during resuscitation of traumatic brain injury may outweigh detrimental increases in oxidative stress and neuroinflammation.

What We Already Know about This Topic

- Although hypoxemia worsens outcomes after traumatic brain injury, hyperoxic resuscitation is controversial

What This Article Tells Us That Is New

- In a model of combined traumatic brain injury and hemorrhagic shock, despite the observation of exacerbated ascorbate depletion and neuroinflammation, hyperoxic resuscitation increased brain tissue oxygen tension and hippocampal neuronal survival

NEUROLOGIC deficits following traumatic brain injury (TBI) are caused by the primary mechanical injury and subsequent cascade of biochemical, metabolic, and cellular derangements encompassing the secondary injury. In civilian and military settings, TBI is often seen not in isolation but in combination with other insults (polytrauma) that exaggerate the secondary injury.[1,2] The occurrence of

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hypotension is associated with doubled mortality and worsened morbidity after TBI.[3] Coupled with reduced cerebral blood flow acutely following TBI,[4–6] hemorrhagic hypotension likely exacerbates cerebral hypoxia.[7,8] Sustained brain tissue oxygen tension (Pbt_{O_2}) levels less than 15 mmHg or a Pbt_{O_2} less than 6 mmHg for any period of time following TBI is associated with increased mortality.[9] Administration of supplemental oxygen after TBI in an attempt to improve oxygenation of vulnerable brain tissue in various settings has been used clinically with mixed results.[10–13] In the setting of TBI with superimposed hemorrhagic shock (HS), however, it is unknown whether administration of supplemental oxygen improves resuscitation and outcomes.

Varying the fraction of inspired oxygen after different types of brain injury is currently under intense debate.[14] Hyperoxic resuscitation of a focal cerebral injury has been shown to be neuroprotective in experimental studies by improving penumbral blood flow and maintaining penumbral oxygenation, decreasing infarct size, and ultimately improving outcomes.[15–20] In clinical stroke studies, early application of normobaric hyperoxia increased magnetic resonance imaging evidence of aerobic metabolism and neuronal stability as well as transient clinical improvement.[21,22] At the other end of the spectrum, however, the ischemia–reperfusion injury that occurs from administration of 100% oxygen on return of spontaneous circulation after cardiac arrest could be detrimental, particularly with regard to neurologic outcomes.[23] Kilgannon *et al.* recently showed arterial hyperoxia to be an independent predictor of in-hospital mortality after resuscitation from cardiac arrest.[24] The surge of reactive oxygen species upon hyperoxic reperfusion leads to neuronal death from damaging lipid and protein peroxidation, impaired mitochondrial oxidative metabolism, and activation of cellular inflammation.[25,26] The effect of hyperoxia on neurologic outcomes after TBI may be similar to a focal cerebral insult; although the mechanically disrupted brain is likely unsalvageable, the surrounding vulnerable tissue may benefit from hyperoxia in the face of reduced cerebral blood flow and altered cerebral hemodynamics.[11]

The combination of TBI and HS that occurs after polytrauma may represent a unique scenario with regard to the use of oxygen because the insult includes both TBI, which may be favorably influenced by supplemental oxygen, and the damaging effects of ischemia–reperfusion. We have developed and previously characterized a unique mouse model of controlled cortical impact (CCI) coupled with a brief, severe, pressure-controlled HS with a clinically relevant mean arterial pressure (MAP)-targeted resuscitation permitting study of acute systemic and cerebral physiology, neuropathology, and long-term functional outcomes.[27] We sought to determine whether treatment with oxygen *versus* room air (RA) influenced resuscitation and neuropathologic outcome in our combined CCI and HS mouse model. In this article, we present results showing that hyperoxia *versus* RA improved cerebral hemodynamics and CA3 hippocampal neuron survival but resulted in worsened oxidative stress and neuroinflammation.

Materials and Methods

Experimental Protocol for In Vivo Studies

The Institutional Animal Care and Use Committee of the University of Pittsburgh approved all experiments. Adult (12–15 weeks of age) C57BL6 male mice (Jackson Laboratories, Bar Harbor, ME) weighing 27.7 ± 0.5 g were housed in controlled environmental conditions and allowed *ad libitum* access to food and water until the start of the study. We used our established mouse combined CCI plus HS model as described by Hemerka *et al.*[27] with four experimental groups: sham RA (burr hole, brain temperature probe, catheters, anesthesia, RA during prehospital and hospital phases, but no CCI or HS), sham oxygenation (craniotomy, catheters, anesthesia, pure oxygen *via* nose cone during prehospital and hospital phases, but no CCI or HS), combined CCI plus HS and RA (RA administered throughout protocol), or combined CCI plus HS and oxygenation (pure oxygen *via* nose cone during prehospital and hospital phases). Naïve mice were also used for the various brain tissue measurements detailed below. Naïve mice were age-, sex-, and vendor-matched C57BL6 mice not exposed to any insult or therapy. Briefly, anesthesia was induced with isoflurane 4% in 2:1 N_2O–O_2 *via* nose cone in spontaneous breathing mice. Maintenance of anesthesia for subsequent surgical procedures was with isoflurane 1.5% and in a 2:1 N_2O–O_2 mixture. Femoral arterial and venous catheter access (modified PE-50 tubing) was *via* inguinal cutdown. Mice were placed in a stereotactic frame (David Kopf Instruments, Tujunga, CA), and a 5-mm left parietal craniotomy was performed using a dental drill with removal of bone flap. After craniotomy, anesthesia was decreased and maintained with 1% isoflurane in RA for a 10-min stabilization period. A mild to moderate CCI to the left parietal cortex was performed *via* a pneumatic impactor (Bimba Manufacturing Co., Monee, IL) with a flat 3-mm tip at a velocity of 5 m/s to a depth of 1 mm. The craniotomy was closed by replacing the bone flap and sealing with dental cement. Next, a 35-min pressure-controlled hemorrhagic "shock" phase (MAP, 25 ± 2 mmHg) was initiated by withdrawing blood (2.3 ml/100 g; citrated for later reinfusion) over 5 min. Continued removal or reinfusion of the autologous blood *via* the IV route in 0.05-ml aliquots was performed to rigorously maintain MAP of 25 ± 2 mmHg during the shock phase. The mice were allowed to spontaneously breathe 0.5% isoflurane in RA during this phase. Thirty-five minutes after CCI and HS (or at an equivalent anesthesia time point in the sham groups), spontaneously breathing mice were randomized to treatment with either oxygen ("hyperoxia") or RA by nose cone administration upon entering the "prehospital" phase. Prehospital resuscitation consisted of an initial IV bolus of 20 ml/kg of lactated Ringer's solution followed by maintenance lactated Ringer's fluid at 4 ml kg^{-1} h^{-1}. Lactated Ringer's boluses (10 ml/kg) were used every 5 min as needed to maintain MAP greater than 70 mmHg for the 90-min duration of prehospital resuscitation. The final "hospital" phase simulated

definitive care by reinfusion of shed blood over 15 min, during which the experimental fraction of inspired oxygen was maintained and administered with 1% isoflurane. After this phase, the femoral catheters were removed, incisions were closed and infiltrated with bupivacaine 0.25% (0.05 cc), and the inhalational anesthetic was discontinued. Mice treated with oxygen were allowed to recover with supplemental oxygen, whereas those in the RA group were allowed to recover on RA. Both groups were returned to their cages after they were fully awake. Resuscitation fluid volumes administered in each phase of the experimental protocol were recorded.

Monitoring

MAP and heart rate were monitored continuously *via* the femoral arterial cannula and recorded at baseline; after CCI; and every 5 min during the shock, prehospital, and hospital phases in all mice. Intracranial pressure (ICP) was similarly monitored and recorded in select mice (n = 14 that were subsequently sacrificed for histologic assessment at 7 days after injury) using a 2-French ultra-miniature pressure transducer (SPR-407; Millar, Houston, TX) inserted into the right frontal cortex (contralateral to the CCI) through a burr hole. $PbtO_2$ was also monitored and recorded in a separate group of mice (n = 8, not used for additional outcome studies) using a microelectrode (Unisense, 50 μm; Arhus, Denmark) stereotactically inserted *via* a burr hole through the cortex into the left dorsal hippocampus (ipsilateral to the CCI; bregma –2.5 AP, –2.0 ML; depth, 2.0 mm). Arterial blood gas measurements were obtained at baseline, at the end of the shock phase (35 min after CCI and HS), after the 90-min prehospital resuscitation (125 min after CCI and HS), and at the completion of the hospital phase (140 min after CCI and HS). Lactate, hematocrit, base deficit, and glucose values were recorded in conjunction with the arterial blood gas measurements (Stat Profile; Nova Biomedical, Waltham, MA). Temperature was monitored in the brain (*via* a separate burr hole in the right frontal lobe bone) and rectum, and brain temperature was controlled at 37.0 ± 0.5°C using a heat lamp throughout the experimental protocol.

Sample Preparation

Brain tissue samples were obtained at 24 h after CCI and HS injuries (or the equivalent anesthesia time point in sham animals) from mice anesthetized with 4% isoflurane and euthanized by transcardial perfusion with 50 ml of ice-cold heparinized saline. Brains were removed and divided into ipsilateral and contralateral hemispheres. Cortices and hippocampi were dissected and snap frozen in liquid nitrogen, and all samples were stored at –80°C until later use.

Assessment of Antioxidant Status and Markers of Oxidative Stress

Brain samples were obtained according to methods described above at 24 h after injury from 21 mice randomized to each of the aforementioned four groups (n = 5 each from sham RA, sham oxygenation, and combined CCI plus HS and RA, and n = 6 from combined CCI plus HS and oxygenation) as well as from five naïve animals. According to methods previously reported by Belikova *et al.*,[28] the brain tissue samples were thawed on ice and homogenized using a Tissue Tearor (BioCold Scientific, Fenton, MO) in lysis buffer (tissue weight/volume ratio of 1:20) consisting of sodium chloride (0.1 M final concentration), Tris chloride (0.01 M final concentration), and Triton X (1%) at pH adjusted to 7.4. Homogenates were centrifuged at 1,000*g* for 10 min and then divided into aliquots for measurement of protein, ascorbate, reduced glutathione, and superoxide dismutase (SOD) activity. Metaphosphoric acid was added to a final concentration of 5% (v/v) to the aliquots for ascorbate measurements, and all samples were stored at –80 °C until use.

At the time of the measurement of ascorbate, samples were thawed on ice and vortexed for 10 s every 2 min at least five times, and then centrifuged at 12,000*g* for 5 min at 4°C. The supernatant was collected into a microcentrifuge tube and the pH was adjusted to 7.0 using 10 M NaOH individually in each sample using pH paper. The volume of base required for the adjustment was recorded. Each sample was divided into two aliquots, each containing 0.1–0.6 mg/ml protein. To ensure the selectivity of the assay toward ascorbate, one of the aliquots (50 μl) was treated with 1 U of ascorbate oxidase for 40 min at room temperature. When all samples were ready, they (20 μl) were transferred onto a 96-well plate along with ascorbate standards (0–1.5 nM). After this, 130 μl of 4-([9-acridinecarbonyl]-amino)-2,2,6,6-tetramethylpiperidine-1-oxyl radical stock aliquots (23.1 μM in phosphate buffer) was added immediately using a multichannel pipette to each well, and samples were incubated for 40 min at room temperature in the dark and analyzed using a Packard Fusion Alpha Multifunctional Plate Reader (PerkinElmer Life Sciences, Boston, MA). Fluorescence was measured using a 390 ± 15-nm filter for excitation and a 460 ± 35-nm filter for emission. Fluorescence readings from the ascorbate oxidase–treated samples were subtracted from nontreated and normalized to protein (corrected for prior dilutions with metaphosphoric acid and NaOH). Samples were analyzed in triplicate.

A fluorescence-based assay using ThioGlo-1, a maleimide reagent (Sigma-Aldrich, St. Louis, MO) that produces a highly fluorescent adduct upon its reaction with sulfhydryl groups, was used to measure levels of low-molecular-weight thiols (LMWTs), reduced glutathione, and protein sulfhydryl groups according to methods described previously.[29] The immediate fluorescence response upon addition of ThioGlo-1 to brain tissue homogenate (10–35 μg of protein) estimated the content of total LMWTs according to a standard curve. The fluorescence response after addition of glutathione peroxidase (1 U) and cumene hydroperoxide (100 μM) for 30 min to an aliquot of the same sample was then measured. Reduced glutathione levels were then determined

by taking the difference between the fluorescence response of the original sample (LMWT) and the glutathione peroxidase–treated sample. Total protein sulfhydryl levels were determined by adding 4 mM sodium dodecyl sulfate to each LMWT sample and measuring the fluorescence response. A Packard Fusion Alpha Plate Reader was used to detect fluorescence at excitation and emission wavelengths of 388 and 500 nm, respectively. Samples were analyzed in triplicate.

SOD activity was measured by the cytochrome *c* reduction method[30] according to the methods described by Walson *et al.*[29] Manganese SOD (MnSOD) was measured in the presence of 1 mM potassium cyanide to inhibit both copper–zinc SOD and extracellular SOD, whereas the total SOD activity was measured in the absence of potassium cyanide. Samples were analyzed in triplicate.

Assessment of Cytokine, Chemokine, and Growth Factor Levels

Cytokine, chemokine, and growth factor levels in brain samples obtained 24 h after injury were quantified with a multiplex microbead array assay using the MILLIPLEX MAP Mouse Cytokine/Chemokine kit (Millipore, Billerica, MA) according to the manufacturer's instructions and previously described methods.[31,32] Brain tissue supernatant from ipsilateral (injury side) and contralateral hippocampi and cortices from 20 mice randomized to each of the aforementioned four groups (sham RA, sham oxygenation, combined CCI plus HS and RA, combined CCI plus HS and oxygenation; n = 5 per group), as well as from four naïve mice, was evaluated for levels of 21 chemokines, cytokines, and growth factors that included the following: monocyte chemotactic protein-1, macrophage inflammatory protein-1α, keratinocyte-derived cytokine (KC), eotaxin, interferon-inducible protein-10, regulated upon activation normal T-cell expressed and secreted (RANTES), interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, interferon-γ, tumor necrosis factor-α, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), and vascular endothelial growth factor. Protein concentrations in the brain supernatants were determined with the BCA Protein kit (Thermo Scientific, Rockford, IL), and all chemokine, cytokine, and growth factor values were normalized.

Histologic Assessment

A total of 18 mice (combined CCI plus HS and RA and combined CCI plus HS and oxygenation; n = 9 per group) were evaluated at 7 day after injury for hippocampal neuronal survival. The animals were anesthetized with 4% isoflurane and killed by transcardial perfusion fixation with ice-cold saline followed by 10% buffered formalin according to methods published previously.[27,33–35] Brain tissue was fixed with 10% buffered formalin and embedded in paraffin. Five-micron-thick sections, 200 μm apart from bregma −1.86 to −2.26, were prepared from each brain and stained with hematoxylin and eosin (Thermo Fisher Scientific, Pittsburgh, PA). Hippocampal neuronal survival was quantified as linear density as the number of cells per 0.1 mm in CA1 and CA3 regions by an evaluator blinded to treatment group using a Nikon Eclipse E600 microscope (Melville, NY) and ImageJ software (National Institutes of Health, Bethesda, MD) as reported previously.[27,33–35]

Statistical Analysis

Two-way repeated measures ANOVA (effects for group, time, and group × time) were used to analyze the physiologic parameters with two-tailed hypothesis testing. *Post hoc* testing using Bonferroni correction for multiple comparisons was performed when group effect differences were identified. Oxidative stress data were compared between groups using ANOVA with *post hoc* testing for multiple comparisons using the Tukey test. Cytokine levels were compared between groups using ANOVA with Student-Newman-Keuls *post hoc* testing for multiple comparisons. Cell counts were compared between RA and oxygen treatment groups using the Student *t* test. All data are provided as mean ± SEM. A difference of $P < 0.05$ was considered significant. SAS software (SAS Institute Inc., Cary, NC) was used for statistical analyses.

Figure 1 diagrams the number of animals used in each arm of the study as well as the variable measured. A total of 53 mice were included in the combined CCI plus HS model and resuscitated with either oxygen (n = 26) or room air (n = 27). A select number of mice from each treatment group (oxygen *vs.* room air) were selected for measurement of ICP (n = 6 and n = 8, respectively) and $Pbto_2$ (n = 4 in each group). At 24 h, mice were sacrificed for measurement of levels of oxidative stress and inflammatory markers. At 7 days, the surviving mice were sacrificed for histologic assessment. Additional mice sacrificed at 7 days for histologic analysis were the surviving mice who had ICP monitoring in the contralateral hemisphere.

Results

$Pbto_2$

Mouse $Pbto_2$ was measured in hippocampus underlying the cortical contusion. The first measurement was obtained upon placement of the oxygen sensor probe immediately after the CCI injury and was 47.9 ± 4.8 mmHg on RA (overall mean for all mice in both groups, n = 8) in isoflurane-anesthetized mice (fig. 2). A second reading was obtained 5 min later (34.3 ± 5.0 mmHg), after which HS was initiated immediately according to the above-described protocol. The addition of HS to the CCI injury reduced hippocampal $Pbto_2$ to an average pressure of 9.2 ± 3.7 mmHg during the 35-min shock phase (overall phase mean for all mice in both groups), although the reduction to 9.2 ± 3.7 mmHg during the shock phase was not significantly different than $Pbto_2$ values immediately after CCI (47.9 ± 4.8 mmHg) or 5 min after CCI (34.3 ± 5.0 mmHg). Randomization of mice to treatment with oxygen, however, produced a marked and

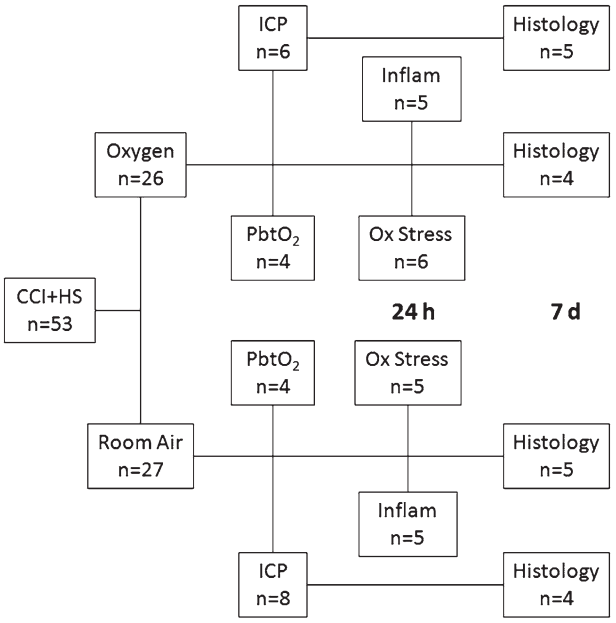

Fig. 1. Flow diagram showing the number of mice used in the combined controlled cortical injury (CCI) and hemorrhagic shock (HS) injury model (CCI+HS) (n = 53) and resuscitated with either oxygen (n = 26) or room air (n = 27). Mice from each treatment group (oxygen *vs*. room air) were selected for measurement of intracranial pressure (ICP) (n = 6 and n = 8, respectively) and brain tissue oxygen tension ($Pbto_2$) (n = 4 in each group). At 24 h, mice were sacrificed for measurement of levels of oxidative stress (Ox Stress) and inflammatory markers (Inflam). At 7 days, the surviving mice were sacrificed for histologic assessment (Histology). Additional mice sacrificed at 7 days for histologic analysis were the surviving mice that had ICP monitoring in the contralateral hemisphere.

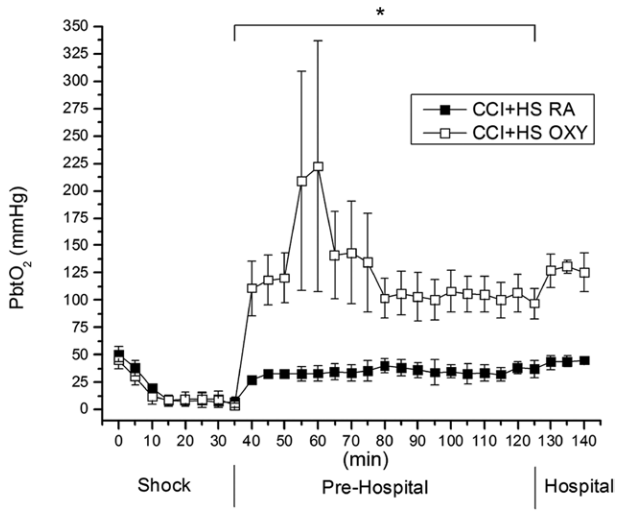

Fig. 2. Brain tissue oxygen tension ($Pbto_2$) (in millimeters of mercury) *versus* time (in minutes) in mice subjected to combined controlled cortical impact (CCI) and hemorrhagic shock (HS) and subsequent resuscitation with oxygen (CCI+HS OXY) (*open squares*) or room air (CCI+HS RA) (*closed squares*) during the prehospital and hospital phases. Time 0 represents $Pbto_2$ measured in hippocampus underlying the cortical contusion area immediately after CCI injury. The shock phase is initiated after the 5-min time point and is accompanied by a reduction in the $Pbto_2$. The onset of the prehospital resuscitation phase at 35 min is represented by increased $Pbto_2$ levels in both treatment groups; however, treatment with supplemental oxygen significantly increased $Pbto_2$ levels in the oxygen group during the prehospital phase compared with room air (*P = 0.0327).

significant increase in the average hippocampal $Pbto_2$ levels compared to treatment with RA during the prehospital phase (average mean prehospital phase value, 106.5 ± 16.9 *vs.* 38.4 ± 5.4 mmHg, respectively; P = 0.0327). During the hospital phase, the difference between the oxygen- and RA-treated groups was close to significant (average mean hospital phase value, 125.5 ± 17.4 *vs.* 45.2 ± 3.3 mmHg, respectively; P = 0.0504) (fig. 2).

Acute Physiology

Hyperoxic resuscitation resulted in highly significant differences in Pao_2 and So_2 during the prehospital and hospital phases compared to RA (P < 0.05) (table 1). There was a significant, albeit modest, decrease in pH in mice treated with oxygen *versus* RA during the prehospital phase (n = 51; P < 0.032) that likely resulted from the significant increase in $Paco_2$ during the prehospital and hospital phases in oxygenated *versus* RA groups (P < 0.001). As expected, blood lactate levels increased and the base deficit worsened during the shock phase, with similar recovery in both treatment groups during the prehospital and hospital phases (table 1).

Fluid requirements to reach and maintain the target MAP (>70 mmHg) during the prehospital resuscitation phase were reduced by treatment with oxygen *versus* RA (154 ± 6 *vs.* 176 ± 4 ml/kg, P = 0.004, respectively) (fig. 3; table 1). Despite reduced fluid requirements, the mean MAP was higher in the oxygen-treated group, although the difference was not statistically significant when compared with the RA group (fig. 3; table 1). Similarly, the mean ICP was lower and resultant mean cerebral perfusion pressure was higher with hyperoxic resuscitation compared to RA; however, the group differences were again not significant (fig. 3; table 1).

Brain Antioxidant Status and Oxidative Stress

Brain antioxidant status and oxidative stress were assessed by measuring hippocampal and cortical ascorbate, LMWTs, reduced glutathione, and protein oxidation, as well as total SOD and MnSOD activity in samples ipsilateral and contralateral to the injury in mice at 24 h after combined CCI and HS. There was a significant decrease in ascorbate levels in hippocampus of mice resuscitated with oxygen *versus* RA (ipsilateral combined CCI and HS and oxygenation *vs.* combined CCI and HS and RA; −19.54 ± 3.20 *vs.* −4.42 ± 1.90 nM/mg protein, respectively; P < 0.001; contralateral combined CCI plus HS and oxygenation *vs.* combined CCI and HS and RA; −11.61 ± 2.41 *vs.* −1.19 ± 2.50 nM/mg protein, respectively;

Table 1. Physiologic Variables at Defined Time Points in the Experimental Protocol

	RA				Hyperoxia			
	Baseline	Shock	Prehospital	Hospital	Baseline	Shock	Prehospital	Hospital
MAP, mmHg	90 ± 1	30 ± 6	60 ± 2	79 ± 1	89 ± 1	30 ± 2	62 ± 2	73 ± 2
ICP, mmHg	5.2 ± 1.3	2.9 ± 0.7	13.5 ± 1.9	19.9 ± 1.5	6.0 ± 1.4	3.3 ± 1.0	13.2 ± 1.8	18.1 ± 1.0
CPP, mmHg	74.8 ± 1.3	23.8 ± 1.4	44.8 ± 2.0	56.9 ± 1.7	78.2 ± 2.1	25.2 ± 3.1	50.0 ± 2.4	55.6 ± 2.4
LR, ml/kg			176 ± 4				154 ± 6*	
pH	7.37 ± 0.01	7.32 ± 0.01	7.39 ± 0.01	7.35 ± 0.01	7.37 ± 0.01	7.29 ± 0.01	7.34 ± 0.01*	7.31 ± 0.01
Pa_{O_2}, mmHg	141.3 ± 9.6	93.5 ± 6.5	81.9 ± 4.6	77.5 ± 3.9	127.8 ± 11.5	83.6 ± 7.5	342.6 ± 36.8*	346.4 ± 37†
Pa_{CO_2}, mmHg	35.3 ± 1.0	24.0 ± 0.7	32.1 ± 0.8	34.7 ± 1.0	35.8 ± 1.1	23.9 ± 0.7	38.3 ± 1.3*	41.9 ± 1.5†
S_{O_2}, %	98.0 ± 0.8	94.7 ± 1.2	93.6 ± 0.9	92.0 ± 0.9	97.9 ± 0.7	94.3 ± 0.8	99.3 ± 0.2*	99.7 ± 0.1†
Hematocrit, %	41.5 ± 0.6	27.6 ± 0.7	18.7 ± 0.5	29.4 ± 0.4	41.8 ± 0.6	27.3 ± 0.6	20.1 ± 0.5	29.4 ± 0.4
Lactate, mM	2.5 ± 0.1	7.4 ± 0.4	2.6 ± 0.1	1.8 ± 0.1	2.5 ± 0.1	8.3 ± 0.5	2.1 ± 0.2	1.6 ± 0.1
Base deficit, mM	4.1 ± 0.5	12.0 ± 0.5	4.6 ± 0.4	5.4 ± 0.4	3.9 ± 0.5	13.1 ± 0.6	4.4 ± 0.4	5.0 ± 0.5
Glucose, mg/dl	167.0 ± 13.6	245.0 ± 18.2	99.4 ± 6.7	88.6 ± 6.5	166.3 ± 15.2	258.2 ± 20.1	108.4 ± 9.3	94.9 ± 7.2

Values are mean ± SEM.

* $P < 0.05$ *vs.* RA in the prehospital phase. † $P < 0.05$ *vs.* RA in the hospital phase, *t* test.

CPP = cerebral perfusion pressure; ICP = intracranial pressure; LR = lactated Ringer's; MAP = mean arterial pressure; Pa_{CO_2} = partial pressure of arterial carbon dioxide; Pa_{O_2} = partial pressure of arterial oxygen; RA = room air; S_{O_2} = oxygen saturation.

$P = 0.005$) (fig. 4). Ascorbate levels were 42.35 ± 3.76 and 46.63 ± 4.06 nM/mg protein in left (ipsilateral) and right (contralateral) hippocampi, respectively, in naïve controls. Although ipsilateral cortical ascorbate levels were lower in oxygen- *versus* RA-treated mice, this difference was not statistically significant. LMWT levels were significantly increased in the ipsilateral hippocampus of mice resuscitated with RA (combined CCI plus HS and RA; 17.37 ± 2.46 nM/mg protein) compared to controls (naïve, sham RA, and sham oxygenated values were 13.59 ± 2.05, 14.79 ± 3.65, and 14.27 ± 1.25 nmol/mg protein, respectively; $P = 0.002$), but not compared to animals resuscitated with oxygen (combined CCI plus HS and oxygenation, 14.32 ± 1.09 nM/mg protein) (fig. 5). There were no differences between groups in LMWT levels in the contralateral hippocampus or either cortex. Reduced glutathione was greater in the ipsilateral hippocampus of naïve mice and injured animals treated with RA (combined CCI plus HS and RA) *versus* both sham groups, but the difference between the oxygen and RA resuscitation groups was not significant (fig. 5). There were no measured differences in cortical reduced glutathione levels across all groups. Protein oxidation was measured by assessing protein sulfhydryl levels in brain samples at 24 h after injury. There was no effect of hyperoxic resuscitation (*vs.* RA) on the level of protein oxidation in ipsilateral or contralateral hippocampus and cortex. Finally, there was no significant difference in the activity of total SOD in the hippocampus or cortex in animals from any group. MnSOD activity was increased in the ipsilateral hippocampus of mice resuscitated with oxygen *versus* naïve animals (combined CCI plus HS and oxygenation *vs.* naïve, 3.92 ± 0.75 *vs.* 2.71 ± 0.22 U/mg protein, respectively; $P = 0.046$), but not compared to RA resuscitation (combined CCI plus HS and RA, 2.96 ± 0.28 U/mg protein) (fig. 5). MnSOD activity in the cortex was not different across groups. In summary, ascorbate levels in the hippocampus were significantly reduced in the oxygen- *versus* RA-resuscitated group. There was no difference in cortical ascorbate levels in the oxygen *versus* RA groups. There was also no effect of oxygen on the levels of LMWT, reduced glutathione, protein oxidation, or MnSOD activity in the hippocampus or cortex compared to RA in mice at 24 h after combined CCI and HS.

Brain Chemokine, Cytokine, and Growth Factor Response

Assessment of chemokines, cytokines, and growth factors revealed a number of significant findings with regard to the effect of combined CCI and HS and the impact of treatment with supplemental oxygen.

Chemokines

Six chemokines were assessed, including monocyte chemotactic protein-1α, macrophage inflammatory protein-1, KC, eotaxin, interferon inducible protein-10, and RANTES. All six chemokines were significantly increased in injured hippocampus versus either naïve or sham groups (fig. 6). The only difference between oxygen and RA treatment groups was for the chemokine KC, with significantly higher levels seen in hippocampus after hyperoxia. In the injured cortex, five of the six chemokines (all except RANTES) were significantly increased *versus* either naïve or sham groups. The magnitude of the increase in cortex was similar to that seen in hippocampus. The only difference between treatment groups in the cortex, however, was a significant increase in KC with RA compared with the hyperoxia group. Overall, chemokines were robustly increased in brain at 24 h after combined CCI plus HS, but KC was the only chemokine

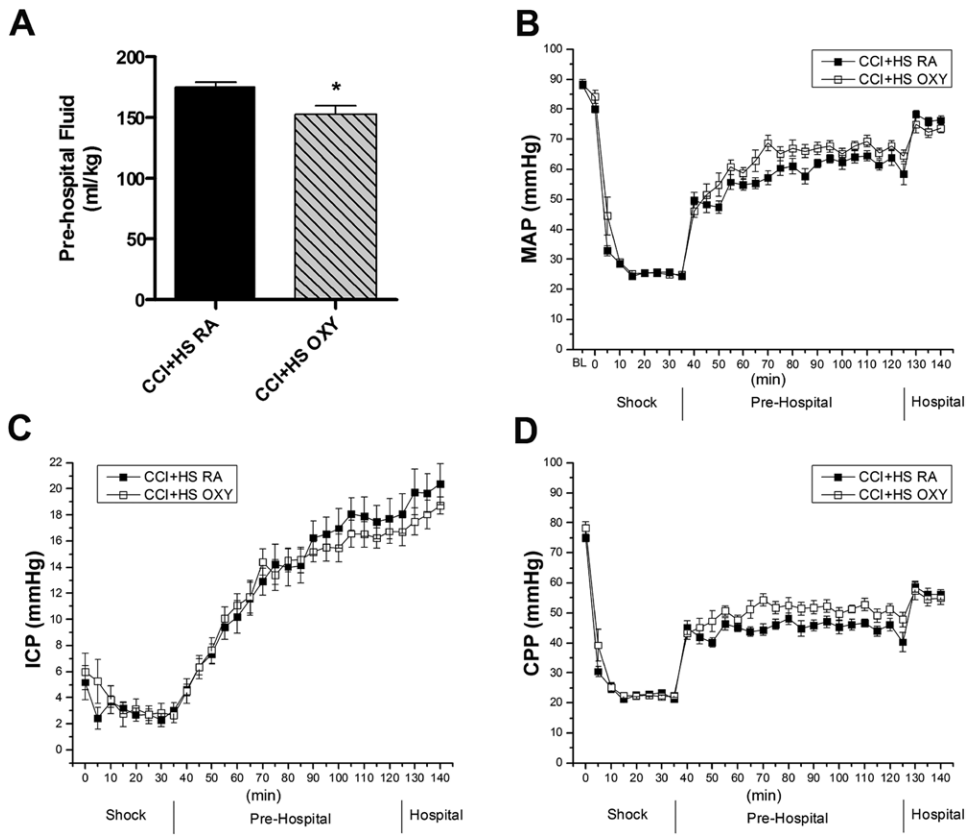

Fig. 3. Systemic and cerebral physiologic variables measured during the combined controlled cortical impact (CCI) and hemorrhagic shock (HS) model followed by lactated Ringer's fluid resuscitation in the prehospital phase and return of shed blood in the hospital phase in mice administered either oxygen or room air (RA) during the resuscitation phases. (*A*) Resuscitation fluid administered during the prehospital phase required to maintain mean arterial blood pressure greater than 70 mmHg was significantly higher in the mice resuscitated with RA (CCI+HS RA) compared to oxygen (CCI+HS OXY) (*P = 0.004). (*B*) Mean arterial blood pressure (MAP) (in millimeters of mercury) *vs.* time (in minutes) in mice with no significant difference between groups resuscitated with either oxygen (CCI+HS OXY, *open squares*) or RA (CCI+HS RA, *closed squares*). (*C*) Intracranial pressure (ICP) measurements were not different between mice resuscitated with either oxygen (CCI+HS OXY, *open squares*) or RA (CCI+HS RA, *closed squares*), although a favorable trend was seen in the oxygen group. (*D*) Calculated cerebral perfusion pressure (CPP) (MAP – ICP) was not different between animals resuscitated with oxygen (CCI+HS OXY, *open squares*) or RA (CCI+HS RA, *closed squares*).

affected by hyperoxia versus RA treatment with opposite responses to oxygen in the hippocampus (increased) and cortex (decreased).

Cytokines

Twelve cytokines were assessed, including tumor necrosis factor-α, interferon-γ, IL-1β, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, and IL-17. In hippocampus, five cytokines were increased after injury *versus* either naïve or sham groups (i.e., IL-1β, IL-2, IL-5, IL-6, and IL-17) (fig. 7). IL-1β was significantly increased in hippocampus of both treatment groups. IL-2 and IL-6 were significantly increased in hippocampus only in the oxygenated group, whereas IL-5 and IL-17 were significantly increased in hippocampus only in the RA group. In cortex, only three cytokines were significantly increased *versus* either naïve or sham groups, namely, IL-4, IL-5, and IL-6. Of these three cytokines, increases in IL-4 and IL-5 were seen after injury only in the RA group. There was no significant difference in cortical IL-6 levels with regard to oxygen or RA treatment. The overall effects of oxygen on cytokine expression was an increase in hippocampal IL-2 and IL-6, whereas hippocampal IL-5 and IL-17 and cortical IL-4 and IL-5 were decreased when compared with RA treatment. Thus, the patterns of increase with these cytokines were highly region and treatment dependent.

Growth Factors

Three growth factors were assessed, including G-CSF, GM-CSF, and vascular endothelial growth factor (VEGF) (fig. 8). In hippocampus, G-CSF was significantly increased in hippocampus after injury *versus* either naïve or sham groups (fig. 8). The increase in hippocampal G-CSF was greater in the oxygen group compared with the RA group. GM-CSF and VEGF were not significantly increased in hippocampus after combined CCI and HS injury. In cortex, G-CSF and VEGF were significantly increased after injury *versus*

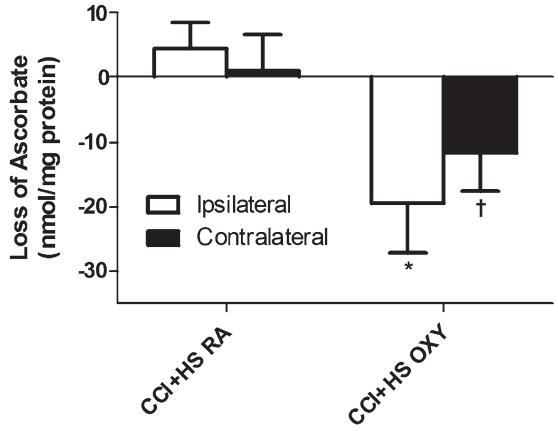

Fig. 4. Assessment of brain ascorbate levels in mice 24 h after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury and resuscitation. Loss of ascorbate was significantly greater in the ipsilateral and contralateral hippocampi of animals resuscitated with oxygen (CCI+HS OXY) compared with room air (CCI+HS RA) (*P < 0.001 and †P = 0.005 *vs.* corresponding brain region in the CCI+HS RA group).

either naïve or sham groups. The increase in cortical VEGF after CCI and HS was significantly greater in the RA *versus* oxygen group; however, there was no observed effect of oxygen on cortical G-CSF *versus* RA. GM-CSF in cortex was not increased *versus* naïve or sham after combined CCI plus HS. The overall effect of oxygen on the growth factors after combined CCI plus HS injury was an increase in hippocampal G-CSF and decrease in cortical VEGF compared with RA treatment.

Neuronal Survival

Surviving neurons in the selectively vulnerable CA1 and CA3 hippocampal regions underlying the cortical contusion were quantified in hematoxylin and eosin–stained sections at 7 days after combined CCI and HS and resuscitation. There was a significantly greater number of surviving neurons in the CA3 region of mice resuscitated with oxygen *versus* RA (21 ± 2 *vs.* 15 ± 2 CA3 cells per 0.1-mm linear density, respectively; P < 0.032) (fig. 9). There was also a trend toward improved survival of CA1 neurons in the oxygenated group; however, the difference from RA was not significant (P = 0.2).

Discussion

Hyperoxic resuscitation after combined CCI and HS in mice reduced fluid requirements and attenuated CA3 hippocampal neuronal death, a vulnerable brain region in our model, despite an increase in oxidative stress as evidenced by ascorbate depletion and a shift toward a more proinflammatory cytokine profile in brain *versus* RA. Contrary to the recent concern with oxygen administration after cardiac arrest, combined CCI and HS represents a scenario where supplemental oxygen is beneficial. Our data mirror those in studies of stroke.[15–22] Evidence also supports titration of therapies to maintain $PbtO_2$ above a critical threshold after severe TBI,[9,36,37] although the threshold remains undefined.[38,39] Similarly, work in TBI suggests that hyperbaric oxygen may have merit,[40] although further study is needed.

We used a mouse model coupling CCI with a pressure-controlled HS followed by MAP-targeted resuscitation. This mimics the prehospital scenario where supplemental oxygen can be provided as recommended

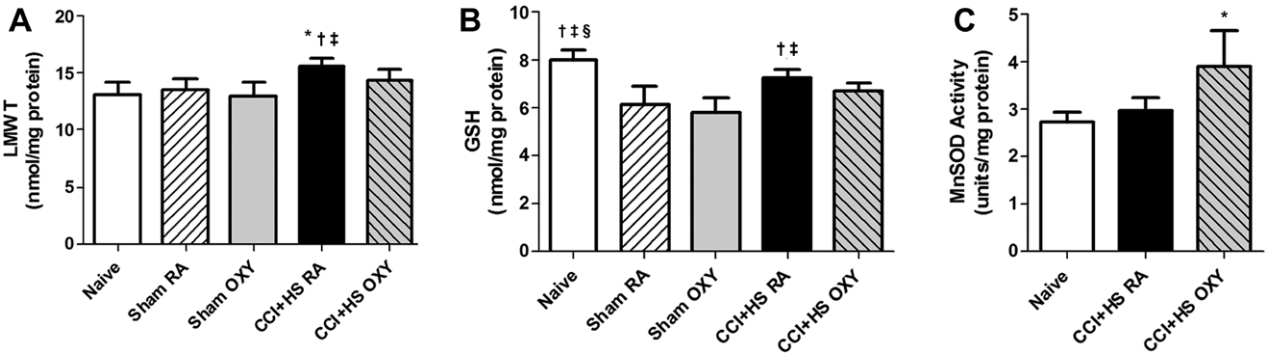

Fig. 5. Assessment of oxidative stress in mice 24 h after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury and resuscitation. (*A*) Levels of low-molecular-weight thiols (LMWT) were found to be significantly increased in the ipsilateral hippocampus of animals resuscitated with room air (CCI+HS RA) compared with naïve and sham animals treated with oxygen (Sham OXY) or room air (Sham RA), but not compared with animals resuscitated with oxygen (CCI+HS OXY). (*B*) Reduced glutathione (GSH) levels were increased in the ipsilateral hippocampus of naïve mice and injured animals treated with RA (CCI+HS RA) *versus* sham groups, but the difference between the oxygen (CCI+HS OXY) and RA resuscitation groups was not significant. (*C*) Manganese superoxide dismutase (MnSOD) activity was increased in the ipsilateral hippocampus in mice after hyperoxic resuscitation *versus* naïve animals (CCI+HS OXY *versus* naïve, respectively), but not compared with RA resuscitation (CCI+HS RA). Mean ± SEM; ANOVA; *P < 0.05 *versus* naïve; †P < 0.05 *versus* Sham RA; ‡P < 0.05 *versus* Sham OXY; #P < 0.05 *versus* CCI+HS RA; §P < 0.05 *versus* CCI+HS OXY.

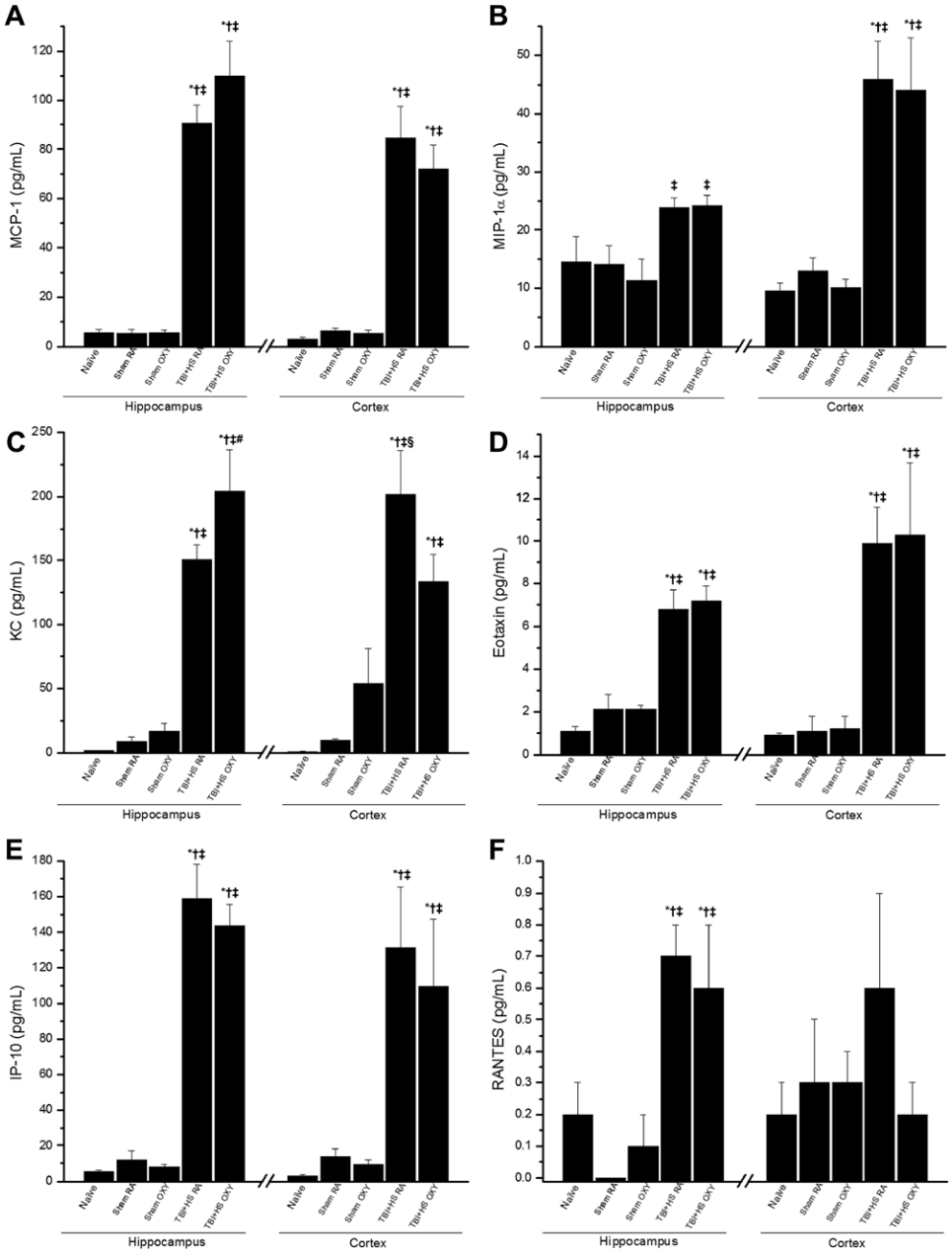

Fig. 6. Chemokine responses in mouse brain homogenates at 24 h after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury and resuscitation. The chemokines analyzed include: (*A*) monocyte chemotactic protein-1 (MCP-1), (*B*) macrophage inflammatory protein-1α (MIP-1α), (*C*) keratinocyte-derived cytokine (KC), (*D*) eotaxin and (*E*) interferon inducible protein-10 (IP-10), and (*F*) regulated upon activation normal T-cell expressed and secreted (RANTES). There were significant increases in levels of all chemokines analyzed in injured hippocampus of mice treated with room air (CCI+HS RA) and oxygen (CCI+HS OXY) *versus* either naïve or sham groups. The only effect of oxygen treatment was an increase in hippocampal KC levels *versus* RA. All of the above chemokines analyzed except RANTES were significantly increased in the injured cortex (CCI+HS RA and CCI+HS OXY) *versus* either naïve or sham groups. In the cortex, however, the level of KC was significantly greater in the RA *versus* oxygen group. Mean ± SEM; ANOVA; **P* < 0.05 *versus* naïve; †*P* < 0.05 *versus* Sham RA; ‡*P* < 0.05 *versus* Sham OXY; #*P* < 0.05 *versus* CCI+HS RA; §*P*< 0.05 *versus* CCI+HS OXY.

by guidelines for prehospital treatment of severe TBI,[41] taking into account that the guidelines do not recommend tracheal intubation if hypoxia can be corrected. Administration of pure oxygen *via* nose cone revealed a greater than threefold increase in the Pa_{O_2} and Pbt_{O_2} *versus* RA. The significantly increased Pa_{O_2} and Pbt_{O_2} levels achieved with oxygen *versus* RA permitted direct comparison. We were surprised by the robust Pbt_{O_2} response to oxygen, considering that the additional oxygen is in the dissolved form.

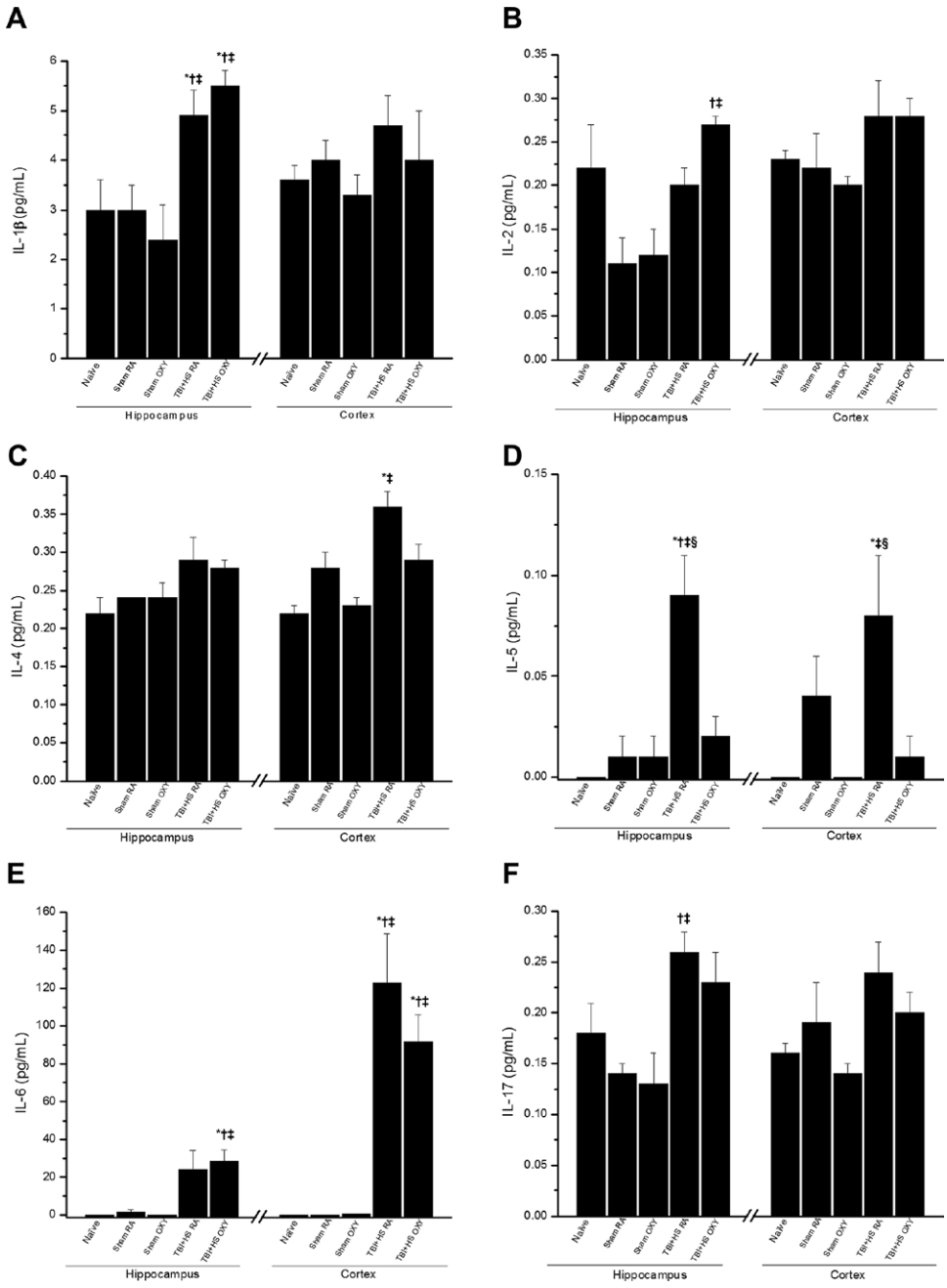

Fig. 7. Cytokine responses in mouse brain homogenates at 24 h after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury and resuscitation. Of the cytokines analyzed, there were significant increases seen in (*A*) interleukin (IL)-1β, (*B*) IL-2, (*C*) IL-4, (*D*) IL-5, (*E*) IL-6, and (*F*) IL-17 after combined CCI plus HS. In injured hippocampus, (*A*) IL-1β was significantly increased in both RA and oxygen groups. Hippocampal (*D*) IL-5 and (*F*) IL-17 were greater in only the RA group, whereas levels of (*B*) IL-2 and (*E*) IL-6 were greater only in the oxygen group. (*C*) IL-4 and IL-5 levels were significantly increased in the injured cortex only after resuscitation with room air (CCI+HS RA), whereas IL-6 was significantly increased in cortex of both RA and oxygen resuscitation groups. Mean ± SEM; ANOVA; **P* < 0.05 *versus* naïve; †*P* < 0.05 *versus* Sham RA; ‡*P* < 0.05 *versus* Sham OXY; §*P* < 0.05 *versus* CCI+HS OXY.

We found that oxygen use after combined CCI and HS in our model reduced resuscitation fluid requirements to reach the target MAP (70 mmHg) during the prehospital phase. Oxygen supplementation increases systemic vascular resistance, mostly because of a hyperoxia-induced decrease in nitric oxide availability.[42–47] Increased systemic vascular resistance is balanced by a decrease in cardiac output secondary to a reflex decrease in heart rate.[48] In shock states, hyperoxia stabilizes hemodynamics and redistributes blood flow to vital organs.[49–51] Our results showed that despite reduced fluid volumes, oxygen administration increased mean MAP *versus* RA, although the difference in MAP was

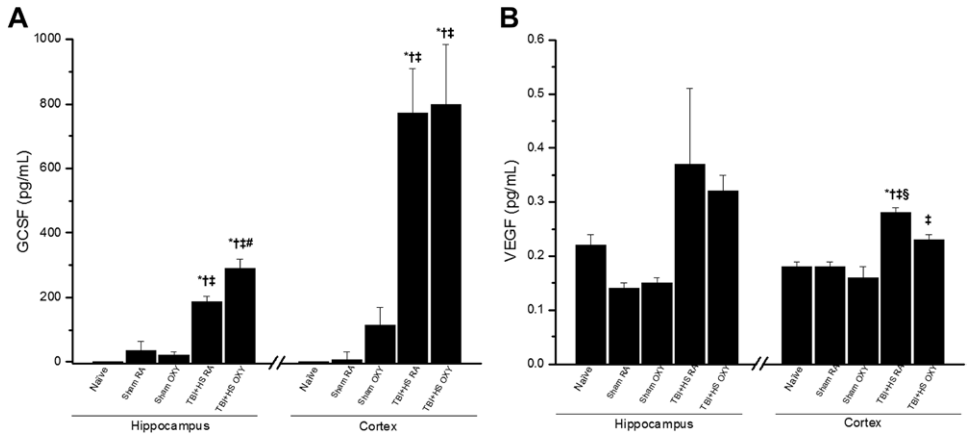

Fig. 8. Growth factor responses in mouse brain homogenates at 24 h after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury and resuscitation. (*A*) Granulocyte colony-stimulating factor (G-CSF) was significantly increased in the injured hippocampus and cortex (CCI+HS RA and CCI+HS OXY) *versus* either naïve or sham groups. There was a greater increase in hippocampal G-CSF in the oxygen group (CCI+HS OXY) *versus* RA, but there was no effect of oxygen on the levels of G-CSF in cortex. Vascular endothelial growth factor (VEGF) was significantly increased in the injured cortex (CCI+HS RA) *versus* either naïve or sham groups, as well as compared with the oxygen resuscitation group (CCI+HS OXY). Mean ± SEM; ANOVA; **P* < 0.05 *versus* naïve; †*P* < 0.05 *versus* Sham RA; ‡*P* < 0.05 *versus* Sham OXY; #*P* < 0.05 *versus* CCI+HS RA; §*P* < 0.05 *versus* CCI+HS OXY.

not significant. Our approach to set a target MAP mimics the clinical scenario where caregivers desire to optimize cerebral perfusion pressure during resuscitation to minimize secondary injury.[52]

Hyperoxia is not a universal vasoconstrictor; its effects vary according to the vascular bed and the disease state.[53] Breathing 100% oxygen causes cerebral vasoconstriction that is offset by an increase in Pbt_{O_2} from increased oxygen delivery.[8,54] Normobaric oxygen administration in patients with severe TBI improves ICP and cerebral metabolism.[11] We noted that oxygen administration in mice yielded a lower mean ICP and increased mean cerebral perfusion pressure after combined CCI and HS; however, these differences were not statistically different from RA treatment.

During resuscitation, pH decreased and Pa_{CO_2} increased in mice treated with oxygen *versus* RA. The hyperoxia-induced decrease in pH and increase in Pa_{CO_2} likely resulted from suppression of the oxygen chemoreflex ventilatory drive.[55] We did not measure respiratory rate; however, we measured the ventilatory response to oxygen in separate uninjured isoflurane-anesthetized mice and found a decreased respiratory rate *versus* RA (data not shown). Increased Pa_{CO_2} likely resulted from decreased ventilation in the oxygen group as reported in TBI[8] and HS.[56] It is intriguing in our study that we observed a lower but not statistically different mean ICP during resuscitation with oxygen *versus* RA despite a higher Pa_{CO_2}.

Ascorbate is depleted and brain antioxidant reserves are decreased after TBI.[57,58] Consistent with our hypothesis, hyperoxia further compromised this brain antioxidant defense after combined CCI and HS *versus* RA. Ascorbate decreased in the hippocampus underlying and contralateral to the cortical contusion at 24 h in mice resuscitated with oxygen. Ascorbate is the first line of defense against oxidative stress, and reduced levels represent a sensitive marker of oxidative injury.[58,59] The decrease in hippocampal ascorbate with hyperoxia is significant but may be within the tolerance limits of the brain; thus, improvement in hippocampal neuronal survival is the most important result of resuscitation with oxygen *versus* RA. The neuroprotective effects of oxygen resuscitation might be further improved by combination of 100% oxygen with an antioxidant in combined CCI and HS. We did not find a significant loss of ascorbate in the cortex, although there was a lower mean cortical ascorbate with oxygen treatment *versus* RA. The cortex is severely injured after the level of CCI imposed, possibly limiting perfusion and oxygen exposure; we did not measure cortical Pbt_{O_2}. In terms of markers of oxidative stress, we did not observe differences in levels of reduced glutathione, LMWT, or SOD after resuscitation with oxygen *versus* RA. It may be that the effect of hyperoxia on brain antioxidant reserves is not as severe as anticipated, or that the duration of oxygen exposure was short. We observed an increase in MnSOD activity in the injured hippocampus of mice resuscitated with oxygen *versus* naïve, but not *versus* RA. MnSOD is the first line of defense against superoxide in mitochondria and is induced after TBI.[60] Increased MnSOD activity in injured hippocampus is interesting given our prior report showing inactivation of MnSOD by neuronal nitric oxide synthase–mediated nitration after TBI.[61] TBI plus HS likely represents a unique insult.

This study builds on our prior report showing robust inflammation after combined CCI and HS in mice dominated by chemokines and selective increases in cytokines and growth factors.[32] We assessed hippocampal and cortical tissue in the injured hemisphere, where changes in these

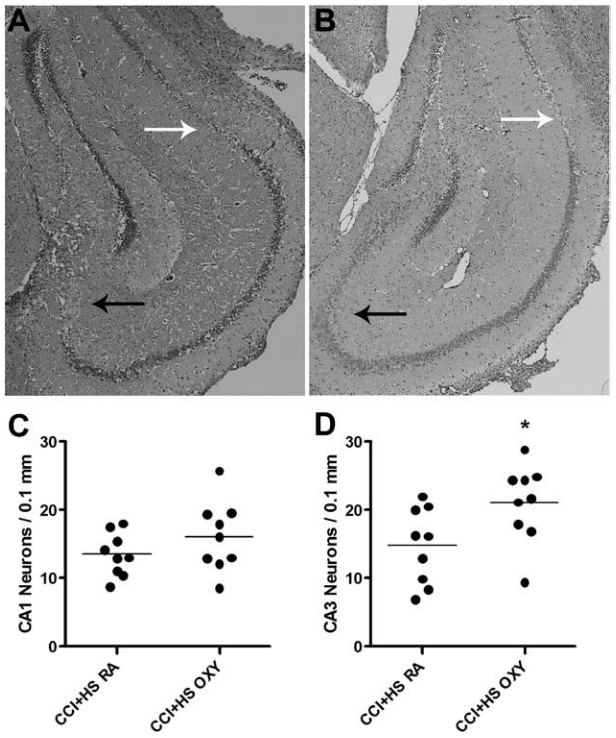

Fig. 9. Neuronal survival was assessed at 7 days after combined controlled cortical impact (CCI) and hemorrhagic shock (HS) injury. Photomicrographs of representative hippocampal sections underlying the cortical contusion stained with hematoxylin and eosin. (*A*) Mouse hippocampus after combined CCI plus HS injury and room air (RA) resuscitation (CCI+HS RA) and (*B*) oxygen resuscitation (CCI+HS OXY). The loss of neurons in the CA1 region (*white arrows*) does not appear to differ between groups on hematoxylin and eosin staining. There is gross degeneration of neuronal tissue in the CA3 region (*black arrows*) of a mouse resuscitated with RA (*A*) compared with oxygen (*B*). Scatter plots of the number of surviving neurons (quantitated as linear density) in both (*C*) CA1 and (*D*) CA3 for CCI+HS RA and CCI+HS OXY groups with median represented by *solid bar* (*$P < 0.032$, *t* test).

mediators are likely to define the impact of oxygen administration. Chemokines represent the predominant inflammatory mediator class after combined CCI and HS in mouse brain and signal cellular infiltration.[61–67] Because oxygen reduced neuronal death but increased oxidative stress, one could not *a priori* suggest that oxygen would exacerbate or attenuate neuroinflammation, despite links between leukocyte infiltration, inflammatory mediators, tissue injury, and oxidative stress.[61,68]

Consistent with our previous work,[32] there was a robust increase in chemokine levels after combined CCI and HS, although oxygen did not consistently increase chemokines to reflect an exacerbation of neuroinflammation. Effects of oxygen on cytokines, however, produced significant effects that are predominantly proinflammatory. Overall, the effect of oxygen on cytokine expression was an increase in hippocampal IL-2 and IL-6, whereas hippocampal IL-5 and IL-17 and cortical IL-4 and IL-5 were decreased *versus* RA. IL-6 and IL-1β are important signaling molecules after TBI, with IL-6 consistently increased in brain such that it is considered a TBI biomarker.[69,70] Thus, whether hyperoxia-induced augmentation of increased IL-6 in injured hippocampus after combined CCI and HS is beneficial or detrimental is unclear. Given that IL-6 overexpression is neurotoxic,[71] it suggests a proinflammatory effect of hyperoxia. IL-1β is also implicated in signaling proinflammatory events and the up-regulation of tropic factors after TBI.[72–76] However, oxygen did not influence IL-1β. Supplemental oxygen blunted increases in IL-4, IL-5, and IL-17 in injured cortex and/or hippocampus. IL-4 plays an antiinflammatory Th2 role in TBI,[76,77] possibly by triggering a neuroprotective profile in microglia,[78] and IL-5 has Th2 antiinflammatory effects.[79] IL-17 may mediate regeneration.[80] Thus, our findings also suggest a proinflammatory effect of supplemental oxygen—reducing antiinflammatory cytokines. However, IL-17 also mediates a proinflammatory Th17 response in brain, exacerbating injury.[80–82] Increases in IL-2 in hippocampus were also magnified by oxygen; IL-2 is a proinflammatory cytokine important for Th1 signaling.

Finally, oxygen blunted the cortical VEGF response after combined CCI and HS. Given the important role of tissue hypoxia in VEGF signaling and the marked increase in $PbtO_2$ by oxygen in our model, this is not surprising. The impact of this finding, however, is unclear and could reflect either a reduction in damage or attenuation of vascular regeneration signaling.

Despite increased evidence of oxidative stress in brain, oxygen administration after combined CCI and HS attenuated hippocampal CA3 neuronal death at 7 days after injury. The moderate CCI we used was designed to leave the hippocampus intact but vulnerable to HS.[33,34] We were surprised to see benefit from oxygen on CA3 rather than CA1, as we propose that the entire hippocampus underlying the cortical injury represents a penumbra. We observed a positive trend in CA1 cell counts with oxygen treatment; a larger sample size is needed to test our hypothesis in CA1.[27] TBI increases metabolic demands in CA3, making it vulnerable to secondary injury, which might benefit from oxygen during hypotension.[83] Thus, TBI plus HS may show beneficial and detrimental effects of supplementation oxygen. This is similar to stroke, where benefit was seen during occlusion but detrimental effects occurred during reperfusion.[84]

Our study has limitations. TBI plus HS is a complex scenario with limitless severity levels. We used a single injury level, a single level of HS; thus, the findings could depend on the clinical presentation. Nevertheless, our model includes a clinically relevant injury and resuscitation. We administered pure oxygen to maximize the potential effect of hyperoxia, but it is unclear what affect titration of the fraction of inspired oxygen would have. We also studied only adult male mice, which are more vulnerable to oxidative stress

than female mice.[85] Studies of behavioral and long-term outcomes are also needed.

Given the controversy regarding supplemental oxygen use after cardiac arrest, stroke, and TBI, we sought to determine whether oxygen influenced resuscitation and neuropathologic outcome in TBI plus HS. Hyperoxia improved cerebral hemodynamics and CA3 hippocampal neuron survival but worsened oxidative stress *versus* RA and possibly increased inflammation. The overall benefit of hyperoxic resuscitation suggests the need to test the addition of an antioxidant strategy to supplemental oxygen or other approaches to enhance oxygen delivery after TBI plus HS injuries.

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