M1 - Immunology, Winter 2008

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http://hdl.handle.net/2027.42/64939
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 (H₂O₂)
  superoxide anion (O₂⁻)
  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

Phospholipase C

Lysophospholipid

+ Arachidonic acid

Diacylglycerol

Arachidonic acid + phosphoryl-R

Diacylglyceride lipase

Cyclooxygenase 1 + Lipooxygenase Products
Cyclooxygenase 2
Cell Membrane Phospholipids

PHOSPHOLIPASE A2

LIPOXGENASE PATHWAY

HETEs (mono & di)

LEUKOTRIENE (SRS-A)

Arachidonic Acid

CYCLOOXYGENASE

PGG2 → PGH2

PGI2 UNSTABLE

PGE2

TXA2 UNSTABLE

6-Keto PGF1α

PGF2α

TXB2
Leukotriene Synthesis

Arachidonic Acid → Lipoxygenase → 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>Thromboxxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacyclin</td>
</tr>
</tbody>
</table>
Biological Function

Cyclooxygenase-derived Products:

Prostaglandin E$_2$/Prostacycllin  
- 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:

Leukotriene B_4
- Neutrophil Activation
- degranulation

Mast cell activation
- 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
Production of Fever

**Hypothalamus**

- Arachidonic Acid
- Prostaglandin E2
- Temperature

**(e.g. Interleukin-1)**

- Activated leukocytes
- Endogenous pyrogen

**Phagocytic leukocytes**

- Viruses
- Bacteria
- Toxins

**Aspirin**

**NSAIDs**

- Shivering
- Sweating
- Vasomotor tone
Rheumatoid Arthritis distorts joints

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

Complement

Anti-altered IgG → Fixation Activation → Altered IgG → Chemotaxis

Phagolysosome

Lysosomal Enzymes

Collagenase Neutral Proteases Phospholipase

Cartilage

Subchondral bone plate

Activated oxygen

(O₂, H₂O₂)

Nonsteroidal Anti-inflammatory Agents

Arachidonic acid → Prostaglandins

Nerve Sensitization

Vasodilation

Source: Undetermined
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 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

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THE HOMEOSTATIC BALANCE

PGI₂
ENDOTHELium

TXA₂
PLATELETS
Thrombus Formation

- Elastic Lamina
- Basement Membrane
- Endothelium
- platelets
- 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.
lipid mediators of Inflammation

Stimulus

+ Phospholipase

Cell membrane
Phospholipids

Arachidonic acid
Acute inflammation: lipid mediators

Stimulus ➔ Phospholipase ➔ Phospholipids ➔ Arachidonic acid ➔

Cell membrane

- 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 → 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
Acute inflammation: lipid mediators

Prostacyclin PGI\(_2\)  

**Endothelium**

**Platelets**

COX-2  

Prostacyclin PGI\(_2\)  

Anti-thrombotic

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 $\text{PGI}_2$

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

**Ibuprofen**

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

Therapeutic targets

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

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

**Vioxx®**

COX-2 blockage leads to decreased Prostacyclin PGI₂, promoting anti-thrombotic effects. COX-1 remains active, leading to increased TXB2, promoting pro-thrombotic effects.
Acute inflammation: lipid mediators

**Prostacyclin PGI\(_2\)**

**TXB2**

Endothelium

Platelets

Aspirin inhibits COX-1 irreversibly

Aspirin inhibits COX-2 irreversibly

All cells but the platelet can resynthesize the enzymes

Prostacyclin PGI\(_2\) → TXB2

Anti-thrombotic → Pro-thrombotic
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  Chemokines
  Interferons
  Tumor Necrosis Factor
  Growth Factors