M1 - Immunology, Winter 2008

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<http://hdl.handle.net/2027.42/64939>
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Arachidonic Acid Metabolites and Inflammation

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

<table>
<thead>
<tr>
<th>PLASMA DERIVED</th>
<th>CELL-DERIVED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COMPLEMENT CASCADE</strong></td>
<td><strong>VASOACTIVE AMINES</strong></td>
</tr>
<tr>
<td>C3a, C5a</td>
<td>histamine, serotonin</td>
</tr>
<tr>
<td><strong>COAGULATION CASCADE</strong></td>
<td><strong>OXYGEN METABOLITES</strong></td>
</tr>
<tr>
<td>Thrombin, plasmin</td>
<td>hydrogen peroxide (H₂O₂)</td>
</tr>
<tr>
<td></td>
<td>superoxide anion (O₂⁻)</td>
</tr>
<tr>
<td></td>
<td>hypochlorous acid (HOCl⁻)</td>
</tr>
<tr>
<td><strong>ARACHIDONIC ACID METABOLITES</strong></td>
<td><strong>CYTOKINES</strong></td>
</tr>
<tr>
<td>cyclooxygenase-derived</td>
<td>Interleukins</td>
</tr>
<tr>
<td>lipoygenase-derived</td>
<td>Chemokines</td>
</tr>
<tr>
<td></td>
<td>Interferons</td>
</tr>
<tr>
<td></td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td></td>
<td>Growth Factors</td>
</tr>
</tbody>
</table>
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 → Phospholipid

**Phospholipase A**
- Lysophospholipid + Arachidonic acid

**Phospholipase C**
- Arachidonic acid + phospholyl-R
  - Diacylglycerol
    - Diacylglyceride lipase
      - Arachidonic acid + HO-CH
        - Cyclooxygenase 1 + Lipoxygenase Products
          - Cyclooxygenase 2
Cell Membrane Phospholipids

PHOSPHOLIPASE A2

LIPOXYGENASE PATHWAY

HETEs
[mono & di]

LEUKOTRIENE
[SRS-A]

Arachidonic Acid

CYCLOOXYGENASE

PGG₂ → PGH₂

PGI₂ ↔ UNSTABLE

PGE₂

TXA₂ ↔ UNSTABLE

6-Keto PGF₁α

PGF₂α

TXB₂
Leukotriene Synthesis

1. Arachidonic Acid → 5-HPETE
2. 5-HPETE → Leukotriene A (LTA)
3. Leukotriene A (LTA) → Leukotriene B (LTB)
4. Leukotriene A (LTA) → Leukotriene C (LTC)
5. Leukotriene C (LTC) → Leukotriene D (LTD)

Enzymes involved:
- 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>Thromboxoxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacyclin</td>
</tr>
</tbody>
</table>
### Biological Function

**Cyclooxygenase-derived Products:**

<table>
<thead>
<tr>
<th>Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandin E₂/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>Thromboxoxane</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 B$_4$</th>
<th>Neutrophil Activation</th>
</tr>
</thead>
<tbody>
<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
Production of Fever

Hypothalamus

Viruses, Bacteria, Toxins → Activated leukocytes → Endogenous pyrogen → Hypothalamus

Arachidonic Acid → Prostaglandin E2 → Temperature

Aspirin, NSAIDs → Shivering, Sweating, Vasomotor tone

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

Source: http://www.nih.gov/
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 cysteinyi 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.
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

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Thrombus Formation

- Elastic Lamina
- Basement Membrane
- Endothelium
- Platelets
- Injury
- ADP
- Thromboxane
- Collagen
- Aggregation
- 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 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.

**Physiologic Stimuli** → COX-1 (constitutive) → PG E₂
(Renal function)

Thromboxane A₂
(Platelet function)

Prostacycline (PGI₂)
(Gastric Protection)

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

Inflammatory Stimuli
lipid mediators of Inflammation

Stimulus

Phospholipase

Cell membrane
Phospholipids

Arachidonic acid
**Acute inflammation: lipid mediators**

Stimulus → Phospholipase → Arachidonic acid

- COX-1+2
  - Prostaglandins
    - Prostaglandin E₂
    - Prostacyclin PGI₂
  - Thromboxanes
    - TXB₂
- COX-1
- Lipooxigenases (5-LO)
  - Leukotrienes
    - LTB₄
    - LTC₄, LTD₄

Cell membrane Phospholipids
Arachidonic acid

Cell membrane
Phospholipids

Stimulus

Phospholipase

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 vs. Platelets**

- **Prostaglandin E2 (PGE2)**
- **Prostaglandin D2 (PGD2)**
- **Prostaglandin I2 (PGI2)**
- **Thromboxane A2 (TXA2)**
- **Thromboxane B2 (TXB2)**

**COX-1** vs. **COX-2**

- COX-1: Pro-inflammatory
- COX-2: Pro-inflammatory

**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

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

- Prostanoids
  - Prostanoids
  - TXB2
  - Prostacyclin PGI2

- COX-1
- Vioxx®
- COX-2

- Therapeutic targets

- Endothelium
- Platelets

- Anti-thrombotic
- Pro-thrombotic
Prostacyclin PGI₂

Aspirin inhibits COX-2 irreversibly

Endothelium

All cells but the platelet can resynthesize the enzymes

Platelets

Aspirin inhibits COX-1 irreversibly

Prostacyclin PGI₂

TXB2

Anti-thrombotic

Pro-thrombotic

Therapeutic targets
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