Oxidative Stress Injuries and the Role of Antioxidants in Newborns

Terry S. Johnson, NNP-BC, MN
Neonatal Nurse Practitioner
Founder
Lodestar Enterprises, Inc., Downer’s Grove, IL

The speaker has signed a disclosure form and indicated she has no significant financial interest or relationship with companies or the manufacturer(s) of any commercial product/service that will be discussed as part of this presentation.

Session Summary

Oxidative stress injuries are a risk factor in neonates. The speaker will discuss how oxygen radicals cause injuries in preterm and term neonates and look at the role of antioxidants. Recommended upper/lower pulse oximeter ranges for preterm infants will also be discussed.

Session Objectives

Upon completion of this presentation, the participant will be able to:

- identify the differences between intraterine and extrauterine oxygenation ranges;
- describe the concept of oxidative stress injuries as a risk factor in newborns;
- describe how oxygen radicals cause injuries in term and preterm infants;
- discuss the benefit of antioxidants in the term and preterm infant;
- identify the recommended upper/lower ranges for POX monitoring on premature infants.

References

References can be found throughout the presentation and the attached outline.

Session Outline

See handout on the following pages.
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Disclosure Statement

• Terry S. Johnson, APN, NNP-BC, MN–I n addition to any clinical practice, education and consulting services I provide
  • I am currently on the speaker’s bureau and/or consult with
    Prolacta Bioscience, Abbott Nutrition Health Institute and the National March of Dimes
  • I receive financial reimbursement for those services
  • Images & photographs used in this presentation
    • Come from publicly accessed sources
  • I will make no recommendations for any off label use of any drug or medical device
  • I am honored to be here with you today

Oxidative Stress

Four billion years ago
  • Earth’s atmosphere contained approximately one part per million of oxygen
  • Cells evolved in an oxygen-free environment
  • Cells generated energy via oxygen-independent pathways
  • Atmospheric oxygen levels rose slowly over millions of years and
  • Cells acquired mitochondria from relationships with proteobacteria
  • Cells developed the process of oxidative phosphorylation


Developmental Aspects

  • Optimum oxygen concentration for mammalian embryonic development is ~ 3-5%
    • Follicular fluid aspirate oxygen tensions varies from <1 to 5.5%
  • First 10-12 weeks of pregnancy transpires without significant maternal blood flow to fetus
  • Placental development
    • ~50% of combined maternal-fetal blood flow does not supply the fetal membranes but is shunted
    • Limits release of oxygen to the fetus


  • Developmental Aspects
    • Fetal circulation
      • Provides developing tissues with hypoxic levels of oxygen
        • Fetal arterial & venous pO2 values rarely > 30 mm Hg
        • Saturation ~65% in UV
    • Hemoglobin development
      • By 30-32 weeks, 90-95% of the infant’s hemoglobin is fetal
      • Fetal hemoglobin has a greater affinity for oxygen than adult hemoglobin
      • Limiting availability of oxygen release to fetal cells

Developmental Aspects

“Making the transition from intrauterine to extra-uterine life is probably the single most dangerous event that most of us will ever encounter in our lifetimes. Our bodies are required to make more radical physiologic adjustments immediately after birth than they will ever have to do again.”

Kattwinkle, Neonatal Resuscitation Program
Oxidative Stress

• Transitional Physiology
  – From placenta to pulmonary based oxygenation
    • First breath dynamics
    • Established respiratory drive
    • Alveolar ventilation
    • Adequate gas exchange
    • ↑ Oxygen content/saturation


Virtually all forms of organ injury start with molecular or structural alteration in cells.

Today there will be approximately 1,400 babies born prematurely in the US at risk for Oxidative Stress Injuries (OSI).

Oxidative Stress Injury

• Classic Definition of Oxidative Stress Injury
  – Physiological stress on cells (and various cellular components), tissues, and organs that is caused by the cumulative damage done by free radicals inadequately neutralized by antioxidants
  – Inadequate or insufficient antioxidant defenses
  – Increased generation of ROS
    • Hyperoxia
    • Reperefusion
    • Inflammation

Jain et al. J Am Coll Nutr 199615:44-48

• Aerobic Organisms
  – Have an oxygen based metabolism
  – Cells require uninterrupted delivery of molecular O2 and a substrate
  – In a process known as cellular respiration, or oxidative phosphorylation, oxygen oxidizes substrates (glucose, fats & protein) into ATP
  – Energy is produced
  – CO2 & water are the metabolic by-products

• Oxidative Stress
  – However, due to the abrupt change to a hyperoxic extrauterine environment
  – pO2 levels are suddenly five times higher than intrauterine values
  – Elevated levels of vitamin E oxidation product in infants cord blood is > than found in maternal serum
  – Normal, full term, healthy infants - even without resuscitation - demonstrate metabolic evidence of oxidative stress


Oxidative Stress

• Radical Oxygen Species (ROS)
  • Produced as a consequence of this oxidative activity
  • Highly reactive atoms or molecules
  • Unstable, unpaired electron in the molecule’s outer orbit
  • Combines sequentially with other non-radicals
  • Initiates & perpetuates cellular chain reactions, induces cellular injury, and promotes inflammation

Targeted Pre-Ductal SPO2 After Birth

1 min 60-65%
2 min 65-70%
3 min 70-75%
4 min 75-80%
5 min 80-85%
10 min 85-95%

Oxidative Stress

- Radical or Reactive Oxygen Species (ROS)
  - Produced during the reduction of oxygen to water
  - ROS’s (Reactive Oxygen Species)
    - Superoxide anion (O$_2^\cdot$)
    - Singlet oxygen (1O$_2$)
    - Hydrogen peroxide (H$_2$O$_2$)
    - Hydroxyl radical (OH$^\cdot$)
  - RNS (Reactive Nitrogen Species)
    - Peroxynitrite (ONOO$^-$)

ROS’s
- Play a role in normal growth and development
  - Fertilization, developing embryos
  - Aerobic metabolism, fetal growth
  - Granulocyte function
- ROS’s
  - Activate complex array of physiologic processes
    - Amplified inflammatory response
    - Outpouring of cytokines, chemokines
    - Increased reactivity of endothelium
    - Promotion of a pro-coagulation state
    - Altered nitric oxide synthesis
    - Stimulates necrotic & apoptotic cell death mechanisms

Oxidative Stress Injury
- Role of Inflammation and Preterm Birth
  - Microbial Invasion of the Amniotic Cavity (MIAC)
    - Most MIAC of these infections are subclinical in nature and cannot be detected without amniotic fluid analysis
    - Frequency of MIAC depends upon
      - Clinical presentation and gestational age
      - Preterm labor with intact membranes, the rate of positive amniotic fluid cultures is 12.8%
      - Preterm labor with intact membranes and deliver a preterm neonate, the frequency is 22%
      - Preterm premature rupture of membranes (PROM), the rate of positive amniotic fluid cultures at admission is 32.4%
      - Frequency of MIAC in women with cervical insufficiency ~41%

- Meta-analysis
  - 17 primary studies comprising 6,270 mother-infant pairs
  - Spontaneous preterm birth was strongly associated
    - Increased levels of interleukin-6 (IL-6) in midtrimester cervicovaginal fluid (OR 3.05, 95% CI 2.00-4.67) (NNT=7 for identifying an additional preterm delivery) and amniotic fluid (OR 4.52, 95% CI 2.67-7.65) (NNT=7)
    - Increased C-reactive protein (CRP) levels in midtrimester amniotic fluid (OR 7.85, 95% CI 3.88-15.87) (NNT=3)
  - Suggests that inflammation at the maternal-fetal interface, rather than systemic inflammation, may play a major role in the etiology of such spontaneous preterm births

Induction of Antioxidant Enzymes (AOE)
- Parallels the maturational pattern of pulmonary surfactant
  - Increases 150% in the last 15% of pregnancy
  - Supports ventilation
  - Surface tension
  - Antioxidant Protection
  - SOD – 3 types
  - Catalase
  - Glutathione peroxidase

ROS’s and Antioxidants
- Superoxide dismutase
- Uric acid
- Vitamin C and E
- B-Carotene
- Catalase
- Glutathione
- Glutathione peroxidase
- Glutathione transferase
• Preterm Birth Exposures
  - Supraphysiologic oxygen
    - Resuscitation, RDS, ventilation
  - Inflammation & infection
    - PARS, radical oxygen species
  - Ischemia & reperfusion
    - Hypotension, hypoperfusion
  - Parenteral solutions
    - Exposed to light
  - Free circulating metals
    - Iron, transfusions
  - Limited available antioxidants

All have been implicated in the origin of certain neonatal diseases

• Oxygen Radical Disease of Neonatology

  • Increased ROS Production
  • Deficient Antioxidant Defenses

  Inflammation
  Ischemia
  Reperfusion

  Cell/Tissue/Organ Injury

  - Retinopathy of Prematurity
  - Chronic Lung Disease/BPD
  - Periventricular Leukomalacia
  - Intraventricular Hemorrhage
  - Necrotizing Enterocolitis
  - Patent Ductus Arteriosus
  - Cerebral Palsy

Trindade & Rugolo. NeoReviews. 2007;8:e522-e632

• Premature Infants

  Insufficient Antioxidant Defense System

  Increased Production of ROS’s

• Management of Oxidative Stress

  Strengthen the infant’s Antioxidant Capacity (AC)

  Avoid Hyperoxia
  Target Oxygen Saturation
  Modulate Inflammation

Antioxidants
  Human Milk & Colostrum
  Carotenoids

• Antioxidants
  - Enzymatic – synthesized endogenously
  - Non-enzymatic – vitamins/carotenoids provided in diet
  - Metal Binding or Complexing Proteins

Antioxidants in Human Milk
  - Breast Milk
    - Neutralizes oxidative stress
    - “Traps” neutrophil/inflammation-generated ROS’s
  - Antioxidants in colostrum
    - Vitamin A (3x mature milk)
    - Vitamin E (2-3x mature milk)
    - Carotenoids (10x mature milk)
      - β-carotene
      - Lycopene
      - Lutein
• Antioxidant activity in fresh human milk
    • 8 samples of human milk
      – From others who delivered at term (38.8 weeks)
    • 8 samples of human milk
      – From mothers who delivered preterm (27.4 weeks)
    • Milk collected within 24 hours of birth
      – Fresh samples immediately tested
      – Remainder stored, later analyzed at 48 hours and 7 days
      – 5 formula samples also tested

• Results
  • No difference in antioxidant activity in milk from mothers who delivered prematurely
  • Antioxidant activity at both refrigeration & freezing temperatures were reduced
    – Freezer > refrigerator; 7 days > 48 hours
  • Significantly lower antioxidant activity in formula than fresh human milk
  • Antioxidant capacity of the formulas was similar

• “Critical Dosage”
  – For the use of Human Milk
    • Definition of “human milk fed”
    • Calculating percentage of human milk feeding vs. formula-based
    • Quality improvement strategies
    • Encouraging mother to provide her milk for her baby
    • Lactation support & use of lactation technologies
    • Prioritizing maintenance of maternal milk volume


• A Pilot Study to Determine the Safety and Feasibility of Oropharyngeal Administration of Own Mother’s Colostrum to Extremely Low-Birth-Weight Infants.
  – Purpose:
    • To determine the safety of oropharyngeal administration of own mother’s colostrum to ELBW infants in first days of life
  – Subjects:
    • 5 ELBW infants
    • Mean BW and GA (657 g and 25.5 weeks respectively)
  – Design:
    • Quasi-experimental, 1 group, pretest-posttest design


• Methods:
  • Subjects received 0.2 ml of own mother’s colostrum administered oropharyngeally every 2 hours for 48 consecutive hours
  • Beginning at 48 hours of life
  • Concentrations of secretory immunoglobulin A and lactoferrin were measured in tracheal aspirates and urine of subjects at completion of the intervention and again 2 weeks later

• Results:
  • All infants completed the entire protocol, each receiving 24 treatments
  • A total of 15 urine specimens were collected and 14 were sufficient in volume for analysis
  • A total of 15 tracheal aspirates were collected, but only 7 (47%) were sufficient in volume for analysis
  • There was a wide variation in concentrations of secretory immunoglobulin A and lactoferrin in both urine and tracheal aspirates
Milk as Medicine

• A Pilot Study to Determine the Safety and Feasibility of Oropharyngeal Administration of Own Mother’s Colostrum to Extremely Low-Birth-Weight Infants.

  – Results:
    • All infants began to suck on the ET tube during the administration of colostrum drops
    • Oxygen saturation measures remained stable or increased slightly during each treatment
    • No episodes of apnea, bradycardia, hypotension or other adverse effects associated with the administration

  – Conclusion:
    • Administration of mother’s own colostrum is easy, inexpensive and well-tolerated


Antioxidants

• Antioxidants in Premature Infants
  – Preterm delivery → ↑ed overall burden of OS
  • Minimal uteroplacental transfer of antioxidants

• Colostrum composition in premature birth
  • Inverse relationship between duration of pregnancy and the concentration of factors
  • Least mature infants - most protective colostrum

  – Colostrum as the transition from amniotic fluid


• Lutein and Zeaxanthin in Human Milk
  – Levels vary significantly by maternal diet
  – 9-country survey on the carotenoid composition among 471 women

  • Breast milk lutein + zeaxanthin was present at levels of 25 ± 19 mcg/l
  • Individual country means varied from a low of 15 ± 5 mcg/l in the U.S. to a high of 44 ± 18 mcg/l in Japan
  • Highest individual lutein concentration measured was 232 mcg/l in China and the lowest was 3 mcg/l in the U.K.


Antioxidants in Colostrum and Mature Milk

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<tr>
<th></th>
<th>Colostrum</th>
<th>Mature Milk</th>
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<tbody>
<tr>
<td>2 ± 1 days (n = 150)</td>
<td>7 ± 3 days (n = 150)</td>
<td>30 ± 3 days (n = 102)</td>
</tr>
<tr>
<td>Total Antioxidant Capacity (μmol/L)</td>
<td>594.6 ± 566.6</td>
<td>815.3 ± 371.4*</td>
</tr>
<tr>
<td>DPPH Radical Scavenging Activity (% in μmol/L)</td>
<td>50.4 ± 19.7</td>
<td>40.8 ± 20.0*</td>
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</tbody>
</table>

Values are presented as Mean ± SD and * indicate significant difference in comparison with colostrums (p<0.05)

Antioxidants in Colostrum and Mature Milk

• Carotenoids
  – Naturally occurring organic pigments (biochromes)
  – Synthesized by bacteria, fungi, green plants

  • Breast milk carotenoids were present at levels of 7 ± 4 mcg/l
  • Individual country means varied from a low of 3 ± 2 mcg/l in the U.S. to a high of 18 ± 9 mcg/l in Japan
  • Highest individual carotenoid concentration measured was 232 mcg/l in China and the lowest was 3 mcg/l in the U.K.

Carotenoids

<table>
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<th>Carotenoids</th>
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<tbody>
<tr>
<td>600 Identified in Nature</td>
<td>~50 in the Human Diet</td>
</tr>
<tr>
<td>~20 Found in Serum</td>
<td>Only 2 are in the Eye</td>
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</table>
• Carotenoids
  – Carotenes – pure hydrocarbons
    • β-carotene
      – Main carotenoid in human milk
      – Precursor of vitamin A
      – Role in antioxidant status of the skin
  – Lycopene
    – Present in HM and acts as an antioxidant
    – Highest concentrations seen in colostrum
  – Xanthophylls – contain oxygen
    • Lutein and Zeaxanthin
    – Selectively accumulate in macular region of the retina
    – Role in protection from oxidative stress by absorbing blue and UV light

• Lutein
  – Lutein concentration is over 1000-fold greater in the eye than in circulating blood
  – Lutein especially concentrates in the macula, a small area of the retina responsible for central vision

The retina is the most metabolically active tissue in the body

The retina and retinal vasculature are the last eye structures to develop in the human fetus/neonate

• Lutein
  – Absorbs blue light, which has high energy and can damage the retina
  – The neonatal retina is susceptible to damage from blue light due to the relative clarity of the lens
  – Lutein protects against oxidative damage in the eye

Reduction of Inflammation in Preterm Infants Supplemented with Lutein
**Antioxidants**

• **STUDY:** Measure serum lutein concentrations among infants fed human milk or formulas with and without added lutein

  - **METHODS:** A prospective, double-masked trial was conducted in healthy term formula-fed infants (n = 26) randomized between 9 and 16 days of age to study formulas containing 20 (unfortified), 45, 120, and 225 mcg/l of lutein. A breastfeeding reference group was studied (n = 14) and milk samples were collected from their mothers. Primary outcome was serum lutein concentration at week 12.

  - **CONCLUSIONS:** Breastfed infants have higher mean serum lutein concentrations than infants who consume formula unfortified with lutein. These data suggest approximately 4 times more lutein is needed in infant formula than in human milk to achieve similar serum lutein concentrations among breastfed and formula fed infants.

**Antioxidants**

• **STUDY:** Skin and Serum Carotenoids in Preterm Infants Fed on a Formula Supplemented With Carotenoids

  - **PI:** University of Utah Dr. Gary Chan

  - **BACKGROUND:**

    - Hylander et al. (Hylander et al 2001) have reported that human milk feeding of preterm infants (<1500 g birth weight) has been associated with a lower incidence of ROP and this association was proposed to be driven by the antioxidant content of human milk. Human milk provides a variety of antioxidants to the breastfed infant, including the carotenoids, lycopene, β-carotene, zeaxanthin and lutein

  - **STUDY:**

    - The primary objectives of this study are to compare the serum and skin concentrations of beta-carotene, lutein, and lycopene in preterm infants fed preterm formulas with mixed carotenoids to serum concentrations in preterm infants fed preterm formulas with no added carotenoids and to human milk fed infants.

    - The secondary objective of this study is to evaluate the effects of dietary carotenoids on the developing eye. Stages and zones of retinopathy of prematurity (ROP), retinal function, and retinal characteristics will also be examined.

    - 70 infants to be enrolled

    - Estimated date of completion March 2012

**Terry S. Johnson, APN,NNP-BC,MN, CLEC**

Neonatal Nurse Practitioner
Founder, Lode Star Enterprises, Inc.

7709 Knottingham Lane
Downers Grove, IL  60516
Phone:  630.881.2606
Fax:   630.725.9371
Email:  lodestar@mindspring.com