COX-2 inhibitors:
A cautionary tale

October 1, 2007

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 acetylating 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

Figure 1. An overview of eicosanoid biosynthetic pathways, as understood from the mammalian background. Major families of eicosanoids include prostaglandins, epoxeicosatrienoic acids and the many lipoxigenase products.

Eicosanoid = 20 carbons (compare icosahedron)

Other GPCRs for prostaglandins signal through calcium

Some prostaglandin receptors are nuclear hormone receptors (e.g. PPAR gamma) that directly activate gene transcription
**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

A role for COX 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>
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<th>NSAID Treatment</th>
<th>Outcome</th>
<th>Reference</th>
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<tr>
<td>Apc+/– mouse</td>
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<td>Sulindac*</td>
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<td>119</td>
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<td>Celecoxib†</td>
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<td>Azoxymethane (ACI)-treated rat</td>
<td>Tumour incidence and multiplicity</td>
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<tr>
<td>Celecoxib‡</td>
<td>Colon carcinoma cell growth</td>
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*Non-selective NSAID
†COX-2-selective NSAID
Why does this work?

Prostaglandin E2 via Wnt/beta-catenin?
Castellone et al. Science 2005 310:1504

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

combined sales worldwide >$4 billion per year
(through 2004)

Work poorly in standard assay
(rat kidney or testis extracts)
Work great in brain extracts
Change in colorectal polyposis for FAP patients on COX-2 inhibitors

Patients receiving 400 mg of Celecoxib 2x daily show improvement in all areas of colon

Patients receiving 100 mg of Celecoxib 2x daily show trend towards improvement in rectum, ascending colon, and cecum

Are these two areas the first targets of COX-2 inhibitor-mediated polyp regression? Is the mechanism different for different dosages?

Steinbach et al., 2000.


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**News Flash**

**September 30, 2004**

**Merck Pulls Vioxx Painkiller From Market, and Stock Plunges**

BY TERENCE NELAN

Merck & 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 5% of the company's revenue. The company's stock price fell by more than 25% to $33. The company's market capitalization was reduced by $50 billion.

Shares of Pfizer, maker of Celebrex, Vioxx's main competitor, gave up most of the gains to close at $20.42, up 42 cents, or 2.1%.

A Merck company spokesman, Tony Foldes, said there were 13 million prescriptions written for the drug in the United States in 2003 and 6 million for Celebrex.

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The New York Times
What happened? Why?

Why does COX-2 inhibition carry cardiovascular risk, while inhibition of both COX-1 and COX-2 does not?

Hypothesis: A COX-2 product (prostaglandin I2) is atheroprotective

Supported in female but not male mice

Egan et al., 2004, Science 306: 1964

Other possibilities: Rofecoxib-specific effects?

Increases susceptibility of LDL and membrane lipids to oxidative modification

Medical vs. policy questions

COX-2 inhibitors have a clear clinical advantage for the subset of arthritis patients likely to experience gastric bleeding

Vioxx and Celebrex were direct-marketed to all arthritis patients without clear explanation of the relevance only to patients at risk for bleeding…millions of otherwise healthy people took an expensive prescription drug instead of an OTC NSAID

Several clinical trials excluded patients with known cardiovascular risk factors; the study patients were not representative of the target population

Should the cardiovascular risk have been clear earlier than September 2004?
Cumulative meta-analysis of published trials


A large Merck-sponsored trial in 2000 (Bombadier et al., “VIGOR” Vioxx gastrointestinal outcomes) showed definitive evidence for increased risk of MI; attributed to a “cardioprotective effect” of naproxen.

Vioxx was pulled from the market only after data from the much smaller Merck-sponsored APPROVe trial (Adenomatous Polyp Prevention on Vioxx) confirmed increased risk relative to placebo.

How can we predict how different people are going to respond to “selective” enzyme inhibitors?

Fries S, Grosser T, Price TS, Lawson JA, Kapoor S, DeMarco S, Pletcher MT, Wiltshire T, FitzGerald, GA

Marked interindividual variability in the response to selective inhibitors of cyclooxygenase-2.


How does altering COX activity affect other important life processes?


Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis.