A dietary supplement is a selective Cox-2 inhibitor both in vitro and ex vivo in healthy human volunteers.

Marc Lemay, Ph.D.*; Mary A. Murray, Ph.D.*; Audra Davies, M.S.*; Haeri Roh-Schmidt, Ph.D.†; Keith Randolph, Ph.D.*

* Nutritional Health Institute, Access Business Group LLC, 5800 Beach Boulevard, Suite 100, Orlando, FL 32819
† Analytical Services, Access Business Group LLC, 1195 Fulton Street East, Ada, Michigan 49310

† Analytical Services, Access Business Group, Inc., 7575 Fulton Street East, Ada, Michigan 49355.

It has not yet been demonstrated that oral consumption of botanical agents can inhibit Cox-2 activity in humans. This 3-stage study was therefore designed by Access Business Group LLC (ABG) to (1) screen a variety of botanical putative anti-inflammatory agents in vitro; (2) formulate a promising ingredient into a prototype dietary supplement for an ex vivo Cox-2 inhibition study using healthy volunteers, and (3) submit this same prototype to a 2-week tolerability study.

**Statistical methods**

In vitro results are shown as means (standard error of the mean) of duplicate treatments assayed in duplicate. Ex vivo results are expressed as group means with standard errors.

**Ex vivo Cox-1 and -2 inhibition caused by plasma samples** was calculated as a percentage of the activity at baseline (before product consumption).

Cox inhibition was calculated as area over the 9-h inhibition curve. Selectivity was calculated by dividing Cox-1 by Cox-2 AOC, and compared to a hypothetical value of 1 with a Wilcoxon Signed Rank Test. Alpha was set at 0.05, 2-tailed. Cox-2 inhibition curves were compared with a One-Way ANOVA, followed by Dunn’s Multiple Comparisons Test. Test material selectivity comparisons between products were calculated with a One-Way ANOVA and Bonferroni’s Multiple Comparisons Test.

**In Vitro Screening**

Method

Botanicals were selected for in vitro testing based on one or more of the following criteria: known Cox-2 inhibitor; known anti-inflammatory action; or traditional for pain relief. The Cox-1 and -2 inhibitory potential of botanicals was assessed in phosphatidylcholine, 12-myristate 13-acetate, PMA (10 nM) stimulated 2 cell line. Cells were preincubated with 10-100 µM of test materials for 4 h prior to stimulation with 50 nM PMA for 20 h, following which cells were incubated with 10 nM formyl LPS in 0.5% acetic acid for 1 h. PGE2 secretion was measured with a PGE2-specific ELISA kit.

Results

Results are presented in Panel A (VCU = 50 µg/ml), B (VCU = 10-50 µg/ml), and C (VCU = 10-50 µg/ml) of the figure. The active control products (in panel C) were markedly effective in inhibiting PGE2 secretion.

**Ex Vivo Clinical Study**

Method

This was a randomized, active-controlled, double-blind parallel-groups study performed at ABG. The protocol was approved by the Western Institutional Review Board (Olympia, WA). Each subject provided written informed consent. Nine healthy adults volunteered, with a mean age (SD) of 41.0 ± 12.4 years (range 22-63), participated.

Dietary Supplement Prototypes

IsoOxygene was formulated into 2 prototypes, one a softgel containing the hops extract oleoresin, and the other a capsule containing the oleoresin converted to power form. Both were standardized for hops alpha acids and had added vitamin E and astaxanthin (10 IU and 0.5 mg respectively per 150 mg alpha acids) as well as rosemary extract (1.5 mg camphor acid or 1.8 mg rosmarinic acid in the softgel and capsule respectively per 150 mg alpha acids). Both test formulas were produced by ABG.

Treatment Groups

After screening for inclusion and exclusion factors, subjects were randomized to receive either: a single dose of ibuprofen 400 mg, or hops alpha acids 450 mg (oleoresin), or hops alpha acids 1200 mg (oleoresin converted to powder) in 4 divided doses of 300 mg, every 2 h for 6 h.

Subjects provided either 7 (resin and ibuprofen) or 14 doses (hops and ibuprofen) of test agents and provided blood samples at timed intervals for 9 h.

**Results**

The potency of test products across the 9-h sampling period is shown in Panel E. Cox-1 and -2 inhibition was achieved within 1 to 2 h with the two formulations. There were no statistically significant differences in Cox-2 inhibitory potency over 9 h (P > 0.05) (Panel F, Table 2).

Cox-2 selectivity

Plasma from subjects who had taken ibuprofen showed Cox-1 selectivity with a ratio of 1.5 over 9 h. Plasma from subjects who had taken either a single dose of hops resin 450 mg, or hops powder 1200 mg in 4 divided doses, showed Cox-2 selectivity which was significantly different from the Cox-1 selectivity of the ibuprofen control group.

The Cox-2 inhibition and selectivity found in the present study with the ibuprofen control and the two hops test groups can be compared to other Cox-1 or Cox-2 selective agents. The 9-h Cox-2 selectivity ratios of hops powder (0.44) and resin (0.42) are comparable to the 8-h Cox-2 selectivity ratios reported for plasma from subjects who had taken the known Cox-2 selective drugs etodolac, meloxicam, and nabumetone (0.36, and 0.71 respectively) [Giuliano et al, Eur J Pharmacol 2001: 426(95-103)]. In Panel D, the AOC is normalized to the mean hourly Cox-2 inhibition; filled symbols are from Giuliano et al, empty symbols from the present study. Plasma from the ibuprofen group inhibited Cox-2 by a mean of about 30% over 9 hours, with a selectivity ratio between 1 and 2. By contrast, plasma from a naproxen-treated group (in Giuliano et al) showed greater mean Cox-2 inhibition over 8 h, but not greater selectivity; and meloxicam proved less potent but more selective than naproxen. Plasma from hops resin subjects showed mean Cox-2 inhibition and selectivity values that overlap with the corresponding values from an etodolac-treated group.

Tolerability Study

Method

After the ex vivo study subjects were randomized for once-daily dosing for 2 weeks with either hops powder or resin or placebo. Subjects were examined and provided blood samples after 4 and 14 daily doses.

Results

In the hops powder group (n = 6), 5 subjects reported 11 adverse events (AEs): 10 were GI-related (bloating, nausea, diarrhea), and 1 was bronchitis. In the hops resin group (n = 7), 7 subjects reported 61 AEs, 57 of which were GI-related. 2 were related to gastrointestinal problems (bad taste in mouth; 2 to a skin rash, and 1 to dizziness. In the placebo group (n = 7), 4 subjects reported 6 AEs, all GI-related.

Discussion

It is well established that selective Cox-2 inhibitors cause fewer GI problems than non-selective drugs. However, selective drugs are cost-effective only for high-risk subjects. Although botanical anti-inflammatory dietary supplements have generally not been found to be as potent as analgesics, it has been suggested that they could at least be used to decrease the consumption of NSAIDs, with concomitant decrease in health care expense and risk of side effects.

A 400-µg dose of ibuprofen is known to provide pain relief, and this same dose in our study caused total Cox-2 inhibition over 9 h that was equivalent to that obtained with either of the hops formulations. While however showed Cox-2 selectivity, in contrast to the Cox-1 selectivity of ibuprofen. While the selectivity difference was modest—with hops showing about a 3-fold greater Cox-2 selectivity than ibuprofen—this small difference may yet yield considerable clinical benefit. Between ibuprofen and meloxicam, there exists in vitro a modest difference in Cox-2 selectivity, which small shift in emphasis of drug action nonetheless yields a considerable safety advantage for ibuprofen.

The time to maximum effect was much shorter for ibuprofen than for the test products. A clinical trial protocol for ibuprofen, however, would require a dose of 400 mg per day to achieve maximum effect.