Scarred for Life

Purines and Fibrosis

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Disclosure

- **GRANT SUPPORT**
  - NIH
  - Takeda
  - Gilead Pharmaceuticals
  - AstraZeneca

- **CONSULTANT**
  - Bristol-Myers Squibb
  - Novartis
  - CanFite Biopharmaceuticals
  - Endocyte
  - Protalex
  - Allos, Inc.
  - Gismo Therapeutics
  - Antares Pharmaceutical

- **EQUITY**
  - Can-fite Pharmaceuticals

- **INTELLECTUAL PROPERTY**
  - A2A adenosine receptor agonists for wound healing
  - A2A adenosine receptor antagonists for fibrosis
  - A1 adenosine receptor antagonists for osteoporosis
  - A1 and A2B adenosine receptor antagonists for fatty liver
  - A2A adenosine receptor agonists to prevent orthopedic prosthesis loosening
  - Blockade of Netrin1 to prevent orthopedic prosthesis loosening

- **BAGELS, ICE CREAM, SNACKS, ETC**
  - Too many

- **PENS**
  - Merck (Vioxx)
  - Amgen (Enbrel)
Adenosine Production

Hypoxia
Oxygen Radicals

ATP → ADP → AMP → Adenosine

Pannexin 1

ATP → ADP → AMP → Inosine

Uric Acid

ENT1

Adenosine → Inosine
Adenosine Receptors Are G Protein Coupled Receptors

- Osteoclast Function
- Increase Tregs
- Promote M1 to M2 Transitions
- Stimulate Mast Cells
- Increase Edema
- Inhibit Inflammation
- Promote Apoptosis
Adenosine-Related Drugs

- Adenosine
- Regadenoson
- Caffeine
- Theophylline
- Aminophylline

- Dipyridamole
- Ticagrelor
- Methotrexate
- Sulfasalazine
- High Dose Salicylates
- Cyclosporin, FK506
Adenosine Drugs in Trials

- Adenosine A2B Receptor Antagonist
  - Sickle Cell Disease (Priapism)
- Adenosine A2A Receptor Antagonist
  - Parkinson’s Disease (Istradefylline, licensed in Japan)
- Adenosine A2B Receptor Antagonist
  - Fatty Liver
- Adenosine A2A Receptor Agonist
  - Coronary Stress Testing
Adenosine A$_{2A}$ Receptor Activation Promotes Wound Healing In Vivo in Healthy Young Mice
Adenosine A_{2A} Receptor Activation Promotes Wound Healing \textit{In Vivo} in Healthy Young Mice

Control

A_{2A} Receptor Agonist

10X

40X

3 Day old wounds
Adenosine $A_{2A}$ Receptor Activation Promotes Wound Healing *In Vivo*

- Increases granulation tissue formation
  - Increases angiogenesis
  - *Increases matrix production*
- Diminishes inflammation
  - Diminished oxidant production
  - Production of extracellular mediators of wound healing
The Adenosine $A_{2A}$ Receptor Agonist, CGS-21680, Increases Collagen Production by Primary Human Dermal Fibroblasts

![Graph showing the effect of CGS-21680 on collagen production with different antagonists]
The Adenosine $A_{2A}$ Receptor Agonist, CGS-21680, Increases Collagen mRNA Expression by Primary Human Dermal Fibroblasts

**Collagen I**

**Collagen III**

* $p<0.05$  ** $p<0.005$
Adenosine A2A Receptor Stimulation Promotes Matrix Production

• Stimulation of growth factors/cytokines that stimulate matrix protein production
  • Growth Factors
    • CTGF
    • TGFβ
  • Cytokines
    • IL-13
• Direct stimulation of matrix protein production
  • Signaling mechanisms?
Adenosine Receptor Activation Diminishes Nuclear Fli1, a Constitutive Repressor of CTGF Expression
Adenosine A$_{2A}$ Receptor Occupancy Stimulates CTGF Production
Adenosine Receptor Stimulation Promotes Expression of Growth Factors, Cytokines That Provoke Matrix Production

- Diminishes Fli1 expression leading to increased CTGF production
- Increases IL-13 expression
- Increase in activated TGFβ expression *in vivo*, no effect on *in vitro* expression
There Are Multiple Pathways for Stimulation of Collagen Production by Adenosine A2AR
Adenosine $A_{2A}$ Receptor Activation Promotes Wound Healing *In Vivo*

- Increases granulation tissue formation
  - Increases angiogenesis
  - Increases matrix production
- Diminishes inflammation
  - Diminished oxidant production
  - Production of extracellular mediators of wound healing

Are adenosine $A_{2A}$ receptors involved in fibrosis?
Skin from ADA\(^{-/-}\) Mice Releases Increased Adenosine
Skin from ADA\textsuperscript{-/-} Mice is Fibrotic
A Model of Adenosine-Mediated Fibrosis

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure}
\caption{Comparison of skin properties between ADA WT, ADA KO, and ADA KO + ZM groups.}
\end{figure}

Skin from ADA\(^{-/-}\) Mice Secretes More IL-13 and Active TGF\(\beta\) than Wild Type Mice and this Effect is Reversed by Adenosine Receptor Antagonism
There is More CTGF in the Skin of ADA^-/- Mice

![Images of histological sections showing CTGF staining]

- ADA WT
- ADA KO
- ADA KO + ZM

Graph showing % CTGF staining/area:
- ADA WT: 1
- ADA KO: 6
- ADA KO + ZM: 3

Statistical significance:
- p<0.01
Bleomycin-induced Dermal Fibrosis Model

PBS/Bleomycin

18 days

A2A WT / A2A KO

Control / ZM-241385

Sacrifice

Skin

Morphometric analysis

Collagen content analysis

Histological analysis
Adenosine A$_{2A}$ Receptor-Deficient Mice Are Protected from Bleomycin-Induced Dermal Fibrosis
Adenosine $A_{2A}$ Receptor Blockade Prevents Bleomycin-Induced Dermal Fibrosis
Adenosine Axis
Endogenous Adenosine Mediates Bleomycin-Induced Dermal Fibrosis

PBS

Bleomycin

C57BL/6

CD39/CD73 KO

C57BL/6

CD39/CD73 KO

Adenosine (nmol/mg tissue)

Hydroxyproline (µg/mg tissue)

Fold (mm)

PBS

BLC

PBS

BLC

PBS

BLC

**

**
Blockade of Adenosine A\textsubscript{2A} Receptors Diminishes Scar Size

Vehicle | ZM241385

Scar area (mm\textsuperscript{2})

**Bar graph showing scar area comparison:**
- **Vehicle:** 25.0
- **ZM241385:** 20.0

Significance level: ***
Adenosine A\textsubscript{2A} Receptor Blockade Alters the Quality of the Scar Tissue Matrix

Unwounded  Vehicle  ZM241385

Collagen I

Collagen 3

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Adenosine A$_{2A}$ Receptor Blockade Diminishes Fibroblast Activation and Vascularization in Scars

Unwounded  Vehicle  ZM241385

α-SMA

CD-31
Adenosine $A_{2A}$ Receptor Blockade Diminishes Fibroblast Activation and Vascularization in Scars

**Myofibroblasts**

![Graph showing the number of $\alpha$-SMA positive cells (% Unwounded) for Un, Vehicle, and ZM conditions.]

**Macrophages**

![Graph showing the number of CD-68 positive cells (% Unwounded) for Un, Vehicle, and ZM conditions.]

**Angiogenesis**

![Graph showing the number of CD-31 positive vessels (% Unwounded) for Un, Vehicle, and ZM conditions.]

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**NYU Langone Medical Center**

**HOSPITAL/INSTITUTE/CENTER**
Adenosine Receptors Play a Central Role in Hepatic Fibrosis
A$_{2A}$ Receptor-Deficient Mice are Protected Against CCl$_4$-Induced Hepatic Fibrosis

Knockout Mice

Littermate Control mice

A$_{2A}^{-/-}$ mice

A$_3^{-/-}$ mice
Adenosine Receptor Blockade Prevents CCl₄-Induced Hepatic Fibrosis

Control

DPCPX (A₁ Antagonist)

Caffeine

ZM241385 (A₂A Antagonist)
Caffeine Prevents Hepatic Fibrosis

• Multiple epidemiologic studies from US, Europe and Japan indicate that coffee drinking is associated with diminished overall mortality and, in particular, mortality from liver disease. Reduction in mortality is dose-dependent.
• Case control study from NIH indicates that caffeine consumption is associated with reduced hepatic fibrosis (Hepatology 51:201-209, 2010).
Deletion of Ecto-5’ Nucleotidase (CD73) Diminishes Adenosine Production In Response to Ethanol and Toxins

![Graph showing adenosine concentration (nM) for PBS, CCL4, Ethanol, and TAA. The y-axis represents adenosine concentration in nM, ranging from 0 to 400. The x-axis represents different treatments: PBS, CCL4, Ethanol, and TAA. The graph includes error bars and indicates statistical significance with P-values less than 0.01 for all comparisons.]
Deletion of Ecto-5’ Nucleotidase Diminishes Hepatic Fibrosis in Response to CCl₄
Adenosine Receptors Play a Central Role in Hepatic Fibrosis
AMP Analogues Are Potent Inhibitors of Viral Reverse Transcriptase

AMP

Adenosine

Tenofovir

Adefovir
The Antiviral Tenofovir Reduces Cirrhosis in Humans with HBV

Tenofovir Abrogates Bleomycin-Induced Adenosine Release in Skin

* p=0.023  
* p=0.038  
ns  
ns
Tenofovir Inhibits Bleomycin-Induced Fibrosis

Vehicle

Bleomycin

Bleomycin + Tenofovir

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Tenovir Inhibits Bleomycin-Induced Change in Collagen Architecture

Vehicle | Bleomycin | Bleomycin + Tenofovir (75mg/kg)

10x

20x

40x

Scar Index

Vehicle | Bleomycin | Bleomycin + Tenofovir

Inhibits Bleomycin-Induced Change in Collagen Architecture
Tenofovir Prevents Thioacetamide-Induced Hepatic Fibrosis

Thioacetamide

Vehicle

Tenofovir (75mg/kg)

Vehicle

Tenofovir (75mg/kg)

Fibrotic area / Hepatic slide area (%)

CTL  TENO  TAA  TENO + TAA

0  0.5  1  1.5  2  2.5  3

Fibro area

Hepatic slide area

10x

40x
Adenosine Production

ATP → ADP → AMP → Adenosine → ENT1

Pannexin 1

Tenofovir?

ATP → ADP → AMP → Adenosine → ENT1 → Inosine

Inosine → Uric Acid

Dipyridamole

Ticagrelor
Tenofovir Treatment Inhibits ATP Release From RAW264.7 Cells
Tenofovir does not inhibit ATP release in Pannexin-1 knockdown cells
Adenosine Production

ATP → ADP → AMP → Adenosine

Pannexin 1

ENT1

Tenofovir

Dipyridamole

Ticagrelor

ATP → ADP → AMP → Inosine

Uric Acid

Adenosine
Conclusion

Cellular release of adenosine, a purine nucleoside produced primarily as the breakdown product of extracellular ATP, directly and indirectly stimulates collagen production via interaction with specific $A_{2A}$ receptors. Either blockade of adenosine $A_{2A}$ receptors or inhibition of adenosine production can reduce fibrosis and may be attractive targets for new anti-fibrotic agents.
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