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

Jeffrey A. Niezgoda, MD
FACHM, MAPWCA, CHWS

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Jeffrey A. Niezgoda, MD, FACHM, MAPWCA
Medical Director 1997 - 2013
Center for Comprehensive Wound Care and Hyperbaric Oxygen Therapy
Aurora Health Care
Milwaukee, Wisconsin
Advancing the Zenith of Healthcare
Regenerative Medicine / Wound Care
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?
Oxygen Free Radical

- 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

$$\cdot \ddot{\text{O}}:\text{H}^-$$  Hydroxyl Radical

$$\ddot{\text{O}}:\ddot{\text{O}}$$  Oxygen

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$$
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)
ROS Pathophysiology

- Once formed oxygen free radicals seek out electrons to form a stable molecule

\[
\begin{align*}
    &\text{Fatty Acid Chain} \\
    &\text{Hydroxyl Radical}
\end{align*}
\]
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
Ionizing Radiation
Endogenous Sources of Free Radicals

Endogenous

- 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)
The electron transport chain occurs in the inner membrane of the mitochondrion (membranes of cristae).

**PROCESS: ELECTRON TRANSPORT CHAIN**

- **Complex I**: NADH dehydrogenase complex
- **Complex II**: Cytochrome b–c₁ complex
- **Complex III**: Cytochrome oxidase complex
- **Complex IV**: ATP synthase

The diagram illustrates the movement of electrons from NADH and FADH₂ through the complexes, with the release of protons (H⁺) and oxygen (O₂) to form water (H₂O). The electron transport chain is essential for generating ATP in cellular respiration.
Mitochondrial ROS

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

Inflammation
Endogenous Sources of Free Radicals

Endogenous

- Cellular Metabolism
  - Glycolysis

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

[Diagram showing the stages of wound healing]

- Wounding
- Coagulation
- Inflammation
- Fibroplasia
- Fibroblasts
- Proteoglycans
- Angiogenesis
- Collagen Deposition
- Collagen Fibril Crosslinking
- Scar Maturation
- Remodeling

TIME
# Wound Healing Physiology

## Tissue Repair Phases

<table>
<thead>
<tr>
<th>Injury</th>
<th>Hematoma</th>
<th>Inflammation</th>
<th>Granulation</th>
<th>Remodeling</th>
<th>Tissue Repair</th>
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</thead>
<tbody>
<tr>
<td>Platelet aggregation, Haemostasis,</td>
<td>Cytokines and Growth factors</td>
<td>Increased vasodilation/vasopermeability lead to increased exudate, Fibrin clots, Increased blood flow, Phagocytosis</td>
<td>Cytokines and Growth factors</td>
<td>Fibroblast/endothelial cell activation, Angiogenesis, Collagen synthesis and extracellular matrix formation, Wound contracture</td>
<td>Resorption of Type III collagen, Orientation of collagen fibers, Type I collagen formation</td>
</tr>
</tbody>
</table>
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.

Chronic Wound Physiology

Excess MMP’s Degrade Extracellular Matrix

Diagram showing protease and matrix proteins.
Excess MMP’s Inactivate Growth Factors
Endogenous

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

- Ischemic Event
- Reperfusion of Tissue

Collard & Gelman, 2001, 1134
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 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
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

Overpressure

Ambient pressure

Extreme hyperbaric exposure

Hypobaric exposure

Time
Bubble Evolution

Overpressure

Time

Extreme hyperbaric exposure

Ambient Pressure

Hypobaric exposure
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

- 4 hours Hypoxia/Hypoglycemia
- 20 hours Normoxia/Normoglycemia
- 1.5 hours HBO at 2.5 ATA
- 18.5 hours Normoxia/Normoglycemia

HUVECs

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

control  hypoxia/hypoglycemia  hypoxia/hypoglycemia HBO 2.5 ATA 1.5 h

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.

**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. L-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

ICAM

WBC

VENULE

β₂ - Integrins

Adhesion

Tissue damage

Arteriolar Vasoconstriction

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

- Tissue damage
- Arteriolar Vasoconstriction

\[ \beta_2 \text{- Integrins} \]

ICAM

WBC

VENULE

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

1. Prolonged ischemia
2. Reperfusion
3. Oxygen ($O_2$) to Oxygen Radical ($O_2^*$)
4. Leukocyte Recruitment
5. Endothelial Damage
6. Sludging
7. Thrombosis
8. No reflow / Necrosis
Do we use HBOT to treat “Bubbles”?
AGE
DCS
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 (WBI), 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 WBI 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 WBI. 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 WBI 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.

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, $\times 40,000$; bar = 200 nm. (B) Formation of vacuoles. Original magnification, $\times 60,000$; bar = 100 nm. (C) Formation of vacuoles. Original magnification, $\times 15,000$; bar = 200 nm. (D) Swelling and thickening of basal membrane, increase of micropinocytotic vesicles, and cytoplasmic extrusion of the endothelial cell. Original magnification, $\times 30,000$; 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. Means ± SD are plotted over 5 days from the time of injury. Light 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 (222.27 ± 8.73 μmol reduced NBT/mg protein, p < 0.001; and 148.79 ± 8.33 μmol reduced NBT/mg protein, p < 0.01, respectively) compared with the control (44.62 ± 2.487 μmol reduced NBT/mg protein) values (Fig. 6). Similarly, local blast injury induced prompt increase in O$_2^−$ generation at 3 hours after trauma (147.87 ± 1.790 μmol reduced NBT/mg protein, p < 0.001) followed by a significantly increased value of 123.90 ± 1.426 μmol reduced NBT/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 PSS/TS 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 = 0.85, p < 0.001) during the observed period.

Malondialdehyde Concentration in Hippocampus

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

Fig. 5. Relationship between blast injury severity (PSS/TS) and active-avoidance response (AAR) expressed as a percentage of total 20 trials over a 5-day period after (A) whole-body (WBBI) blast exposure (r = −0.78, p < 0.01), and (B) local/ chest (LBI) blast exposure (r = −0.79, p < 0.01). Data points refer to individual animals.

alterations in MDA concentration in the hippocampus were reported in rats after WBBI or LBI at different times after injury. MDA was significantly increased at 3 hours after trauma (1.445 ± 0.099 μmol/mg protein, p < 0.01 after WBBI; 1.238 ± 0.010 μmol/mg protein, p < 0.05 after LBI) compared with control values (1.047 ± 0.014 μmol/mg protein), which was followed by its maximal concentration recorded 24 hours after both types of blast injury (1.632 ± 0.023 μmol/mg protein, p < 0.01 after WBBI; and 1.679 ± 0.019 μmol/mg protein, p < 0.001 after LBI). In both the WBBI and LBI groups, MDA concentration was near control values 5 days after injury.

Superoxide Dismutase Activity in Hippocampus

Figure 8A reports total SOD activity in the hippocampus of animals subjected to whole-body or local (chest) blast exposure. In comparison with the controls (7.829 ± 0.197 U/mg protein), total SOD activity was significantly increased in both the WBBI and LBI groups, persisting for the entire 5-day study period. In the WBBI group, total SOD activity significantly (r = 0.69, p < 0.01) correlated with PSS/TS representing blast injury severity. Significant (r = 0.66, p <
Concussion

- Hemorrhage
  - subcortical contusions and lacerations
  - intracranial bleedings
    - subarachnoid hemorrhage and subdural hematoma
- Diffuse Axonal Injury (DAI)
- Chemical Biomarkers (ROS)
SAM SHIELDS INJURY: UPDATES ON PACKERS CB'S CONCUSSION AND RETURN
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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