IsoOxygene™

A Botanical Anti-Inflammatory COX-2 Selective Ingredient

Question: How doses IsoOxygene compare to the prescription COX-2 inhibitors (Vioxx and Celebrex) and the OTC NSAID pain relievers such as aspirin and ibuprofen?

Answer: In-vivo human oral dosing studies conducted with ***IsoOxygene*** demonstrated excellent potency and selectivity for COX-2. Oral dosing of ***IsoOxygene*** (1 gram) produced a 56% reduction in COX-2 versus a 62% reduction for a 400 mg. (two tablets) dose of the OTC pain reliever Ibuprofen. However, the selectivity of ***IsoOxygene***, which is calculated as the COX-1 IC-50/ COX-2 IC-50, was 0.25 versus 1.41 for Ibuprofen. Therefor, ***IsoOxygene*** is of equivalent potency to Ibuprofen, but much more selective for the COX-2 form of the enzyme than COX-1. Based on numerous clinical studies done with COX-2 selective inhibitors, a COX-2 selective inhibitor is expected to be gentler on the stomach than a non-selective COX inhibitor such as Aspirin or Ibuprofen.

The prescription COX-2 inhibitor Celebrex, produced a 45% reduction in COX-2 activity in our human oral dosing study, versus 56% for ***IsoOxygene***, making ***IsoOxygene*** more potent than Celebrex. The selectivity of Celebrex (COX-1/COX-2) was 0.45 versus 1.41 for ***IsoOxygene***, making ***IsoOxygene*** not only more potent than Celebrex, but also more selective for COX-2 inhibition. Celebrex has resulted in gastric bleeding in some individuals, and is not more potent than the OTC pain relievers such as Aspirin. Because Celebrex was the first COX-2 inhibitor to be approved by the FDA, it achieved sales in excess of $3 billion, even though it does not offer any better efficacy than the typical NSAIDs.

Vioxx (Rofecoxib), the second COX-2 inhibitor to be approved by the FDA, and also a blockbuster drug which achieved over $3 billion in sales, is highly selective for COX-2. Published human oral dosing studies with Vioxx (Eur J Clin Pharmacol, 2000, 56: 167-174) demonstrate that Vioxx produced an almost complete inhibition of COX-2, with no inhibition of COX-1. The COX-2 selectivity for Vioxx is therefore almost 1:100, in other words, Vioxx virtually shuts down COX-2, blocking all down stream production of prostaglandins. It is known that some prostaglandins such as prostacyclin (PGI-2) are important for cardiovascular health, whereas pro-inflammatory prostaglandins, such as PGE-2, are responsible for inflammation.

The natural anti-inflammatory patent pending ingredient ***IsoOxygene***, derived from hops (humulus Lupulus L.) alpha acids, is therefor believed to be an effective pain reliever with equivalent potency (efficacy) to synthetic drugs, but with a stomach friendly side-effect profile, especially when used on a chronic basis. Individuals, who suffer from chronic pain such as osteoarthritis, are driven to consume pain relievers on a daily basis due to the chronic nature of the inflammation. Chronic use of dual inhibitors of both COX-1 and COX-2 will produce gastric erosion. Conversely, too much selectivity and potency for COX-2 may result in other side-effects, such as potentially harmful

cardiovascular events associated with the complete inhibition of prostaglandin production.

By producing about a 56% reduction in COX-2, IsoOxygene does not completely shut down the production of all prostaglandins, but does inhibit the production of pro-inflammatory PGE-2 enough to provide pain relief equivalent to OTC and prescription pain relievers, but with better selectivity for COX-2. By inhibiting COX-1 by about 10-15%, IsoOxygene provides for some anti-platelet aggregation, while also preserving prostacyclin production, which is beneficial for cardiovascular health.

IsoOxygene™ "The Gastro and Cardio friendly anti-inflammatory ingredient"

- Preserves prostacyclin production
- Anti-platelet aggregation via COX-1 inhibition
- Stomach friendly due to good COX-2 selectivity

IsoOxygene™ Human Oral Dosing/In-Vivo Studies

Two human oral dosing clinical studies have been conducted with IsoOxygene. These studies were designed to assess the COX-2 and COX-1 inhibition of IsoOxygene in vivo. The two most important things that need to be determined when screening for new COX-2 inhibitor anti-inflammatory compounds are the potency and selectivity. The potency is determined by the magnitude of COX-2 inhibition from baseline, and the selectivity is determined from the ratio between COX-2 and COX-1. If the COX-2 inhibition is divided by the COX-1 inhibition, a number is obtained, and the lower the number, the more selective the compound is for COX-2 inhibition relative to COX-1 inhibition.

Cyclooxygenase Inhibiting Activity Of *IsoOxygene™*:

Oral Dosing Study In Humans :

Summary of Results

Study Objective

This study was designed to assess the potential of IsoOxygene, to inhibit cyclooxygenase-1 and –2, (COX-1, COX-2) compared to a control product with known COX inhibiting and pain-relieving properties (400mg ibuprofen). The criterion for success was comparable COX inhibition and selectivity equal to or greater than the ibuprofen control.

Method

Twenty four healthy subjects were enrolled in the study following screening for inclusion and exclusion criteria. Twelve subjects were assigned to receive IsoOxygene (1,000 mg.).

Twelve subjects were assigned to the ibuprofen control group; dosing in the control group was a single two tablet dose (400 mg.).

Cox-2 inhibitory potency and selectivity

The effectiveness of a Cox-2 inhibitor can be expressed in terms of its **potency**, and in terms of its **selectivity**.

Cox-2 potency refers to the ability of a product to reduce the amount of Cox-2 enzyme in the blood by a given percent. For example, a product which inhibits Cox-2 enzyme by a maximum of 50% within a given sampling period is more potent than one that inhibits it by 30%.

Cox-2 selectivity refers to the degree to which a product is able to inhibit Cox-2 without inhibiting Cox-1. Selectivity is calculated by dividing the *integrated Cox-1 potency* by the *integrated Cox-2 potency* (see below). The smaller the number below one, the greater the Cox-2 selectivity.

Integrated potency is potency over time: for example, if a product inhibits Cox-2 by 50% over 4 hours, the integrated potency equals 200 (50 X 4). If the same product inhibits Cox-1 by 25% over 4 hours, its integrated Cox-1 potency will be 100, and its Cox-2 selectivity will be 100 divided by 200, or 0.5.

Blood samples were taken immediately before the dosing of test products, as well as at various time points after the dose. The subjects in the control group gave blood samples 6 hours before as well as immediately before dosing, and 0.5, 1, 2, and 3 hours after dosing.

Results

Plasma from each of the blood samples were evaluated for potency and selectivity in a validated ex vivo assay. It should be noted that the only way to measure COX-2 is by the protocol of Giuliano, F. et al, in Br J Pharmacol 126, 1824-30; 1999, in which the test compounds are administered orally, blood is drawn, and COX-1 and COX-2 are measured Ex-Vivo.

Product effects over the sampling time are expressed as a percentage of baseline Cox-1 and –2 activity in **Figure 1**. The maximum Cox inhibition within the 9 hour (active products) or 3 hour (ibuprofen control) sampling time represents each product's Cox-2 inhibitory potency. The Cox-2 inhibitory potency of IsoOxygene™ (56%) was in the range of the Cox-2 inhibitory potency of a single 2-tablet dose of ibuprofen (62.2%). As expected from published reports[1] and in previous unpublished studies, plasma from subjects in the ibuprofen group (bottom right panel) inhibited Cox-1 and Cox-2 by 80% and 60%, respectively. The potency and selectivity results are shown in **Table 1.**

Table 1. Integretated Cox potency and selectivity values. Means +- standard error.

	Integrated Potency		Cox-2 Selectivity	Statistics	
Product	Cox-1	Cox-2	Ratio	within products	between products
IsoOxygene	90.09 ± 51	354.2 ± 23	0.25	**	a
Ibuprofen	233.5 ± 12	166.3 ± 5	1.40	*	b

Integrated Potency: Increasing number indicates increasing inhibition of Cox-1 or Cox-2. Cox-2 selectivity Ratio: the smaller the number below 1, the greater the selectivity. Selectivity comparisons within products: * = P < 0.05, ** = P < 0.01, n.s. = not significant. Selectivity comparisons between products: ratios not sharing a letter are significantly different.

IsoOxygene showed statistically significant Cox-2 selectivity. (Ibuprofen, by contrast and as expected, was selective towards Cox-1.) The Cox-2 selectivity of IsoOxygene was significantly different ($p<0.01$) from that of ibuprofen

Discussion

A 2-tablet dose of ibuprofen (400 mg) is known to provide pain relief, and this same dose in our study caused a 62% reduction in Cox-2 activity. By comparison, IsoOxygene (1 gram) caused a 56% reduction in Cox-2 activity. These results suggest the hypothesis that IsoOxygene would provide pain relief comparable to a single 2-tablet dose of ibuprofen.

IsoOxygene/Celecoxib Comparison Study

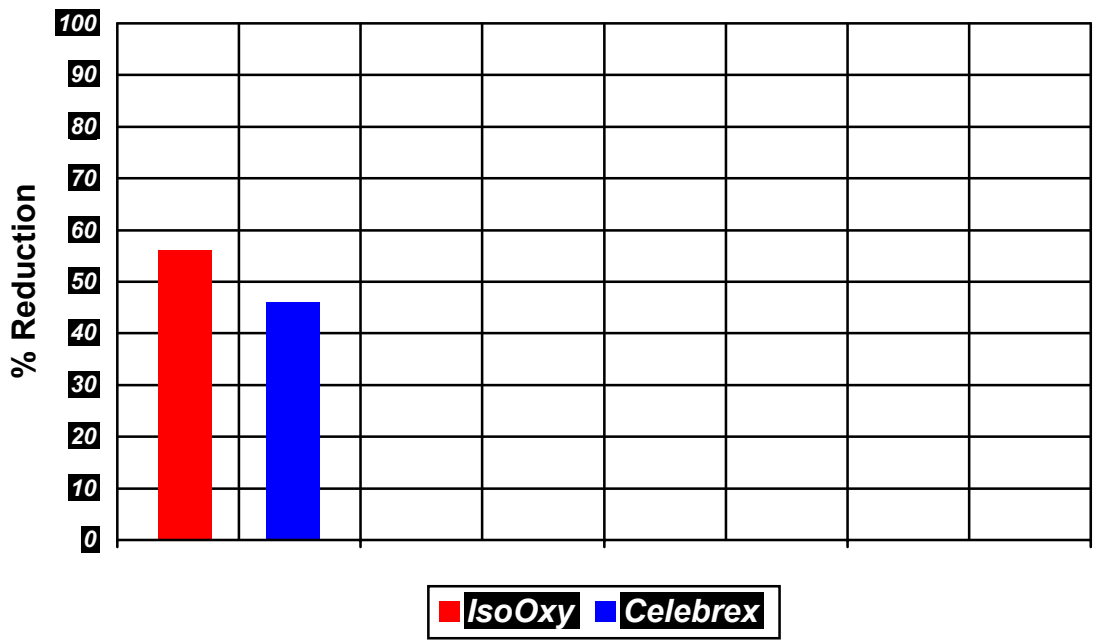
A comparison study was conducted to compare the effects of IsoOxygene™ and Celecoxib (Celebrex®). Six subjects were dosed with IsoOxygene (1,000 mg.) and six subjects with Celecoxib and COX inhibition activity was measured in plasma according to the previous protocol. Additionally, six subjects were given a 400 mg. dose of Ibuprofen for comparison. Results of the study are summarized below.

Table 2. Maximum Cox-2 reduction from baseline over 3 hours, by product, and Cox-2 specificity, by product. Comparative data from separate Celebrex and Ibuprofen pilot study included.

	IsoOxy	Ibuprofen	Ibuprofen from 2	Celebrex from validation study§
% reduction from baseline Cox-2	56%	62%	69%	46%
Cox-2 selectivity	0.25	1.41	1.53	0.45
Cox-2 selectivity	0.25	1.41	1.53	0.45

Cox-2 selectivity: Cox-1 AUC / Cox-2 AUC; the smaller the number below 1, the greater the Cox-2 selectivity.

The results of this study indicate that the ***selectivity*** of IsoOxygene for COX-2 was in the range of that found with Celebrex. In this light, it is interesting to note that IsoOxygene had a COX-2 inhibitory potency (56%) in the range of the potency of a single 100 mg dose of Celebrex (46%).



In-Vivo Human Oral Dosing; IsoOxygene™ versus Celebrex®

Not only did IsoOxygene reduce or inhibit COX-2 equivalent to Celebrex, it had a much better selectivity for COX-2. The COX-2 selectivity for IsoOxygene was 0.25, whereas the COX-2 selectivity for Celebrex® (celecoxib) was 0.45. COX-2 selectivity = COX-1 AUC/COX-2 AUC (AUC is the area under the curve). Better selectivity for COX-2 means less potential for gastric bleeding.

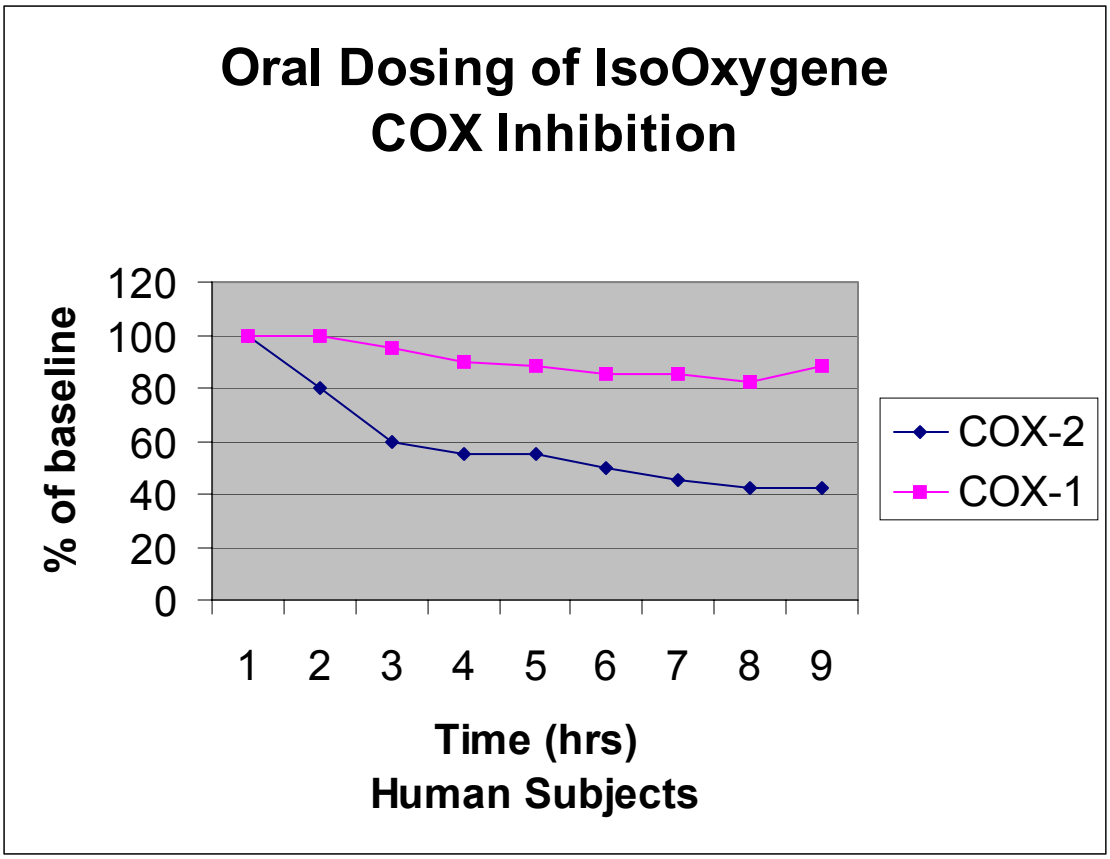


Figure 1.

Conclusion

IsoOxygene had a Cox-2 inhibitory potency comparable to that obtained from a single 2-tablet dose of ibuprofen, while possessing superior Cox-2 selectivity. In addition, comparison of data from a validation study suggests that IsoOxygene would provide Cox-2 inhibitory potency comparable or better than a single dose of Celebrex, with superior selectivity. The present study suggests that IsoOxygene is clearly a selective and potent Cox-2 inhibitor, comparable to ibuprofen and Celebrex in efficacy, but with superior selectivity.

Reference

1. Van Hecken, A. *et al.* Comparative inhibitory activity of rofecoxib, meloxicam, diclofenac, ibuprofen, and naproxen on COX-2 versus COX-1 in healthy volunteers. J Clin Pharmacol **40**, 1109-20 (2000).