Effects of Drinking a Water Product with Anti-Oxidant Activities *In Vitro* on the Blood Levels of Biomarker Substances for the Oxidative Stress

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The *in vivo* effects of drinking a water product, which had been confirmed to have antioxidant activities *in vitro*, were preliminarily studied by monitoring the blood concentrations of oxidative stress marker substances in the two group subjects who ingested the same quantity of the water product and a tap water solution during the same time. The results indicated that hydrogen gas and reductive vanadium ions as the components responsible for the antioxidant activities *in vitro* cannot enhance the scavenging ability for reactive oxygen species *in vivo* after being drunk and absorbed into the human body, although it was suggested that an ingestion of a greater quantity of water than usual gives a slight reduction, overall, in the oxidative stress.

Key words —— antioxidant activity, water product, oxidative stress, lipid peroxide, biopyrrin

INTRODUCTION

It has been generally accepted that many diseases are caused by the actions of reactive oxygen species (ROS).[1)] In recent years, many companies have developed and sold various water products, some of which are advertised to have antioxidant activities by scavenging harmful ROS in the human body. Many people are taking them (aqueous solution systems) as a kind of supplement drinks, although only a few investigations have been made on their real existence and actual effects. We already studied the physico-chemical properties *in vitro* of four such aqueous solution systems and elucidated that all of the examined samples really have antioxidant activities *in vitro* and that the I'm Fine (IF), a product of Nihon Trim Co. Ltd. (Osaka, Japan), has the strongest effect among them.[2)] It was also revealed that the antioxidant activities *in vitro* of these aqueous solution systems are derived from hydrogen gas and/or reductive cations, especially such vanadium (V) ions as V^{+2} and V^{+3}.[2)] As a preliminary study to elucidate the actual effects *in vivo* of such aqueous solution systems, we monitored the blood levels of oxidative stress marker substances in the two group subjects who took IF and a tap water (TW) solution as the control.

MATERIALS AND METHODS

Subjects —— Fourteen healthy persons (5 males and 9 females, 21–23 years old) were divided into the IF group (2 males and 5 females) and the control group (3 males and 4 females). The classification made by us using a lottery system was not shown to the subjects until the last blood specimen was collected as will be described later. Informed consent was obtained from all of the subjects, and the design of the experimental procedures were permitted by the ethics committee of Faculty of Health Sciences, Kyorin University.

Experiments of Drinking IF or the TW Solution —— All of the subjects were directed to drink 1 liter per 1 day of IF (7 subjects in the IF group) or the TW solution (the other 7 subjects in the control group) during 5 successive days. Both the IF purchased from Nihon Trim and the TW solution obtained on the Hachioji campus of Kyorin University were stored in same-type bottles and provided to individual subjects every morning (at 10:30–11:00) of the 5 days during the drinking period without

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Table 1. Changes in the Serum Lipid Peroxide (Malondialdehade) Level in the IF Group and the Control Group Subjects

Date of Blood Sampling	IF Group	The Control Group
The 1st Day	3.90 ± 1.48 nmole/ml	3.43 ± 1.35 nmole/ml
(at 11:00–12:00)[a)]	($n = 7$)	($n = 7$)
The 5th Day	2.29 ± 0.96 nmole/ml	2.42 ± 0.87 nmole/ml
(at 16:00–17:00)[b)]	($n = 7$)	($n = 7$)
The 9th Day	4.11 ± 1.75 nmole/ml	4.67 ± 1.98 nmole/ml
(at 11:00–12:00)	($n = 7$)	($n = 7$)
The 15th Day	2.97 ± 1.21 nmole/ml	3.08 ± 1.25 nmole/ml
(at 11:00–12:00)	($n = 7$)	($n = 7$)

a) Just before the drinking period started, *b*) Immediately after the drinking period was over (see text). Note that in both of the IF and the control groups, the mean (± S.D.) values were the lowest for the samples taken immediately after the drinking period was over, although no significant difference was detected ($p < 0.05$) between the values obtained on the four sampling days in each group and between those for the two groups on each sampling day.

opening the caps, in order to prevent them from recognizing to which group they belonged. In the morning of the second, third, fourth and fifth days, the empty bottles were recovered from all of the 14 subjects and exchanged for new ones filled with either the IF or the TW solution. Blood specimens for preparation of the serum samples were taken from all of the subjects at 11:00–12:00 on the first day and 16:00–17:00 in the fifth day during the drinking period, as well as at 11:00–12:00 on 4 and 10 days after the drinking period was over (namely 9 days and 15 days after the drinking period started). After the last blood specimen was collected, all of the subjects were requested to answer whether they recognized IF or the TW solution they had been drinking and then told the right answer. During these 15 days, they were required to keep regular hours without excessive eating and drinking.

Biochemical Examinations of the Blood Oxidative Stress Markers —— The serum lipid peroxide (LPO) level, which reflects the degree of oxidative alterlation of blood lipoproteins,[3)] was obtained as the content of malondialdehyde.[4)] The concentrations in the serum of biopyrrins (BP), the oxidation products of bilirubin,[5)] were measured as previously reported.[6)]

Mineral Analysis —— All of the serum samples, as well as IF and the TW solutions, were quantitated for 53 cation minerals as previously reported.[2)]

RESULTS AND DISCUSSION

Changes in the serum LPO and BP concentrations in the subjects of the two groups during the 5 days of the drinking period and the subsequent 10 days are summarized in Tables 1 and 2, respectively. As shown in Table 1, no significant difference in the serum LPO concentration was observed for each sampling day between the subjects in the IF group and the control group, as well as in the subjects of each group between the four sampling days. However, in the serum samples prepared from the blood specimens obtained immediately after the drinking period was over, the mean values (± S.D.) of the LPO concentration were the lowest in both of the two groups (Table 1). A similar trend was also found in the changes in the mean values (± S.D.) of the serum BP concentration (Table 2).

The results of the mineral analysis of the IF and the TW solution indicated that the concentration ratio (the former/the latter) for V was 25.3 (22.5 μg/l in the IF and 0.9 μg/l in the TW solution), which was the highest among that for all of the 53 minerals analyzed. According to the HP of Nihon Trim, IF is produced by sampling an alkaline solution near the cathode after the electrolysis of an underground water solution containing various minerals, especially calcium (Ca) and V, collected in the area around Mt Fuji. It is well known that the electrolysis affords hydrogen-gas rich solutions near the cathode (electrolyzed-reduced waters, ERWs) and that in such reducing solutions, V is dissolved mainly as the reductive ions (V^{+2} and/or V^{+3}).[7)] In this study, on each sampling day, the mean value (± S.D.) of

Table 2. Changes in the Serum Biopyrrin Level in the IF Group and the Control Group Subjects

Date of Blood Sampling	IF Group	The Control Group n
The 1st Day	24.1 ± 12.1 μg/ml	21.6 ± 11.4 μg/ml
(at 11:00–12:00)[a)]	($n = 7$)	($n = 7$)
The 5th Day	18.0 ± 8.3 μg/ml	16.8 ± 7.5 μg/ml
(at 16:00–17:00)[b)]	($n = 7$)	($n = 7$)
The 9th Day	26.8 ± 14.1 μg/ml	25.4 ± 9.2 μg/ml
(at 11:00–12:00)	($n = 7$)	($n = 7$)
The 15th Day	23.7 ± 10.3 μg/ml	27.6 ± 12.9 μg/ml
(at 11:00–12:00)	($n = 7$)	($n = 7$)

a) Just before the drinking period started, *b*) Immediately after the drinking period was over (see text). Note that in both the IF group and the control group, the mean (± S.D.) values were the lowest for the samples obtained immediately after the drinking period was over, although no significant difference was observed ($p > 0.05$) between the values obtained on the four sampling days in each group and between those for the two groups on each sampling day.

the serum V concentration in the IF group was not different from that of the control group, and on each group, there was also no difference between the mean (± S.D.) values on the four sampling days (data not shown). Indeed, in all of the subjects, the serum V content was revealed to be strictly maintained within a narrow range of 0.5–2.0 μg/l and their changes were independent of drinking IF or the TW solution (data not shown).

It was reported that the oxidative stress in the human body is significantly reduced by drinking aqueous solution systems with dissolved antioxidants, such as vitamins C[8)] and E,[9)] polyphenols,[3)] *etc*. On the other hand, the above-presented results of this study suggest that hydrogen gas and reductive V ions as the components responsible for the antioxidant activities *in vitro* of IF cannot enhance the scavenging ability for ROS *in vivo* after being drunk and absorbed into the human body. The causes of this phenomenon may be explained as follows. Hydrogen gas and reductive cations are weaker antioxidants than the above-mentioned excellent scavengers for ROS. In addition, the human body has homeostatic functions to maintain the blood concentrations of various gasses and minerals within the narrow ranges specific for each component. Therefore, it is difficult to expect that hydrogen gas and reductive cations may be adequately absorbed into the blood plasma and developed into the tissues where their scavenging ability for ROS *in vivo* is required.

When the drinking period was over, 6 out of 7 subjects in both of the two groups replied that they had been taking the TW solution, and the only other one in each group replied that he (she) had been drinking IF. So, it is considered that the effects of IF in this study were not positively influenced by the mental factor based on the placebo effects, although this study was performed without employing a double blind test.

It is also noteworthy that in both the IF group and the control group, the mean values (± S.D.) in the serum levels of both LPO and BP were the lowest immediately after the drinking period was over (Tables 1 and 2). Earlier workers described that taking ERWs prepared from TW solutions or aqueous Ca lactate gave a clinical improvements in patients with abdominal complaints.[10)] According to them, the results of a double blind test showed a significant difference in an alleviation of clinical improvements between patients drinking 500 ml per day of the ERWs for one month (the ERW group) and the control subjects taking a same volume of the TW solutions (the TW group) during the same period.[10)] So, it must not be neglected that such preferable effects are actually caused by the functions of the solute(s) of ERWs. However, in their report, the value of the alleviation ratio of the clinical symptoms was 50/79 (= 63.3%) for the TW group, which was not remarkably different from that for the ERW group of 64/84 (= 76.2%).[10)] This trend can be partly explained by the placebo effects, although their data may also show that ingesting a considerable volume of water can provide some preferable effects on the functions of the human body. The above-presented results in this study also suggest that taking a greater quantity of water than usual gave a slight reduction, on the whole, in the oxidative stress in the human

body, possibly due to an increase in the volume of tissue water and/or the blood plasma as the solvent of internal aqueous solution systems. However, in order to conclude that taking such aqueous solution systems with the antioxidant activities *in vitro* gives no effects *in vivo*, further investigations should be performed on the basis of examinations to detect clinical improvements in patients with diseases associated with the oxidative stress and/or a reduction in the mental stress-induced oxidative stress in normal subjects. Some oxidative stress markers should be monitored not only in the blood but also in the urine, since the oxidative damage is reflected also by increased excretions of BP and oxidation products of guanosine.[11,12] Effects of drinking a greater quantity of water than usual should also be quantitatively studied.

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