



Insights into the Anti-inflammatory Actions of Soluble Epoxide Hydrolase Inhibitors through a Systems Biology Based *In Silico* Modeling Approach

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Introduction:

The arachidonic acid (AA) cascade represents a critical juncture in inflammation, pain, and multiple pathological states. Metabolism of AA by cyclooxygenase (COX) and lipoxygenase (LOX) leads to the production of inflammatory prostaglandins and leukotrienes while cytochrome P450 (CYP450) mediated metabolism results in the production of anti-inflammatory epoxyeicosatrienoic acids (EETs) which are then degraded by soluble epoxide hydrolase (sEH) to the inactive dihydroxyeicosatrienoic acids (DHETs). Previous *in vivo* studies have demonstrated that use of sEH inhibitors (sEHI) attenuated the effects of LPS induced inflammation without affecting the prostacyclin to thromboxane ratio. Furthermore, concurrent use of a COX inhibitor demonstrated synergistic effects. The goal of this study was to validate a dynamic *in silico* platform with experimental data and use it to probe the mode of action of the anti-inflammatory effects of the sEHI.

Methodology:

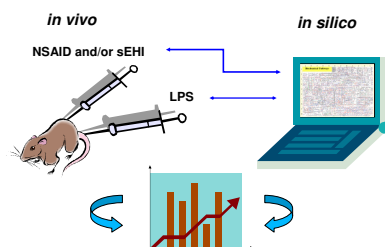


Figure 1. *In vivo* and *In Silico* methodology

Table: IC50 values of COX1, COX2 and sEHI inhibitors

Inhibitors	COX1 IC50	COX2 IC50	sEH IC50
Refecoxib	Beyond 100 uM	0.5 uM	-
AUDA-BE	-	-	50-100nM

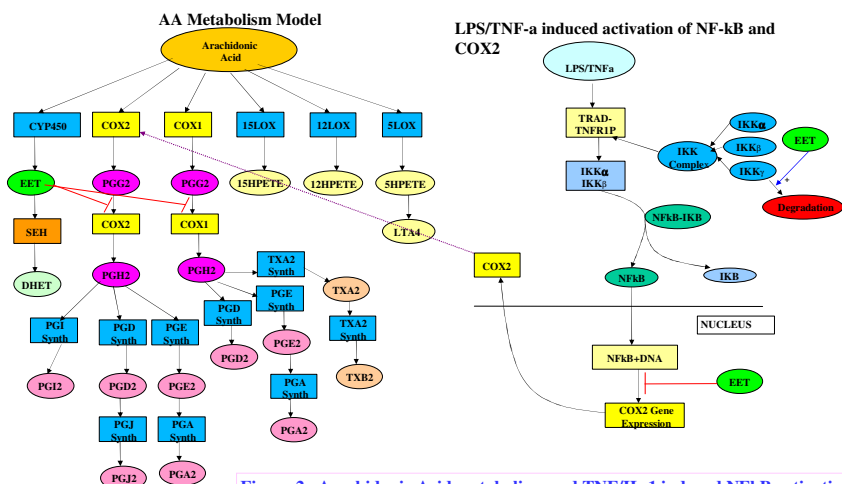


Figure 2. Arachidonic Acid metabolism and TNF/IL-1 induced NFkB activation

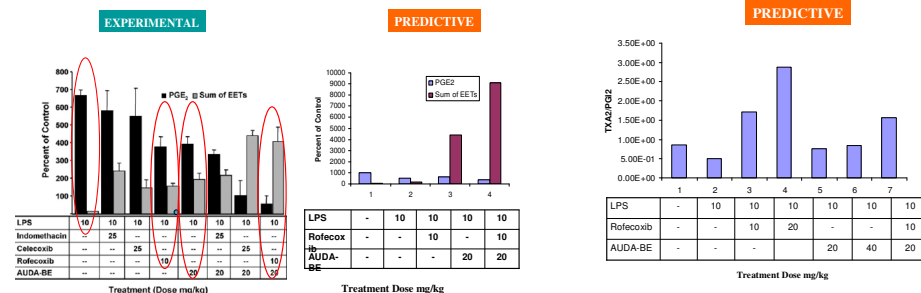


Figure 3. Synergistic reduction of PGE2 plasma levels by combined treatment of COX and sEHI and simultaneous increase of EETs.

Figure 4. Effect of COX2 and sEH inhibitors on TXA2/PGI2 ratio

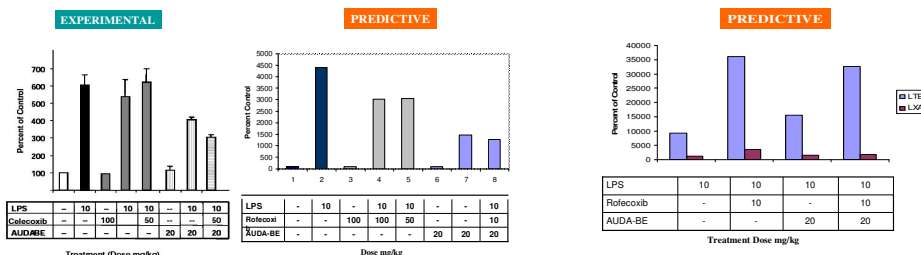


Figure 5. Effect of sEHI on COX2 expression

Figure 6. Effect of COX2 and sEH inhibitors on LTB4 & LXA4

Key Findings:

- *in vivo* administration of sEHI leads to reduced PGE2 (Figure 3a), COX2 (Figure 5a), 5-LOX (data not shown) metabolites *in vivo* administration of sEHI lead to increased EETs (Figure 2a)
- Co-administration of sEHI and NSAID demonstrates synergistic results (Figures 3a & 5a)
- *in silico* results align with *in vivo* results (Figures 3b, 5b)
- *in silico* results shows COX2 inhibition by EET, this inhibition takes place via inhibition of NFkB by EET (Figure 5)
- *In silico* analysis predicts increased LXA4, LTB4 levels following sEH or COX2 inhibition (Figure 6)
- *In silico* analysis predicts effect of COX2 and sEH inhibitors on TXA2/PGI2 (Figure 4)

Results and Conclusions:

Both *in vivo* and *in silico* analyses indicate that an increase in EETs may mediate the inhibition of COX2. The *in silico* results also suggest that the EETs mediated reduction in expression of COX2 and inflammatory cytokines may occur through inhibition of nuclear factor kappa B transcription. This study shows that *in silico* analysis of AA metabolism is consistent with *in vivo* results and that predictive analysis is particularly useful and highly practical for streamlining hypothesis generation and testing.

Selected References:

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