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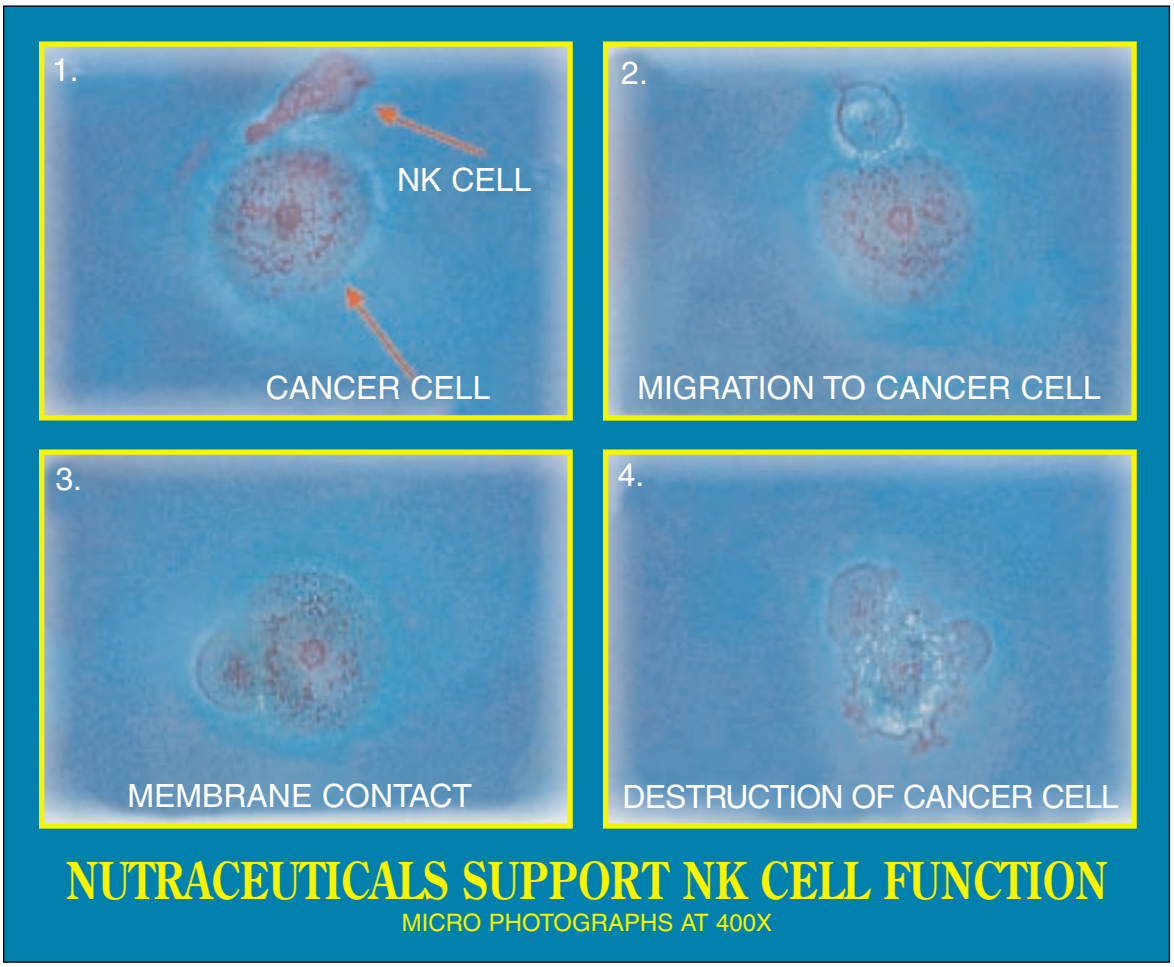

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ORIGINAL RESEARCH

Pilot Study: Effect of PDS-2865 on Natural Killer Cell Cytotoxicty

[1]Steven E. Chavoustie, MD, FACOG,* [2]Pedro P. Perez, PhD, [3]Mary Ann Fletcher, PhD, [3]Kevin J. Maher, PhD, [1]Alberto A. Mitrani, MD, [1]Robert H. Thomas, MD

1. Executive Women's Health, Coral Gables, Florida

2. Director, New Products Research and Development
Innovation Laboratories, Inc., Miami, Florida

3. E. M. Papper Clinical Immunology Laboratory
University of Miami School of Medicine, Miami, Florida

ABSTRACT

Objective: To determine if a dietary supplement (PDS-2865) can enhance NK cell cytotoxicity.

Methods: Five healthy patients, 2 men and 3 women, between 18–68 years of age were enrolled in the pilot study. Baseline blood samples were drawn from all participants at the same time and were hand delivered to the reference lab at the University of Miami Clinical Immunology Laboratory. Following a daily dose of 3 grams of the study product for two weeks, blood was again drawn and sent to the reference laboratory to measure natural killer cells (NKC) cytotoxicity as compared to baseline. Cytotoxicity was measured using the ^{51}Cr release assay.

Results: Post treatment results demonstrated a clinically and statistically significant increase of 17.08%. As shown in Table 1, natural killer cell cytoxocity increased nearly threefold from a mean of 10.1% (range 5.5% to 18.7% at baseline) to 27.2%, (range 13.0% to 45.3% post treatment), $p< 0.05$. NK cell cytotoxicity (NKCC) increased in all 5 study participants. There were no reported adverse effects.

Conclusion: As PDS-2865 demonstrated its ability to serve as a potent immunostimulator in the 5 patients that participated in this pilot study, the results were significant enough to warrant an expanded controlled study that has enrolled 20 patients and will begin in June 2003.

INTRODUCTION

An open label pilot study was conducted to study the effectiveness of the dietary supplement PDS-2865 as a potential immunostimulator of natural killer cell cytotoxicity (NKCC) in vivo. Natural killer cells are another type of lethal lymphocyte, which lyses certain virus-infected, tumor-derived, or selected normal cells without the benefit of previous immunization. They are a possible first line defense against tumor development. Like cytotoxic T cells, they contain granules filled with potent lytic agents that target tumor cells and protect against a wide variety of infectious microbes. Natural killer cells may also contribute to immunoregulation by secreting high levels of influential lymphokines.

PDS-2865 is a naturally obtained hemicellulose (high molecular weight polysaccharide complex) containing arabinoxylan, arabinogalactan, and a fatty acid mixture extracted by a proprietary process from a natural blend of

* *Correspondence:*
Steven E. Chavoustie, MD, FACOG
Clinical Director, Executive Women's Health
283 Catalonia Avenue
Coral Gables, Florida 33134
Phone: 305-753-7561 Fax: 305-668-2542
Email: schav@compusource.net

Table 1. Natural killer cell cytotoxicity n = 5

Patient	Baseline		2weeks		%change
# 1	18.7%		45.30%		26.60%
# 2	6.9%		17.70%		10.80%
# 3	12.0%		26.30%		14.30%
# 4	5.50%		13.00%		7.50%
# 5	7.40%		33.60%		26.20%
Mean	**10.1%**		**27.18%**		**17.08%**

***** % change is computed as the difference between 2 weeks and baseline calculation

Graminiae, Poaceae, and/or Dioscoreaceae family of plants. The research history is well documented and began in the 1940s. Studies revealed that the laboratory extraction process broke down or "pre-digested" polysaccharide molecules into smaller components called hemicellulose. Hemicellulose compounds are more water soluble and digestible, making them an ideal food supplement.[1-3] Of these hemicellulose compounds, the arabinoxylanes are the most potent immunostimulators.

PATIENT SELECTION PROFILE

Five healthy patients, 2 men and 3 women between 18–68 years of age, were enrolled in this initial study. Participants did not change their daily routines during the study period, including dietary or nutritional supplement habits.

METHOD

The study was conducted as an open label, single center study to evaluate the effectiveness of PDS-2865 at increasing natural killer cell (CD56) cytotoxicity. Five healthy participants had baseline blood drawn at the same time of the morning. Blood samples were hand delivered to the reference laboratory within one hour for analysis.

NKC cytotoxicity was measured using the chromium release assay protocol at the Univiverstiy of Miami Clinical Immunology Laboratory. The whole blood protocol used relates the cytolytic activity of the blood sample to the number of cells in the sample that are phenotypically NK cells, as determined by flow cytometry and lymphocyte count. Release of radioactive sodium chromate from labeled target cells is still the most commonly used marker of target cell lysis. Features of ^{51}Cr-release assays are: incubation of sensitized lymphocytes with ^{51}Cr-labeled target cells, collection by centrifugation of the ^{51}Cr-containing supernatant fluids and quantitation of the amount of ^{51}Cr released with a gamma counter. The whole blood assay was selected for this study since it more closely reproduces physiological conditions.

Preparation of chromium labeled cells: Target cells for the assay were the K562 cell line grown in culture to log phase, then labeled with ^{51}Cr. Labeling of target cells required 100 µCi of ^{51}Cr for each 20x10^6 cells. These were incubated 1 hour at 37°C in 5% humidified atmosphere with gentle shaking every 15 minutes, then washed 4 times and resuspended to make dilutions of labeled target cells to final concentrations of 2, 1, 0.5, and 0.25 x 10^6 cells/ml.

Plating: Dispense 150 µl of whole blood with repeat dispenser with sterile tips to one row of 12 wells: for spontaneous release control dispense 150 µl of assay medium to one row of 12 wells; for total release control dispense 150 µl of 1% Triton X-100 to one row of 12 wells. Dispense 50 µl to each of 3 wells for each dilution of ^{51}Cr-labeled target cells; cover microtiter plate and centrifuge 10 minutes at room temperature at 400 x g; and incubate for 4 hours at 37°C in 5% CO_2 humidified atmosphere

Harvesting: Terminate incubation by dispensing 100 µl chilled (4°C) assay medium to each well corresponding to either specimen, spontaneous release, or total release control wells; cover the plate and centrifuge for 10 minutes at 400 x g. Carefully transfer 100 µl of supernatant fluid to counting tubes (do not disturb the cell pellet) in the following order: first spontaneous release control, then total release control followed by patient samples; count supernatant fluids in gamma counter.

Lymphocyte count: In order to relate the cytotoxic potential of the blood sample to the cells in the sample, which are phenotypically NK cells, it is necessary to have a lymphocyte count. This is most conveniently and accurately done with an electronic hematology analyzer. This analysis also gives the hematocrit that is needed in the final calculation.

Flow cytometry: Lymphocytes that are CD56+ but CD3- comprise the bulk of cells capable of NK cytotoxic activity. Four-color flow cytometry done on each sample permits the determination of the percent of NK cells in the sample. The percent of CD56+CD3- cells times the number of lymphocytes gives the number of NK cells per ^{3}mm.

Calculations*:* Subtract background counts from all experimental, spontaneous, and total counts; and calculate % cytotoxicity (CYT) for each dilution of labeled target cells as follows: %CYT = ((ER-b) – (SR-b)/(TR-b – (SR-b)) x 100 where ER = mean cpm of experimental release; SR = mean cpm of spontaneous release; TR = mean cpm of total release; Vt = total volume in well; Vb = volume of blood in well; HCT = hematocrit of blood sample; and b = gamma counter instrument backgound in cpm. Using a regression analysis, % CYT at an effector to target ratio of 1:1 is calculated, where the number of effector cells is defined as CD3-CD56+ lymphocytes in each well.[4]

Each participant enrolled in the study underwent a physical examination and provided a complete history as well as baseline CBC, platelets, metabolic profile, and flow

Table 2. Flow cytometry measuring NK cells before and after treatment. n = 5

Baseline cells/uL	2 weeks after treatment cells/uL	% change
375	372	-.8%
52	62	19.2%
107	123	14.9%
63	116	84.1%
393	310	-21.1%
		Mean increase =19.3%

cytometry to measure natural killer cells (CD 56) and T-lymphocytes (CD3), T helper/inducer, T-cytotox/suppressor, CD4+/CD8+ ratio, and B lymphocytes (CD19). Following initial examination, the study group was given a two-week supply of PDS-2865 supplement to begin taking at the dosage of 3 grams per day in divided doses. The PDS-2865 extract (active material) was obtained from a natural blend of Graminiae, Poaceae, and/or Dioscoreaceae family of plants and produced by Innovation Laboratories, Inc. (Miami, Florida, Innovation lot 1000-03062-A), with a practical yield of 26% by weight and then formulated by Nature's Value, Inc, (Bayshore, New York) in 00 gelatin capsules containing 750 mg of PDS-2865. The 3 gram/day dose in the present study was chosen based on previous studies demonstrating an efficacy at the 15 mg–45/kg/day dosage.[5] Blood was drawn again at the two-week endpoint. All blood analysis for NKC cytoxicity was drawn at the same time of the morning as the initial draw because of circadian variation in NKC activity. Blood samples were hand delivered to reference lab within one hour from draw and analyzed for NK cell cytotoxicity, flow cytometry, and CBC. All blood samples were analyzed at the University of Miami Clinical Immunology laboratory.

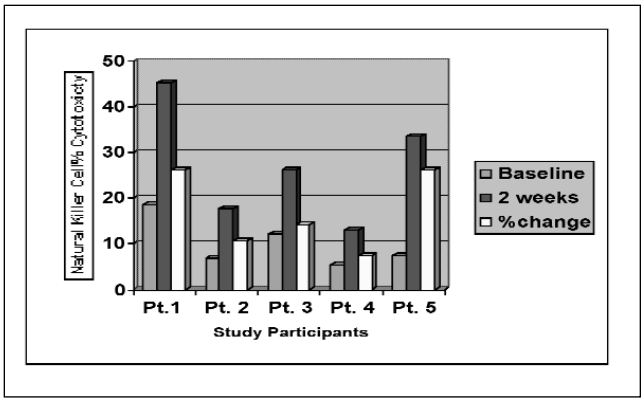
RESULTS

Initial data for the five participants are summarized in Table 1. The mean percent NKCC at baseline was 10.1% (N = 24% to 53%). After two weeks on supplement, the mean percent NKCC was 27.18%. The overall mean NKCC increased 17.08%.

In Table 2, flow cytometry was used to evaluate the quantitative number of CD56+CD3 cells (NK cells). Results showed a mean increase of 19.3% in NK cells after treatment with PDS-2865 as compared to before treatment.

In Figure 1, the bar graph analysis demonstrates the overall percent change of NKCC after 2 weeks on PDS-2865 supplementation. The results demonstrate that NKCC increased in all 5 study participants. These results are statistically significant (P<.05). No participants reported any adverse effects at the 3 gram/day dose.

Figure 1. NK cell % cytotoxicity, measured at baseline and 2 weeks on PDS-2865 with % change.



STATISTICAL METHODS

Assessment of the significance of the mean changes (absolute and percentage) from baseline in the NKCC and flow cytometry variables was made within the treated group, using paired t-tests demonstrating the effects were significant ($P < 0.05$). All analyses were performed using Version 6.06 of the SAS® System.

DISCUSSION

In the present investigation, we demonstrated that PDS-2865 enhanced NKCC over a 2-week period of time. The mechanism by which PDS-2865 augments the NKCC is not fully understood, but may involve enhancement of the binding capacity of NK cells to tumor cells and increased production of interferon-y and tumor necrosis factor.

Studies show other modalities that augment NK cell activity include various nutritional supplements such as MGN-3, echinacea, and ginseng. Stress management and relaxation have been shown to enhance NKCC, as has massage therapy. [6-9] Further investigation looking into potential beneficial effects of PDS-2865 on patients with cancer, chronic fatigue syndrome, hepatitis, and HIV will be considered.

CONCLUSION

The results from this pilot study are encouraging as an increase in NKCC occurred in all study participants. The response generated in this initial pilot study was significant enough to warrant further investigation in an expanded, controlled study that will begin on 20 participants in early June 2003. In conclusion, this pilot study on PDS-2865 demonstrated it to be a safe and effective immunopotentiator with no known adverse effects.

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