Ischemia Reperfusion Injury: The New Frontier for Wound Healing & HBOT

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Vascular Medicine / Hyperbaric Oxygen
Overview

- Definitions
- Oxygen Free Radicals
- Pathophysiology of ROS
- ROS & Compromised Wound Healing
- Ischemia Reperfusion Injury
- Common Mechanism of Action for HBOT?
A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. Free radicals are highly reactive due to the presence of an unpaired electron. Oxygen Free Radical = Reactive Oxygen Species

\[
\begin{align*}
\text{Oxygen} & \quad \text{Fe}^{2+} + \text{H}_2\text{O}_2 & \quad \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^- \\
\end{align*}
\]
Reactive Oxygen Species (ROS)

- Hydroxyl radical (OH·)
- Superoxide Anion (O2·)
- Singlet oxygen
- Ozone (O3)
- Hydrogen peroxide (H2O2)
- Nitric Oxide: Peroxynitrite (ONOO–)
- Carbon Based: Peroxyl Radicals (·O2CCl3).
- Thiol compounds (RSO2·)
ROS Production

- Environmental
  - Air Pollution
- External - Exogenous
  - Smoking
- Direct Ionizing Radiation
  - Environmental-Therapeutic
- Cellular Metabolism
- Inflammation
Redox Reactions

- Oxidation is gain of oxygen
- Reduction is loss of oxygen

Oxidation is loss of electrons (Hydrogen)
Reduction is gain of electrons (Hydrogen)
Once formed oxygen-free radicals seek out electrons to form a stable molecule.

Fatty Acid Chain

\[ \text{H} \quad \text{C} = \text{C} - \text{C} = \quad \text{H} \]

Hydroxyl Radical

\[ \cdot \text{O} : \text{H}^- \]
ROS Effects & Damage

- Oxygen Free Radical ATTACK on molecules results in Oxidation Reactions
  - Lipids (LIPID PEROXIDATION)
  - Amino acids in proteins
  - Enzymes by oxidation of co-factors

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoproteins.

- PUFAs allow for fluidity and transport across cellular membranes.
- When oxygen free radicals that attack PUFAs the result is damage to cellular membranes

- LIPID PEROXIDATION
ROS Cause Chain Reactions

- Initiation
- Propagation
- Termination

- two free radicals combine to form a more stable species

Antioxidant Defenses

- Antioxidants give up their own electrons to free radicals rendering ROS inactive
- Oxygen Free Radicals are stabilized
  - lipid peroxidation ceases
  - chain reaction of oxidation is broken
Antioxidants

- Vitamin E
- Beta-carotene
- Coenzyme Q
- Intracellular Antioxidant Scavengers
  - vitamin C, superoxide dismutase, catalase
Oxidative Stress

Free Radicals
Oxidative Stress

- Excessive ROS
  - Deficient termination reactions
  - Lack of endogenous scavengers / antioxidants
  - Production exceeds reduction reactions

Oxidative Stress (Systemic)

- Atherosclerosis
- Parkinson's disease
- Heart Failure
- Myocardial Infarction
- Alzheimer's disease
- Chronic fatigue syndrome
- Aging

Gems D, Partridge L (March 2008). "Stress-response hormesis and aging: "that which does not kill us makes us stronger"." Cell Metab. 7 (3): 200–3.
Chronic Inflammation
The Silent Killer

Cardiovascular Disease
Neurological Disease
Diabetes
Arthritis
Alzheimer's Disease
Cancer
Auto Immune Disease
Oxidative Stress (Cellular)

- Cell Wall Disturbance (PUFAs)
- Enzyme Disruption
- DNA Damage
- Apoptosis (Cellular Death)
- Tissue Necrosis
- COMPROMISED WOUND HEALING

Exogenous Sources of Free Radicals

Exogenous Sources

- Environmental
- External
Air Pollution / Cigarette Smoke

AIRWAY EPITHELIUM

DEP

allergen

Cigarette smoke

ALVEOLI

neutrophil

T cell

Macrophage

Danger signals, TNFα
IL1-β, IL-6, IL-8, MCP-1
TSLP↑, GM-CSF↑, Eotaxin↑

Oxygen radicals

Major basic protein

Eosinophil↑

Leukotrienes

Histamine

Prostaglandin D₂

Airway hyperresponsiveness

Airway wall remodeling

Goblet cell hyperplasia↑

FceRⅠ

Mast cell

CD80/86↑

IL-4↑

IL-5↑

IL-13↑

Th proliferation↑

Th↑

IgE↑

IgG↑

B-cell↑

Plasma cell

LYMPH NODE
Ionizing Radiation
Endogenous Sources of Free Radicals

Endogenous

(shared) Cellular Metabolism

- Glycolysis
Cellular Metabolism

- Glycolysis
- Mitochondria
- Krebs Cycle
- ATP Production

In each of these drops, energy is transferred to energy-storing molecules ATP, NADH, and FADH$_2$. 

Change in free energy, $\Delta G$ (in kcal/mol): Glycolysis, Pyruvate Processing and Citric Acid Cycle.
The electron transport chain occurs in the inner membrane of the mitochondrion (membranes of cristae).

**Process: Electron Transport Chain**

- **Intermembrane space**
- **Mitochondrial matrix**
- **Complex I** (NADH dehydrogenase complex)
- **Complex II** (Cytochrome b–c₁ complex)
- **Complex III** (Cytochrome oxidase complex)
- **Complex IV** (ATP synthase)

The electron transport chain involves the following reactions:

- **NADH** → **FADH₂**
- **FADH₂** directly contributes to **Complex II**
- **NAD⁺** is produced from **NADH**
- **O₂** is reduced to **H₂O**

The chain is summarized with the following reactions:

- **2e⁻ + 2H⁺ + ½O₂ → H₂O**

The diagram illustrates the movement of electrons and protons through the various complexes, facilitating the production of ATP.
Mitochondrial ROS

- Submitochondrial localization of ROS generating sites
  - Cytochrome b5 reductase
  - Monoamine oxidases
  - Dihydroorotate dehydrogenase
  - Dehydrogenase a-glycerophosphate
  - Succinate dehydrogenase
  - Aconitase
  - a-Ketoglutarate dehydrogenase complex

Endogenous Sources of Free Radicals

Endogenous

- Cellular Metabolism
  - Glycolysis

- Inflammation
  - Phagocytosis – Respiratory Oxidation
  - Protease Induction
Wound Healing Physiology
# Wound Healing Physiology

## Tissue Repair Phases

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<th>Granulation</th>
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<th>Tissue Repair</th>
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<td>Increased vasodilation/</td>
<td>Cytokines and</td>
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<td>Wound contracture</td>
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Inflammatory Phase

- Polymorphonuclear Neutrophils (PMN)
  - White Blood Cells
- Action
  - Cleanse the wound by secreting proteases
  - Phagocytize debris and bacteria
  - Kill bacteria
    - free radicals
    - respiratory or oxidative burst

Respiratory (Oxidative) Burst

- PMNs and Macrophages
- Degradation of internalized particles
- Formation free radicals
- Rapid release of reactive oxygen species
  - ROS
  - superoxide radical and hydrogen peroxide

BACTERIAL KILLING


OXIDATIVE BURST
Neutrophils kill microbes by producing reactive oxygen species, demonstrated here with the dye nitroblue tetrazolium (NBT)
**ROS and Proteases**

- Proteolytic enzymes are the second line of defense against the ROS in that they degrade and eliminate the damaged molecules.
- Proteolytic process preferentially degrade oxidatively modified and damaged proteins.
- ROS may activate cellular proteases or damage protease inhibitors and promote indiscriminate proteolysis.
- Studies have suggested an increased activity of erythrocyte proteolytic enzymes in degrading oxidant damaged hemoglobin in diabetes mellitus.

Excess MMP's Degrade Extracellular Matrix

Protease → Matrix Proteins
Excess MMP’s Inactivate Growth Factors

Protease  Matrix Proteins  Growth Factors
Sources of Free Radicals

Endogenous

- Cellular Metabolism
  - Glycolysis
- Inflammation
  - Phagocytosis – Respiratory Oxidation
  - Protease Induction
- Ischemia Reperfusion Injury
Ischemia Reperfusion Injury
Ischemia Reperfusion Injury

- Ischemic Event
- Reperfusion of Tissue
Ischemia Reperfusion Injury

- Ischemic Event
  - Direct cellular injury due to tissue hypoxia
  - ROS production via Xanthine Oxidase system
Ischemia Reperfusion Injury

- **Ischemic Event**
  - Direct cellular injury due to tissue hypoxia
  - ROS production via Xanthine Oxidase system

- **Reperfusion of Tissue**
  - Neutrophil Activation
    - Binding and Vasculature Adhesion
    - ROS Production
  - Growing evidence that neutrophil mediated free radical production may be more important than xanthine oxidase in ischemia-reperfusion injury
Neutrophil Adhesion
Neutrophil Adhesion

- Following 4 hours of ischemia, there is an increase in the number of neutrophils that adhere to post-capillary venules.
- This was maintained throughout a 3-hour reperfusion observation period.
- Venule walls became ill defined and disruption of endothelial basement membranes adjacent to adherent neutrophils was observed.
Neutrophil Adhesion
PMN Adhesion & Vascular Insult

- Vasoconstriction
PMN Adhesion & Vascular Insult

- Vasoconstriction
- Endothelial damage
PMN Adhesion & Vascular Insult

- Vasoconstriction
- Endothelial damage
- Vessel leakage and tissue edema
- Vascular sludging
- ROS damage to perivascular tissue

ADDITIONAL

- Tissue ischemia and hypoxia
- ROS Cascade
Terminal Result

Immune Response
Free Radical Production
Tissue Destruction
IRI - ROS
Clinical Correlations
Anaerobic Metabolism
IRI and Anaerobic Respiration

- **Aerobic Conditions**
  - ROS due to glycolysis

- **Anaerobic Conditions**
  - Exercise induces increased formation ROS
  - Additional increase in post-exercise ROS free radical production mediated by xanthine oxidase

Surgical Procedures
Flaps & Grafts
Transplantation
Reimplantation
Surgical Bypass
Fasciotomy
Crush Injury
Endovascular
Acute Hypotensive Episodes
Wounds
Burn Physiology

Evolutionary process over 3-5 days

- Central area zone of coagulation
- Surrounding zone of stasis
- Outer zone of hyperemia

Zone of coagulation
- Can increase 10x in 1st 48hrs

Hemo-concentration
Platelet microthrombi

Edema formation in area of injury & distant areas as well

Larger "transition area" than mechanical trauma
Zone of Stasis

- Vasogenic and Cytogenic Edema
- Platelet microthrombi and hemoconcentration in postcapillary venules
- RBC and WBC adhesion and activation
- Inflammatory mediators liberated
- Sludging and loss of integrity of microvasculature
- Tissue desiccation and thrombosis of capillaries
- Tissue death and progression of Zone of Necrosis/Coagulation
Bubble Related Events
IRI and Intravascular Gas

- Acute Gas Embolism
- Decompression Illness
DCS - Pathophysiology

- Caused by the rapid reduction in environmental pressure sufficient to cause formation of bubbles from inert gases (nitrogen) in the body tissue.

- **Bubbles**
  - Intracellular (myelin sheaths, nerve cells)
  - Extracellular (CSF, anterior chamber)
  - Intravascular
DCS - Pathophysiology

- Vessel sludging and ischemia/hypoxia
- Platelet adhesion to bubbles
  - Activation bradykinins, serotonin, histamine
- Oxygen free radical formation
Is There Another Etiology of Bubble Formation?

Evolved Gas

Blast Injury
Overpressurization Injury

Original Concept Credited to Tom Fox CHT
Emboli in Blast Injury

- Over pressurization and over distention
- Evolved gas
  - related to duration & pressure
- Compressible turbulence of multiple hypersonic waves
- Nano cavitation
  - pressure reductions associated with shock waves can produce cavitation nuclei and bubble excitation*

Pressure-time Curve
Air Blast Overpressurization
Retroreflective Shadowgraphy and High Speed Videography

Photograph courtesy of Gary S. Settles. Penn State University
Bubble Evolution

Overpressure

Extreme hyperbaric exposure

Ambient Pressure

Hypobaric exposure

Time
Unrecognized Blast Injury

Photograph courtesy of Gary S. Settles. Penn State University
Bubble Embolization

Decompression Event

Blast Event
Do “Bubbles” cause Ischemia Reperfusion Injury?
YES!

- **Complete Arterial Occlusion**
  - Early
  - Large

- **Compliment Activation**
  - Sludging
  - Lo-Flow/No-Flow
What is the clinical manifestation of Bubble Induced Ischemia Reperfusion Injury?
Macro Vasculature
Micro Vasculature
Blast Injury
Historical “Silent Wounding”

- DaCosta’s syndrome*
- Effort syndrome
- Irritable Heart*
- Battle Fatigue
- Tristesse Sombre*
- Soldier’s Heart*
- Shell Shocked
- Combat Fatigue
- PTSD
- Mild TBI

Silent Wounding has been reported since the beginning of the use of gunpowder… 1768

Since that time it has perplexed those seeking to care for the wounded or explain deaths with no evidence of external wounds*
Therapeutic Options and Management Strategies
Therapeutic Target & Goals

- Oxidative Stress: Limit
- Excessive Oxygen Free Radicals: Control
- Ischemia Reperfusion: Limit
- Endogenous Imbalances: Control
**ROS Management Strategies**

- **Endogenous Antioxidant Pathways**
  - Nutritional Support
  - Antioxidant Therapy (caution)
    - Beta-carotene, Vitamin C, and Vitamin E
    - Zinc, Selenium
    - Phytochemicals
    - Glutathione
    - Melatonin
ROS Management Strategies

- Endogenous Antioxidant Pathways
- Hyperbaric Oxygen Therapy
HBOT Down-regulates Endothelial Cell Adhesion Molecules in IRI

- In vitro endothelial cell model
- HBO 2.5 ATA prevented IRI by decreasing or preventing induced E-selectin, ICAM-1 and VCAM-1

Conclusions:
- Demonstrates positive role of HBO on endothelium
- Demonstrates WBC anti-adhesive effect of HBO

Experimental Design
I/R injury and HBO treatment

HUVECs

4 hours
Hypoxia/Hypoglycemia

1.5 hours
HBO at 2.5 ATA

18.5 hours
Normoxia/Normoglycemia

20 hours
Normoxia/Normoglycemia

HBO down-regulates hypoxia/hypoglycemia-induced VCAM-1 expression

Effect of HBO on PMN Adherence

HBOT - Mitigates IRI

HBOT mitigates ischemia-reperfusion (IR) injury via a nitric oxide mechanism that is nitric oxide synthase (NOS) dependent. HBOT increases eNOS.


Cabigas BP, Su J, Hutchins W, Shi Y, Schaefer RB, Recinos RF, Nilakantan V, Kindwall E, Niezgoda JA, Baker JE.

Abstract

OBJECTIVE: The relative contributions of the fraction of inspired oxygen (FIO2) and atmospheric pressure (ATM) to cardioprotection are unknown. We determined whether the product of FIO2 x ATM (oxygen partial pressure) controls the extent of hyperoxic-hyperbaric-induced cardioprotection and involves activation of nitric oxide synthase (NOS).

METHODS: Adult Sprague Dawley rats (n = 10/gp) were treated for 1 h with (1) normoxia+normobaria (21% O2 at 1 ATM), (2) hyperoxia+normobaria (100% O2 at 1 ATM), (3) normoxia+hyperbaria (21% O2 at 2 ATM) and (4) hyperoxia+hyperbaria (100% O2 at 2 ATM).

RESULTS: Infarct size following 25 min ischemia and 180 min reperfusion was decreased following hyperoxia+normobaria and normoxia+hyperbaria compared with normoxia+normobaria and further decreased following hyperoxia+hyperbaria treatment. I-NAME (200 microM) reversed the cardioprotective effects of hyperoxia+hyperbaria. Nitrite plus nitrate content was increased 2.2-fold in rats treated with normoxia+hyperbaria and hyperoxia+hyperbaria. NOS3 protein increased 1.2-fold and association of hsp90 with NOS3 four-fold in hyperoxic+hyperbaric rats.

CONCLUSIONS: Cardioprotection conferred by hyperoxia+hyperbaria is directly dependent on oxygen availability and mediated by NOS.
HBOT Prevents Vasoconstriction Post Ischemia

Zamboni WA. “The microcirculation and ischemia-reperfusion: basic mechanisms of hyperbaric oxygen.” Hyperbaric Medicine Practice, 1999; Best Publishing, Flagstaff AZ, pages 779-794
HBOT - Antioxidant

- Significant increase in the synthesis of Heat Shock Protein 70 (HSP70) post HBOT
- HSP70 has protective effect in oxidative stress.

HBOT in Ischemia Reperfusion

- HBO decreases WBC adherence following ischemia.
- The leukocyte adhesion molecule is Beta-2-integrin.
- Hypoxia and sepsis can cause membrane guanylate cyclase to trigger the neutrophil β2-integrin.
- HBO appears to act via NO to inhibit neutrophil β2 integrin function.
- HBO does not induce vasoconstriction in post ischemic muscle.
Ischemia Reperfusion

**β₂ - Integrins**

**Adhesion**

**Tissue damage**

**Arteriolar Vasoconstriction**

ICAM

WBC

VENULE

Zamboni. Post Ischaemic Leucocyte sequestration (‘93) Plas Recon Surg; 91(6) 1112
HBOT in Ischemia Reperfusion

- ICAM
- WBC
- Arteriolar Vasoconstriction
- Tissue damage
- Adhesion
- $\beta_2$ - Integrins

Zamboni. Post Ischaemic Leucocyte sequestration (‘93) Plas Recon Surg; 91(6) 1112
Oxygen Paradox

1. Prolonged ischemia
2. Reperfusion
3. Leukocyte Recruitment
4. Endothelial Damage
5. Sludging
6. Thrombosis
7. No reflow / Necrosis
Do we use HBOT to treat “Bubbles”?
Can we use HBOT to treat Bubble Induced IRI?
HBOT Primary

- Mechanical
  - Crush the Bubble
  - Table 6 – 165fsw
- Hyperoxygenation
HBOT Secondary

- Complement formation
- Vessel sludging
- Ischemia Reperfusion Injury???
HBO Mechanisms

- Enhanced WBC Killing
- Growth Factor Stimulation
- Decreased Edema
- Tissue Hyperoxygenation
- Cellular Proliferation
- Platelet Deformability
- Neovascularization
- Antioxidant
- Prevents IRI
List of HBOT Indications

- DCS
- CO/CN
- AAI
- DFU
- Compromised Flaps & Grafts
- CRAO
- ISSH
- CRO
- Crush Injury / Compartment Syndrome
Is There a Primary (or common denominator) Mechanism of Action for HBOT?
Ischemia Reperfusion Injury
HBOT Indications (IRI ???)

- AGE, DCS, Blast Injury
- Carbon Monoxide Poisoning
- Thermal Burns
- Compromised Flaps & Grafts
- Acute Ischemia
- Compartment Syndrome
- CRAO
- ISSHL
- TBI
- Post Concussive Injury
Traumatic Brain Injury
Post Concussion Syndrome
Injury Generation from Blast Event

Pressure Deviations
Projectile / fragmentation Wounding
Injuries of Inertia and 3 axis G- Force transmission
Burns and injuries involving toxins

Compound Injury: Combinations of these types of blast injuries maybe present in the blast casualty

Silent Wounds

- No evidence of external injury
  - Emboli formation due overpressure (8.526 psi)
  - Goes untreated as the subtle signs and symptoms may not present until 36 hours
  - Primary cause of immediate death due to blast exposure

- Pronounced evidence of external injury
  - Penetrating wound requires immediate attention of physicians along the chain of evacuation
  - Evacuation dictated by severity of the wound and length of time to return to duty

- Pronounced evidence of external injury
  - "Traditional TBI brought about by inertia. Specialty treatment required"
  - Torsion and shear
  - Evacuation dictated by severity of the wound and length of time to return to duty

- Pronounced evidence of external injury
  - Evacuation dictated by severity of the burn body surface area, or degree of toxic exposure and length of time to return to duty
Ultrastructural and Functional Characteristics of Blast Injury-Induced Neurotrauma

Ibolja Cernak, MD, PhD, Zhengguo Wang, MD, PhD, Jianxin Jiang, MD, PhD, Xiuwu Bian, MD, PhD, and Jovan Savic, MD, PhD

Objective: The present study investigates whether whole-body or local (chest) exposure to blast overpressure can induce ultrastructural, biochemical, and cognitive impairments in the brain.

Methods: Male Wistar rats were trained for an active avoidance task for 6 days. On day 6, rats that had acquired the avoidance response were subjected to whole-body blast injury (WBBI), generated by large-scale shock tube (n = 40); or local (chest) blast injury (LBI), induced by blast overpressure focused on the right middle thoracic region and generated by small-scale shock tube (n = 40) while the heads of animals were protected. At the completion of cognitive testing, rats were killed at 3 hours, 24 hours, and 5 days after injury. Ultrastructural changes in the hippocampus were analyzed electron microscopically. Parameters of oxidative stress (malondialdehyde and superoxide anion generation) and antioxidant enzyme defense (superoxide dismutase and glutathione peroxidase activity) were measured in the hippocampus to assess biochemical changes in the brain after blast.

Results: Ultrastructural findings in animals subjected to WBBI or LBI demonstrated swellings of neurons, glial reaction, and myelin debris in the hippocampus. All rats revealed significant deficits in performance of the active avoidance task 3 hours after injury, but deficits persisted up to day 5 after injury only in rats subjected to WBBI. Oxidative stress development and altered antioxidant enzyme defense was observed in animals in both groups. Cognitive impairment and biochemical changes in the hippocampus were significantly correlated with blast injury severity in both WBBI and LBI groups.

Conclusion: These results confirm that exposure to blast overpressure induces ultrastructural and biochemical impairments in the brain hippocampus, with associated development of cognitive deficits.

Blast Injury – Overpressurization

- Cernak and colleagues exposed rats and rabbits to primary blast, delivering the blast via a "shock tube"
- The animals were constrained to prevent secondary-tertiary injuries.
- Histological examination of the hippocampus and brainstem indicated that the blast exposure resulted in histological changes in the central nervous system (CNS), indicating neural injury.
  - expansion of perineuronal spaces
  - cytoplasmic vacuoles
  - changes in myelin structure
  - axoplasmic shrinkage
- Changes are consistent with diffuse axonal damage
- Noted impaired performance on an active avoidance task and biochemical changes indicative of oxidative stress

Fig. 3. Electron micrograph of the hippocampus, taken from rats 24 hours (A and B) and 5 days (C and D) after local (chest) blast exposure. (A) Formation of vacuoles. Original magnification, ×40,000; bar = 200 nm. (B) Formation of vacuoles. Original magnification, ×60,000; bar = 100 nm. (C) Formation of vacuoles. Original magnification, ×15,000; bar = 200 nm. (D) Swelling and thickening of basal membrane, increase of micropinocytotic vesicles, and cytoplasmic extrusion of the endothelial cell. Original magnification, ×30,000; bar = 200 nm.
Fig. 4. Effects of whole-body (WBBI) and local/chest (LBI) blast exposure on the performance of active-avoidance response (AAR) expressed as a percentage of total 20 trials. Mean ± SD are plotted over 5 days from the time of injury. Statistically significant differences (p < 0.001) in AAR performance were observed between injured and control animals. Statistically significant (p < 0.05) difference between WBBI and LBI animals.

−0.78, p < 0.01; Fig. 5A) and local (chest) exposure (r = −0.79, p < 0.01; Fig. 5B).

Superoxide Anion Radical Generation in Hippocampus

Whole-body blast exposure caused statistically significant increase in \( O_2^- \) generation 3 hours and 24 hours after injury (222.272 ± 6.733 μmol/min/mg protein, p < 0.001, and 148.750 ± 5.833 μmol/min/mg protein, p < 0.01, respectively) compared with the control (44.628 ± 2.483 μmol/min/mg protein) values (Fig. 6). Similarly, local blast injury induced prompt increase in \( O_2^- \) generation at 3 hours after trauma (147.873 ± 1.790 μmol/min/mg protein, p < 0.001) followed by a significantly increased value of 123.905 ± 1.426 μmol/min/mg protein (p < 0.01) 24 hours after injury (Fig. 6). However, the peak in the \( O_2^- \) generation measured in the WBBI group was more prominent compared with the values in the LBI group at 3 hours after trauma (p < 0.05). At day 5 after trauma, the levels of \( O_2^- \) generation were near normal values in both WBBI and LBI groups.

A statistically significant positive linear relationship was found between the SSS/SIS showing blast injury severity and the superoxide anion generation in the hippocampus after both whole-body (r = 0.75, p < 0.01) and local blast exposure (r = 0.75, p < 0.01) during the observed period.

Malondialdehyde Concentration in Hippocampus

Changes in MDA concentrations measured by the methods according to Placer et al. and Andreuva et al. showed comparable trends. Therefore, in this study, only results obtained with the last method will be demonstrated. In Figure 7.
Concussion

- Hemorrhage
  - subcortical contusions and lacerations
  - intracranial bleedings
    - subarachnoid hemorrhage and subdural hematoma

- Diffuse Axonal Injury (DAI)

- Chemical Biomarkers (ROS)
Concussions could threaten Sam Shields' career

Ryan Wood, USA TODAY NETWORK-Wisconsin 6:08 p.m. CT Sept. 16, 2016

GREEN BAY - It's more than the sheer volume of Green Bay Packers cornerback Sam Shields' concussion history that could threaten his career.

Shields wasn't "blown up" in a high-speed, open-field collision Sunday. His helmet didn't slam against the field. There was no blindside hit. No unexpected whiplash.

The fifth documented concussion of Shields' football career came on a hard but routine tackle. His head collided against Jacksonville running back T.J. Yeldon's shoulder, a softer blow than his helmet.

There is no "magic number" for football players to know when it's time to walk away, Dr. Vernon Williams of the Kerlan-Jobe Center for Sports Neurology told USA TODAY NETWORK-Wisconsin. More troubling, Williams said, is a pattern that develops with each subsequent brain injury.

"If we see a pattern where each concussion takes longer to get better," Williams said, "the symptoms are more severe, or if we see a pattern where a person is more and more easily concussed. Like, if the first time it takes a big blow, an 80-G impact, a
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