RESEARCH INDICATES

ALL

VITAMIN C FORMS

ARE

NOT CREATED

EQUAL

The Future Generation of Vitamin C

These notes contain a compilation of our research, and rationale for BioActive-C™ and the previous generations which consisted of the following names: Generation Vitamin C, Beyond C™, and Bio En'R-G'y* C.

Research is still ongoing, and thoughts, ideas and rationale, pathways and theoretical prospecting are still being evaluated.

We have tried to convey to you where we are and to give you some insight into the research and testing behind our product, what research shows us, and why it may be working as feedback shows it has been.

These Research Reports are for Health Professional information only and NOT intended as a basis for advertising statements or marketing. They are not intended for use in diagnosis or treatment.

They apply **only** to the researched product **Beyond C** and the RNA enhanced version **Bio En'R-G'y* C and BioActive-C™** (for which **Beyond C** is the basic Vitamin C used with **Ribose Nucleotide Activation).**

These tests were NOT done by Advanced BioNutrional Research a division of ABR Enterprises LLC but were contracted through an independent research group 3rd party and accomplished blind with the people involved at 3rd party direction.

These statements have not been evaluated or approved by the Food and Drug Administration

***** Research Report RPN 9911 December 17, 2004 Silliker Inc.

CHALLENGE TEST OF A VITAMIN C PRODUCT

Objective

The objective of this study was to conduct a challenge test to assess the microbiological stability of a product when challenged with one strain each of yeast, mold, lactic acid bacteria, *Salmonella*, and *Staphylococcus aureus*.

Conclusions

The AIBMR Life Sciences Beyond C Enhanced Vitamin C Powder with GMS Metaboliter stored at ambient room temperature (73-77°F) had a microbiological shelf-life of 28 days and was microbiologically stable against challenge with *Salmonella*, *Staphylococcus*, lactic acid bacteria, yeast and mold for 28 days.

*******Stability Testing for shelf life. -Initial Assay and 12 week accelerated –equivalent to 3 years shelf life. We use only a 2-year expiration date**

ABC Testing

Advanced Botanical Consulting & Testing, Inc.

15401 Redhill Ave., Suite E, Tustin, CA 92780, Phone: (714) 259-0384 Fax: (714) 259-0385

Client Sample ID: Beyond C™ 200 g Bottle
Accelerated stability--Initial
Lot #: G 4077, Exp. 07/06
Lab #: 006197

Received Date: 11/22/2004

Report Date: 11/26/2004

Analyses	Results
Per serving (6. 5 g) contains:	
Vitamin C (HPLC)	**4221. 96 mg**
Vitamin C (USP)	**4065. 25 mg**

Methods: ALC091A, USP27/ND22 (Titration)

Client Sample ID: Beyond C™ 200 g Bottle
Accelerated stability—12 week at 40° C/75% RH
Lot #: G 4077, Exp. 07/06
Lab #: 006202

Received Date: 11/22/2004

Report Date: 02/23/2005

Analyses	Results
Per serving (6. 5 g) contains:	
Vitamin C (HPLC)	**3967. 8 mg**
Vitamin C (USP)	**3972. 3 mg**

Methods: ALC091A, USP27/ND22 (Titration)

*******Acute oral toxicity study at 2000 mg/kg body weight. No Adverse Effects Noted**.

Acute oral toxicity study of Beyond C™
with 14-day post-treatment observation period in the rat (limit test)
(Study code: PCDL-0410)

SUMMARY

General information:

Single oral limit dose of 2,000 mg/kg body weight of Beyond C™ (Lot number: G 4077) was applied to rats orally by gavage. Animals were observed for lethality and toxic symptoms for 14 days.
Gross pathological examination was carried out on the 15th day.

Body weight:

The body weight of the animals corresponded to their species and age throughout the study.

Evaluation:

No death occurred after oral administration of Beyond C™ at 2,000 mg/kg dose.
No toxic clinical symptoms were observed.
Scheduled autopsy carried out on Day 15 revealed no toxic gross pathological changes.

Conclusion:

No adverse effects were noted at single oral dose of 2,000 mg/kg Beyond C™ in male and female rats.

*****Study–Inhibition of ROS formation by "Beyond C". Report #054 76 032805

Objective: To evaluate whether the reportedly high ORAC value of this product translates into a physiological effect in a cell-based assay.

Background for this study: **Oxidative stress is a condition in cells which is characterized by an excess of reactive oxygen species (ROS)**. An **excess** of these **molecules leads to oxidative damage**, which plays a role in many disease processes. Many products are marketed to help combat oxidative damage, often relying on a standardized ORAC value for marketing claims. The ORAC test is a chemical assay that measures the capacity for neutralization of oxygen radicals.

Assay principle: Based on standard immunological assays, we have modified an existing method for measurement of ROS production to study natural products with respect to their ability to inhibit ROS formation in a cell-based assay. **This method may supplement a standard ORAC test by documenting an anti-inflammatory effect in a cellular system.**

The method is based on challenging human cells with an inflammatory stimulus to produce damaging reactive oxygen radicals. Changes in the oxidative stress level in each cell are monitored by a ROS-sensitive dye, DCF-DA.

Method: Freshly purified human neutrophils were preincubated with a botanical extract over a wide range of dilutions, loaded with DCF-DA, and then challenged with a high dose of hydrogen peroxide to induce severe oxidative stress.

Conclusion:

Beyond C demonstrated a substantial inhibitory effect on the ROS formation in human neutrophil cells. Beyond C displayed a ***maximum effect at a concentration of 1 ppm (v/v).*** At that dose, Beyond C **inhibited approximately 68% of the oxidative stress** caused by the peroxide challenge. A non monotonic dose response was observed but is typical in this type of assay, where a blend of active ingredients results in multiple factors contributing to intracellular oxidative stress. **The level of ROS formation was not brought back to baseline at any of the dilutions tested, including 0.1 parts per trillion**.

NOTES FROM PRIVATE MEETING WITH RESEARCHERS FROM ROS TESTING LAB REGARDING RESULTS AND FUTURE PLANNED TESTING. NOW UNDER WAY.

As was evident from the meetings we had with NIS Labs in Las Vegas last month, they found your product most interesting. **The ROS tests they already performed indicate that the compounds in the product are available to the interior of the cells**. The possible effect of cellular energy metabolism would make it interesting to **study** a broad panel of assays monitoring different types of cellular behavior, **using both healthy and malignant cell types**.

Given its composition, we believe we need to **examine MODULATION instead of DIRECT**

ACTIVATION of immune reactions. We believe the approach should be to study several well-known reactions in the presence versus absence of the product.

In consultation with the lab, we propose to assess the modulatory effect of the product on:

1. Cox-2 and PGE-2 induction by the known inflammatory stimulus LPS;
2. Basic immune reactions to known compounds (PHA-induced proliferation, IL-2 induced NK activation);
3. **Tumor cell growth in the presence of product (select four easy-to-grow human leukemia cell lines of different types).**

*****Study -Macrophage phagocytosis assay

Bioassay Research Laboratory

Bioassay Report

Sample	:	'Beyond C'	**Report No.**	:	MP/0504
Batch no	:	Lot # K4774	**Date**	:	24/10/2005

Assay Performed : Macrophage phagocytosis assay

Introduction:

Neutrophils/macrophages play a major role in phagocytosis of microorganisms and other foreign entities that enter the body. Compounds that increase the phagocytic capacity of these cells are potent immunostimulators. Thus this assay can be used to gauge the immunostimulatory effect of a substance.

Remarks:

The sample Beyond C at 0.5-250 µg/ml demonstrated no significant effect on macrophage infection but it appeared to cause an increase in number of yeasts engulfed per macrophage. This effect seemed to be dose dependent in nature.

*****Study –Lymphocyte proliferation assay

Bioassay Research Laboratory

Bioassay Report

Sample	:	'Beyond C'	**Report No.**	:	LPA/0504
Batch no	:	Lot # K4774	**Date**	:	23/08/2005

Assay Performed : Lymphocyte proliferation assay

Introduction:

Lymphocyte proliferation assay (LPA) is a measure of immune activation/stimulation. It measures the ability of lymphocytes placed in short term tissue culture to undergo clonal proliferation when exposed to a foreign substance/mitogen. This assay helps in the evaluation of the immunostimulatory/immunosuppressive activity of a mitogen.

Remarks:

The sample 'Beyond C' did not show any effect on lymphocyte proliferation at the concentrations tested (0.5-500 µg/ml) at a 24 hr assay point. LPS (5 µg/ml), the positive control for the assay, demonstrated a 1.5 fold increase in lymphocyte proliferation over cell controls. The latter result is in keeping with the data usually obtained with LPS in this assay.

*****Study –Nitric Oxide assay

Bioassay Research Laboratory

Bioassay Report

Sample	:	'Beyond C'	**Report No.**	:	NO/0504
Batch no	:	Lot # K4774	**Date**	:	21/09/2005
Assay Performed	:	Nitric oxide assay			

Introduction:

Nitric oxide (NO) is an inorganic, free radical that functions as an intracellular messenger and effector molecule. It is produced during the conversion of arginine to citrulline and is catalyzed by the enzyme nitric oxide synthase (NOS)[1]. NOS has three isoforms – NOS I, II and III. Out of these three isoforms, only NOS II is inducible and is produced during macrophage activation[2].

Remarks:

The sample Beyond C did not show any effect on NO release by J774A.1 macrophages at the concentrations tested (10-250 µg/ml) at a 48 hr assay point. LPS (5 µg/ml), the positive control for the assay, demonstrated a 4.93 fold increase in NO release over cell controls. Beyond C (100-250 µg/ml) demonstrated a significant dose dependent inhibition of LPS induced nitric oxide in this assay; suggesting a probable anti-inflammatory role of the compound.

*****Background and proposed work – some in progress some almost completed. Preliminary results follow.

Our previous testing on BeyondC showed that the product inhibits Reactive Oxygen Species formation in healthy human neutrophils. The effect in this protocol is also an **indication that the antioxidant compounds in BeyondC are available and can penetrate to the interior of healthy intact human immune cells.**

As a result, we must **assume** that **the product is available to penetrate into cytoplasm, mitochondria and nuclei of** healthy and **malignant cell types**.

We propose to test the following:

1. Support for anti-inflammatory action beyond a simple anti-oxidant effect:
    a. Cox-2 induction
    b. PGE-2 production
2. Effects on known immune reactions:
    a. PHA-induced lymphocyte proliferation
    b. IL-2 induced NK cell activation

Effects on human tumor cell growth.

PRELININARY FINDINGS

The lab has finished testing Beyond C and several things worked as expected, in terms of Beyond C modulating responses to known stimuli. **Beyond C synergizes with IL-2 in several tests**.

In addition, the lab got a big surprise. **Beyond C directly induces much higher levels of the interleukin-2 receptor (CD25) than IL-2 does on its own.** They had not expected a direct effect, only modulation to other responses.

A small pilot test was performed on Beyond C/BCL-2. The **data suggests that possibly Beyond C protects healthy lymphocytes from apoptosis.**

Additional testing at the lab put a new approach on the conclusions on the **highest dose of BeyondC (10g/L).** It **makes all lymphocyte subsets highly sticky, and severely interferes with immunophenotyping.** Even though **the researcher at the lab** personally **interpreted this is a result of activation**, they can not distinguish activation markers from

negative controls – all are sky-high. Therefore, we must **conclude that there is a direct effect**, but the apparent expression of activation markers must be interpreted with caution.

The most interesting **data are for the dose 0.1g/L**. Here **we have a re-direction of NK cell programming,** and a direct effect of at least a subset of T cells. There is inhibition of PHA-induced cell proliferation, and the increased expression of the IL-2receptor CD25 is synergistic with IL-2.

Update Report on further Testing Immunomodulation by Beyond C on Human Lymphocytes in vitro in a different Lab by another assay procedure for continued research. Report February 2006

Test types: **IMMUNE MODULATION:**

Lymphocyte Proliferation assay, human

- Modulation of PHA-induced proliferation
- Mitogenic activity of product

NK activation assay, human

- Modulation of IL-2-induced NK cell activation
- Direct activation of NK cells

Addendum: **ANTI-APOPTOTIC EFFECTS**

Changes in intracellular levels of BCL-2 protein – pilot

Objective

To evaluate possible immunomodulating effects of BeC on human lymphocytes in vitro.

Background for this study

Our previous testing on BeC showed that the product inhibits Reactive Oxygen Species formation in healthy human neutrophils. The effect in this protocol is also an indication that the antioxidant compounds in BeC are available and can penetrate to the interior of healthy intact human immune cells. As a result, we must assume that the product is available to penetrate into cytoplasm, mitochondria and nuclei of healthy and malignant cell types. It can be speculated that cellular energy metabolism would be affected, and that this central activity could be seen as a measurable change in many different types of cell behavior. This project aimed at testing this in a select series of in vitro assays, where the immune response to well-known activators would be compared in the presence versus absence of BeC.

REMARKS:

We found that BeC alone did not induce lymphocyte proliferation. There is a slight loss of membrane dye at the lowest dose of BeC, but this was similar to untreated cells. **This was seen in three experiments, all performed in triplicate**. Due to several other aspects of this analysis, this was interpreted as an **indirect indication that the higher doses of BeC may have protective effects against spontaneous apoptosis in the cell cultures,** and that the lowest dose of BeC did not display this protective effect, leading to the slight loss of membrane dye. **Further evaluation of the possible protection from apoptosis is warranted.**

----We conclude that **BeC is non-mitogenic** alone.

----It exhibits **strong immunomodulation** in this experimental model

--- **BeC inhibits the IL-2-induced expression of CD69 on activated NK cells in vitro**

--------- BeC 0.1 g/L: We still observed a strong inhibition of IL-2-induced CD25 expression, even stronger than at the higher dose of BeC.

----------BeC 0.001g/L: Inhibition of IL-2-induced CD25 was seen

Note: The highest dose of 10g/L BeC lead to unexpected staining patterns of human lymphocytes (T, B, and NK cells). As no cell death was observed at this high concentration of BeC, we presume that this is due to activation. However, we cannot conclude whether the activation markers are expressed or whether the cells are expressing novel cell surface antigens, recognized by the control antibody. The effect is significant ($p < 0.00002$).

Several observations pointed towards a **possible effect on protection from apoptosis.** We propose that this is further addressed in a small series of tests on healthy and malignant T cells in vitro. A protocol is attached to describe this proposed further work.

PROPOSED FUTURE TESTS, WHICH WE ARE PREPARING FOR.

1. Protocol 3: BeyondC – Possible anti-apoptotic effects. Continuation of in vitro work.

Purpose

The purpose of this **study** is to perform an assessment of **possible protective effects of BeyondC** on **spontaneous and induced programmed cell death (apoptosis)** of human lymphocytes ex vivo/in vitro and the Jurkat T cell line.

In this present protocol, we propose to test the following:

1. Effects of BeyondC on BCL-2 expression in healthy human T and B cells in vitro.
2. Effects of BeyondC on apoptosis:
    a. Spontaneous apoptosis of
        i. Freshly purified lymphocytes from healthy donors;
        ii. Jurkat T cell line;
    b. Fas-induced apoptosis on
        i. Healthy human lymphocytes cultured with PHA;
        ii. Jurkat T cell line.

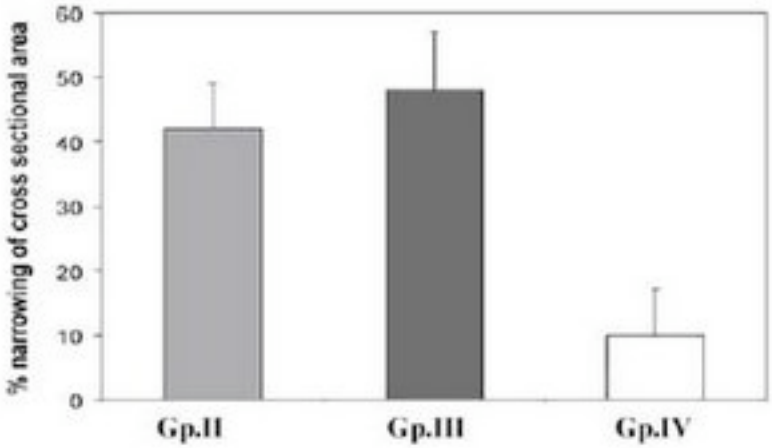
2. A bioavailability and plasma level study in comparison to Ascorbic acid and Ester C. This has already been done in two Guinea Pig studies (using a 9 pig study and confirming it with a 30 pig study) and one human study using a considerably lower metabolite percentage than that used in the Beyond C which showed approximately twice the amount of Vitamin C in plasma levels as Ester C and with a longer half life curve.

3. A Cholesterol Study–to see how Beyond C rates compared to statins and regular ascorbic acid as indicated below.

High-Dose Vitamin C Eradicates Cholesterol From Artery Walls

Researchers in New Delhi, India now demonstrate the cholesterol-eradicating effect of high-dose vitamin C in animals. Using rabbits that were **force fed a high-cholesterol diet, or a high-cholesterol diet plus low or high-dose vitamin C, the researchers conclusively showed the power of vitamin C to prevent narrowing of arteries with cholesterol plaque.**

The low-dose group of rabbits were given the human equivalent of about 350 milligrams of vitamin C, and the high-dose group the human equivalent of 11,000 milligrams of vitamin C per day. The chart below shows the percentage of arterial narrowing by cholesterol. Group II was fed cholesterol, no vitamin C. Group III was fed cholesterol + low-dose vitamin C. Group IV was fed cholesterol + high-dose vitamin C. Arterial narrowing declined from about 40-50% to ~10% with high-dose vitamin C. **The study was published in the advanced online Feb. 14 issue of Molecular & Cellular Biochemistry 2006**



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They apply **only t**o the researched product **Beyond C** and the RNA enhanced version **Bio En'R-G'y* C** for which **Beyond C** is the basic Vitamin C used with **Ribose Nucleotide Activation.**

*** Bio En'R-G'y – Biologically Enters Ribose Gateway**

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