A tale of two enzymes: Aspirin and COX-2 inhibitors

October 1, 2004

Molecular interventions in human disease...
An approach as old as human civilization.

With whom the herbs have come together
Like kingly chiefs unto the gathering,
That Brahman is called a “healer” (bhisaj),
A demon-killer, a plague-dispeller.

From the Rg Veda (1500-900 BCE)

Datura stramonium
Vol. 2, plate 28 from the
Hortus Indicus Malabaricus
Published 1679
Source of scopolamine and atropine
Aspirin from willow bark

Written records of willow bark use in ancient Greek and Arabic medical documents
Traditional medication for pain and fever throughout much of the world

“Ethnobotany” - study of the role of plants in human societies
“Zoopharmacognosy” - self-medication in animals (best documented in primates)

Development of aspirin

1830s: Salicin purified from willow bark - too irritating for human use
1850s: Acetylsalicylic acid synthesized - analgesic, antipyretic, anti-inflammatory
1897: Felix Hoffmann synthesizes acetylsalicylic acid for his arthritic father; Bayer gets the patent
1971: John Vane finds that aspirin inhibits prostaglandin synthesis (Nobel Prize 1982)
1970s: Aspirin shown to act by acetylation of Serine 530 of cyclooxygenase (prostaglandin synthase)
1988: Physician's Health Study shows daily aspirin reduces incidence of a first heart attack by 40%
Various prostaglandins, cell types, cell responses...

Regulate smooth muscle contraction
  (lowering blood pressure, stimulating labor)
Mediators of pain and inflammation
Inhibit gastric acid secretion, protect against gastric ulcers

Many effective drugs affect signaling pathways; high level intervention may be more efficient?
COX reactions

requires heme, tyrosyl radical formed

second active site on same polypeptide

COX = cyclooxygenase
(aka prostaglandin synthase)

Aspirin mechanism

In vitro, aspirin acetylates many targets

Serine 530:
Near active site, NOT involved in catalysis
Contrast penicillin, protease inhibitors (via acyl-enzyme intermediate)

Note COVALENT attachment to enzyme, so irreversible inhibition
Important in platelets: no new gene expression (no nucleus), so one
dose of aspirin is effective for the life of the platelet
Drawback: can contribute to serious bleeding
Low-dose aspirin regimens recommended for decreasing likelihood of stroke and myocardial infarction in at-risk patients
COX structure with indomethacin

Heme

Cyclooxygenase active site

NSAID = non-steroidal anti-inflammatory drug
Ibuprofen, naproxen, sulindac, others.
NONCOVALENT, competitive enzyme inhibition

But wait, there’s more...

1991: Two different COX enzymes
   COX-1 expressed constitutively in many tissues
       Generates protective prostaglandins in the GI tract
       Stimulates platelet activation via thromboxane synthesis
   COX-2 induced at sites of injury and inflammation

2002: COX-3 (splice variant of COX-1)
   expressed in CNS, selectively inhibited by acetaminophen (works for pain and fever, no effect on inflammation)
COX-2-specific inhibitors identified by drug screening

**Rofecoxib**
(methyl sulfone)  
(Vioxx)

**Celecoxib**
(sulfonamide)  
(Celebrex)

combined sales worldwide >$4 billion per year

Work poorly in standard assay  
(rat kidney or testis extracts)  
Work great in brain extracts
News Flash

Merk & Company announced today that it was immediately pulling its arthritis and acute pain medication Vioxx from the worldwide market after data from a clinical trial showed that the drug produces an increased risk for heart attacks and strokes.

"We are taking this action because we believe it best serves the interests of patients," Merck's chairman, president and chief executive officer, Raymond V. Gilmartin, said.

"Although we believe it would have been possible to continue to market Vioxx with labeling that would incorporate these new data, given the availability of alternative therapies, and the questions raised by the data, we concluded that a voluntary withdrawal is the responsible course to take," Mr. Gilmartin said in a statement posted on the New Jersey-based company's Web site.

The recall represents a big blow for Merck, with Vioxx accounting for 10 percent of its sales. Shares plunged at the opening bell on the New York Stock Exchange to as low as $12.96, and closed only slightly higher, at $33, down 20 percent for the year. The company's market capitalization was reduced some $32 billion. Shares of Pfizer, maker of Celebrex, Vioxx's main competitor, gave up most of that gain to close at $30.69, up 42 cents, or 1.4 percent.

A Merck company spokesman, Tony Flaherty, said there were no indications that 84 million prescriptions had been written for the drug in the United States, and that 70 percent of those were for patients older than 60. The drug was available in 67 countries, with 25 percent of sales in Europe and the rest in the United States.

A role for COX(-2) inhibitors in treatment of colorectal cancer

1983: Polyps regress in patients taking NSAIDS
1991-1993: Large scale studies show prophylactic effect of NSAIDS (40-50% risk reduction) and effectiveness in patients with APC deficiency (familial adenomatous polyposis)

<table>
<thead>
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<th>NSAID Treatment</th>
<th>Outcome</th>
<th>Reference</th>
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<tr>
<td>Apa** mouse</td>
<td>Polyph multiplicity</td>
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<tr>
<td>Sulindac*</td>
<td>Polyph multiplicity</td>
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<td>Piroxicam*</td>
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<td>Celecoxib*</td>
<td>Polyph multiplicity</td>
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<tr>
<td>Apa** mouse</td>
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<td>Azoxymethane (AXM)-treated rat</td>
<td>Tumour incidence and multiplicity</td>
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<td>Celecoxib*</td>
<td>Colon carcinoma cell growth</td>
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*Non-selective NSAID
**COX-2 selective NSAID

Table 1 | Effects of NSAID treatment in animal models of colorectal cancer
Why does this work?

Paper 1:
Trial for Cox-2 inhibitor treatment of FAP patients

Maybe targets other than COX?

note: NFκB only relevant for aspirin, NOT COX-2 inhibitors

Paper 2:
Aspirin acetylates IKK

Many familiar players...