

【Original】

Immunostimulating effects of oxygen nano-bubble water on human body

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ABSTRACT

The aim of the present study is to investigate the effects of oxygen nano-bubble water on human immunity in a randomized, double-blinded, placebo-controlled clinical trial. The participants consisted of twelve healthy volunteers. Six participants were given 500ml of oxygen nano-bubble water (Aqua Nano, Nano Science Inc ; Chiyoda-ku, Tokyo) to ingest orally daily for six weeks and the remaining six ingested unlabeled bottled water (Crystal Geysler, Ohtsuka Foods Co., Ltd) similarly. The immune status of the participants before and after the 6-week ingesting period was assessed by inspecting the subsets of T cells and T cell indices, which are known to decrease because of aging, stress, and disease. The numbers of naïve T cells were observed to increase significantly ($p=0.039$) in the participants ingesting oxygen nano-bubble water. Furthermore, the score of immunological vigor, which is a comprehensive scale of immunity determined from the numbers of T cell, naïve T cell, CD8⁺CD28⁺T cell, CD4/CD8 T cell ratio, and naïve/memory T cell ratio, increased by 13.4% with a marginal significance ($p=0.060$) in the participants ingesting oxygen nano-bubble water. These results suggest that oxygen nano-bubble water has an immunostimulating effect on the human body.

keywords

T cell immunity, scoring system, clinical trial, RCT

Introduction

The immune system functions as a defense mechanism of biological beings. When the function of the immune system declines, one's risk of contracting a disease increases. Thus, it is preferable for us to maintain and improve our immunity for our healthy life.

The immunological function is known to decrease with increasing age, and this phenomenon may be associated with the increasing the risk of infection and cancer among the elderly people^{1, 2)}. It is also known that immunity declines because of stress or disease as well as aging²⁾. Because factors such as stress or aging are unavoidable, we are always under risk of immunological deterioration. Therefore, we might have

a benefit by restoring our immunological function with the use of an immunostimulatory supplement.

The authors have focused on the strong physiological activity of oxygen nano-bubble water^{3, 4)}. Oxygen nano-bubble water contains nanometer-sized oxygen bubble nuclei and is expected to have a conditioning effect on the immune system because of its physiological activating effect on the living organism. However, there have been no reports on the effects of oxygen nano-bubble water on human immunity.

The aim of this study was to investigate the effects of oxygen nano-bubble water on human immunity in a randomized, double-blinded, placebo-controlled trial. Because there is a broad range of immunological

parameters, comprehensive understanding of human immunity is difficult. To assess the immune status of trial participants, we used an objective method of scoring human immunity that was recently developed by Hirokawa and Utsuyama⁵⁾.

Methods

The present clinical study was conducted in compliance with the Declaration of Helsinki and approved by the ethical committee at Seishin-kai Medical Association, Shinagawa, Tokyo. Written informed consents were obtained from the participants.

Participants

Twelve healthy volunteers (5 men and 7 women, age : 39.6 ± 10.4 y.o. [mean \pm SD]) were enrolled. We chose healthy volunteers who had felt daily fatigue subjectively because we intended that persons whose immunity was very strong would not enter into the trial, though so far there was no clear relationship between human immunity and his/her fatigue.

Test group

The participants were randomly assigned into two groups, the oxygen nano-bubble water intake group (3 males and 3 females, age 39.7 ± 13.6 y.o.) and the control group (2 males and 4 females, age 39.5 ± 7.4 y.o.), so that the average and SD of age, scoring of immunological vigor (described below), and the subordinate immunity parameter (the number of T cells, CD4/CD8 T cell ratio, naïve T cells, naïve/memory T cell ratio, and CD8⁺CD28⁺T cells) would have no significant difference between the two groups. Statlight #11 (Yukms Co. Ltd.) was used for the assignment.

Products under test

The oxygen nano-bubble water intake group ingested 500ml of oxygen nano-bubble water (Aqua Nano, Nano Science Inc.) a day, whereas the control group ingested 500ml of bottled water (Crystal Geysler, Ohtsuka Food Co., Ltd) a day. All the bottles were unlabeled and sent to participants' home by delivery service so

that testers and participants were not able to know the ingredients.

Test Protocol

The participants were instructed to ingest the test products daily in the given manner. There were no restrictions about when to intake the water. The intake period was 6 weeks. Inspection was held two times : just before intake and 6 weeks after starting intake.

Inspection items

Assessment of immunity was performed according to the method proposed by Hirokawa and Utsuyama⁵⁾. In their method, the parameters of immune functions that are most affected by aging, stress, and disease were measured to determine score of immunological vigor (SIV). Accordingly, we observed the immunostimulating effect of the test material when one's immunity has declined due to those factors. The score of immunological vigor (SIV), the comprehensive immunity score, was determined in the following manner : first, the numbers of T cells, CD8⁺CD28⁺ T cells, and naïve T cells, CD4/CD8 T cell ratio, and naïve/memory T cell ratio are individually scored as three levels (1 is "needs improvement", 2 is "needs observation", and 3 is "safe"). Next, the SIV was determined from the sum of the 5 scores, and the SIV therefore ranged from 5 to 15. We also estimated the T lymphocyte age, which is calculated from the number of CD8⁺CD28⁺T cells, the parameter describing one's immunity in terms of age.

Statistical analysis

A paired t-test was used to determine whether the difference of the immunological parameters between before and after 6 weeks of intake were significant. Also, the differences between the 2 groups of the changes between before and 6 weeks after intake were compared using a t-test. A p-value less than 0.05 was considered to be significant, and a p-value less than 0.10 but larger than 0.05 was considered to have marginal significance. These analyses were performed using statistical software IBM SPSS for Windows

ver. 18.0.

Results

All the participants completed the clinical trial and there was no adverse incidents ; therefore, data from all the participants were used in the analyses. Although there was no significant differences in subjective complaints such as fatigue, some of the immunological parameters of the oxygen nano-bubble water changed from baseline.

Table 1 shows the mean \pm S.D. of immunological parameters measured before and after 6 weeks. Fig.1 shows the SIV and naïve T cell number at before and 6 weeks after intake, and Fig.2 shows the changes in the SIV, naïve/memory T-cell ratio, lymphocyte, T-cell, CD8⁺T-cell, and the naïve T-cell of the Oxygen nano-bubble water intake group and control groups between before and 6 weeks after intake.

1) Oxygen nano-bubble water intake group

In the oxygen nano-bubble group, we observed significant change in the mean number of naïve T cells ($p=0.039$) and naïve/memory T cell ratio ($p=0.023$) . A marginal significance was observed in the CD8⁺T cells ($p=0.073$) and the SIV ($p=0.060$) . Although statistically significant changes were not observed in other immunological parameters, all these parameters also showed beneficial changes.

2) Control group

In the control group, there was no significant change in the immunological parameters.

3) Between-group comparison

Although there were no significant difference in the changes of the immunological parameters before and after 6 weeks of intake between the two groups, there was marginal significance for the changes in parameters, including the mean numbers of lymphocyte ($p=0.040$) , T-cells ($p=0.072$) , CD8⁺ T cells ($p=0.073$) , naïve T-cells ($p=0.081$) , naïve/memory T-cell ratio ($p=0.057$) , and SIV (p

$=0.071$) . The differences in the changes in these parameters were large for the oxygen nano-bubble group than for the control group.

Discussion

We examined the immunostimulating effect of six-week oral ingestion of oxygen nano-bubble water on healthy humans. We estimated the immune vigor by measuring the numbers of T cells and other subordinate parameters of the T cells that tend to decrease with aging, disease, and stress. Each immune parameter measured on average showed beneficial changes in the participants ingesting oxygen nano-bubble water compared with the participants ingesting plain bottled water. Particularly, the number of naïve T-cells and the naïve/memory T cell ratio showed significant changes before and after 6 weeks of ingestion of oxygen nano-bubble water.

The naïve T-cells had no information of the antigen, whereas the memory T-cells had proper information of the antigen. An individual with a large naïve/memory T-cell ratio would respond more rapidly to an unknown antigen. Therefore, the results of this study suggest that oxygen nano-bubble water may strengthen immune resistance to infectious disease and/or cancer. We also found that the increase in SIV, the overall measure of immune strength, tended towards significance in the nano-bubble group but not in the control group, where there was little change in immune parameters. This suggests that oxygen nano-bubble water has an immunostimulating effect on humans.

However, the in vivo mechanisms of action of oxygen nano-bubble water on human body, what organs are affected, and how it is absorbed, are still unknown. Future studies are warranted further elucidate the effects of oxygen nano-bubble water on human immunity.

Conclusions

Immune parameters that tend to decline in accordance

with aging, disease and other stresses showed beneficial changes after six weeks of oral ingestion of oxygen nano-bubble water. The results suggest that oxygen nano-bubble water has an immunostimulatory effect on humans.

References

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Table1 Mean values of Immunological items and results of the paired t-test.

Measurement Item	Standard Range	Group	Initial		6 weeks after		p
			M	SD	M	SD	
Neutrophil	1320/ul - 6750/ul	Nano Bubble	2987.9	1215.1	2420.1	591.1	0.264
		Control	2675.8	499.3	2983.6	570.7	0.163
Lymphocyte	594/ul - 4410/ul	Nano Bubble	1608.4	149.7	1875.4	377.5	0.105
		Control	1662.6	367.3	1516.7	378.5	0.243
T-cell (CD3 ⁺) ^a	1360/ul -	Nano Bubble	1161.2	83.8	1324.4	214.6	0.121
		Control	1019.5	187.3	936.0	198.2	0.378
CD4 ⁺ T-Cell (CD4 ⁺)	835/ul -	Nano Bubble	707.9	90.7	820.5	173.6	0.157
		Control	588.5	117.0	609.6	159.9	0.771
CD8 ⁺ T-Cell (CD8 ⁺)	418/ul -	Nano Bubble	358.2	130.0	421.7	149.7	0.073 [†]
		Control	296.5	106.7	292.5	121.0	0.824
CD4 / CD8 ratio ^a	1.30 - 2.55	Nano Bubble	2.25	0.93	2.19	0.90	0.578
		Control	2.20	0.88	2.27	0.84	0.696
Naïve T cell (CD4 ⁺ CD45RA ⁺) ^a	330/ul -	Nano Bubble	294.9	64.8	417.9	128.0	0.039 [*]
		Control	261.0	105.1	265.2	82.9	0.923
Memory T cell (CD4 ⁺ CD45RA ⁻)	459/ul -	Nano Bubble	413.1	73.8	402.6	108.1	0.799
		Control	327.5	62.0	344.4	105.1	0.677
N / M ratio ^a	1.0 -	Nano Bubble	0.74	0.21	1.08	0.33	0.023 [*]
		Control	0.82	0.32	0.80	0.25	0.894
CD8 ⁺ CD28 ⁺ T-cell ^a	238/ul -	Nano Bubble	221.7	68.6	263.7	108.9	0.120
		Control	225.9	66.5	221.9	97.0	0.844
T-lymphocyte Age	not available	Nano Bubble	46.7	13.7	44.3	15.5	0.264
		Control	47.0	5.7	46.3	6.5	0.618
Scoring of Immunological Vigor	13 -	Nano Bubble	11.2	1.0	12.7	1.9	0.060 [†]
		Control	11.2	1.0	11.0	1.1	0.771

^a : Item used calculating Score of Immunological Vigor. ^{**} : p < 0.01, ^{*} : p < 0.05, [†] : p < 0.10 vs Initial.

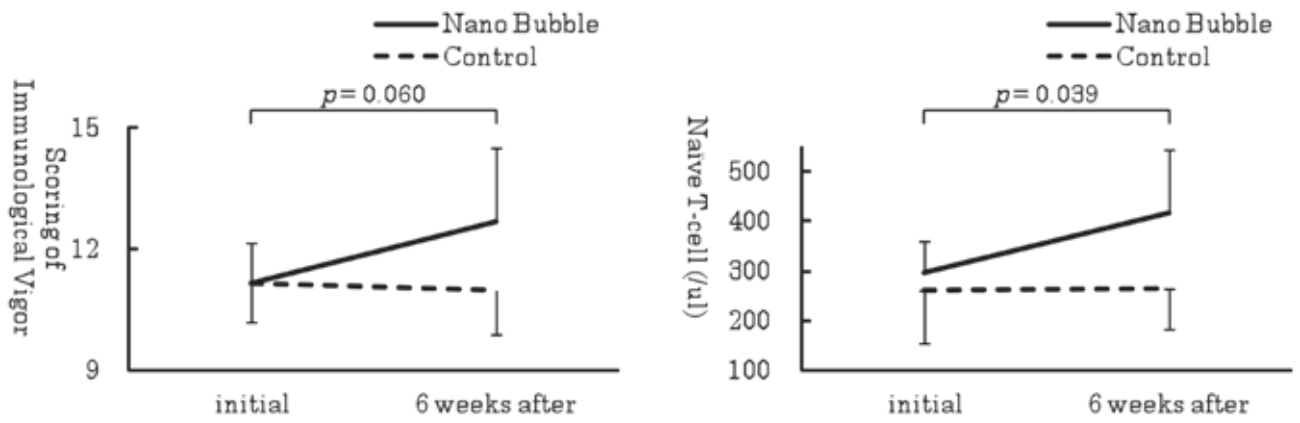


Fig. 1 SIV and naïve T-cell at before and after the intake.

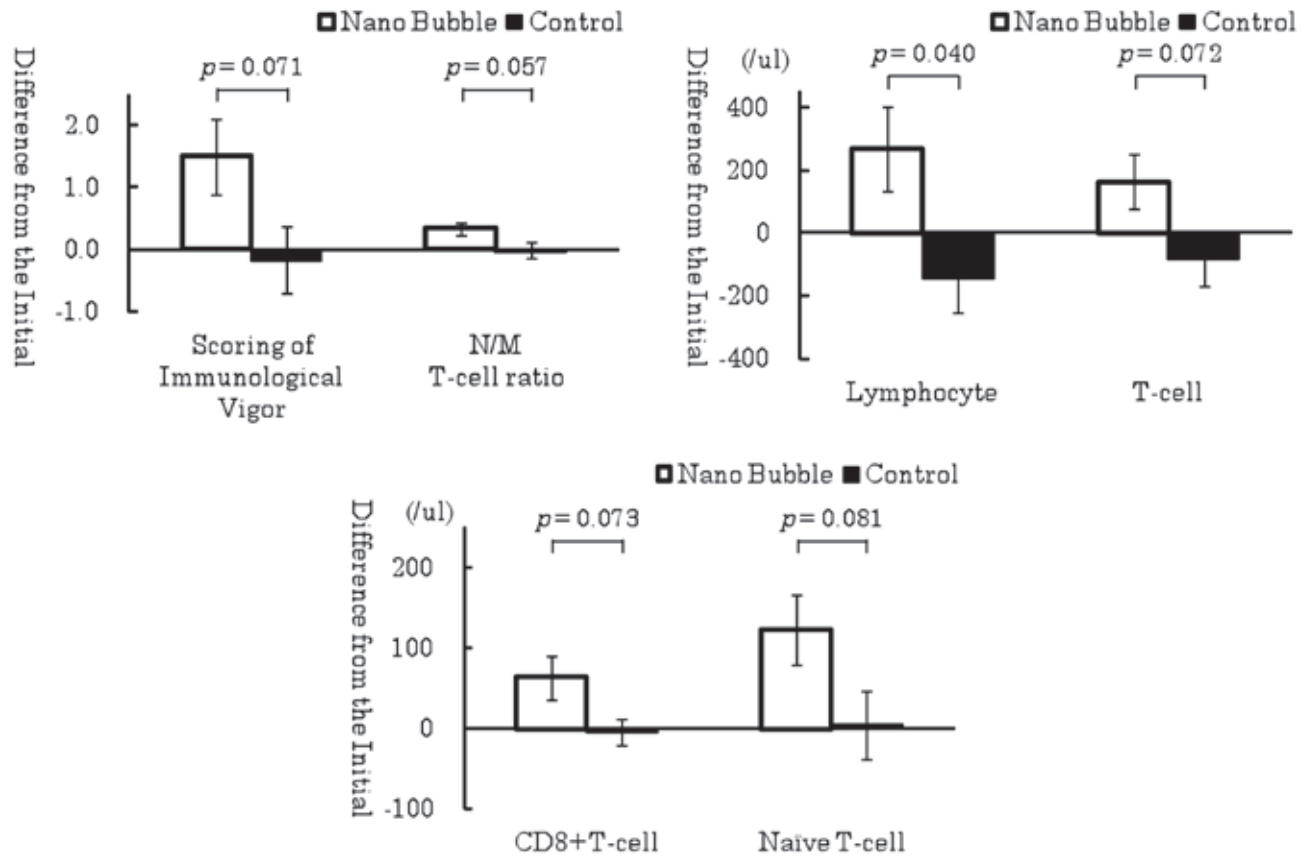


Fig. 2 Differences of SIV, N/M ratio, Lymphocyte, T-cell, CD8⁺T-cell, and naïve T-cell between before and after 6 weeks of intake.