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The hemicellulose preparation, (Natramune™ (PDS-2865)®, increases macrophage phagocytosis and nitric oxide production and increases circulating human lymphocytes levels

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Hemicellulose containing nutritional supplements demonstrate benefits to the immune system *in vitro* and *in vivo*. Here we show that Natramune (PDS-2865) stimulates phagocytosis, nitric oxide production and boosts viability in J774A.1 murine machrophages. We also show that dietary supplementation with Natramune (PDS-2865) significantly increases the levels of circulating lymphocytes in human subjects.

Material/Methods: In order to measure the beneficial effects of Natramune (PDS-2865) on cells of the immune system, phagocytosis was measured by J774A.1 uptake of fluorescently labeled *E. Coli* bioparticles, nitric oxide production was measured by the formation of nitrite, and cell proliferation and viability was measured by NADH reduction of WST-8. The effect of Natramune (PDS-2865) on human circulating leukocyte levels was measured in 18 volunteers after an 8 week regimen of two 250 mg doses daily after which blood was collected and blood cell number and types were counted.

Results: Natramune (PDS-2865) stimulated phagocytosis, nitric oxide production and promoted proliferation/viability in J774A.1 cells by 65%, 517%, and 155% respectively. Further, Natramune (PDS-2865) did boost human circulating total lymphocyte levels (18%) in a statistically significant manner and while all lymphocyte subtype levels also increased, the individual subtype increases were not statistically significant.

Conclusions: Dietary supplementation with Natramune (PDS-2865) enhances immune system function and vitality.

key words: **natramune (PDS-2865) • hemicellulose • nitric oxide • phagocytosis • supplementation • cell proliferation**

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BACKGROUND

Natramune (PDS-2865) is a hemicellulose mixture containing amino acids, oligosaccharides, glycoproteins, polyphenols and fatty acids which have been extracted from mushrooms and plants in the gramineae, poaceae, and dioscoreaceae families. Hemicelluloses are fungal and plant cell wall polysaccharides which are available to human metabolic enzyme activity. Dietary supplementation with hemicelluloses such as Natramune (PDS-2865), arabinoxylan, arabinogalactan, and other plant and fungal cell wall polysaccharides and derivatives have been shown to have a positive impact on the function of cells of the immune system. For example, an arabinoxylan derivative from rice bran (MGN-3) has been shown to increase macrophage phagocytosis [1,3], stimulate lymphocyte cytokine production [2], and inhibit p24 production and syncytia formation in HIV-1 infected T-cells [4]. Further the Juzen-Taiho-To (JTT) herbal extract contains cell wall polysacharide components and has also been shown to enhance phagocytosis, cytokine production and antibody secretion [5,6] as well as have activity against malignant glioma through antiangiogenic activity [7]. Further, the hemicellulose nutrient mixture, Natramune (PDS-2865) has been shown to elevate lymphocyte cytokine production, increase natural killer cell destruction of cancer cells and reduce xenobitic-induced T-cell mediated inflammatory damage [8,9].

Macrophages play an important role in the host-mediated destruction of infectious micro-organisms as well as in the activation of the specific immune response. In both of these cases, through the process of phagocytosis, macrophages engulf exogenous cells and materials and process them through a series of endocytic vesicles ultimately geared toward the lysis and destruction of the engulfed material. Nitric oxide (NO) has been implicated in this mechanism of phagocytosis in a variety of the, afore mentioned stages. For example, lippopolysaccharide and interferon gamma activate macrophage-mediated NO release [10]. Further, nitric oxide release in as important signaling molecule in the initiation of phagocytosis by retinal pigmented epithelia cells and inhibition of nitric oxide production has been shown to reduce phagocytic activity in Kupffer cells and macrophages [12,13]. Additional roles for macrophage-derived nitric oxide have also been described and include direct toxicity to pathogens as well as increased macrophage migration and inflammation [14 and reviewed in 15].

Here we investigate the effect of the hemicellulose nutrient mixture, Natramune (PDS-2865) on macrophage phagocytic activity, nitric oxide production and proliferation/viability. We also measure circulating leukocyte levels in volunteers after eight weeks of Natramune (PDS-2865) supplementation.

MATERIAL AND METHODS

Clinical studies

The 18 volunteers (15 healthy and 3 immunosuppressed with either Cancer, HIV or Hepatitis C) enrolled in this open label; single center study had an average age of 45 years (ranging from 18–75 years). They were scheduled for four visits, maintained a regular diet and recorded times of supplementation and meal consumption daily in product use diaries. Volunteers could not be using another immunity boosting product, alternative therapies, or dietary supplements 8 weeks prior to or during the study. Subjects were assigned to take two 250 mg (twice daily) (Natramune™ (PDS-2865®) treatment with instructions to consume the coded supplement capsules two in the morning and two in the evening daily for 8 weeks. Weight, medical history, brief physical, blood pressure, patient symptom checklist, CBC, platelets, basic metabolic panel, and flow WBC, NK cells, T-lymphocytes, T helper/inducer, T cytotoxic/suppressor, B lymphocytes, natural killer cell cytotoxicity, creatinine, and blood glucose were measured at baseline and then at 4 and 8 weeks. All diaries, study capsules, capsules container were collected at each clinic visit. Compliance with treatment supplement regiment was determined by a capsule count and product use diary review.

Phagocytosis

J774A.1 murine macrophages were plated in 96 well plates on day 0 at 1×10^5 cells/well in DMEM medium containing 10% FBS. These plates were incubated for one hour at 37°C in a CO2 incubator. Natramune (PDS-2865) and Levamizole hydrochloride were added at the various and indicated concentrations and incubated for an additional hour at 37°C. After 1 hour, 25 µl of fluorescent bioparticles were added to the macrophage cultures and incubated for an additional two hours. After two hours the cell culture supernatant was discarded and trypan blue was added to the cell pellet and this was incubated for one minute and the plates were reader on a Floustar fluorescent plate reader at 485 nm and 520 nm. The fluorescence measured with no treatment was considered 100% of background phagocytosis.

Nitric oxide production

J774A.1 murine macrophages were plated in 96 well plates at 5×10^5 cell/ml in 200 µl/well in DMEM containing 10% FBS and incubated overnight at 37°C in a CO2 incubator. After the overnight incubation, the old media were discarded at 100 µl fresh media was added to each well. Natramune (PDS-2865) was added at the various indicated concentrations as well as LPS as a positive control to bring the total concentration in each well to 200 µl. The cells were then incubated for 48 hours, the supernatant was collected for nitric oxide production, and the cell pellets were used to measure proliferation as described below. For nitric oxide, 50 µl of the cell media supernatant were added to 96 well plates. To this 50 µl of Greiss reagent was added. The plates were then incubated at room temperature for 10 minutes in the dark and the plate was read in a micro plate reader for absorption at 540 nm.

Cell proliferation and viability

Cell proliferation and viability was measured using the WST-8 assay. Briefly, the cell pellet from the nitric oxide assay above was resuspended in the wells in 100 µl of fresh medium and 10 ml of the WST-8 reagent was added to each well. The cells were incubated for an additional two hours and then the plates were read for optical density on a micro plate reader at 450 nm.

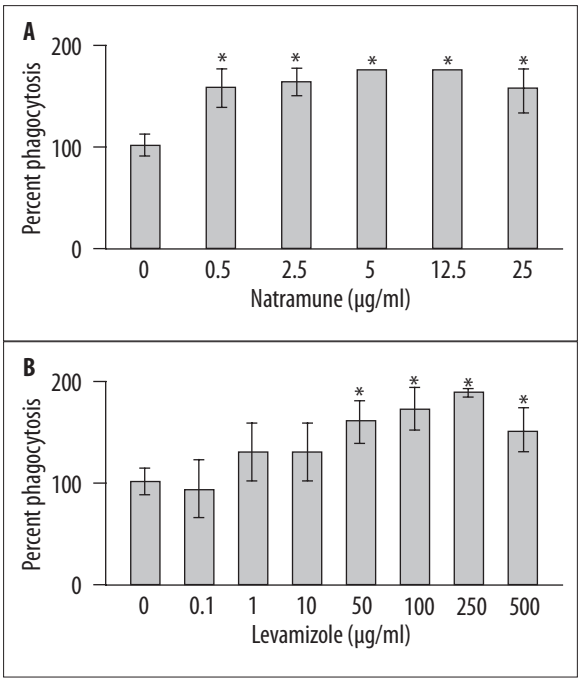

Figure 1. Natramune (PDS-2865) stimulates J774A.1 murine macrophage phagocytosis. (**A**) Natramune (PDS-2865) (**A**) and Levamizole (**B**) induced phagocytosis is expressed as a percentage of control which were untreated cells. Error bars represent SD of four replicates per sample. Asterisks indicate statistical significance compared to the control (untreated) ($P \leq 0.001$).

Results

Phagocytosis was measured by internalization of fluorescently labeled *E. Coli* bioparticles in J774A.1 murine macrophages. Phagocytosis was increased statistically significant fashion with a 0.5 µg/ml Natramune (PDS-2865) treatment (Figure 1A). Further increases in phagocytosis were also significant with a 2.5 and 25 µg/ml treatment increasing phagocytosis by 62% and 65% respectively and maximal stimulation at 5 µg/ml and 12.5 mg/ml treatments which both showed a 71% increase (Figure 1A, Table 1). All Natramune (PDS-2865) treatment level did give a statistically significant increase when compared to untreated controls (Figure 1A, Table 1). The increases in phagocytosis observed with Natramune (PDS-2865) are similar to those observed with levamizole, a known activator of phagocytosis. The maximal increases in phagocytosis with levamizole were 71% (Figure 1B).

Natramune (PDS-2865) also stimulated nitric oxide release by the J774A.1 macrophages in a dose dependant fashion (Figure 2). When treated with less than 0.5 µg/ml Natramune (PDS-2865), nitric oxide production was not increased, however, at and above this level a statistically significant increase in nitric oxide production was measured (Figure 2). A maximum of slightly over a five-fold (518%) increase in nitric oxide production was observed with 25 µg/ml l Natramune (PDS-2865) treatment (Figure 2, Table 1). This compares to slightly over a 9-fold increase in nitric oxide production with the LPS control treatment (Figure 2).

Table 1. The benefits of Natramune™ (PDS-2865®) on immune system cell numbers and function.

	Effect of Natramune (PDS-2865)	
	(% change)	Reference/Study
***In vivo* Circulating cell levels**		
Total leukocytes	+14.5	Present study
Total lymphocytes	+18.0*	Present study
B-lymphocytes	+10.1	Present study
T-Lymphocytes	+6.8	Present study
Cytotoxic T-Lymphocytes	+18.3	Present study
Helper T-Lymphocytes	+10.0	Present study
Natural Killer Cells	+14.4	Present study
***In vitro* cellular function**		
Killer cell response rate	+15.0*	Chavoustie et al. [1]
Killer cell cytotoxicity	+26.0*	Chavoustie et al. [1]
Phagocytosis	+71.0*	Present study
Inflammation (interaction with matrix)	–80.0*	Weeks et al. [9]
Nitric oxide production	+518.0*	Present study
Monocyte viability	+155.0*	Present study

* Statistically significant changes when compared to pre-supplementation and pretreatment values.

Cell viability and proliferation was also increased by treatment with Natramune (PDS-2865) (Figure 3). A statistically significant increase in viable cell number a population of J774A.1 macrophages treated for 48 hours with 2.5 µg/ml of Natramune (PDS-2865), with a maximum of a 155% increase in viable cell numbers with a treatment of 25 mg/ml of Natramune (PDS-2865) (Figure 3, Table 1).

In the cohort of 18 volunteers who were supplemented with Natramune (PDS-2865) for eight weeks, an analysis of variance (ANOVA) was used to assess pre- and post-treatment levels of circulating total leukocytes and leukocyte subpopulations. We observed a significant increase in circulating total lymphocyte levels, in both the healthy subjects and those with impaired immunity ($p=0.039$) (Table 1). Further, increases were observed in the number of total leukocytes, and T-cell, B-cell and NK cells subsets, but these changes were not statistically significant changes (Table 1). These results compare to earlier studies in which Natramune (PDS-2865) supplementation in volunteers did not increase circulating NK cell levels but did lead to increases in both NK cell activity and NK cell response rate ($p=0.025$) (Table 1).

Discussion

Dietary supplementation with hemicellulose mixtures has been shown to enhance immune system function and activity. Consequently, hemicellulose formulations have received

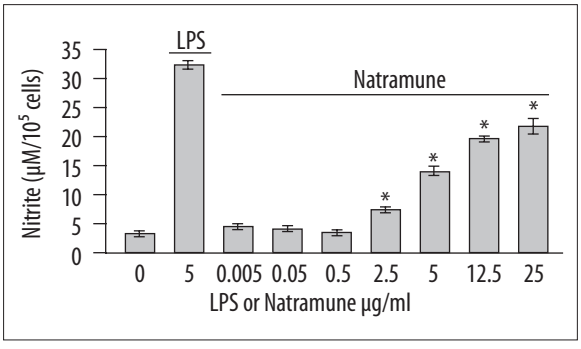

Figure 2. Natramune (PDS-2865) stimulates nitric oxide release in J774A.1 murine macrophages. LPS was added as a positive control. Error bars represent SD of four replicates per sample. Asterisks indicate statistical significance compared to the control (untreated) (P≤0.001).

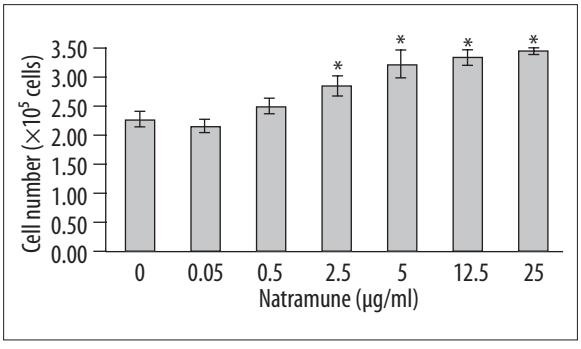

Figure 3. Natramune (PDS-2865) increases cell proliferation and viability in J774A.1 murine macrophages. The number of cells respiring cells was determined as described in the Materials and Methods section. Error bars represent SD of four replicates per sample. Asterisks indicate statistical significance compared to the control (untreated) (P≤0.001).

a great deal of attention as clinically beneficial nutraceuticals. For example, supplementation with either plant or fungal hemicelluloses as well as bacterial exocelluloses have been shown to enhance immune system clearance of fungal and viral diseases. This hemicellulose-enhanced immune system activity is associated with increased antibody and cytokine production, increased phagocytosis, reduced tissue damage, and antioxidant and free radical scavenging activity [1–4,10–15]. Many dietary fiber supplements are commercially available which contain various forms of hemicelluloses in order to help people take advantage of these dietary benefits. Natramune (PDS-2865) is one such product and has been shown to increase immune cell cytokine production, increase natural killer cell destruction of cancer cells and reduce inflammatory damage [8,9].

Here we show that Natramune (PDS-2865) supplementation increases circulating levels of lymphocytes in humans. Further, we find that Natramune (PDS-2865) stimulates macrophage phagocytic activity, nitric oxide production and viability. These data extend the observations that hemicellulose formulations can boost immune system function and can provide people with a dietary way to increase immune system function and enhance resistance to disease.

Conclusions

Natramune (PDS-2865) supplementation significantly increases human circulating lymphocyte levels. Further, Natramune (PDS-2865) stimulates macrophage viability and nitric oxide production and consequently phagocytic activity. Therefore, Natramune (PDS-2865) supplementation is a healthy and natural way to enhance immune system function.

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