CONFIDENTIAL

Multi-Functional Anti-Inflammatory Drugs (MFAIDs) for the Treatment of Inflammatory/Allergic Diseases:

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Scientific Background and Concept
Phospholipase A$_2$ (PLA$_2$) Initiates the Production of the Inflammation Lipid Mediator (ILM) Cascade.

- Phospholipid (PL) $\rightarrow$ PLA$_2$ $\rightarrow$ Arachidonic acid (AA) + LysoPL
  - Prostaglandins
  - Leukotrienes
  - Thromboxanes
  - PAF
  - LysoPA
  - LysoPC
  - LysoPS
Double Edge of Steroid Anti-inflammatory Activity
PLA$_2$ → LysoPL → Cell membrane

PL hydrolysis

PLA$_2$

Outside

Toxins
Allergens
Inflammatory Mediators

Inside

COX I
Aspirin®
Celebrex®/Vioxx®

COX II

AA

LOX
Zyflo®/Singulair®

Inflammation
Allergy

Celebrex®/Vioxx®
ILMs form an expanding tree

“Current approaches are “too little, too late”…”
Problems with prevalent approach

1. Mediators of the same process are produced by alternative pathways

<table>
<thead>
<tr>
<th>Pathology</th>
<th>AA-Derivatives</th>
<th>Lyso-PL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>COX pathways</td>
<td>LOX pathways</td>
</tr>
<tr>
<td>IBD</td>
<td>↑ TXA&lt;sub&gt;1&lt;/sub&gt;</td>
<td>↑ LTB&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Asthma</td>
<td>↑ PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>↑ CysLT LTB&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Skin inflammation</td>
<td>↑ COX-2 Derivatives</td>
<td>↑ LTB&lt;sub&gt;4&lt;/sub&gt; LTB&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>↑ TXA&lt;sub&gt;1&lt;/sub&gt;</td>
<td>↑ LTB&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
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</table>
Blocking One Pathway Diverts the AA Pool to the Other Pathway

Vioxx®, Celebrex®, Bextra®
2. Agonists (mediators) and **Antagonists** of the same process are produced by the same pathway

- **PGI2** (Anti-thrombotic) & **TXA2** (Pro-thrombotic)
- **PGE2** (airway dilator) & **PGD2** (airway constrictor)

3. The **same** eicosanoids exert **opposing** effects in different organs and tissues.

- **PGE2**: respiratory function (good) vs. other organs (bad)
3. Systemic treatment is a problem: The same eicosanoids exert opposing effects in different organs and tissues.

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Pro-inflammatory</th>
<th>Anti-inflammatory / protective</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2</td>
<td>• intestinal inflammation&lt;br&gt;• arthritis&lt;br&gt;• fever&lt;br&gt;• chronic pain&lt;br&gt;• bone-loss associated with inflammation&lt;br&gt;• sepsis&lt;br&gt;• brain injury&lt;br&gt;• IBD&lt;br&gt;• colorectal cancer</td>
<td>• smooth muscle cells relaxation&lt;br&gt;• reduction macrophages inflammatory activation&lt;br&gt;• broncho-dilation&lt;br&gt;• cytoprotective effects&lt;br&gt;• neuroprotective</td>
</tr>
<tr>
<td>PGD2</td>
<td>• pro-thrombotic&lt;br&gt;• vaso- constriction&lt;br&gt;• broncho-constriction&lt;br&gt;• lung inflammation</td>
<td>• reducing IBD in rats</td>
</tr>
</tbody>
</table>
Yedgar S, Krimsky M, Cohen Y, Flower RJ.

Treatment of inflammatory diseases by selective eicosanoid inhibition: a double-edged sword?

Target the tree-trunk

- PLA2
- LysoPL
- AA
- TXs
- LTs
- PGs
- COXs
- LOXs
- PAF
- LPA
- LPS
- LPC
PLA2 enzymes, mainly the secretory sPLA2, are involved directly and indirectly in the progression and initiation of many, diverse pathological conditions.
sPLA$_2$s

Expressed primarily, and play pivotal roles in inflammatory/allergic processes (Atherosclerosis, Asthma, R.A., CNS, IBD, Lung surfactant disorders and RDS, Sepsis, Skin inflammation, Metastasis…).

Not involved in homeostatic functions (exception: bacteriolyis in tears).

Extracellular sPLA2
Acts as a receptor ligand to Induces Cell signaling; vascular and airway contraction; proliferation of normal and cancer cells.

Synergizes with ROS and other injurious stimuli to induce tissue damage
sPLA$_2$s act primarily on the cell surface membrane.

“Cell-impermeable inhibitor, which protects the cell from extracellular sPLA$_2$, without direct interference with intra-cellular PLA$_2$ activities, is desirable”.

Synergism between H2O2 & PLA2 in cell damage: H2O2 degrades cell surface glycosaminoglycans (GAG, left) and renders the cell to lysis by sPLA2 (right); GAG Protect the Cell from Injurious Agents.

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Fig. 1. Release of ArAc by the sequential treatment of cells with H2O2 and PLA2. BGM cells, prelabelled with [3H]ArAc were washed and treated with H2O2 or GO for 1 h. The cells were then washed again and treated with Crotalus atrox PLA2 for 1 h. The release of [3H]ArAc was determined as described in section 2. * and ▲, significant at P < 0.005; ▲, significant at P < 0.01.

Fig. 3. Degradation and release of cell surface GAG by H2O2. Cultured BGM cells were labelled overnight with $^{35}$SO$_4$, 1.2 mCi per 24 well plate, then washed and treated with GO for 1 h, as in the experiment of Fig. 1. The GAG released into the culture medium were chromatographed and determined as described in section 2. ●, Cultured medium of H$_2$O$_2$-treated cells; ○, culture medium of untreated cells.
Role of GAG in cell membrane protection

Glycosaminoglycans (GAG) are macro-molecules that protect the cell membrane from a multitude of extra-cellular stimuli and agents such as:

- Free radicals (ROS)
- Exogenous PLA2
- Interleukins and other inflammatory mediators
- Allergens
- Growth factors
- Degrading / invasion promoting enzymes (heparinase, collagenase, heparanase, hyaluronidase)

GAG enrichment assists in cell protection
The Solution

Comprehensive (rather than selective) control of ILM production/action, by Cell-Impermeable PLA_2 Inhibitors

Combined with Enrichment of cell-surface GAG
Multi-Functional Anti-Inflammatory Drugs (MFAID), composed of GAG-conjugated PLA2 inhibitors, address Both Needs:

2. Enrichment of cell surface GAG
1. Inhibiting sPLA2 activity at the cell membrane

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**PLA2 inhibiting moiety**

I. Broad-range PLA2 inhibitor
II. Anchors GAG to cell surface

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**GAG-tail**

I. Enriches cell surface with protective GAG
II. Eliminates interference with homeostatic intracellular events by preventing internalization of sPLA2 inhibitor.
Broad Upstream Anti-Inflammatory Activity At Target
Protection against:

- **PLA$_2$**
- Toxins
- Allergens
- Inflammatory Mediators

**Cell membrane**

Inflammatory cascade not induced

Reduction in inflammation
Selected Publications

Inhibition of LPS-induced chemokine production in human lung microvascular endothelial cells

Control of capillary formation by membrane-anchored extracellular inhibitors of phospholipase A2

Modulation of IFNγ-induced immunogenicity (vascular endothelial cells, kidney epithelial cells, lymphocytes).

Amelioration of endotoxin-induced sepsis in rats

Amelioration of TNBS-induced colon inflammation in rats.

Suppression of central nervous system inflammation in rats and mice.

Amelioration of OVA-induced asthma in rats, by Inhalation: preventer and reliever

Ingber et al, IJIP March 2007
Treatment of Contact Dermatitis in patients by Topical Application:
A Double-Blind Placebo-Controlled Pilot Study

MMP production in human fibrosarcoma cells and their invasiveness are regulated by Group IB secretory Phospholipase A2 receptor-mediated activation of Cytosolic Phospholipase A2.

Mruwat et al., PLOS One 8: e76641, 1-9, 2013 :
Phospholipase A2 in Experimental Allergic Bronchitis: A Lesson from Mouse and Rat Models..

Mruwat et al., Am J Rhinology & Allergy 29: e122-e128, 2015
Phospholipase A2-dependent release of inflammatory cytokines by superantigen-stimulated nasal polyps of patients with chronic rhinosinusitis.

Yedgar et al., Biochim Biophys Acta 1761:1373-1382, 2006 (Review)
Control of Phospholipase A2 Activities for the Treatment of Inflammatory Conditions.

Yedgar et al., TRENDS in Pharmacological Sciences (TiPS) 28:459-464, 2007 (Review)
Treatment of inflammatory diseases by selective eicosanoid inhibition: a double-edged sword?.

Mast et al., J. Am. Coll. Cardiol. 58:1205-1214, 2011
Phospholipase A2 dependent production of hydrolytic products in human and murine vascular endothelial cells.

Yedgar et al., Crit. Care Med. 40:2239-2249, 2012
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Conclusion

For the treatment of pathological conditions involving lipid mediators, the *comprehensive (rather than selective) control of lipid mediator* production/action is desirable, i.e. inhibition of PLA\(_2\).

*sPLA\(_2\)* enzymes play a key role in the pathophysiology of lipid mediator-related diseases, by their direct action and by providing the precursors for these mediators.

*Cell-impermeable PLA\(_2\)* inhibitors are desirable for the treatment of these diseases.

*Enrichment of cell-surface GAG* is desirable for protecting cells.

*The GAG-lipid conjugates*, providing combined control of lipid mediator production and cell surface GAG enrichment, introduce a promising prototype of Multi-Functional Anti-Inflammatory Drug.
Our Technology

Multi Functional Anti-Inflammatory Drugs (MFAIDs)

- Inhibit secretory PLA2 enzymes: key players in inflammation
- Enriches the protective GAG layer at the cell surface
- Unique and superior to past industry attempts (Eli Lilly, Wyeth)
- Clinically effective
- Excellent safety profile (animal + man)
- Novel, first-in-class platform of compounds based on proprietary platform
- Synthetic + flexible formulation options
Summary of Results and Current Status:

See Separate Attachment