healt$H_2$0
Research Report

Submitted January 27, 2009

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EXHBIT A

healtH_20 report on the anti-inflammatory activity of its enhanced water product.

Introduction

Enhanced water is the term used in this report for the aqueous bioreactor product of healtH_20. The company healtH_20 has documented numerous case reports of health promoting activity of its enhanced water in individuals suffering from severe asthma, chronic obstructive pulmonary disease, post-surgical low blood oxygen levels and increased wound healing time all of which are associated with inflammation. The interest of the University of Texas M.D. Cancer Center with healtH_20's enhanced water product has to do with the mission of M.D. Anderson which is to eliminate cancer in Texas, the nation and the world through patient care, research and education. Cancer is an inflammatory disease and any product that can help reduce or modify the body's inflammatory response may be beneficial to patients with cancer. Initial discussions between healtH_20 principles and Robert A. Newman, Ph.D. , co-Director of the M.D. Anderson Cancer Center, Pharmaceutical Development Center and Analytical Core Laboratory, now retired, regarding the health promoting activity of healtH_20 enhanced water with cancer patients and patients with breathing problems (i.e. COPD, asthma, reduced blood oxygen levels) led to some limited analytical survey work by the Analytical Core Center. The initial assay was to try and identify compounds present in the enhanced water. Of specific interest to healtH_2O was the possible presence of cytokines, chemokines, archael peptides or other known anti-inflammatory molecules. While these initial investigations were unable to document the presence of these compounds, sufficient evidence was obtained to warrant additional investigations regarding the reported anti-inflammatory activity of the enhanced water product. Dr. Newman and his associates during the past 10 years have developed a unique mass spectrometry method for the detection and quantification of bioactive lipids associated with inflammation. This method has been published in peer reviewed scientific publications and has been used to evaluate the anti-inflammatory activity of numerous pharmacologic agents and nutraceutical supplements. A limited study to evaluate the anti-inflammatory activity of healtH_20 enhanced water using this method was done. This report will present the results of that study together with recommendations for additional investigations on healtH_20's enhanced water product.

Scope

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 healtH_20'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 A2
prostaglandin D3	**leukotriene B4**
prostaglandin E3	14,15-diHete
17-trans-prostaglandin E2	8,15-diHete
prostaglandin D2	17,18-diHete
prostaglandin E2	5,15-diHete
13,14-dihydro-15-keto-prostaglandin D2	12-HHTrE
13,14-dihydro-15-keto-prostaglandin E2	13-Oxo
20-hydroxy-leukotriene B4	13-Hode
13,14-dihydro-15-keto-prostaglandin E1	15-deoxy-prostaglandin J2
13,14-dihydro-15-keto-prostaglandin F2alpha	12-Hepe
prostaglandin F2 alpha	5-Hepe
prostaglandin E1	15-Hepe
20-carboxy-leukotriene B4	11-Hepe
20-hydroxy-prostaglandin E2	5-Hete
6-keto-prostaglandin F1 alpha	12-Hete
leukotriene B5	15-Hete
prostaglandin J2	

The RBL1 and A549 cells were grown in standard cell culture media at 37°C in a 5 % CO_2 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).

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 healthH_20 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

RBL1 cell line

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
healthH2O 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
healthH2O media	BLQ	15.3	8.2
% change	NA	-80	-88

A549 cell line

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
healthH2O 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
healthH2O 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 healthH_20'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 thought 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 healthH_20 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 healthH_20 enhanced water. Oddly, LTB4's cellular concentration increased compared to control following incubation in healthH_20 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.

Conclusion

The overall conclusion from these data is that healthH_20 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.

Recommendations

Based on the results of these initial experiments it is recommended that a follow-up analysis be conducted to confirm these conclusions. The follow-up analyses should incorporate concentrated healtH_20 enhanced water (2 to 4X) to more definitively ascertain the concentration depend affect of this product on eicosanoid production and better define its relative activity as an anti-inflammatory agent

In addition, any follow-up investigation should address the possible affect of Houston city water which is used in the preparation of healtH_20's enhanced water. The standard water for cell culture media utilizes highly purified, deionized water free of heavy metals, minerals and organic compounds. By contrast Houston City water is hard, has high levels of carbonates and minerals. Houston municipal water may also contain low levels of heavy metals and organic compounds which could affect cellular physiology and enzymatic activity with respect to bioactive lipid production.

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