Innovative Trial for QOL Assessment from Analog System to Digital One - in Case of Remodeled Water -

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Key words: QOL, digital assessment, constitution, remodeled water, leukocyte subset, macrophage, emotional hormone, immunological factors, anti-oxidative activity, diabetes meritus

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Abstract

Around 60% of the body weight consisted with water. So we tried to characterize the quality of the water for human physical factors. Lots of fundamental and clinical studies reported that many kind of water plays an important role in the physiological maintenance of humans as well as animals. This report was design to assess the influence of administration by remodeled water (RMW) on the development of physiological functions in human and and experimental animal model. RMW was different from many other water, different from molecule its self. RMW was proved to absorbed human odor molecule in vitro and in vivo. The animal model of experiment by immune-suppressive agent, RMW recovered the function of phagocytic cell in DM model. In a human trial for intestinal model were shown to increased lactobacillus group other than bacillus. More about odor factor, RMW decreased main odor elements ammonia and hydrosulfate to decrease the odor in the exhaustive faces even after 7days. The important thing was also digital way of assessment, not by in vitro technique but in vivo rout of approach. Most important function of oxidative process is a degradation of the microorganism in cytoplasm of phagocytic cell, phagosome. This function is fundamentally important for infectious agent for what the minimum level of activity is required. So the assessment is necessary discuss in an in vivo system in phagocyte. The digital approach of anti-oxidative assessment was also showed in this study.

Introduction

There are many kinds of medicine including Ayurvetic Medicine in the world, therefore it is not many who can receive benefit from the Western medicine like us in Japan. The World Health Organization classifies 65~85% of the world health control business into the “traditional medicine” . In other words, if these traditional medicines are practiced in the West, it is classified into the alternative medicine [1]. The medicine practiced in today’s Japan is called the Japanese medicine, and it is thought as one of the Far Eastern medicine like Chinese
medicine, African medicine and Uyghur medicine [2]. The important thing was also digital way of assessment, not by in vitro technic but in vivo rout of approach. Most important function of oxidative process is a degradation of the microorganism in cytoplasm of phagocytic cell, phagosome. This function is fundamentally important for infectious agent for what the minimum level of activity is required. So the assessment is necessary discuss in an in vivo system in phagocyte. The digital approach of anti-oxidative assessment was also showed in this report (3-13).

METHOD
Preparation of Remodeled Water
Commercially available remodeled water (RMW) labeled SOSEI SUI was purchased with (Sosei World Co, Ltd, Ueda/Tokyo, Japan). Conventionally available purified water that prepared with reverse dialyzed by the laboratory in Kanazawa Medical University was set up as control [14].

Absorbance/Solvent Test
RMW have many character in the molecule its self, exhibiting one eminent effect of RMW. The routine experiment were made for absorption test for odor factor gas elements, ammonia, Tri-Methyle Amine, Methyl Mercaptan, xylem, toluene etc. As shown in Fig 2, ammonia (Fig 2a) and Tri-Methyle Amine (Fig 2b) were immediately absorbed by RMW but not for Methyl Mercaptan Fig2c). Beside the material in the figure, Hydro Sulfate, Acetaldehyde, Formaldehyde were could absorbed by RMW but not xylem and Methyl Mercaptan (assayed and reported by Japanese Food Analysis Center, Foundation, Tokyo).  

Animal Experiment
Ten female seven-week-old ddY mice, were used for the acute oral toxicity study. The tests were carried out according to Ethics of the Organization for Economic Co-operation and Development (OECD) Test Guideline 401. The animals were kept at 24 ± 1°C, 50% relative humidity.

Animal Model for diabetes mellitus by Streptzotosin as immune-compromised host
In the mice model in immuno-competency reduction, male ddY mice, aged 7-8 weeks, were injected with Streptozotocin (5mg/kg) to induce diabetes mellitus in mice. Then, RMW was administered at freely for 30 consecutive days orally. The sterilized *conventional water* was chosen as controls. Four week later, their blood were withdrawn from their tail vain. Then, the number of leukocytes was counted in Bürker-Türk solution.

**Assessment of Anti-Oxidative Activity**

**Experimental Animals**

Eight week-old female C57BL/6 were purchased from animal Laboratory Service Corporation (Shizuoka, Japan). All mice were kept under specific pathogen-free conditions. Mice food and EMW and control water were freely accessible for each mouse. Housing temperature and humidity were controlled 24˚ C ± 1˚ C and 50%

**Reagents and Preparation**

As for the basic medium, HEPES buffer (HEPES 17 mM, NaCl 120 mM, Glucose 5 mM, KCl 5 mM, CaCl2 1 mM, MgCl2 1 mM) was prepared and sterilized by filtration. Phorbol 12-myristate 13-acetate (PMA, Sigma, USA) was diluted to 10− 6 M by dimethyl sulfoxide (DMSO, Sigma, USA) and used as a stimulant for super oxide anion generation for murine peritoneal exudates cells. Cytochrome c (Sigma, USA) was diluted to 1 mM by HEPES buffer. Since cytochrome-c reduced by super oxide showed maximum absorbance at 550 nm, we used cytochrome-c to measure the amount of super oxide anion generation through spectro-photometrical technique. Oyster Glycogen (Type II, Sigma, USA) was diluted in the purified water (10% w/v, Wako, Japan) and autoclaved at 120˚ C for 20 min. This solution was used for intraperitoneal injection.

**Recovery of Macrophage Activity, Phagocytosis**

Cells from peritoneal exudates were collected from the peritoneal cavity of bone marrow-suppressed mice. Phagocytes were purified using adherent technique to get cell suspensions which contained more than 95% of phagocytes. The same cells suspension was purified by adherent technique for phagocyte, which produces cells contained more than 95% of phagocytes. The purified cells were adjusted to 1x10^4 cell/cm^2 and mixed with latex beads that
are 5um in granule with fluorescence isochianate. After 90 min of incubation, remained granule were washed out from the glass slide. Number of phagocytic cell and their ability to catch up the latex beads were automatically measured by ACAS system, which outputs the result in a digital form (Adherent cell activity evaluating system; Shimazu, Kyoto, Japan)

HUMAN TRIAL: Participants

Sixteen participants were selected in this study (age 65±6.7). They were given self questionnaire on their blood sugar levels etc prior to investigation. After noting their medical history, we selected individuals who would participate in the experiment voluntarily. All participants gave their written informed consent prior to participation. The two group were selected for randomized study of this trial.

Statistical Analysis
Data are expressed as means ± standard deviations. The differences between RMW-treated and non-treated conditions were compared using a one-tailed analysis of variance. A P value < 0.05 was considered to be statistically significant.

RESULTS
Animal Test for RMW
Animal Safety Test for RMW
Single and Multiple Dose Toxicity Study for Conventional Water and RMW
Nine female seven-week-old ddY mice were used for the acute oral toxicity study. All mice were weighted at 0 - 8 days after administration of each water sample, and clinical observations were made once a day. Necropsy was tried to all mice on eight days after administration. No animal died or abnormalities of body weight, water and food consumption, or coat condition were seen in both group of mice. Necropsy evaluation of the mice did not reveal any significant differences in thymus, liver, spleen, kidney, adrenal gland and testicle weights between the control group and RMW group.

The Comparison of Generated Super Oxide
Antioxidant Assay is Necessary to put into \textit{in vivo} or \textit{ex vivo} System: The Comparison of Generated Super Oxide Anion in RMW

The temporal disappearance in human blood plasma of endogenous antioxidants in relation to the appearance of various classes of lipid hydro peroxides measured by HPLC post column chemiluminescence detection has been investigated under two types of oxidizing conditions. Exposure of plasma to aqueous peroxyl radicals generated at a constant rate leads immediately to oxidation of endogenous ascorbate and sulfhydryl groups, followed by sequential depletion of bilirubin, urate, and alpha-tocopherol. Stimulating polymorph nuclear leukocytes in plasma initiates very rapid oxidation of ascorbate, followed by partial depletion of urate. Once ascorbate is consumed completely, micromolar concentrations of hydro peroxides of plasma phospholipids, triglycerides, and cholesterol esters appear simultaneously, even though sulfhydryl groups, bilirubin, urate, and alpha-tocopherol are still present at high concentrations. None-sterified fatty acids, the only lipid class in plasma not transported in lipoproteins but bound to albumin, are preserved from per oxidative damage even after complete oxidation of ascorbate, most likely due to site-specific antioxidant protection by albumin-bound bilirubin and possibly by albumin itself. Thus, in plasma ascorbate and, in a site-specific manner, bilirubin appear to be much more effective in protecting lipids from per oxidative damage by aqueous oxidants than all the other endogenous antioxidants. Hydro peroxides of linoleic acid, phosphatidylcholine, and cholesterol added to plasma in the absence of added reducing substrates are degraded, in contrast to hydro peroxides of trilinolein and cholesterol linoleate. These findings indicate the presence of a selective peroxidase activity operative under physiological conditions. Our data suggest that in states of leukocyte activation and other types of acute or chronic oxidative stress such a simple regimen as controlled ascorbate supplementation could prove helpful in preventing formation of lipid hydro-peroxides, some of which cannot be detoxified by endogenous plasma activities and thus might cause damage to critical targets. Extracellular release of superoxide anion and hydrogen peroxide during the respiratory burst of porcine neutrophil/phagocyte was studied by using diacetyldeuteroheme-substituted horseradish peroxidase as a trapping agent for these oxygen derivatives. The method permitted simultaneous measurement of oxygen consumption and formation of both $\text{O}_2$ and $\text{H}_2\text{O}_2$ in a single reaction mixture. When neutrophils/phagocytes were stimulated
with phorbol myristate acetate in the presence of the home-substituted peroxidase, a rapid accumulation of compound, a complex of the enzyme with O$_2$, was observed accompanying an increase in oxygen consumption. During the process, amounts of compound formed and oxygen consumed were stoichiometric, and no compound II, an indicator of H$_2$O$_2$ formation, was observed. These results establish that neutrophil/phagocyte stimulated with the phorbol ester produce exclusively O$_2$ as the primary oxygen metabolite and release it into the extracellular medium. In case of activated water SOSEISUI (Sosei World, Co. Ltd, Nagano, Japan), anti-oxidative activity was exhibited compared than conventional water. (Fig 3, Fig 4).

When a limited amount of opsonized zymosan was used as the stimulus, compound formation was also observed but it ceased at an early stage of oxygen consumption. When a sufficient amount of azide was included in the system, however, formation of compound II was noted in the later stage of oxygen consumption. The findings suggest that O$_2$, formed during phagocytosis, is converted to H$_2$O$_2$ within phagosomes and then diffuses out into the extracellular medium when its decomposition by catalase and peroxidases are blocked by chelating agent [15-46]. Since the anti-oxidative effects of herbal medicine were reported by us, stressing to employ animal phagocytic cell other than in vitro system [15]. We investigated the significance of this way to confirm by animal cell, phagocyte. Since RMW include active hydrogen molecule in the structure, expecting the enhancement of its anti-oxidative effects. The generated super oxide anion after one week administration of conventional water and RMW, were 2.9±0.6 and 1.6±0.6 × 10$^{-5}$ mmol/ml, respectively, positive control =2.8±0.2. The value obtained by RMW was a half level of control is required level of active oxygen level in the phagocyte, minimally need for the phagocyte against foreign micro organism against infection. (Fig3, Fig4).

Recovery of Macrophage Activity, Phagocytosis by RMW in Experimental DM mice
Our data, Fig 5 showed that Streptozotosin clearly suppressed the phagocytic activity of mice both in number and function (Fig 5a). After the treatment of RMW, the mice recovered their phagocytic activity to normal range. With a precise observation, the recovery activity of RMW was almost normal level among the four formulae as to augmentation in number and function of phagocytes (Fig 5b).
Recovery of Lymphocyte Subset by RMW in Cancer Chemotherapeutic Agent in mice

It had been well known that severe immune depressive status had accompanied by injection of cancer chemotherapeutic agent. In this script, Streptozotosin was employed as cancer chemotherapeutic agent. This was well known drug that established experimental diabetes meritus. The trial had been made to detect lymphocyte subset as in number and function after administrating RMW. As a result of this experiment, all the lymphocyte subsets as revealed by CD number, recovered after administrating RMW in number and functions.

Fig 6

CLINICAL FINDINGS
Detecting Biochemical Factors, Ammonia and Sulfide in the Feces after RMW

The volunteers were healthy subject, with no drastic change for health problem. After informed and consented by the Ethics Committee in Kanazawa Medical University, they were administered RMW 3 weeks. Every one week after the start, the amount of both ammonia and sulfide were detected. As shown in Fig 7, From 7 days after the start, both ammonia and sulfide were significantly reduced in their feces. However, stop after the administration both level were up to the level at start, indicating close causal relationship to RMW administration. Fig 7

Effect on population of both goody bacillus and villainous bacillus

The volunteers were healthy subject, with no drastic change for health problem. After informed and consented by the Ethics Committee in Kanazawa Medical University, they were administered RMW 3 weeks. Every one week after the start, the amount of both ammonia and sulfide were detected. As shown in Table 1, 7 days after the start, both ammonia and sulfide were significantly reduced in their feces. As a representative of intestinal flora, Bifidobacterium, bacteroides, Escherichia Coli and Streptococcus were selected for this tracing. RMW was compared to the health promoting standard, yogurt, Chitosan, Oligosaccharide and Lactulose. The results were shown in Table 1a, Table 1b, Table 1c and Table 1d.
respectively. From the result of this trial, RMW was the best material to up-regulating goody bacillus. On the same trend was evident that RMW was the best one for down-regulation of villainous bacillus number in the intestine. Table 1a, Table 1b, Table 1c and Table 1d

Dynamics of Intestinal Flora after RMW

As was the same trend of trial for the analysis of intestinal flora, four groups of bacteria were selected as this marker, Vifidobacterium, Bacteroide, Clostridium and others. The relative value of Vifidobacterium was increased in the intestine significantly after 7 days and maximum to after 14 days. However, stop after the RMW administration gradually reduced in number. On the other hand anaerobic bacteria Chlostridium were reduced after 7 days and almost negligible after 14 days. This analysis was also causative relationship to RMW administration. Fig 6

Dividing Subjects According to Constitution into Two Groups, G-type and L-type by Granulocyte and Lymphocyte Proportion

For the total evaluation by administrating RMW, 10 volunteer were informed and contented to join atrial for this observation. The volunteer were collected blood from fore arm vein before and 30 days after the trial. Peripheral leukocyte subset and lymphocyte subsets were estimated by authorized institution on the county (Ishikawa Institute for Preventive Medicine, Foundation, Kanazawa, Japan). In Table 2, volunteer were divided as G-rich type or L-rich type. As a results, G-rich type tend to decrease granulocyte yet, lymphocyte were increased. On the other hand, L-rich type were shown as vice versa. Table 2

Dividing Subjects into Two Groups, G-type and L-type by Lymphocyte Subsets Proportion

For the total evaluation by administrating RMW, 10 volunteer were informed and contented to join atrial for this observation. The volunteer were collected blood from fore arm vein before and 30 days after the trial. Peripheral lymphocyte subsets were estimated by authorized institution on the county (Ishikawa Institute for Preventive Medicine, Foundation, Kanazawa, Japan). In Table 3, volunteer were divided as G-rich type or L-rich type. As a results, G-rich type tend to T-cell series of lymphocyte, yet, lymphocyte were decreased.
Discussion

The Alternative and Complementary Medicine Society defines alternative and complementary medicine in Japan, the modern Western medicine which has not been verified scientifically and practiced at the daily clinic. In U.S., it is called the alternative medicine or the Complementary and Alternative Medicine (CAM). Anyway it is the medicine that is not lectured at the medical school and practiced at the daily clinic. The medicine can be classified into either the conventional medicine or the unconventional medicine. The conventional medicine is also called the modern Western medicine, scientific medicine or technical medicine, and the unconventional medicine is called the alternative, complementary, natural or fringe medicine. In Japan, the alternative medicine contains the traditional Chinese medicine, acupuncture and judo reduction etc. established as the Eastern medicine and they have their own independent long history [1]. Since the herbal medicine is not insured in Japan, some insist it is not belong to the alternative medicine. However it is considered as alternative medicine in the West. Thus the position of the alternative medicine is various, so it might be recognized uniformly. There are many kinds of medicine including Ayurvedic Medicine in the world, therefore it is not many who can receive benefit from the Western medicine like us in Japan. The World Health Organization classifies 65~85% of the world health control business into the “traditional medicine”. In other words, if these traditional medicines are practiced in the West, it is classified into the alternative medicine. The medicine practiced in today’s Japan is called the Japanese medicine, and it is thought as one of the Far Eastern medicine like Chinese medicine, Tibetan medicine and Korean medicine other than Uyghur medicine [5-13].

In this article, we select one of the simple compound remodeled water, hence the over 60% of body weight. This specialized water was eminent molecule that autonomally activated within a water molecule. This water was as safe by animal safety test. Moreover, the regulatoional effect was obtained in peripheral leukocyte that we propose that this facto is the best scale for access the physiological condition not by analog but digital one, preclinical and bedside test. In order to compare the each traditional medicine in the each country, a common digital evaluation system is necessary. We propose that the best way for the sale is an immunological factor and compare the
results as constitution dependent manner [23-45]. As a result of CAM sample exhibiting efficacy through immunological factor by hot spring hydrotherapy, light exercise, floor heating and TCM etc. In this report we confirmed that RMW was also regulated immunological factor through life related complains, especially in senile. Our results showed that within 24 hours after hot spring hydrotherapy, the white blood cells in peripheral blood had changed significantly, not only in cell count but also cell function. We hope that our work will attract more attention to the mechanisms of which hot spring hydrotherapy regulates the human immune system. Abo reported that according to the lymphocyte subset content, lymphocyte rich type showed over 40% on the other hand granulocyte rich type show over 60% of granulocyte [15], [16], [17]. Each type exhibited different character even in the same age, sexuality and each age. In the Figures, within the same age and the sex, even in mankind can sorts as G-rich type (granulocyte 60%), and L-rich type (lymphocyte 40%). On the other hand, as a stand point of sex difference, the lady belongs to L-rich type. The contents are the result from the data showing the too much number individual tend to downy regulate, on the other hand lower number one is up-regulated and suitable number is left alone. This kind of regulation showed within a 24 hours, for the leukocyte subsets, granulocyte and lymphocyte are changed even under circadian rhythm as a constitution dependent manner. So for purpose of evaluating leukocyte deviation, we adjust the point for evaluation at the same time-zone from the first set of evaluation in order to consider circadian rhyme. Under these condition, we got a reproducible results that the whole number of leukocyte, and its subset, granulocyte and lymphocyte also regulated within 24 hours.

CONFLICT OF INTEREST

We declared that there was no conflict of interest in this study

ACKNOWLEDGEMENT

Declared none
Table 1 Constitution dependent regulation of leukocyte by RMW, Volunteer were divide according to their constitution base on their granulocyte/Lymphocyte Ratio. The data represented the value obtained 30 days after RMW administration

REFERENCES


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**Figure Legend**

**Figure 1. Conceptual structure of Remodeled Water.**
Precise explanation was found the reference # in this report. General experimental design to evaluate each CAM therapy. General style for the experiment was designed to collect factors before and after the CAM menu. However, the impotent sings to collect the factor have to keep the time lag is 24 hours in order to avoid the circadian rhythm. Twenty-four hours change of leukocyte counts on the bases of group comparison between pre/post therapy. We sampled peripheral blood from each volunteer before and after CAM therapy, at the same time on each day, in accordance with the consideration of circadian rhythm of leukocyte in this figure, we tried to show the date simply pooled and make mean, then compared.
Figure 2. Absorbance character of RMW
One of the character of RMW was absorb capacity, especially odor element molecule. The representative s were shown in this figure. Both Ammonia and Tri Methyle Amine were quickly absorbed with this RMW as time dependent manner. Some of the molecule ,such as Methyl Mercaptane was not.

Figure 3. Experimental Protocol for *exvivo* system for Anti-oxidative Activity
Hydro peroxides of linoleic acid, phosphatidylcholine, and cholesterol added to plasma in the absence of added reducing substrates are degraded, in contrast to hydro peroxides of trilinolein and cholesterol linoleate. These findings indicate the presence of a selective peroxidase activity operative under physiological conditions. Our data suggest that in states of leukocyte activation and other types of acute or chronic oxidative stress such a simple regimen as controlled ascorbate supplementation could prove helpful in preventing formation of lipid hydro-peroxides, some of which cannot be detoxified by endogenous plasma activities and thus might cause damage to critical targets. Extracellular release of superoxide anion and hydrogen peroxide during the respiratory burst of porcine neutrophil/phagocyte was studied by using diacetyldeuteroheme-substituted horseradish peroxidase as a trapping agent for these oxygen derivatives. The method permitted simultaneous measurement of oxygen consumption and formation of both $O_2$ and $H_2O_2$ in a single reaction mixture. When neutrophils/phagocytes were stimulated with phorbol myristate acetate in the presence of the home-substituted peroxidase, a rapid accumulation of compound, a complex of the enzyme with $O_2$, was observed accompanying an increase in oxygen consumption. During the process, amounts of compound formed and oxygen consumed were stoichiometric, and no compound II, an indicator of $H_2O_2$ formation, was observed.

Figure 4 Experimental Protocol for *exvivo* system for Anti-oxidative Activity
Exvivo system prepared by mouse peritoneal macrophage shown in this figure. RMW include active hydrogen molecule in the structure, expecting the
enhancement of its anti-oxidative effects. The generated super oxide anion after one week administration of conventional water and RMW, were $2.9 \pm 0.6$ and $1.6 \pm 0.6 \times 10^{-5}$ mmol/ml, respectively, positive control = $2.8 \pm 0.2$.

Figure 5 Digital Representation for *ex vivo* system for Macrophage Phagocytosis

**Fig 3** Digital Representation of Lymphocyte Subset for the Effect of Hydro Therapy according to the Constitution

Figure 6 Populational analysis of intestinal flora after RMD

The populational analysis of intestinal flora after RMD administration, four groups of bacteria were selected as this marker, Vifidobacterium, Bacteroide, Clostridium and others. The relative value of Vifidobacterium was increased in the intestine significantly after 7 days and maximum to after 14 days. However, stop after the RMW administration gradually reduced in number. On the other hand anaerobic bacteria Chlostridium were reduced after 7 days and almost negligible after 14 days. This analysis was also causative relationship to RMW administration.

Figure 7 Total Evaluation of host immune factors by RMW

Streptozotosin was employed as cancer chemotherapeutic agent in order to prepare immune-suppressed mice. This agent was well known drug that established experimental diabetes meritus. The trial had been made to detect lymphocyte subset as in number and function after administrating RMW. As a result of this experiment, all the lymphocyte subsets as revealed by CD number, recovered after administrating RMW in number and functions.

Table 1 Comparison of Goody Bacillus administered RMW

The volunteers were healthy subject, with no drastic change for health problem. They were administered RMW for 3 weeks. Every one week after the start, the amount of both ammonia and sulfide were detected. 7 days after the start, both ammonia and sulfide were significantly reduced in their feces. As a representative of intestinal flora, Bifidobacterium, bacteroides, Escherichia Coli and Streptococcus were selected for this tracing. RMW was compared to the health promoting standard, yogurt, Chitosan, Oligosaccharide and Lactulose.
**Table 2. The constitution/condition dependent regulation by RMW**

The constitution/condition dependent regulation and presentation by linear slant and judging by their tangent value/ co-efficiency. A graphical presentation for the summary of the hot spring hydrotherapy. We tried to express the effect of peripheral total leukocyte number by individual level of change and plot in the X-axis as in each age. Variations in leukocyte subpopulations in the peripheral blood before and after each CAM therapy.
Table 2. The constitution/condition dependent regulation by RMW
The constitution/condition dependent regulation and presentation by linear slant and judging by their tangent value/ co-efficiency. A graphical presentation for the summary of the hot spring hydrotherapy. We tried to express the effect of peripheral total leukocyte number by individual level of change and plot in the X-axis as in each age. Variations in leukocyte subpopulations in the peripheral blood before and after each CAM therapy.

Abbreviations

ACAS: Adherent Cell Assay System, Digitally representation of macrophage function, phagocytosis through fluorescent-activated bead intake as digital summarization in Fig 5

CAM: Complementary and alternative medicine, beside the western medicine, there are many traditional medicine and/or health promoting menu all over the world

CD: Cluster of differentiation. Each lymphocyte has name that expressed CD number, for example CD2, CD4, etc.

DM: Diabetes mellitus

Emotional Hormone: Adrenaline, Dopamine and Cortisone were selected as emotional hormones for this trial.
FCM: Flow Cytometry

G-rich type: The individual that exhibit over 60% of granulocyte in peripheral blood, finding many in young gentleman might be kept warm around the nee but the Head kept rather cool

L-rich type: The individual that exhibit over 40% of lymphocyte in peripheral blood, finding lot in ladies and senile

MHC: Major histocompatibility antigen; Self marking constructions that express almost all the cell surface. Immune response can start with coincides of same MHC. tumor cell and virus-infected cell lose this MHC that can attacked by tumor cell and virus-infected cell.

QOL: Quality of life

RMW: Remodeled Water, a main subject of this paper that molar designed as H$_2$O$_2$, shown in Fig1.

VAS: Visual analog scale
Structural Concept of RMW

![Diagram of RMW structure with hydrogen and oxygen atoms connected by arrows]

Fig 2 Absorbance Character of RMW

Fig 2a Ammonia

Fig 2b Tri-Methyl Amine

Fig 2c Methyl Mercaptane
Fig 3 In Vivo anti-oxidative Assay

<Preparation of Murine Peritoneal Exudative Cells >

- Octe glycerin 2ml i.p.
- After cervical dislocation, peritoneal exudative cells were obtained in 5 ml HBSS (Hank's balanced salt solution).
- Wash with HBSS, Centrifuge 1500rpm 5min at 4°C
- Wash with HBSS buff, Centrifuge 1500rpm 5min at 4°C

<Measurement of Generated Super Oxide >

- 100μl Hepes 0.1mM
- Cyt c 100μg
- Cell suspension 1.00g (prepared to 8 x 10^6 cells/ml)
- FPIA 10μg
- Incubate at 37°C for 20min

Stop reaction on ice water bath

Centrifuge 1500rpm 5min at 4°C

OD of supernatant was measured at 550nm and 540nm

Fig 4
Anti-oxidant Activity of RMW
Fig 5a Phagocytosis Positive Cell, Normal

Fig 5b Phagocytosis Positive Cell, + RMW
Fig 6  Dynamics of Intestinal Flora by RMW

14 days after start

- Other: 37%
- Bacteroides...: 63%
- Others: 23%

7 days before start

- Others: 23%
- Bifidobacterium: 10%
- Bacteroides: 63%

7 days after pause

- Others: 10%
- Others: 20%
- Bacteroides...: 63%

7 days after start

- Others: 20%
- Bifidobacterium...: 63%
- Bacteroides...: 63%
Fig 7  Lymphocyte Subset Analysis
### Table 1 Comparison of Goody Bacillus after Administration of RMW

**Comparative to Bifidobacterium**

(Unit: x10000)

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>14 days after</th>
<th>Subtraction</th>
</tr>
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<tbody>
<tr>
<td>RM Water</td>
<td>645,654</td>
<td>1,819,800</td>
<td>(+)1,174,046 (181.8%)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>776,247</td>
<td>1,288,249</td>
<td>(+)512,002 (65.9%)</td>
</tr>
<tr>
<td>※Chitosan</td>
<td>1,096,478</td>
<td>389,045</td>
<td>(+)707,433 (64.5%)</td>
</tr>
<tr>
<td>Oligosaccharide</td>
<td>741,310</td>
<td>2,754,228</td>
<td>(+)2,012,918 (271.5%)</td>
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<td>Lactulose</td>
<td>487,778</td>
<td>2,454,708</td>
<td>(+)1,964,930 (401.1%)</td>
</tr>
</tbody>
</table>

**Comparison of Villainous Bacillus After Administration**

**Combative to Bacteroides**

(Unit: x10000)

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>14d After</th>
<th>Subtraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM Water</td>
<td>4,168,693</td>
<td>3,890,451</td>
<td>(-)378,232 (149.8%)</td>
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<tr>
<td>Yogurt</td>
<td>3,981,071</td>
<td>3,891,071</td>
<td>-</td>
</tr>
<tr>
<td>※Chitosan</td>
<td>6,760,829</td>
<td>5,754,399</td>
<td>(-)1,006,430 (14.8%)</td>
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<tr>
<td>Oligosaccharide</td>
<td>2,398,832</td>
<td>3,162,277</td>
<td>(+)763,445 (31.8%)</td>
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<tr>
<td>Lactulose</td>
<td>4,897,778</td>
<td>2,454,708</td>
<td>(-)2,443,070 (49.8%)</td>
</tr>
</tbody>
</table>

**Comparative to E. Choli**

(Unit: x10000)

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>14d After</th>
<th>Subtraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM Water</td>
<td>6,025</td>
<td>5,623</td>
<td>(-)402 (6.6%)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>19,952</td>
<td>6,165</td>
<td>(-)13,787 (69.1%)</td>
</tr>
<tr>
<td>※Chitosan</td>
<td>58,884</td>
<td>97,723</td>
<td>(+)38,839 (65.9%)</td>
</tr>
<tr>
<td>Oligosaccharide</td>
<td>16,982</td>
<td>11,748</td>
<td>(+)5,234 (30.8%)</td>
</tr>
<tr>
<td>Lactulose</td>
<td>24,547</td>
<td>6,165</td>
<td>(-)18,381 (74.8%)</td>
</tr>
</tbody>
</table>

**Comparative to Streptococcus**

(Unit: x10000)

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>14d After</th>
<th>Subtraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM Water</td>
<td>300,199</td>
<td>1,380</td>
<td>(-)38,819 (95.4%)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>19,952</td>
<td>31,622</td>
<td>(-)11,670 (58.4%)</td>
</tr>
<tr>
<td>※Chitosan</td>
<td>24,547</td>
<td>97,723</td>
<td>(-)33,176 (298.1%)</td>
</tr>
</tbody>
</table>
Table 2 Constitution dependent regulation of leukocyte by *RMW*

<table>
<thead>
<tr>
<th></th>
<th>G type individual</th>
<th>L type individual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before RMW</td>
<td>After RMW</td>
</tr>
<tr>
<td>Total WBC (x 10^3μl)</td>
<td>6.37</td>
<td>5.97</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>23.6</td>
<td>27.9</td>
</tr>
<tr>
<td>Granulocyte (%)</td>
<td>68.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>63.2</td>
<td>63.3</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.6</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 2 Constitution dependent regulation of leukocyte by *RMW*, Volunteer were divide according to their constitution base on their granulocyte/Lymphocyte Ratio. The data represented the value obtained 30 days after RMW administration.

Table 3 Constitution dependent regulation of lymphocyte by *RMW*

<table>
<thead>
<tr>
<th>CD</th>
<th>G type individual</th>
<th>L type individual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (%)</td>
<td>After (%)</td>
</tr>
<tr>
<td>CD2</td>
<td>73.6</td>
<td>75.66</td>
</tr>
<tr>
<td>CD4</td>
<td>17.64</td>
<td>20.34</td>
</tr>
</tbody>
</table>
Table 3 Constitution dependent regulation of leukocyte by RMW, Volunteer were divide according to their constitution base on their Lymphocyte Subpopulation expressed as CD positive Cell. The data represented the value obtained 30 days after RMW administration.

<table>
<thead>
<tr>
<th></th>
<th>CD8</th>
<th>CD11</th>
<th>CD14</th>
<th>CD16</th>
<th>CD19</th>
<th>CD56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>36.45</td>
<td>72.45</td>
<td>0.05</td>
<td>63.66</td>
<td>8.11</td>
<td>1.44</td>
</tr>
<tr>
<td>SD</td>
<td>41.33</td>
<td>74.32</td>
<td>0.07</td>
<td>59.59</td>
<td>8.35</td>
<td>1.65</td>
</tr>
<tr>
<td>Min</td>
<td>28.67</td>
<td>62.11</td>
<td>0.04</td>
<td>53.67</td>
<td>8.65</td>
<td>1.43</td>
</tr>
<tr>
<td>Max</td>
<td>29.33</td>
<td>68.94</td>
<td>0.07</td>
<td>49.34</td>
<td>7.41</td>
<td>2.57</td>
</tr>
</tbody>
</table>