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 ($H_2O_2$)
  - 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

Phospholipid

Phospholipase A

Lysophospholipid + Arachidonic acid

Phospholipase C

Arachidonic acid + phosphoryl-R

Diacylglycerol

Diacylglyceride lipase

Arachidonic acid + HO-CH

Cyclooxygenase 1 + Lipoxygenase Products

Cyclooxygenase 2
STIMULI

Cell Membrane Phospholipids

[Diagram showing the conversion of arachidonic acid through various pathways involving lipoygenase and cyclooxygenase]

Lipoxygenase Pathway
HETEs [mono & di] + Leukotriene (SRS-A)

Arachidonic Acid

PGG<sub>2</sub> → PGH<sub>2</sub>

PGI<sub>2</sub> unstable → 6-Keto PGF<sub>1α</sub>

PGE<sub>2</sub> + TXA<sub>2</sub> unstable + TXB<sub>2</sub>

PGF<sub>2α</sub>
Leukotriene Synthesis

Lipoxygenase

Arachidonic Acid

5-HPETE

Leukotriene A (LTA)

Glutathione-S-transferase

Leukotriene B (LTB)

Leukotriene C (LTC)

Leukotriene D (LTD)
## CELL DEPENDENT END-PRODUCT SPECIFICITY OF ARACHIDONIC ACID-DERIVED PRODUCTS

<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 + Leukotrienes</td>
</tr>
<tr>
<td>Platelets</td>
<td>Thromboxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacyclin</td>
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</tbody>
</table>
ARACHIDONIC ACID

LIPOXYGENASE PATHWAY

5-HYDROPEROXYEICOSATETRAENOIC ACID (5-HPETE)

- LTA₄ (UNSTABLE)
- LTB₄
- LTC₄
- LTD₄

CYCLOOXYGENASE PATHWAY

- PGG₂ → PGH₂
  - PGF₂
  - PGE₂
  - TXA₂ (UNSTABLE)
  - TXB₂
## Biological Function

### Cyclooxygenase-derived Products:

<table>
<thead>
<tr>
<th>Product</th>
<th>Effects</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:

Leukotriene $B_4$
- Neutrophil Activation
- Mast cell activation
  - degranulation
  - degranulation

Leukotriene C,D,E (SRS-A)
- Causes smooth muscle contraction
- Increases vascular permeability
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

- Shivering
- Sweating
- Vasomotor tone

(e.g. Interleukin-1)

- Aspirin
- NSAIDs

Viruses
Bacteria
Toxins

Phagocytic leukocytes

Activated leukocytes
Endogenous pyrogen
Rheumatoid Arthritis distorts joints

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

Complement

Anti-altered IgG

Altered IgG

Chemotaxis

Fixation Activation

Phagolysosome

Activated oxygen

(O₂, H₂O₂)

Lysosomal Enzymes

Collagenase Neutral Proteases Phospholipase

Nonsteroidal Anti-inflammatory Agents

Arachidonic acid Prostaglandins

Nerve Sensitization Vasodilation

Cartilage

Subchondral bone plate

Source: Undetermined
Chemotactic Activity of LTB4

PMN

Vascular endothelium

PMN

PMN

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 ACETYLATED 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
THE HOMEOSTATIC BALANCE

PGI₂ ENDOTHELIUM

TXA₂ PLATELETS
Thrombus Formation

- Elastic Lamina
- Platelets
- Basement Membrane
- Endothelium
- Injury
  - ADP
  - Thromboxane
  - Collagen
- Aggregation
  - THROMBUS
- 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) ➔ Prostaglandin E<sub>2</sub> (Renal function) ➔ Thromboxane A<sub>2</sub> (Platelet function) ➔ Prostacyclin (PGL<sub>2</sub>) (Gastric Protection) ➔ Pro-inflammatory PGs and other inflammatory mediators ➔ Inflammation

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

Stimulus

Phospholipase

Cell membrane
Phospholipids

Arachidonic acid
Acute inflammation: lipid mediators

Stimulus

- Phospholipase

Cell membrane
Phospholipids

Arachidonic acid

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

COX-1
- Thromboxanes
  - TXB₂

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

Stimulus

Phospholipase

Cell membrane

Phospholipids

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

- Prostaglaycin \( \text{PGI}_2 \)
- Thromboxane \( \text{TXB}_2 \)
- Anti-thrombotic
- Pro-thrombotic

Endothelium → Prostaglaycin \( \text{PGI}_2 \) → Anti-thrombotic

Platelets → Thromboxane \( \text{TXB}_2 \) → Pro-thrombotic
**Acute inflammation: lipid mediators**

**Therapeutic targets**

- **Endothelium**
  - Prostacyclin \( \text{PGI}_2 \)
  - COX-2

- **Platelets**
  - TXB2
  - COX-1

**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**
  - Inhibits both COX-1 and COX-2

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

Therapeutic targets

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

Platelets
- TXB2
- COX-1
- Pro-thrombotic

Vioxx®
Acute inflammation: lipid mediators

**Therapeutic targets**

**Endothelium**

- **Aspirin** inhibits COX-2 irreversibly
- Prostacyclin PGI$_2$

**Platelets**

- All cells but the platelet can resynthesize the enzymes
- Aspirin inhibits COX-1 irreversibly
- TXB2

**Anti-thrombotic**

**Pro-thrombotic**
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CELL-DERIVED

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  histamine, serotonin

• OXYGEN METABOLITES
  hydrogen peroxide (\(\text{H}_2\text{O}_2\))
  superoxide anion (\(0_2^-\))
  hypochlorous acid (\(\text{HOCl}^-\))

• ARACHIDONIC ACID METABOLITES
  cyclooxygenase-derived
  lipoxygenase-derived

• CYTOKINES
  Interleukins
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  Interferons
  Tumor Necrosis Factor
  Growth Factors