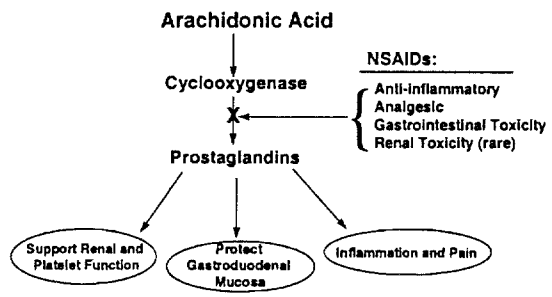
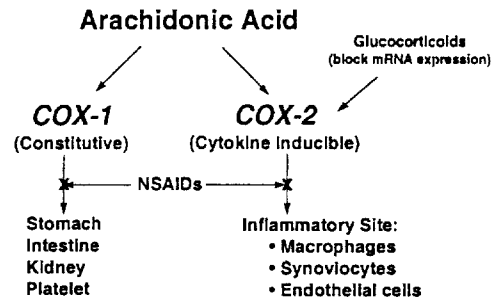
Mechanism of Action of NSAIDs: The Vane Hypothesis



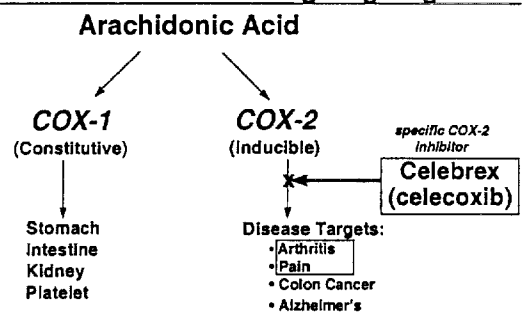
1

1990 Hypothesis: 2 COX Isoforms Exist



2

COX-2 Inhibitors: Mechanism Based Drug Targeting



3

Breakthroughs in Anti-Inflammatory Therapy

- 1897 Invention of aspirin
- 1963 Development of NSAIDs
- 1971 Mechanism of aspirin
- 1990-91 Paradigm shift - Discovery of COX-2
- 1992 Rational drug development begins
- 1998 Delivery of a new class of drugs
  - Delivers the effectiveness of NSAIDs in arthritis and pain
  - Provides greater safety than the NSAIDs
  - Differentiated class with a clear therapeutic index

4

COX-2: Targeted Drug Discovery

Scientific Objectives:

- Mechanism of Specificity
- Evaluation of the COX-2 Hypothesis
- Efficacy and Safety Profile Consistent with COX-2 Specific Mechanism

→ **Clinical Evaluation**

5

Identification and Evaluation of COX-2 Inhibitors

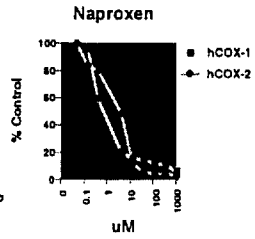
- ***In vitro* pharmacology**
  - Recombinant human COX-1 and COX-2
  - Cells
- ***In vivo* pharmacology**
  - Selectivity
  - Anti-inflammatory and analgesic activity
- **Safety**
  - Acute and chronic

6

Enzyme Inhibition Profiles of Celecoxib and NSAIDS

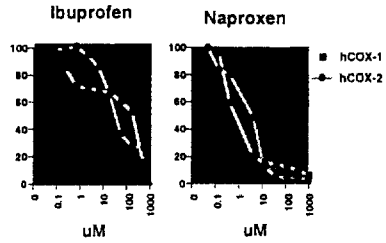
Enzyme + Drug
↓
+ Arachidonate
↓
Measure
Prostaglandin

Recombinant human enzymes assayed *in vitro*



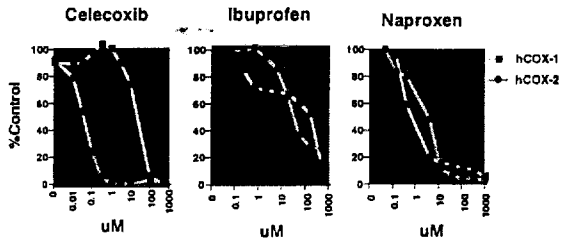
7

Enzyme Inhibition Profiles of Celecoxib and NSAIDS



8

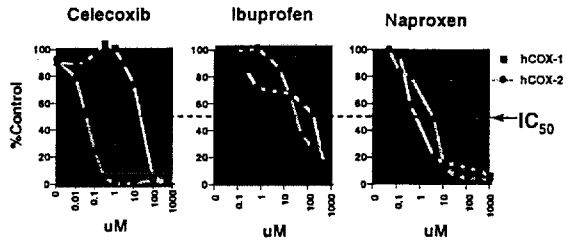
Enzyme Inhibition Profiles of Celecoxib and NSAIDS



Glarse et al, Biochem. J. 305:479, 1995
Penning et al, J.Med.Chem. 40:1347, 1997

9

Enzyme Inhibition Profiles of Celecoxib and NSAIDS



10

Inhibition of Cyclooxygenases *in vitro*

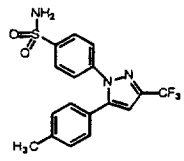
	IC_{50} (μM)		
	COX-1	COX-2	COX-1/COX-2
Diclofenac	0.03	0.01	3
Etodolac	>100	54	>2
Ibuprofen	38	117	0.4
Nabumetone (6-MNA)	82	>1000	<0.1
Naproxen	32	235	0.1

Glarse et al, Biochem. J. 305:479, 1995

11

Inhibition of Cyclooxygenases *in vitro*

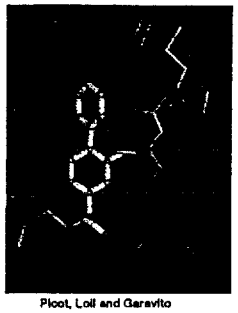
	IC_{50} (μM)		
	COX-1	COX-2	COX-1/COX-2
Celecoxib	15	0.04	375



Penning et al, J.Med.Chem. 40:1347, 1997

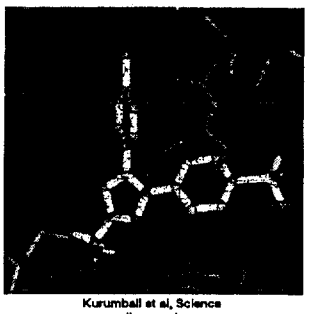
12

COX-1 with flurbiprofen in the active site



Picot, Loll and Garavito
Nature 367:243, 1994

COX-2 with celecoxib in the active site



Kurumbail et al, Science
(in press)

Mechanism of Celecoxib Enyzme Selectivity

- Binds to a side pocket unique to the COX-2 active site
- Selectivity is due to a novel mechanism:
  - Low affinity, competitive inhibition of COX-1
  - High affinity, non-competitive inhibition of COX-2; very slowly reversible
  - → Duration of action longer than pharmacokinetic $T_{1/2}$

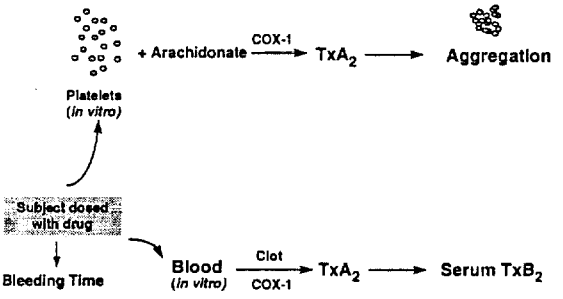
14

Identification and Evaluation of COX-2 Inhibitors

- *In vitro* pharmacology
  - Recombinant human COX-1 and COX-2
  - Cells
- *In vivo* pharmacology
  - Selectivity
  - Anti-inflammatory and analgesic activity
- Safety
  - Acute and chronic

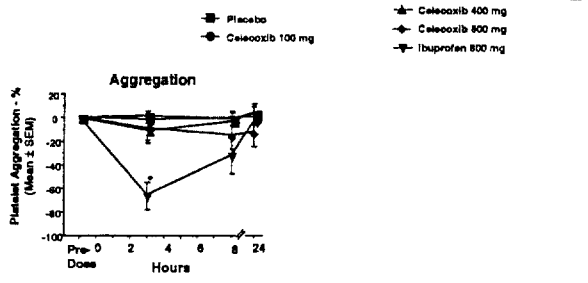
15

Assessment of Specificity with Human Platelets



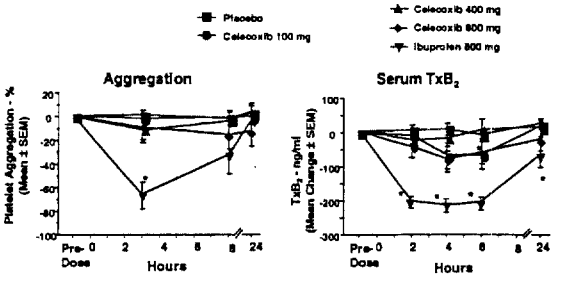
16

Effect of Single Dose Celecoxib on Human Platelet Aggregation and Serum TxB_2



17

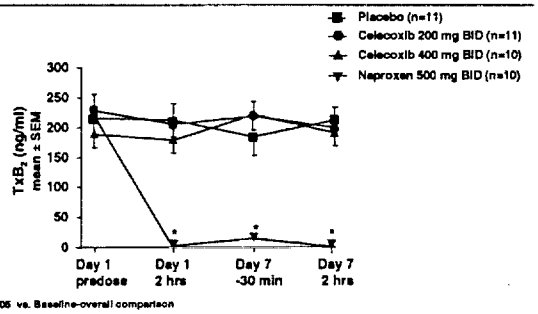
Effect of Single Dose Celecoxib on Human Platelet Aggregation and Serum TxB_2



McAdam et al, PNAS (in press)

18

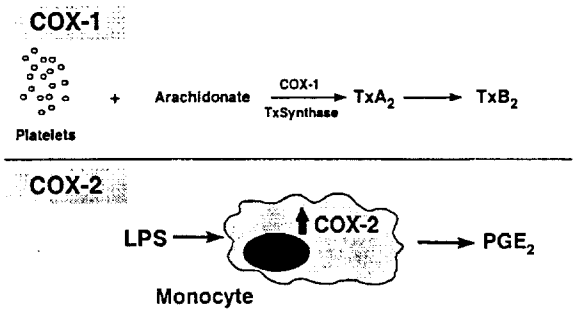
Effect of High Dose Celecoxib on Serum TxB_2



*p<0.05 vs. Baseline-overall comparison

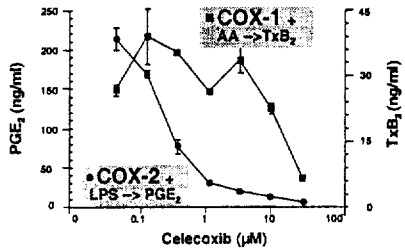
19

Whole Blood Assays of COX-1 and COX-2



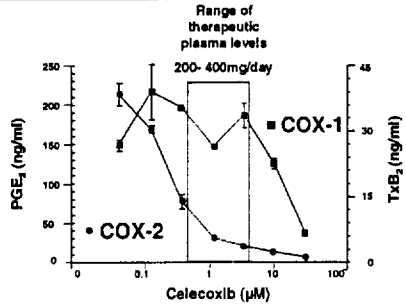
20

Whole Blood Assay of Celecoxib *ex vivo*



21

Relationship of COX Inhibition to Celecoxib Doses in Humans



22

Celecoxib Platelet Effects

- **No effect of celecoxib at 2X the maximum therapeutic dose on:**
  - Platelet aggregation
  - TxB_2 production
  - → COX-1 sparing

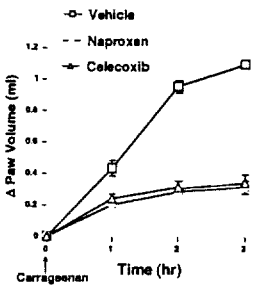
23

Identification and Evaluation of COX-2 Inhibitors

- ***In vitro* pharmacology**
  - Recombinant human COX-1 and COX-2
  - Cells
- ***In vivo* pharmacology**
  - Selectivity
  - Anti-inflammatory and analgesic activity
- **Safety**
  - Acute and chronic

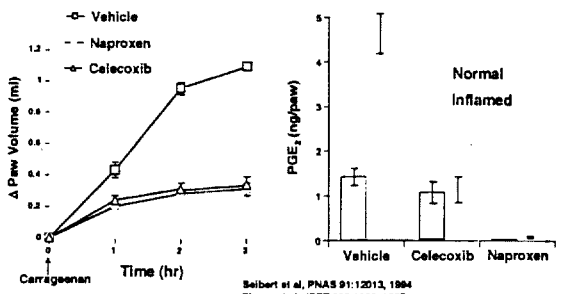
24

Effect of Celecoxib on Acute Inflammation



25

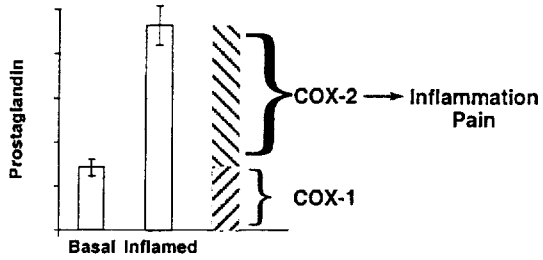
Effect of Celecoxib on Acute Inflammation



Seibert et al, PNAS 91:12013, 1994
Zhang et al, JPET 283:1069, 1997

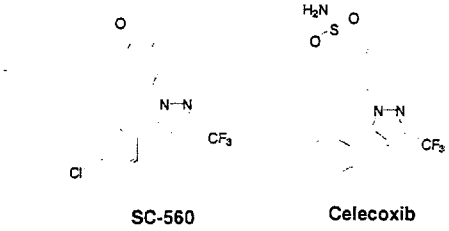
26

Cyclooxygenases in Acute Inflammation



27

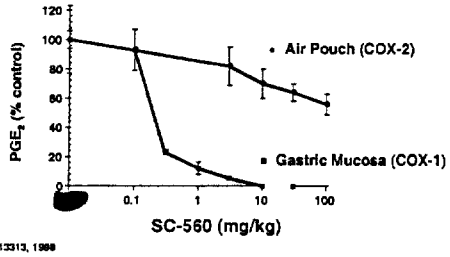
Specific COX-1 Inhibitor



IC_{50}: (uM)	COX-1	0.009	15
	COX-2	6.3	0.04

28

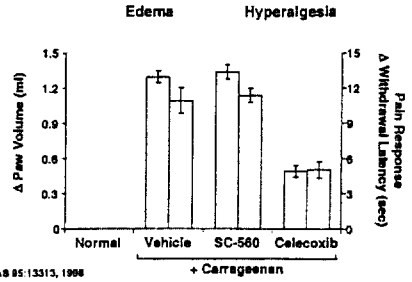
In vivo Specificity of SC-560



Smith et al, PNAS 95:13313, 1998

29

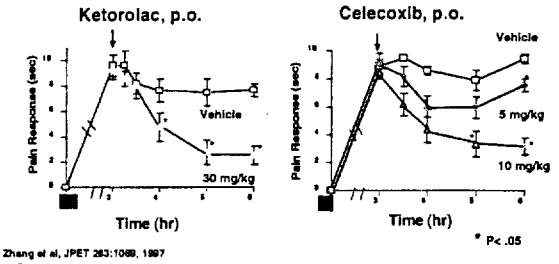
Inhibition of COX-1 is Not Anti-inflammatory or Analgesic



Smith et al, PNAS 95:13313, 1998

30

Rapid Reduction of Pain by Celecoxib

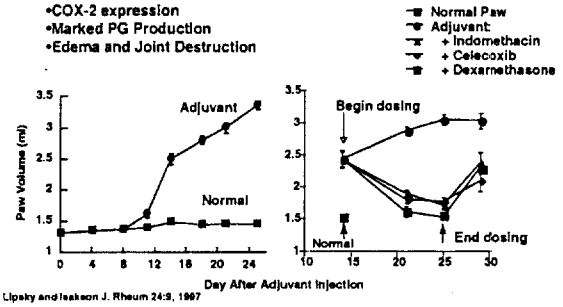


Zhang et al, JPET 283:1089, 1997

31

Rat Adjuvant Arthritis

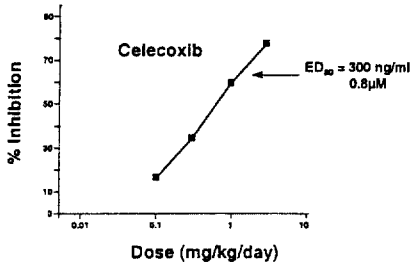
- Mononuclear Cell Infiltrate
- COX-2 expression
- Marked PG Production
- Edema and Joint Destruction



Lipsky and Isakson J. Rheum 24:9, 1997

32

Effect of COX Inhibitors on Rat Adjuvant Arthritis



33

Conclusions

- Specific inhibition of COX-2 by celecoxib results in the same maximal efficacy as inhibition of both COX-1 and COX-2 by NSAIDs
- *Therefore, in animal models COX-2 is the therapeutic target for NSAIDs*

34

Identification and Evaluation of COX-2 Inhibitors

- *In vitro* pharmacology
  - Recombinant human COX-1 and COX-2
  - Cells
- *In vivo* pharmacology
  - Selectivity
  - Anti-inflammatory and analgesic activity
- Safety
  - Acute and chronic
    - Rat (6 months to 2 years)
    - Dog (1 year)

35

Two Forms of Cyclooxygenase

Arachidonic Acid

COX-1	*COX-2*
PGs	PGs
G.I. Tract Platelet (TxA) Kidney Most tissues	Inflammatory Cells *Female Reproduction* *Kidney* *Brain*

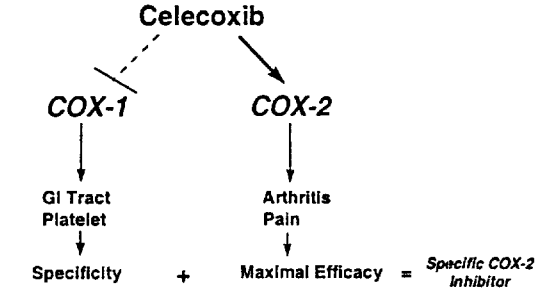
36

Preclinical Toxicology of Celecoxib Relevant to Mechanism

- **Pregnancy**
  - No effect on ovulation or fertility
  - Effects on embryo viability (implantation) at exposures 6-12 fold therapeutic
- **No adverse effects related to:**
  - Bleeding
  - CNS
- **Renal**
  - No renal papillary necrosis
  - Transient anti-natriuresis in rats
- **Gastrointestinal**
  - Chronic safety established at exposures 3 to 6 fold therapeutic in sensitive species (rat and dog)

37

COX-2 Inhibitors: Mechanism Based Drug Targeting



38