Molecular Regulation of Cytochrome P4501A1 Induction by Hyperoxia in Human Pulmonary Cells

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Bronchopulmonary Dysplasia

Oxygen therapy

Reactive oxygen species

Hyperoxic lung injury

CYP1A1

Infection
Chorioamnionitis

Impaired alveogenesis
Deranged repair and fibrosis

Injury & Inflammation

Bronchopulmonary dysplasia (BPD)

Mechanical ventilation

Barotrauma
Volutrauma
CYP1A1 reduces hyperoxic injury

Methylchloanthrene (MC)

CYP1A1

95% Oxygen

Arylhydrocarbon Receptor pathway (AHR)

CYP1A1

ANF, AHR ko

CYP1A1

Hyperoxic injury

Mansour 1988, Moorthy 2000
CYP1A1 regulation: Arylhydrocarbon Receptor (AHR) pathway

- **Ah/O₂**
- **ROS-?**
- **AhR**
- **HSP90**
- **Inducible Pr (CYP1A1)**

**CYTOPLASM**

- **mRNA**
- **ARNT**

**NUCLEUS**

- **Ah/O₂**
- **AhR**
FICZ: (6-formylindolo[3,2-b]carbazole)

- Tryptophan derivative - UVR
- Suggested physiologic AhR ligand
- High affinity $K_d$ 0.07nM
- Feedback loop

AhR responsive element (AhRE) sequence ($5'-T/GCGTG-3'$)

Induction of AhR responsive gene products

+1 at 3' end of exon
Hypothesis:
Hyperoxia Responsive Element (HRE) in CYP1A1 promoter

Induction of CYP1A1 gene

AhRE consensus sequence (5’-T/GCGTG-3’)
Mutated sequence (5’- A/CGGTG-3’)

+1 at 3’ end of exon
pGL4 luciferase reporter plasmid

- Mutated CYP1A1 promoter (WT, 974, ...)
- Firefly luciferase gene
- pGL4 vector sequence
- Firefly luciferase
- Hyperoxia, MC, FICZ

pGL4 Renilla luciferase - null promoter - used as control
H441 cell transfection & Dual luciferase assay

H441 lung adenocarcinoma cell
(96 well microplate x 3 wells)

pGL4 Firefly luciferase
(CYP1A1 WT, Δ974, Δ1047, Δ45 ....)

pGL4 Renilla luciferase
(null internal control)

MC, FICZ, Hyperoxia,

Firefly/Renilla luciferase ratio

CYP1A1 promoter activity

Dual luciferase assay
Mutated Δ974 blocks CYP1A1 induction by MC

Fold of increase 7.7 vs 2.8, p < 0.01
FICZ induces CYP1A1 promoter in pGL4-WT
pGL-Δ974 mutation blocks induction by FICZ

(13.1 vs 6.7, p<0.01)
Mutated Δ974 blocks CYP1A1 induction by Hyperoxia

Luciferase activity

H441 cells with wild type or mutated CYP1A1 promoter vector

- pGL4-WT
- pGL4-Δ974
- pGL4-Δ1373

0.19 vs 0.22, p = 0.25

0.33 vs 0.5, p = 0.03
Electrophoretic Mobility shift assay

Mutation of -974 diminished the AHRE/AhR band in EMSA

Wildtype promoter

Mutated -974 promoter

Shifted AhRE/AhR band

Cold probe

DMSO

MC2h

MC6h

MC8h
Chromatin Immunoprecipitation assay

\[ \Delta 974 \text{ mutated CYP1A1} \]

Control RA O2 MC

Wild Type CYP1A1

inverted colors
Conclusions

- Mutated (-Δ974) blocks CYP1A1 induction by MC(AhR pathway) and hyperoxia
- Hyperoxia induces CYP1A1 by AhR pathway
- FICZ induces CYP1A1 by AhR pathway
- AhRE(-Δ974) is a likely site for the proposed HRE segment in the CYP1A1 promoter
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Future directions and implications

• Novel pharmacological agents influencing Δ974 site need to be investigated for effect on hyperoxic cell/lung injury

• Investigating Nuclear factor 1 binding site for regulatory role in delayed CYP1A1 suppression
References:


References:


Chromatin Immunoprecipitation assay

\[ \Delta 974 \text{ mutated CYP1A1} \]

O2  MC

Control  RA  O2  MC

Wild Type CYP1A1
Transcription Regulation Elements in CYP1A1 Promoter

- Consensus sequence (5'-T/GCGTG-3')
- Mutated sequence (5'- A/CGGTG-3')
Schematic depiction of pGL4 constructs

WT CYP1A1 promoter  Firefly luciferase  SV40 poly(A)

SV40 minimal promoter  Renilla luciferase  SV40 poly(A)

\textit{Vs.}

Mutated CYP1A1 promoter  Firefly luciferase  SV40 poly(A)

SV40 minimal promoter  Renilla luciferase  SV40 poly(A)
Hyperoxia induces endogenous CYP1A1 f/b suppression on sustained hyperoxia

CYP1A1 EROD assay fold of increase O2/RA 0-72 hrs
Possible sites of AhR repression of inflammation mediated transcription

1. cytoplasmic sequestration
2. nuclear sequestration
3. transrepression
4. block synergistic TF interaction or sequester co-factor
Hypothesis:

Hyperoxia Responsive Element (HRE) in promoter region CYP1A1

Induction of CYP1A1 gene

AhRE core consensus sequence (5'-T/GCGTG-3')
NF1 mutation maintains cyp1a1 response to 24-36 hr hyperoxia

![Bar chart showing luciferase activity for different conditions and time points.](chart.png)
NF1 mutation blocks late suppression of cyp1a1 on sustained hyperoxia
Conclusions B

NF1 response element mutation
- suppresses constitutive CYP1A1 expression
- does not interfere AHR pathway
- does not alter CYP1A1 induction in early hyperoxia (24-36hrs)
- Blocks CYP1A1 suppression by late hyperoxia (48-60hrs)