

In-Vitro tests show anti-inflammatory properties. Read Excerpts from the Reports.

A world renown independent lab in Houston, Texas was contracted by Health2o Products, LLC to conduct *in vitro* tests on the Archaea Active™ formula. Based on the data, they concluded that initial testing indicates the formula has similar metabolic properties to those compounds traditionally associated with anti-inflammatory activity. Results from the testing were communicated to Health2o in 2008.

Following are excerpts from their research report.

SCOPE OF THE RESEARCH

The scope of this investigation was to utilize two cell lines (a) the A549 human non-small cell lung adenocarcinoma and (b) the RBL1, a rat basophilic leukemia as cellular models for measuring bioactive lipids associated with inflammation. Previous studies using these cell lines as models have proven to be very useful in evaluating the relative anti-inflammatory activity of numerous compounds The objective of this study was to measure the change in concentration of bioactive lipids associated with inflammation caused by healtH20's enhanced water product.

METHODOLOGY

The bioactive lipids measured in this study (table 1) were prostaglandins, leukotrienes, and mono-hydroxy eicosanoid lipids. The prostaglandins in this panel are known to be associated with cell signaling in inflammation. The leukotrienes and mono-hydroxy eicosanoids are associated with activation and adhesion of leukocytes, neutrophil aggregation and are commonly associated with respiratory distress in asthma and other pulmonary diseases.

Table 1. Inflammatory panel of bioactive lipids (compounds listed in order of detection):

15-keto-prostaglandin E2
prostaglandin D3
prostaglandin E3
17-trans-prostaglandin E2

prostaglandin D2
prostaglandin E2
13,14-dihydro-15-keto-prostaglandin D2
13,14-dihydro-15-keto-prostaglandin E2
20-hydroxy-leukotriene B4
13,14-dihydro-15-keto-prostaglandin E1
13,14-dihydro-15-keto-prostaglandin F2alpha
prostaglandin F2 alpha
prostaglandin E1
20-carboxy-leukotriene B4
20-hydroxy-prostaglandin E2
6-keto-prostaglandin F1 alpha
leukotriene B5
prostaglandin J2
prostaglandin A2
leukotriene B4
14,15-diHete
8,15-diHete
17,18-diHete
5,15-diHete
12-HHTrE
13-Oxo
13-Hode
15-deoxy-prostaglandin J2
12-Hepe
5-Hepe
15-Hepe
11-Hepe
5-Hete
12-Hete
15-Hete

The RBL1 and A549 cells were grown in standard cell culture media at 37°C in a 5 % CO2 enriched atmosphere until ready for use in this study. The endogenous, or baseline, and exogenous, or stimulated, concentration of lipids was determined for both cell lines. The bioactive lipids from table 1 were extracted from the cells and then quantified using mass spectrometry (the specific details of the analytical procedures are not included in this report for reasons of brevity).

AN OUTLINE OF THE EXPERIMENT IS AS FOLLOWS:

Healthy RBL1 and A549 cells initially grown in standard culture media prepared with highly purified water were removed from the standard conditions and incubated for 2 hours with identical standard media prepared with healt$H_2$0 enhanced water with and without addition of 10mM arachidonic acid. Following incubation cells were harvested and lipids extracted. Quantification of bioactive lipids was done using a calibration/quantification regression analysis curve constructed from known concentrations of reference lipid standards purchased from Cayman Chemical Company.

RESULTS:

Table 2. RBL1 exogenous results (values normalized as pg per million cells)

Exogenous sample description	PGE2	PGD2	13,14-dihydro-15-keto-PGE2	PGE1	LTB4	5-Hete	15-Hete
control media	22.9	750.5	24.8	16.2	29.5	1638	177
healtH2O media	17.1	479	35.2	8.6	38.1	1531	113
% change	-25.3	-36.2	41.9	-46.9	29.2	-6.5	-36.2

Table 3. RBL1 endogenous results (values normalized as pg per million cells)

Endogenous sample description	PGE2	PGD2	PGE1
control media	BLQ	75.8	69
healtH2O media	BLQ	15.3	8.2
% change	NA	-80	-88

Table 4: A549 exogenous results (values normalized as pg per million cells)

Exogenous sample description	PGE2	13,14-dihydro-15-keto PGE2	PGE1
media control	250	800	320
healtH2O media	220	1230	140
% change	-12	53	-54

Table 5: A549 endogenous results (values normalized as pg per million cells)

Endogenous sample description	PGE2	13,14-dihydro-15-keto PGE2	PGE1
media control	BLQ	490	BLQ
healtH2O media	BLQ	620	BLQ
% change	N/A	27	N/A

Discussion

The objective of this limited study was to document changes in bioactive lipid concentrations in A549 and RBL1 cell lines that could be attributed to the activity of health$H_2$0's enhanced water product. In general, in these model cell lines compounds that cause a reduction in the concentration of bioactive lipids either endogenously or exogenously are considered to have anti-inflammatory activity. The A549 exogenous lipid levels show a reduction of PGE2 and 5-Hete consistent with the activity of an anti-inflammatory agent (table 4). The endogenous PGE2 and 5-Hete were also reduced by the healthH_2O enhanced water , but the results were below the level of quantification of this analytical assay (table 5). The increased production of 13,14-dihydro-15-keto-PGE2, the primary metabolite of PGE2, in the healthH_2O enhanced water media is consistent with the reduction in PGE2. It should be noted that the level of overall lipid production in the control following stimulation with AA was relatively low. Previous assays have routinely shown 10-20X higher levels for these lipids upon AA stimulation. The cause of this reduction is not known. One possibility is the carrier molecule used as a transport vehicle to convey AA to the A549 cells may have been inadequate so that the amount of AA reaching the cells was insufficient to initiate a greater lipid production. Nonetheless, even though the production of lipid was low, the data shows a reduction of lipid concentrations consistent with the activity of an anti-inflammatory agent.

The RBL1 endogenous lipid concentrations for PGE2, PGD2 and PGE1, all of which bind to the prostaglandin E1 and E2 receptors (EP1, EP2) important in inflammatory response, show a reduction in concentration when the cells were incubated in the health$H_2$0 enhanced water. The endogenous PGE2 levels were below the level of quantification so the actual values are not shown in the table, but are expressed as BLQ (table 3).

The RBL1 exogenous data showed a reduction of the PGE2, PGD2, PGE1, 5-Hete and 15-Hete cellular concentrations upon incubation in health$H_2$0 enhanced water. Oddly, LTB4's cellular concentration increased compared to control following incubation in health$H_2$0 enhanced water concentration (table 2). This increase might suggest shunting of the AA from the cyclooxygenase to the lipooxygenase metabolic pathway, except for the reduction in both 5-Hete and 15-Hete concentrations. At present, no firm hypothesis is available to explain this observation. The increase in the PGE2 primary metabolite, 13,14-dihydro-15-keto-PGE2, is consistent with reduction of the PGE2 and was similarly observed in the A549 cells. As in the A549 cells, with the exception of LTB4, all the lipid marker concentration reductions are consistent with anti-inflammatory activity.

The overall conclusion from these data is that health$H_2$0 enhanced water product has anti-inflammatory properties as measured by its ability to reduce both endogenous and exogenous concentrations of eicosanoid lipids in the model cell lines A549 and RBL1.