M1 - Immunology, Winter 2008

Fantone, J.; Pietropaolo, M. T.
Arachidonic Acid Metabolites and Inflammation

Joseph Fantone, M.D.
Host Defense 2/12  10-11:00am
**INFLAMMATORY MEDIATORS**

**PLASMA DERIVED**
- **COMPLEMENT CASCADE**
  - C3a, C5a
- **COAGULATION CASCADE**
  - Thrombin, plasmin

**CELL-DERIVED**
- **VASOACTIVE AMINES**
  - histamine, serotonin
- **OXYGEN METABOLITES**
  - hydrogen peroxide (H$_2$O$_2$)
  - superoxide anion (O$_2^-$)
  - hypochlorous acid (HOCl$^-$)
- **ARACHIDONIC ACID METABOLITES**
  - cyclooxygenase-derived
  - lipoxygenase-derived
- **CYTOKINES**
  - Interleukins
  - Chemokines
  - Interferons
  - Tumor Necrosis Factor
  - Growth Factors
Intended Learning Outcomes: To Understand The

• Primary inflammatory mediators derived from the metabolism of arachidonic acid including their primary cellular source and biological activity.

• Effects of nonsteroidal anti-inflammatory compounds on blocking the production of arachidonic acid metabolites during disease

• Mechanism of aspirin therapy and diets rich in fish containing high levels of omega 3 fatty acids as potentially important in lowering the incidence of cardiovascular disease.
YOU ARE WHAT YOU EAT
ARACHIDONIC ACID

LIPOXigenase Pathway

5-Hydroperoxyeicosatetraenoic Acid (5-HPETE)

LTA₄ (UNSTABLE)

LTB₄

LTC₄

LTD₄

LTE₄

Cyclooxygenase Pathway

PGG₂ → PGH₂

PGI₂ (UNSTABLE)

PGE₂ (UNSTABLE)

TXA₂ (UNSTABLE)

PGF₂α

5-Keto PGF₁α

COOH
Cell Membrane Phospholipids

\[ \text{PHOSPHOLIPASE A2} \]

LIPIDHYDROXYGENASE PATHWAY
HETEs [mono & di]
LEUKOTRIENE (SRS-A)

\[ \text{Arachidonic Acid} \]

\[ \text{PGG}_2 \rightarrow \text{PGH}_2 \]

\[ \text{CYCLOOXYGENASE} \]

\[ \text{PGI}_2 \text{ UNSTABLE} \]

\[ \rightarrow \text{PGE}_2 \]

\[ + \]

\[ \text{TXA}_2 \text{ UNSTABLE} \]

\[ \rightarrow \text{TXB}_2 \]

\[ \text{6-Keto PGF}_{1\alpha} \]
Leukotriene Synthesis

Arachidonic Acid

Lipoxygenase

5-HPETE

Leukotriene A (LTA)

Glutathione-S-transferase

Leukotriene B (LTB)

Leukotriene C (LTC)

Leukotriene D (LTD)
<table>
<thead>
<tr>
<th>CELL</th>
<th>PRODUCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Leukotrienes</td>
</tr>
<tr>
<td>Macrophage/Monocyte</td>
<td>Prostaglandins +</td>
</tr>
<tr>
<td></td>
<td>Leukotrienes</td>
</tr>
<tr>
<td>Platelets</td>
<td>Thromboxxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacyclin</td>
</tr>
</tbody>
</table>
ARACHIDONIC ACID

LIPOXGENASE PATHWAY

5-HYDROPEROXIDEICOSATETRAENOIC ACID (5-HPETE)

- LTA₄ (UNSTABLE)
- LTB₄
- LTC₄
- LTD₄

CYCLOOXYGENASE PATHWAY

- PGG₂ → PGH₂

- PGI₂ (UNSTABLE)

- PGE₂

- PGF₂

- 5-Keto PGF₁α

- TXA₂ (UNSTABLE)

- TXB₂
Biological Function

Cyclooxygenase-derived Products:

Prostaglandin E₂/Prostacyclin
- Immunoregulatory
  - Inhibits immune cell activation
  - Inhibits cytokine production
  - Inhibits mast cell activation
- Blocks platelet aggregation
- Increases vasodilation
- Stimulates adenylate cyclase

Thromboxane
- Causes vasoconstriction
- Induces platelet aggregation
# Biological Function

## Lipoxygenase-derived Products:

<table>
<thead>
<tr>
<th>Product</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene $B_4$</td>
<td>Neutrophil Activation</td>
</tr>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
<tr>
<td>Mast cell activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
<tr>
<td>Leukotriene C,D,E</td>
<td>Causes smooth muscle contraction</td>
</tr>
<tr>
<td>(SRS-A)</td>
<td>Increases vascular permeability</td>
</tr>
</tbody>
</table>

In Vivo Effects of Arachidonic Acid Derived Products

• Regulates Thermostatic Set Point (Fever)
• Regulates Pain (Interacts with pain receptors)
• Regulates Blood Flow
• Regulates Leukocyte Activity
Hypothalamus

Production of Fever

Viruses
Bacteria
Toxins

Arachidonic Acid
Prostaglandin E2
Temperature

Aspirin
NSAIDs

(e.g. Interleukin-1)

Phagocytic leukocytes

Activated leukocytes
Endogenous pyrogen

Shivering
Sweating
Vasomotor tone
Rheumatoid Arthritis distorts joints

Source: http://www.nih.gov/
Immunopathology of Rheumatoid Arthritis

- Complement
  - Fixation
  - Activation

- Antigen-altered IgG
  - Altered IgG

- Chemotaxis

- Phagolysosome
  - Lysosomal Enzymes
  - Collagenase
  - Neutral Proteases
  - Phospholipase

- Nerve Sensitization
  - Vasodilation

- Nonsteroidal Anti-inflammatory Agents
  - Arachidonic acid
  - Prostaglandins

- Cartilage

Source: Undetermined
Chemotactic Activity of LTB4

BY: Greg Luerman

GNU 1.2
Pharmacologic Regulation of Arachidonic Acid-Derived Products

• Modulate Phospholipase activity:
  – Suppress the release of arachidonic acid (no substrate available)
  – Blocks both COX and LO-derived products

• Modulate Cyclooxygenase Activity:
  – Blocks Cyclooxygenase-derived products
  – COX-1 and COX-2 inhibitors

• Modulate specific enzymes down-stream from COX:
  – Thromboxane synthetase inhibitors

• Modulate lipoxygenase activity:
  – Block 5-lipoxygenase enzyme
  – Small molecule receptor antagonists for cysteiny1 leukotrienes
Non- Steroidal Anti-Inflammatory Compounds

- Aspirin (acetylsalicylic acid)
- Ibuprofen (propionic acid derivatives)
- Indomethacin (indole derivatives)
- Tylenol (Acetaminophen)
- COX-2 Inhibitors (Vioxx, celebrex, Bextra)
COX-2 Inhibitors

• **CELEBREX** (Celecoxib) Pfizer-(Pharmacia)
• **BEXTRA** (Valdecoxib) Pfizer
• **VIOXX** (Rofecoxib) Merck

Osteoarthritis
Rheumatoid arthritis
Primary dysmenorrhea
Pain management
Complications!!
INHIBITS CYCLO-OXYGENASE ENZYME IRREVERSIBLY BY ACETYLATING THE ENZYME AT THE ACTIVE SITE, THUS THE PRODUCTION OF ENDOPEROXIDES AND THEIR DERIVATIVES, INCLUDING PROSTAGLANDINS, THROMBOXANES, AND PROSTACYCLINS WILL BE INHIBITED.
BOTH INHIBIT CYCLO-OXYGENASE ACTIVITY BY BINDING REVERSIBLY TO THE ACTIVE SITE OF THE ENZYME, THUS BLOCKING THE FORMATION OF PROSTAGLANDINS, THROMBOXANES, AND PROSTACYCLINS.
AN ASPIRIN A DAY

Roughly 80 million aspirin tablets are consumed daily in the USA
Of those:
72% are taken for disease prevention
28% are taken for pain
Reduce the risk of heart attack or stroke with…….

Aspirin

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THE HOMEOSTATIC BALANCE

PGI₂
ENDOTHELium

TXA₂
PLATELETS
Can Aspirin Act As An Anti-thrombogenic Agent?

- Inhibits platelet aggregation by blocking platelet-derived thromboxane production

- Blocks platelet cyclooxygenase for the life of the platelet, as no new protein synthesis occurs

- Blocks endothelial cell-derived prostacyclin

- Suppression of endothelial cell-derived prostacyclin is short lived as endothelial cells can generation new cyclooxygenase enzyme

- Platelet activity is blocked more than endothelial cell activity
COX-2 inhibitors work by blocking COX-2 enzyme which is involved in the inflammation pathway. By sparing COX-1 gastrointestinal toxicity is reduced.
lipid mediators of Inflammation

Stimulus

+ Phospholipase

Cell membrane
Phospholipids

Arachidonic acid
Arachidonic acid

Cell membrane
Phospholipids

Phospholipase

Stimulus

+ Arachidonic acid

Prostaglandins
Prostaglandin E₂
Prostacyclin PGI₂

COX-1+2

COX-1

Lipooxigenases (5-LO)

Thromboxanes
TXB₂

Leukotrienes
LTB₄
LTC₄, LTD₄
Acute inflammation: lipid mediators

Stimulus → Phospholipase → Phospholipids

Cell membrane Phospholipids → Arachidonic acid

Arachidonic acid →

COX-1+2 → Prostaglandins
- Prostaglandin E₂
- Prostacyclin PGI₂

COX-1 → Thromboxanes
- TXB₂

Lipooxigenases (5-LO) → Leukotrienes
- LTB₄
- LTC₄, LTD₄

Vasodilation, Increase vascular permeability, Control platelet aggregation, Chemotaxis, Pain, Fever
Acute inflammation: lipid mediators

An important role in vascular homeostasis

Endothelium

Prostacyclin PGI₂

Anti-thrombotic

Platelets

TXB₂

Pro-thrombotic
Acute inflammation: lipid mediators

Therapeutic targets

Endothelium

COX-2

Prostacyclin PGI₂

Anti-thrombotic

Platelets

COX-1

TXB2

Pro-thrombotic

NSAIDs inhibit both COX-1 and COX-2; COXIBs inhibit COX-2
Acute inflammation: lipid mediators

**Therapeutic targets**

- **Endothelium**
  - COX-2
  - Prostacyclin PGI$_2$
  - Anti-thrombotic

- **Platelets**
  - COX-1
  - TXB2
  - Pro-thrombotic

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* Classical NSAID, it inhibits both COX enzymes
Acute inflammation: lipid mediators

- **Therapeutic targets**
  - **Endothelium**
    - COX-2
    - Prostacyclin PGI₂
  - **Platelets**
    - COX-1
    - TXB2

- **Vioxx®**
  - Anti-thrombotic
  - Pro-thrombotic
Acute inflammation: lipid mediators

**Therapeutic targets**

- **Aspirin** inhibits COX-2 irreversibly
- **Aspirin** inhibits COX-1 irreversibly

**Endothelium**

- Prostacyclin PGI₂

**Platelets**

- TXB₂

All cells but the platelet can resynthesize the enzymes.

Anti-thrombotic

Pro-thrombotic
INFLAMMATORY MEDIATORS

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• COAGULATION CASCADE
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  superoxide anion (O$_2^-$)
  hypochlorous acid (HOCl$^-$)
• ARACHIDONIC ACID METABOLITES
  cyclooxygenase-derived
  lipoxygenase-derived
• CYTOKINES
  Interleukins
  Chemokines
  Interferons
  Tumor Necrosis Factor
  Growth Factors