M1 - Immunology, Winter 2008

Fantone, J.; Pietropaolo, M. T.

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

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

Arachidonic Acid \xrightarrow{\text{Lipoxygenase}} 5-HPETE \xrightarrow{\text{Glutathione-S-transferase}}

Leukotriene A (LTA) \xrightarrow{\text{Glutathione-S-transferase}}

Leukotriene B (LTB) \xrightarrow{\text{Glutathione-S-transferase}} Leukotriene C (LTC) \rightarrow 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>Thromboxxane</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Prostacycllin</td>
</tr>
</tbody>
</table>
ARACHIDONIC ACID

LIPOXGENASE PATHWAY

5-HYDROPEROXIDEICOSATETRAENOIC ACID (5-HPETE)

\[ \text{LTA}_4 \text{ (UNSTABLE)} \]

\[ \text{LTB}_4 \]

\[ \text{LTC}_4 \]

\[ \text{LTD}_4 \]

\[ \text{LTE}_4 \]

CYCLOOXYGENASE PATHWAY

\[ \text{PGG}_2 \rightarrow \text{PGH}_2 \]

\[ \text{PGI}_2 \text{ (UNSTABLE)} \]

\[ \text{PGE}_2 \]

\[ \text{PGF}_2 \]

\[ 6\text{-Keto PGF}_1 \text{a} \]

\[ \text{TXA}_2 \text{ (UNSTABLE)} \]

\[ \text{TXB}_2 \]
### Biological Function

#### Cyclooxygenase-derived Products:

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Functions</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>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>Product</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene B&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Neutrophil Activation</td>
</tr>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
<tr>
<td></td>
<td>Mast cell activation</td>
</tr>
<tr>
<td></td>
<td>- degranulation</td>
</tr>
<tr>
<td>Leukotriene C,D,E (SRS-A)</td>
<td>Causes smooth muscle contraction</td>
</tr>
<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

(e.g. Interleukin-1)

- Aspirin
- NSAIDs

- Shivering
- Sweating
- Vasomotor tone
Rheumatoid Arthritis distorts joints

Source: http://www.nih.gov/
Chemotactic Activity of LTB4

LTB4

PMN

Vascular endothelium

PMN

PMN

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.
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

By: Chaval Btasil
http://creativecommons.org/licenses/by-sa/3.0/deed.en
THE HOMEOSTATIC BALANCE

PGI$_2$  
ENDOTHELium

TXA$_2$  
PLATELETS
Thrombus Formation

Elastic Lamina

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 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.
Stimulus

+ Phospholipase

Cell membrane
Phospholipids

Arachidonic acid

lipid mediators of Inflammation
Acute inflammation: lipid mediators

Stimulus

Cell membrane
Phospholipids

+ Phospholipase

Arachidonic acid

COX-1+2
Prostaglandins
Prostaglandin E₂
Prostacyclin PGI₂

COX-1
Thromboxanes
TXB₂

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

Stimulus

Cell membrane
Phospholipids

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 PGI$_2$

Anti-thrombotic

Platelets

TXB2

Pro-thrombotic
Acute inflammation: lipid mediators

Therapeutic targets

Endothelium

COX-2

Prostacyclin PGI₂

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 PGI₂

Anti-thrombotic

Platelets

COX-1

Ibuprofen*

TXB2

Pro-thrombotic

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

**Therapeutic targets**

- Endothelium
- Platelets

**COX-2**
- Prostacyclin PGI$_2$
- Vioxx®
- Anti-thrombotic

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

**COX-2 inhibition**

- Vioxx® targets COX-2 to inhibit prostacyclin PGI$_2$ production.
**Acute inflammation: lipid mediators**

- **Prostacyclin (PGI₂)**
- **TXB2**

**Therapeutic targets**

**Aspirin**
- Inhibits COX-2 irreversibly
- Inhibits COX-1 irreversibly

**Endothelium**

- **Prostacyclin (PGI₂)**
- All cells but the platelet can resynthesize the enzymes

**Platelets**

- **TXB2**
- **Aspirin**

**Anti-thrombotic**

**Pro-thrombotic**
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 ($0_2^-$)
  hypochlorous acid (HOCl$^-$)
• ARACHIDONIC ACID METABOLITES
  cyclooxygenase-derived
  lipoxygenase-derived
• CYTOKINES
  Interleukins
  Chemokines
  Interferons
  Tumor Necrosis Factor
  Growth Factors