Targeting GSK-3β to Prevent Experimental BPD

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Background

• Antenatal inflammation and subsequent exposure to postnatal hyperoxia in preterm infants are crucial factors in the pathogenesis of “New” BPD:
  ➢ Decreased alveolarization
  ➢ Impaired vascular development
  ➢ Increased vascular remodeling (severe BPD)

• Glycogen synthase kinase-3 beta (GSK-3β) is a key regulator of inflammatory response
Background

Infection
Oxidative stress
Mechanical stretch

GSK-3β

TDZD-8

↑ Gene expression of inflammatory mediators

Chemokines
VCAM
ICAM-1

Cytokines
IL-1, 6
TNF-α

Growth factors
TGF-β
GM-CSF

Enzymes
SOD-1,2
MMP-9
Background

• Our previous study showed that inhibition of GSK-3β prevents lung injury induced by severe hyperoxia (90% O₂) in newborn rats.

• Whether GSK-3β inhibition, attenuates lung injury in a model of BPD induced by antenatal inflammation and moderate postnatal hyperoxia is unknown.
Hypothesis

• Inhibition of GSK-3β would prevent lung injury induced by antenatal inflammation and moderate postnatal hyperoxia (70% O₂)
Objective

• To determine the effects of inhibition of GSK-3β in antenatal inflammation and moderate postnatal hyperoxia (70% O₂)-exposed newborn rats on:
  ➢ Lung inflammation
  ➢ Alveolarization
  ➢ Vascular development
  ➢ Vascular remodeling
Study Design

Pregnant rats

- **Normal saline** ip injection
  - Gestation day 19&20
  - Newborn rats
  - Postnatal day 1- RA

- **LPS (0.5mg/kg)** ip injection
  - Gestation day 19&20
  - Newborn rats
  - Postnatal day 1- **70% O₂**

- RA + PL (Control)
- RA + TDZD
- LPS+O₂ + PL
- LPS+O₂ + TDZD

- NS exposed rat pups were placed under RA and LPS exposed rats were placed under hyperoxia (**70% O₂**)

- Rat pups were randomized to receive Placebo (PL) or TDZD (5 mg/kg) by daily ip injection for 14 days
Analyses

• Daily survival and weight gain

• Lung inflammation:
  ➢ Bronchoalveolar lavage (BAL)
  ➢ Expression of inflammatory cytokines
  ➢ Expression of p-NFκB-p65 and NFκB-p65

• Alveolar development: mean linear intercept (MLI)

• Vascular development: vascular density

• Vascular remodeling: muscularization of peripheral vessels

• Data expressed as mean ± SD and analyzed by ANOVA
Inhibition of GSK-3β Improves Weight Gain

*** p<0.001 vs RA+PL
### p<0.001 vs LPS+O2+PL
n= 9-13/group
GSK-3β Inhibition Decreases Inflammatory Cell Counts in BAL

*** p<0.001 vs RA+PL
# p<0.05 vs LPS+O2+PL
n= 4-6/group
GSK-3β Inhibition Decreases Inflammatory Cell Counts in BAL

**Macrophage Count (x10^3)**

- Placebo
- TDZD

**Neutrophil Count (x10^3)**

- Placebo
- TDZD

*** p<0.001 vs RA+PL
# p<0.05 vs LPS+O2+PL
n= 4-6/ group

*** p<0.001 vs RA+PL
## p<0.005 vs LPS+O2+P
n= 4-6/ group
Inhibition of GSK-3β Decreases Expression of Inflammatory Cytokines

**IL-6 Gene Expression**

- RA
- LPS+O₂

* p<0.05 vs RA+PL
# p<0.05 vs LPS+O₂+PL
n= 5-6/group

**TNF-α Gene Expression**

- RA
- LPS+O₂

*** p<0.001 vs RA+PL
### p<0.001 vs LPS+O₂+PL
n= 5-6/group
Inhibition of GSK-3β Decreases NFκB-p65 Phosphorylation

Western Blot for p-NFκB-p65 and NFκB-p65

Expression of p-NFκB-p65

*** p<0.001 vs RA+PL
### p<0.001 vs LPS+O2+PL
n= 4 / group
GSK-3β Inhibition Improves Alveolar Development

Mean Linear Intercept

RA + Placebo               RA + TDZD
LPS+O2 + Placebo       LPS+O2 +TDZD

* p<0.05 vs RA+PL
# p<0.05 vs LPS+O2+PL
n= 5-6/ group
GSK-3β Inhibition Increases Vascular Density

RA + Placebo   RA + TDZD

vWF vWF

RA
LPS+O2

LPS+O2 +Placebo  LPS+O2 +TDZD

Vascular Density (/HPF)

*** p<0.001 vs RA+PL
### p<0.01 vs LPS+O2+
n=5/group
GSK-3β Inhibition Decreases Pulmonary Vascular Muscularization

RA + Placebo              RA + TDZD
LPS+O2 + Placebo        LPS+O2 +TDZD

Muscularized Vessels (%)

*** p<0.001 vs RA+PL
### p<0.001 vs LPS+O2+PL
n= 5/ group
GSK-3β Inhibition Decreases Fibronectin Gene Expression

** p<0.01 vs RA+PL
## p<0.01 vs LPS+O2+PL
n= 5/ group
Summary/Speculation

• Treatment with TDZD, a pharmacological inhibitor of GSK-3β in neonatal rats exposed to antenatal LPS and postnatal moderate hyperoxia (70% O₂):
  ➢ Reduced lung inflammation
  ➢ Attenuated alveolar damage
  ➢ Increased vascular development
  ➢ Decreased pulmonary vascular remodeling

• GSK-3β inhibition did not alter normal neonatal lung development

• Inhibition of GSK-3β may provide a novel strategy to prevent BPD in preterm infants
Thank you...
Questions ??
Questions

• What is your future steps or directions?
• Why Some worsening in NS+ TDZD Group?
Stages of Lung Development in Rat and Humans

- **Embryonic**
  - Day 13-14
  - Week 3.5-6

- **Pseudo-glandular**
  - Day 15-18
  - Week 6-17

- **Canalicular**
  - Day 18-20
  - Week 15-34

- **Saccular**
  - Day 19-birth
  - Week 24-36

- **Nasal**
  - Day 4-14
  - Week 36-2 years

- **Alveolar**
  - Day 14-28
  - 2-8 years

- **Proliferation**
- **Expansion**
GSK-3 Pathway

Conditions activating GSK-3β

Upregulation of proinflammatory pathways

Downregulation of antiinflammatory pathways

β-catenin

Proteosomal degradation

Reduced inhibition of NFκB-pathway

Increased proinflammatory responses

MLK3

STAT3/5

JNK-pathway

p65

CBP

CREB

CBP

p50

p65

p50

p65

IL-6

TNF-α

MCP-1

IL-10
TDZD

- Serine/threonine protein kinase glycogen synthase kinase 3 (GSK-3)
- TDZD-8 is a selective inhibitor of GSK-3
- Thiadiazolidinone derivative, non-ATP competitive inhibitor of GSK-3
- TDZD-8 has been proposed to bind to the kinase site of GSK-3β
- First described as a component of the metabolic pathway for glycogen
Role of GSK-3B Inhibition in Human

- Alzheimer’s:
  - A phase II trial of Tidegusib, a GSK-3 inhibitor was recently completed to evaluate its effect on Alzheimers “A Phase II Trial of Tidegusib (GSK-3 Inhibitor) in Alzheimer's Disease”

- Cancer
  - “Maintaining Glycogen Synthase Kinase-3 Activity Is Critical for mTOR Kinase Inhibitors to Inhibit Cancer Cell Growth”
  - Potential in cancer treatment in various organs “Glycogen Synthase Kinase-3 (GSK3) Inhibition Induces Prosurvival Autophagic Signals in Human Pancreatic Cancer Cells”

- Pulmonary hypertension:

- Improve Hepatic Injury
  - “GSK-38 Inhibition Attenuates CLP-Induced Liver Injury by Reducing Inflammation and Hepatic Cell Apoptosis”

- Improves cardiac injury and remodeling
  - “Cardiomyocyte-specific deletion of Gsk3α mitigates post-myocardial infarction remodeling, contractile dysfunction, and heart failure”