It has not yet been demonstrated that oral consumption of botanical agents can inhibit Cox-2 activity in humans. The aim of the study was to evaluate botanical extracts identified as possessing cyclooxygenase-1 (Cox-1) sparing activity *in vitro* in an *ex vivo* clinical intervention and then in a tolerability study. Seventeen putative botanical anti-inflammatories or pain-relievers were evaluated *in vitro* for Cox-1 and -2 inhibitory potency and selectivity. Two different formulations of a standardized hops extract (resin and powder) were compared with an ibuprofen control in a double-blind, randomized, human *ex vivo* study. Subjects provided blood samples before and at timed intervals for 9 h after the first dose. Plasma was analyzed in a Cox-1 and -2 inhibition assay. There were no differences between treatments or control in Cox-2 inhibition, as indicated by 9-hour Cox-2 Area over the Inhibition Curve (AOC). Hops powder or hops resin extract produced a 9-hour Cox-1 / Cox-2 AOC ratio of about 0.4, compared to 1.5 for ibuprofen. The hops extracts exhibited Cox-2 inhibition over 9 hours equivalent to ibuprofen 400 mg but had significant Cox-1 sparing activity. In the tolerability study, one formulation caused stomach upset in most subjects, but the other formulation was as well tolerated as placebo. Hops extracts may thus represent a safe alternative to ibuprofen for non-prescription anti-inflammation.

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

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There is a need for therapeutic alternatives in the management of benign pain. Ibuprofen and other non-steroid anti-inflammatory drugs (NSAIDs) are associated with injury to the gastrointestinal (GI) mucosa, with sometimes serious consequences. NSAIDs cause injury by the unselective inhibition of the enzyme cyclooxygenase (Cox). Agents which inhibit Cox-2 selectively should provide pain relief and anti-inflammation with less risk of gastric damage.

Several botanicals have shown Cox-2 inhibition *in vitro*, and there have been promising results from clinical trials (e.g., ginger and willow), but it has not yet been demonstrated that botanicals 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 and selectivity were compared with a One-Way ANOVA, followed by Dunn's Multiple Comparisons Test. 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 phorbol 12-myristate 13-acetate-(PMA) stimulated Caco-2 cells.Cells were pretreated in triplicates with 0-1000 µg/ml of test materials for 4 h prior to stimulation with 50 ng/ml PMA for 20 h, following which cells were incubated with 10 µM arachidonic acid for 1 h. PGE2 secretion was measured with a PGE2-specific ELISA kit.

Results

Results are presented in **Panel A** (IC_{80} > 50 µg/ml), **B** (IC_{80} = 10-50 µg/ml), and **C** (IC_{80} <10 µg/ml) of the **Figure**. The active control products (all in **Panel C**) were markedly effective in inhibiting PGE_2 secretion

Table 1

Test Product	Scientific Name	Standardization	COX-2 (PGE2 secretion) IC_{80} (µg/ml)	COX-2 (PGE2 secretion) IC_{50} (µg/ml)	COX-1 (TXB2 secretion) IC_{80} (µg/ml)	COX-1 (TXB2 secretion) IC_{50} (µg/ml)
Ibuprofen	Ibuprofen	Ibuprofen	0.6-0.7	2-3	0.1-0.2	>100
Aspirin	acetylsalicylic acid	acetylsalicylic acid	1-2	8-9	0.3-0.4	>100
Celebrex	celecoxib	celecoxib	0.05-0.06	0.8-0.9	3-4	>100
Grape Extract-1	*Vitis Vinifera*	grape skin, seeds, stem extract, 40% polyphenols	>1000	>1000	1000	>1000
Grape Extract-2	*Vitis Vinifera*	grape skin, seeds, stem extract, 25% polyphenols	>1000	>1000	1000	>1000
Resveratrol	*Vitis Vinifera*	grape stem extract, 20% resveratrol oligostilbenes	3-4	9-10	5-6	>100
IsoOxygene Supercritical Hops Extract	*Humulus lupulus*	30% alpha acids	1-2	3-4	20-30	>100
Chinese Skullcap	*Scutellaria baicalensis*	dried root powder	20-30	>100	>100	>100
Grains of Paradise	*Aframomum stipulatum*	ethanolic extract of ground seeds	<3	<3	5-6	>30
Habanero Chili	*Capsicum frutescens*	ground dried fruit	70-80	>1000	400-500	>1000
Rosemary Extract	*Rosmarinus officinalis*	50% carnosic acid	30-40	100-200	>1000	>1000
Turmeric	*Curcuma longa*	raw root powder	<3	3-4	7-8	700-800
Curcumin	*Curcuma longa*	95% Curcuminoids	2-3	10-20	10-20	800-900
Sage Extract	*Salvia officinalis LINN.*	dried powder	>1000	>1000	>1000	>1000
Cayenne	*Capsicum annuum*	dried powder	200-300	>1000	>1000	>1000
Thai Dragon	*Capsicum annuum var. annuum*	dried powder	300-400	>1000	>1000	>1000
Devil's Claw	*Harpagophytum procumbens*	5% harpagoside	>1000	>1000	1000	>1000
Myrrh	*Commiphora myrrha*	resin	6-7	9-10	900-1000	>1000
Frankincense	*Boswellia Carteri*	resin	30-40	80-90	200-300	>1000
Oregano	*Origanum vulgare LINN*	dried powder	6-7	20-30	90-100	>1000
Wild Blueberry	*Vaccinium angustifolium*	20% Anthocyanidins	<3	3	50-60	>1000

(**Table 1**). Of the botanicals, turmeric root, *A. stipulatum*, wild blueberry extract, and a proprietary, supercritical hops extract, IsoOxygene, were most effective.

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.Nineteen, healthy adult volunteers, with a mean age (± SD) of 41.0 ± 12.4 years (range 22-63), participated.

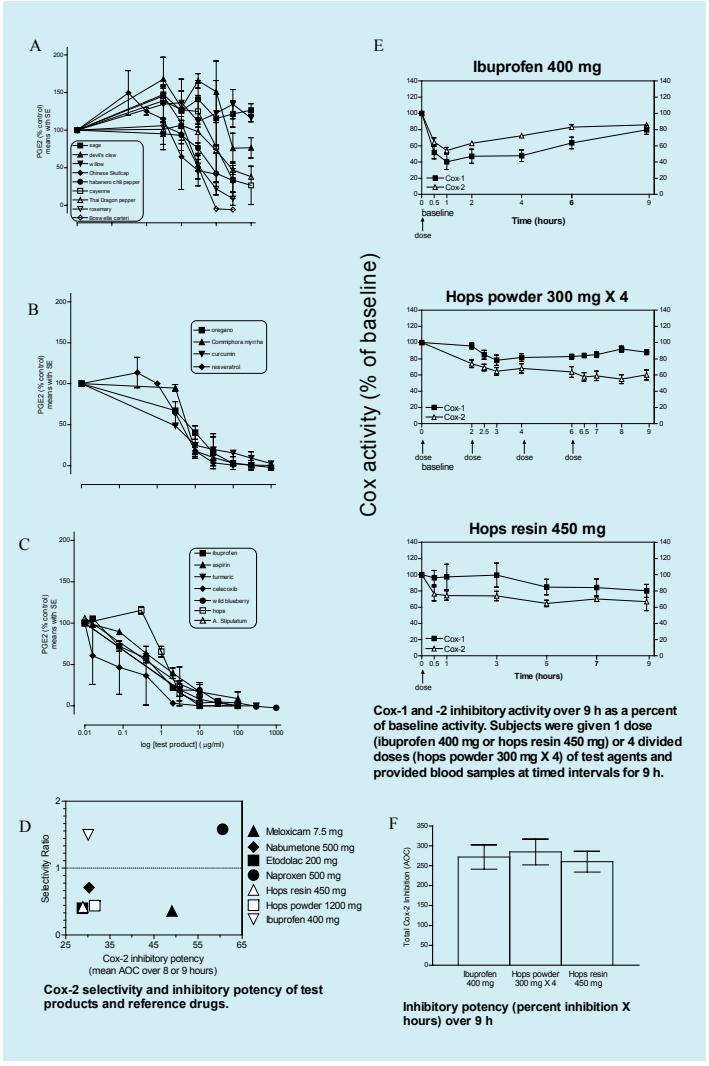
Dietary Supplement Prototypes

IsoOxygene was formulated into 2 prototypes, one a softgel containing the hops extract oleoresin, and the other a capsule containing the oleoresin converted to powder 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 carnosic acid or 1.8 mg rosmarinic acid in the softgel and capsule respectively per 150 mg alpha acids). Both were test formulas 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 groups) or 9 (powder group) blood samples and took 1 or 4 doses of product over 9 h. Placebos were matched to the 2 products and each subject took two products, an active and a placebo.



Cox-2 selectivity and inhibitory potency of test products and reference drugs.

Cox-1 and -2 inhibitory activity over 9 h as a percent of baseline activity. Subjects were given 1 dose (ibuprofen 400 mg or hops resin 450 mg) or 4 divided doses (hops powder 300 mg X 4) of test agents and provided blood samples at timed intervals for 9 h.

Inhibitory potency (percent inhibition X hours) over 9 h

Results

Cox-2 Inhibition

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 hops formulations. There were no differences in Cox-2 inhibitory potency over 9 h (P = 0.88) (**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 (0.40, 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 Giuliani 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 greated 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 (burping, nausea, diarrhea), and 1 was bronchitis. In the hops resin group (n = 7), 7 subjects reported 62 AEs, 57 of which were GI-related; 2 were related to gustatory problems (bad taste in mouth); 2 to a skin rash, and 1 to dizziness. In the placebo group (n = 6), 4 subjects reported 6 AEs, all GI-related.

Table 2. Area Over the Inhibition Curve (AOC) values obtained from the curves shown in Fig. 2. Increasing number indicates increasing inhibition of Cox-1 or Cox-2. The smaller the ratio, the greater the Cox-2 selectivity.

Treatment	AOC			Statistics: Cox Selectivity	
	Cox-1	Cox-2	Ratio	Within products	Compared to control	Between products
Hops powder 300 mg X 4	113.5 ± 18.2	284.5 ± 32.2	0.44 ± 0.09	*	*	a
Hops resin 450 mg	129.8 ± 70.3	260.2 ± 26.2	0.42 ± 0.18	*	*	a
Ibuprofen 400 mg	383.0 ± 69.9	271.7 ± 30.6	1.50 ± 0.31	*	(control)	b

* = P < 0.05, ** = P < 0.01, n.s. = not significant. Selectivity comparisons between products: One-Way Anova = 0.01; ratios not sharing a letter are significantly different.

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 analgesic drugs, it has been suggested that they could at least be used to decrease the consumption of NSAIDs, with a concomitant decrease in health care expense and risk of side effects.

A 400-mg 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 test products, which 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 threefold greater Cox-2 selectivity than ibuprofen—this small difference may yet yield considerable clinical benefit. Between ibuprofen and aspirin, as well, 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 would be required to test for possible differences in pain relief from different times to maximum effect.

The resin-based formulation was not well tolerated, but between hops powder and placebo the number and severity of AEs during the 2-week tolerability study were comparable.

Hops (*Humulus lupulus* L., Cannabinaceae) has long been used to impart bitterness and aroma to beer, in which it also acts as a preservative. These effects are due to the presence in hops cones of a volatile oil, composed chiefly of the so-called alpha and beta acids, or humulone and lupulone respectively. Humulone has previously been reported to be a potent inhibitor of TNF-alpha-induced Cox-2 gene induction in a murine osteoblastic cell model. However, the present study represents the first time that human oral consumption of a high-alpha acid hops dietary supplement has been shown to exert Cox-2 inhibition, with a 9-h potency comparable to that obtained with a dose of ibuprofen 400 mg, but with a theoretically more favorable selectivity, comparable to known Cox-2 selective drugs.