2008-09

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

$\downarrow$ PHOSPHOLIPASE A2

Lipoxygenase Pathway

HETEs

[mono & di]

$+$

Leukotriene

[SRS-A]

$\rightarrow$ Arachidonic Acid

$\downarrow$ Cyclooxygenase

PGG$_2$$\rightarrow$PGH$_2$

$\downarrow$

$\rightarrow$ PGI$_2$ Unstable

$\rightarrow$ PGE$_2$

$\rightarrow$ TXA$_2$ Unstable

$+$

TXB$_2$

$\rightarrow$ PGF$_2\alpha$

$\rightarrow$ 6-Keto PGF$_{1\alpha}$
Leukotriene Synthesis

Arachidonic Acid

5-HPETE

Leukotriene A (LTA)

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>Thromboxoxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacyclin</td>
</tr>
</tbody>
</table>
ARACHIDONIC ACID

LIPOXGENASE PATHWAY

5-HYDROPEROXYEICOSATETRAENOIC ACID (5-HPETE)

LTA₄ (UNSTABLE)

LTB₄

LTC₂

CYCLOOXYGENASE PATHWAY

PGH₂ → PGI₂ (UNSTABLE)

PGE₂

PGF₂α

TXA₂ (UNSTABLE)

TXB₂

6-Keto PGF₁α
### Biological Function

**Cyclooxygenase-derived Products:**

<table>
<thead>
<tr>
<th>Prostaglandin E₂/Prostacyclin</th>
<th>Immunoregulatory</th>
</tr>
</thead>
<tbody>
<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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thromboxane</th>
<th>Causes vasoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induces platelet aggregation</td>
</tr>
</tbody>
</table>
Biochemical Function

Lipoxygenase-derived Products:

- **Leukotriene B$_4$**
  - Neutrophil Activation
  - Degranulation
- **Leukotriene C,D,E**
  - Mast cell activation
  - Degranulation
- **Leukotriene C,D,E**
  - 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 $\rightarrow$ Prostaglandin E2 $\rightarrow$ Temperature

Aspirin NSAIDs

Shivering Sweating Vasomotor tone

Viruses Bacteria Toxins

Activated leukocytes $\rightarrow$ Endogenous pyrogen

Phagocytic leukocytes

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

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

- Complement
- Fixation Activation
- Lysosomal Enzymes
- Collagenase Neutral Proteases Phospholipase
- Cartilage
- Subchondral bone plate

- Activated oxygen $(O_2, H_2O_2)$
- Nonsteroidal Anti-inflammatory Agents
- Arachidonic acid Prostaglandins
- Nerve Sensitization Vasodilation

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
Thrombus Formation

Elastic Lamina

Injury

ADP

Thromboxane

Collagen

Aggregation

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.
Stimulus

Phospholipase

Cell membrane
Phospholipids

+ Arachidonic acid

lipid mediators of Inflammation
Acute inflammation: lipid mediators

Stimulus

Arachidonic acid

Cell membrane
Phospholipids

Phospholipase

+ Stimulus

Prostaglandins

COX-1+2

Prostaglandin E$_2$
Prostacyclin PGI$_2$

COX-1

Thromboxanes

TXB$_2$

Lipoxygenases (5-LO)

Leukotrienes

LTB$_4$
LTC$_4$, LTD$_4$
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

Prostaglandin E₂                 Thromboxanes                    Leukotrienes

Acute inflammation: lipid mediators

An important role in vascular homeostasis

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

**Platelets**
- TXB2
- Pro-thrombotic

Prostaglandin E1 (PGE1) and prostacyclin (PGI$_2$) are anti-thrombotic, while thromboxane A2 (TXA2) acts as a pro-thrombotic mediator.
Acute inflammation: lipid mediators

Therapeutic targets

Endothelium

COX-2

Prostacyclin PGI$_2$

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

**COX-1**

Platelets

**Ibuprofen**

Classical NSAID, it inhibits both COX enzymes

**TXB2**

pro-thrombotic

**Therapeutic targets**

Ibuprofen

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

Therapeutic targets

Endothelium

Platelets

COX-2

Vioxx®

COX-1

Prostacyclin PGI₂

TXB2

Anti-thrombotic

Pro-thrombotic

Prostacyclin PGI₂

TXB2
Prostacyclin PGI\textsubscript{2} and TXB\textsubscript{2} are key lipid mediators in acute inflammation.

- Aspirin inhibits COX-1 irreversibly.
- Aspirin inhibits COX-2 irreversibly.

All cells but the platelet can resynthesize the enzymes.

**Therapeutic targets**

- **Endothelium**
  - Prostacyclin PGI\textsubscript{2}
  - Anti-thrombotic

- **Platelets**
  - TXB\textsubscript{2}
  - Pro-thrombotic
INFLAMMATORY MEDIATORS

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  Chemokines
  Interferons
  Tumor Necrosis Factor
  Growth Factors