M1 - Immunology, Winter 2008

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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
Cell Membrane Phospholipids

PHOSPHOLIPASE A2

LIPOXYGENASE PATHWAY

HETEs [mono & di] +

LEUKOTRIENE [SRS-A]

Arachidonic Acid

CYCLOOXYGENASE

PGG₂ → PGH₂

TXA₂ UNSTABLE

PGI₂ UNSTABLE

PGE₂ + TXB₂

PGF₂α
Leukotriene Synthesis

Arachidonic Acid → 5-HPETE → Leukotriene A (LTA) → Leukotriene B (LTB) → Leukotriene C (LTC) → Leukotriene D (LTD)

Lipoxygenase

Glutathione-S-transferase
<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

LIP OXYGENASE PATHWAY

5-HYDROPEROXYEICOSATETRAENOIC ACID
(5-HPETE)

- LTA₄ (UNSTABLE)
  - LTB₂
  - LTC₂
  - LTD₄
  - LTE₄

CYCLOOXYGENASE PATHWAY

- PGG₂ → PGH₂
- PGI₂ (UNSTABLE)
- PGE₂
- PGF₂
- 5-Keto PGF₁α

- TXA₂ (UNSTABLE)
- TXB₂
## Biological Function

### Cyclooxygenase-derived Products:

<table>
<thead>
<tr>
<th>Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin $E_2$/Prostacyclin</td>
<td>Immunoregulatory</td>
</tr>
<tr>
<td></td>
<td>• Inhibits immune cell activation</td>
</tr>
<tr>
<td></td>
<td>• Inhibits cytokine production</td>
</tr>
<tr>
<td></td>
<td>• Inhibits mast cell activation</td>
</tr>
<tr>
<td></td>
<td>Blocks platelet aggregation</td>
</tr>
<tr>
<td></td>
<td>Increases vasodilation</td>
</tr>
<tr>
<td></td>
<td>Stimulates adenylate cyclase</td>
</tr>
<tr>
<td>Thromboxxane</td>
<td>Causes vasoconstriction</td>
</tr>
<tr>
<td></td>
<td>Induces platelet aggregation</td>
</tr>
</tbody>
</table>
## Biological Function

**Lipoxygenase-derived Products:**

<table>
<thead>
<tr>
<th>Leukotriene B₄</th>
<th>Neutrophil Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
<tr>
<td>Mast cell activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leukotriene C,D,E (SRS-A)</th>
<th>Causes smooth muscle contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></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

- Arachidonic Acid
- Prostaglandin E2
- Temperature
  - Aspirin
  - NSAIDs
  - Shivering
  - Sweating
  - Vasomotor tone

Viruses
Bacteria
Toxins

Activated leukocytes
Endogenous pyrogen

Phagocytic leukocytes

(e.g. Interleukin-1)
Rheumatoid Arthritis distorts joints

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

- Complement
  - Anti-altered IgG
  - Altered IgG
- Fixation Activation
- Chemotaxis
- Lysosomal Enzymes
  - Collagenase
  - Neutral Proteases
  - Phospholipase
- Arachidonic acid
  - Prostaglandins
  - Nerve Sensitization
  - Vasodilation
- Activated oxygen
  - \( \text{O}_2, \text{H}_2\text{O}_2 \)
- Cartilage
- Subchondral bone plate

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 cysteinyl leukotrienes
Non-Steroidal Anti-Inflammatory Compounds

• Aspirin (acetysalicylic acid)
• Ibuprofen (propionic acid derivatives)
• Indomethacin (indole derivatives)
• Tylenol (Acetominophen)
• 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.
INDOMETHACIN

IBUPROFEN

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
THE HOMEOSTATIC BALANCE

PGI₂
ENDOTHELium

TXA₂
PLATELETS

BY: Gretaz
Thrombus Formation

Elastic Lamina

Injury

ADP

Thromboxane

Aggregation

Collagen

platelets

Basement Membrane

Endothelium

Organization

Plaque
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 generate 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.

**Physiologic Stimuli**

- COX-1 (constitutive)
  - \( \text{PG E}_2 \) (Renal function)
  - Thromboxane A\(_2\) (Platelet function)
  - Prostacycline (PGI\(_2\)) (Gastric Protection)

**Inflammatory Stimuli**

- COX-2 (inducible)
  - Pro-inflammatory PGs and other inflammatory mediators
  - Inflammation
lipid mediators of Inflammation

Stimulus

\[ + \]

Cell membrane
Phospholipids

Phospholipase

Arachidonic acid
Acute inflammation: lipid mediators

Stimulus

Cell membrane
Phospholipids

Arachidonic acid

Phospholipase

COX-1+2
Prostaglandins
Prostaglandin E₂
Prostacyclin PGI₂

COX-1
Thromboxanes
TXB₂

Lipooxigenases (5-LO)
Leukotrienes
LTB₄
LTC₄, LTD₄
Acute inflammation: lipid mediators

Stimulus

Phospholipase

Cell membrane
Phospholipids

Arachidonic acid

COX-1+2
- Prostaglandins
  - Prostaglandin E$_2$
  - Prostacyclin PGI$_2$

COX-1
- Thromboxanes
  - TXB$_2$

Lipooxigenases (5-LO)
- Leukotrienes
  - LTB$_4$
  - LTC$_4$, LTD$_4$

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

An important role in vascular homeostasis

- Endothelium
  - Prostacyclin PGI$_2$
  - Anti-thrombotic

- Platelets
  - TXB2
  - Pro-thrombotic

Pro-thrombotic

Anti-thrombotic
Acute inflammation: lipid mediators

Therapeutic targets

Endothelium

- Prostacyclin PGI$_2$

- COX-2

- Anti-thrombotic

Platelets

- TXB2

- COX-1

- Pro-thrombotic

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

**Therapeutic targets**

- **Endothelium**
  - COX-2
  - Prostacyclin PGI₂
  - Anti-thrombotic

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

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* Classical NSAID, it inhibits both COX enzymes

**Ibuprofen**

- Inhibits both COX-1 and COX-2 enzymes,
Acute inflammation: lipid mediators

**Therapeutic targets**

**Endothelium**
- COX-2
- Prostacyclin PGI₂
- Anti-thrombotic

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

**Vioxx®**
Aspirin inhibits COX-2 irreversibly

Prostacyclin $\text{PG}I_2$

All cells but the platelet can resynthesize the enzymes

$\text{TXB}_2$

Aspirin inhibits COX-1 irreversibly

Endothelium

Platelets

Anti-thrombotic

Pro-thrombotic

Therapeutic targets
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  cyclooxygenase-derived
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  Chemokines
  Interferons
  Tumor Necrosis Factor
  Growth Factors