Ion Channel Targets of Dexamethasone and Sex Steroid Hormones in Airway Disease

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SARS-CoV-2 binds the receptor (ACE2) and enters the host cell, removing the protein shell.

Viral DNA integrates host DNA

Viral RNA reverse transcription to generate DNA

Synthesis of virus gene

Assemble process of virus RNA

Synthesis of protease

Synthesis of structural protein

Background to the clinical use of dexamethasone in treating severe COVID-19

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Dexamethasone (DEX) is a synthetic glucocorticoid (1957) used in a wide range of inflammatory diseases particularly in occlusive airway conditions such as asthma.

Recent studies report that DEX can be applied to the treatment of ARDS in COVID-19 and could reduce deaths among patients with serious (ICU) morbidity of COVID-19.

The overreaction of the immune system - ‘Cytokine Storm’ - often occurring in patients with serious COVID-19 can be suppressed by DEX.

The RECOVERY Collaborative Group: Dexamethasone in Hospitalized Patients with COVID-19
DEX in COVID-19

Covid-19 Death rates drop by 1/3 among patients on ventilators

and by 1/5 among patients receiving oxygen with no artificial ventilation

DEX treatment can shorten ICU period
Dexamethasone: Anti-inflammatory and Pro-resolution of inflammation in COVID-19

Beneficial anti-inflammatory, pro-resolution effects of Dexamethasone in COVID-19

Airway epithelial cell

Repression of Pro-inflammatory Cytokines
IL-1, IL-2, IL-6, IL-8, MCP-1, TNF, IFNγ – ‘Cytokine storm’

Expression of Anti-inflammatory Cytokines
IL-10, lipocortin-1

Expression of Pro-resolving lipids
Resolvin-D1, Lipoxin A4
Dexamethasone in COVID-19 and Pulmonary Oedema

- Do the anti-inflammatory actions of DEX account for all of its beneficial effects on COVID-19 lung pathology?

- Covid-19 mortality due to pulmonary oedema and poor oxygen transfer

- Beneficial effects of fluid restriction and pulmonary drainage (pronation) point to therapeutic effects of limiting airway secretion

- Can DEX inhibit airway secretion and airway surface liquid height?
Pulmonary oedema is still one of the main causes of mortality in Covid-19. Severe Covid-19 is associated with lung hypersecretion / fluid accumulation. There was concern at the very start of the pandemic for flash-pulmonary oedema so fluid resuscitation was kept to a minimum.

Increased extravascular lung water index (EVLWI) reflects long ICU and mortality in COVID-19 associated ARDS.

Pulmonary Oedema in COVID-19 Patients: Mechanisms and Treatment Potential

SARS-CoV-2 invasion and alveolar epithelial cells and vascular epithelial cells damage

Formation of minimal thrombus, blood circulation disorder and increased pulmonary venous pressure

Increased vascular permeability and massive fluid exudation

Increased Airway Surface Liquid height ‘flooding’ of the airways

Pulmonary edema

PFC failure and decreased AFC

Abnormal lung humoral metabolism

Hyaluronic acid

RAS

AQP

Bradykinin

Ion channels

DEX

Adapted from Cui X et al. Front. Pharmacol., 07 June 2021
DEX Targets in COVID-19 Pulmonary Oedema

Adapted from Cui X et al. Front. Pharmacol., 07 June 2021
Ion Channels

Some Basic Concepts

Airway Fluid Secretion
Airway Surface Liquid height

Salt and water

Air side
Airway Surface Liquid

Ion Channels

NaCl + H_2O
Blood side

Steroid Analogues
Dexamethasone

Steroid Hormones
Estrogen
Airway Surface Liquid – ASL
And Mucociliary Clearance

Cystic Fibrosis
Mucus plugging of the airways

Low ASL
Dyskinetic ciliary beat
Inefficient mucociliary clearance

Normal ASL
Effective ciliary beat
Efficient mucociliary clearance

Covid-19 ARDS
Pulmonary edema

High ASL
Ineffective ciliary beat
Inefficient mucociliary clearance

Air side

Blood side
Regulation of Airway Surface Liquid height
A fine balance between $\text{Na}^+$ absorption, $\text{Cl}^-$ secretion and water transport

Air side: $\text{H}_2\text{O}$, $\text{Na}^+$, ENaC, $\text{Cl}^-$

Blood side: $\text{Cl}^-$, $\text{H}_2\text{O}$, Na$^+$, ASL (8μm cilia)
Cl and K ion channels in Airway Secretion

Air side

Blood side

Airway Epithelial Cell
Generation of the optimal Airway Surface Liquid height

https://www.youtube.com/watch?v=aATTi1ONjrl
Effect of activating ENaC Na ion channels on Airway Surface Liquid height

Air side

**DEX**

ENaC

$\text{Na}^+$

Na/K pump

KATP

$\text{K}^+$

Blood side

$\text{H}_2\text{O}$

$\text{Na}^+$

CFTR

$\text{Cl}^-$

NKCC

$\text{Na}^+$ $\text{Cl}^-$ $\text{K}^+$

KCNQ1

$\text{K}^+$
Effect of activating CFTR Cl ion channels on Airway Surface Liquid height

**Blood side**

- KATP
- Na/K pump

**Air side**

- ENaC
- Na/K pump

- NKCC
- CFTR

- Na+/K+ pump

- CAMP

- H2O

- ASL
Effect of activating CACC Cl ion channels on Airway Surface Liquid height

Air side

Blood side

ENaC

KATP

Na/K pump

NKCC

KCNN4

Ca²⁺

ASL

H₂O

Na⁺

K⁺

Cl⁻
Effect of inhibition of K⁺ channels on Airway Surface Liquid height

- **Air side**:
  - ENaC
  - CFTR
  - NKCC
  - KCNQ1
  - KCNN4
  - Na⁺
  - Cl⁻

- **Blood side**:
  - K⁺
  - Na/K pump
  - KATP
  - DEX

- **H₂O**
  - ASL
Dexamethasone stimulates airway Na\(^+\) absorption and inhibits TNF inactivation of ENaC to enhance ASL absorption.

Inhibitory effect of Dexamethasone on Cl⁻ secretion via inhibition of K⁺ channels

cAMP activated secretion via CFTR and KCNQ1

Adapted from Hynes and Harvey. Steroids 151 (2019) 108459
Inhibitory effect of Dexamethasone on Cl⁻ secretion via inhibition of K⁺ channels

ATP - Calcium activated secretion via CACC and KCNN4

Adapted from Hynes and Harvey. Steroids 151 (2019) 108459
Regulation of Airway Ion Channels and Airway Surface Liquid height by Dexamethasone

Dexamethasone

ENaC

H_{2}O

Na^{+}

CFTR
cAMP

Cl^{-}

K^{+}

cAMP

KCNQ1

K^{+}

KCNN4

Ca^{2+}

Lowering of Airway Surface Liquid

NaCl + H_{2}O

ASL

Dexamethasone
Relative sensitivity of K ion channels and Cl⁻ secretion pathways to DEX inhibition

Adapted from Hynes and Harvey. Steroids 151 (2019) 108459
Cell signaling pathways for inhibitory effect of Dexamethasone on Cl⁻ secretion

Adapted from Hynes and Harvey. Steroids 151 (2019) 108459
Receptor transduction pathways for DEX inhibition of KCNN4 and KCNQ1 channels

Sexual Dimorphism

Adapted from Hynes and Harvey. Steroids 151 (2019) 108459
Therapeutic value of Dexamethasone in COVID-19

Dexamethasone has proved a useful therapy in treating severe COVID-19 ARDS and reducing mortality in ICU patients

**To combat pulmonary oedema**
- Reduce Airway secretion – inhibit K ion channels and Cl secretion
- Stimulate Airway fluid absorption – activate ENaC and Na absorption
- Reduce Airway Surface Liquid height

**To fight inflammation**
- Inhibit inflammatory response – NFκB, TNFα, IL-6, cytokines
- Pro-resolution with lipoxins and resolvins

**Problem:** Prolonged systemic use of Dexamethasone can cause severe side-effects: hypokalemia, hypotension, cardiac arrhythmia, renal failure, and weight, bone & vision loss

**Solution:** Aerosol delivery of Dexamethasone to patients in ICU with oxygen & active ventilation
Local delivery of dexamethasone directly to the lung in the form of liquid aerosol administered into the pipe of the ventilator or with oxygen supplement appears as a suitable strategy while reducing its dosage and systemic side effects.

Dexamethasone nanoparticles for aerosol delivery to the airways

Sexual Dimorphism in COVID-19

- From the very beginning of the pandemic spread of SARS-CoV-2, it became evident that more men than women suffer severe COVID-19 disease and fewer women die from the disease.

- This finding of men succumbing to more severe disease and dying, was also a feature in the two previous, smaller coronavirus diseases, Middle East Respiratory System (MERS-CoV) in 2012 and SARS-CoV in 2002.

- For SARS-CoV-2, with its global reach and high infectivity, the continued analysis of large global data sets of sex-disaggregated data has been possible, and the data are clear; women fare better with COVID-19.

Sexual Dimorphism in COVID-19

COVID-19 ICU Admissions 28 September 2021
The-COVID-19-Sex-Disaggregated-Data-Tracker-Update.pdf

- Norway
- France
- Estonia
- Greece
- Sweden
- Finland
- New Zealand
- Spain
- Northern Ireland
- England
- Wales
- Scotland
- Denmark
- Austria
- Israel
- Canada
- Colombia
- Chile
- USA

Male | Female
---|---

0% | 10% | 20% | 30% | 40% | 50% | 60% | 70% | 80% | 90% | 100%
Estrogen Targets in Sexual Dimorphism of COVID-19

- Immune response
- Inflammation
- Lung ASL
+ Cardiovascular dynamics
+ Liver metabolism
+ Pancreas Insulin
+ RAAS
+ GI defense
+ ECF volume
+ Wound healing

17β-estradiol ($E_2$)
Sexual Dimorphism in Airway Disease

Cystic Fibrosis
- Boys fare better than girls
- Estrogen reduces already low ASL
- Reduces mucociliary clearance
- Exacerbates PsA mucoidy
- Testosterone hypoallergenic?
- Estrogen increases and testosterone decreases Th2-mediated allergic airway inflammation.
- Females have increased IL-17A-mediated airway inflammation compared to males.

Asthma
- Girls fare better than boys pre-puberty
- Men fare better than women post-puberty
- Boys more hyperallergic
- Lung development differences
- Boys more hyperactive airways

COVID-19
- Women fare better than men
- Estrogen suppresses ACE2-R expression
- Reduces SARS-Cov-2 cellular invasion
- Testosterone increases ACE2-R
- Testosterone immuno-suppressant
- X-linked genes immuno-protective
- Estrogen inhibits inflammatory cytokines
- Estrogen reduces ASL
- Estrogen activates protective RAS
Molecular Mechanisms of Sexual Dimorphism in COVID-19

Estrogen effects on ACE2 expression

• Estrogen inhibition of SARS-Cov-2 cell transfection via ACE2-R
• Estrogen activation of protective arm of RAS

Estrogen and gender effects on the immune response

• Estrogen inhibition of inflammatory transcription factors (NFκB)
• Estrogen inhibition inflammatory cytokines (IL-8)
• Estrogen activation of anti-inflammatory cytokines (IL-4, IL-10)
• Estrogen increasing helper T-cells and B-cells
• X-linked immune genes (TLR7)
• Behaviour, nutrition, comorbidities

Estrogen effects on airway surface liquid volume

• Estrogen activation of ENaC ion channels – stimulation of Na ion absorption
• Estrogen inhibition of KCNQ1 – decrease in Cl ion secretion
• Estrogen inhibition of CFTR expression – decrease in Cl ion secretion
• Estrogen reduces ASL height and volume
Multi-organ pathologies – heart, kidney, liver

Hypertension

Inflammation

Men have higher ACE2-R than women
  → More ACE2-R available for viral cellular invasion

SARS-CoV-2 reduces active ACE2 in RAS
  → Activates vasoconstriction arm of RAS

Estrogen activates the protective arm of RAS

Estrogen reduces the expression of ACE2 mRNA
  → Less ACE2-R available for viral cellular invasion

SARS-CoV-2

ACE2-R

RAS
Sexual Dimorphism in Immune Response to SARS-Cov-2

• SARS-Cov-2 causes a cytokine storm in the lung - IL-6, IL-8, IL-1β, TNFα, along with infiltration of chemokines.

• This ‘Cytokine Storm’ and infiltration of monocytes and neutrophils produce lung injury and respiratory difficulties.

• High estrogen levels in females (even higher in pregnancy) help to suppress proinflammatory cytokine production by macrophages and prevent migration of monocytes and neutrophils into inflamed tissues.

• Repurposing selective estrogen receptor modulators (SERMs) as anti-viral drugs
Sexual Dimorphism in Immune Response to COVID-19

XX Immunoprotective genes

XY Proinflammatory genes

Estrogen suppresses lung inflammation

IL-8 levels are associated with in-hospital death in severe/critical COVID-19 patients
Li Hui et al. Medicine: **2021, vol 100 (11) - p e23656**
Estrogen inhibits NFkB/IL-8 inflammatory response in lung

Estrogen regulation of ion channels to reduce Airway Surface Liquid height

- Observations from Cystic Fibrosis ‘Gender Gap’. Female CF patients have more severe lung exacerbations and lower life expectancy than male CF.

- ASL height is lower and disrupted in CF lung.

- Estrogen makes this worse and lowers ASL still further.
Estrogen reduces ASL height in Normal and CF lung

Estrogen reduces airway surface liquid height – positive effect in pulmonary oedema

Mechanisms
- Estrogen activation of ENaC – stimulation of Na ion absorption
- Estrogen inhibition of KCNQ1 – decrease in Cl ion secretion
- Estrogen inhibition of CFTR expression – decrease in Cl ion secretion

O’Mahony & Harvey J Biol Chem 2007
Alzamora et al. J Physiol 2011
Rapetti-Mauss et al. J Physiol 2013
Saint-Criq & Harvey PLoS 2013
Saint-Criq & Harvey Steroids. 2014
Estrogen – Anti-Secretion and Pro-Absorption in the Lung
When Physiology informs Pathophysiology

Fluid Retention & Endometrial Expansion in the Implantation Window

Blastocyst Implantation
Endometrial expansion

Whole-body fluid retention

Anti-secretion
Pro-absorption

Elevated 17β-estradiol levels during the implantation window

Beneficial effect
In COVID-19 ARDS
Pulmonary Oedema

O’Mahony and Harvey. J Physiol 2009
Regulation of Airway Surface Liquid height by Dexamethasone and Estrogen.


Further Resources

• Videos
• Webinars
• Blogs
• And more!
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