

1. Clinical evaluation of alkaline ionized water for abdominal complaints: Placebo controlled double blind tests

Hirokazu Tashiro, Tetsuji Hokudo, Hiromi Ono, Yoshihide Fujiyama, Tadao Baba (National Ohkura Hospital, Dept. of Gastroenterology; Institute of Clinical Research, Shiga University of Medical Science, Second Dept. of Internal Medicine)

"Effect of alkaline ionized water on abdominal complaints was evaluated by placebo controlled double-blind tests. Overall scores of improvement using alkaline ionized water marked higher than those of placebo controlled group, and its effect proved to be significantly higher especially in slight symptoms of chronic diarrhea and abdominal complaints in cases of general malaise. Alkaline ionized water group did not get interrupted in the course of the test, nor did it show serious side effects, nor abnormal test data. It was confirmed that alkaline ionized water is safer and more effective than placebos.

Summary

Effect of alkaline ionized water on abdominal complaints was clinically examined by double-blind tests using clean water as placebo. Overall improvement rate was higher for alkaline ionized water group than placebo group and the former proved to be significantly more effective than the other especially in cases of slight symptoms. Examining improvement rate for each case of chronic diarrhea, constipation and abdominal complaints, alkaline ionized water group turned out to be more effective than placebo group for chronic diarrhea, and abdominal complaints.

The test was stopped in one case of chronic diarrhea, among placebo group due to exacerbation, whereas alkaline ionized water group did not stop testing without serious side effects or abnormal test data in all cases.

It was confirmed that alkaline ionized water is more effective than clean water against chronic diarrhea, abdominal complaints and overall improvement rate (relief of abdominal complaints) and safer than clean water.

Introduction

Since the approval of alkaline ionized water electrolyzers by Pharmaceutical Affairs Law in 1966 for its antacid effect and efficacy against gastrointestinal disorders including hyperchylia, indigestion, abnormal gastrointestinal fermentation and chronic diarrhea, they have been extensively used among patients.

However, medical and scientific evaluation of their validity is not established. In our study, we examined clinical effect of alkaline ionized water on gastrointestinal disorders across many symptoms in various facilities. Particularly, we studied safety and usefulness of alkaline ionized water by double-blind tests using clean water as a control group.

Test subjects and methods

163 patients (34 men, 129 women, age 21 to 72, average 38.6 years old) of indigestion, abnormal gastrointestinal fermentation (with abnormal gas emission and rugitus) and abdominal complaints caused by irregular dejection (chronic diarrhea, or constipation) were tested as subjects with good informed consent.

Placebo controlled double-blind tests were conducted using alkaline ionized water and clean water at multiple facilities. An alkaline ionized water electrolyzer sold commercially was installed with a pump driven calcium

dispenser in each of the subject homes. Tested alkaline ionized water had pH at 9.5 and calcium concentration at 30 ppm.

Each subject in placebo group used a water purifier that has the same appearance as the electrolyzer and produces clean water.

The tested equipment was randomly assigned by a controller who scaled off the key code which was stored safely until the tests were completed and the seal was opened again.

Water samples were given to each patient in the amount of 200 ml in the morning with the total of 500 ml or more per day for a month. Before and after the tests, blood, urine and stool were tested and a log was kept on the subjective symptoms, bowel movements and accessory symptoms. After the tests, the results were analyzed based on the log and the test data.

Conclusion

As a result of double-blind clinical tests of alkaline ionized water and clean water, alkaline ionized water was proved to be more effective than clean water against chronic diarrhea, abdominal complaints (dyspepsia) and overall improvement rate (relief from abdominal complaints). Also, safety of alkaline ionized water was confirmed which clinically verifies its usefulness.

2. Effect of Electrolytic Water (Ionized Water) Intake on Lifespan of Autoimmune Disease Prone Mice

Research from Texas University

Recent studies on electrolyzed water indicate that anode or acidic water is most effective as disinfectants; whereas, reduced or alkaline water processed through cathode is used as safe drinking water. The present drinking water study was undertaken in two strains of autoimmune disease prone mice to establish the spontaneous disease process and longevity. Weanling MRL/lpr and NZBxNZW [B/W] F1 female mice were provided daily with (1) tap water [pH ~7.5, oxygen reduction potential (ORP)~600+] (2) electrolyzed water with pH of ~9.0 and ORP ~400- and (3) hyper-reduced water with pH~10.0 and ORP~600-. Mice were provided H₂O and chow diet ad libitum and weekly body weights and spontaneous deaths were recorded. The mean survival data recorded as days for MRL/lpr mice [25 mice/group] is as follows: (1) tap water 235±25, (2) reduced water 287±40 and (3) hyper-reduced water 346±45 days [<0.05]. In the case of B/W mice [25 mice/group], (1) tap water 269±16, (2) reduced water 298±19 and (3) hyper-reduced 302±18 days. A significantly decreased (<0.05) serum lipid peroxides were observed in mice fed hyper-reduced H₂O. Also, the source of water did not alter lymphocyte subsets or their response to mitogens. In summary, hyper-reduced water with pH~10.0 appears to inhibit autoimmune disease of MRL/lpr mice whereas only a modest increased lifespan was noted for B/W mice. The increased lifespan by electrolyzed H₂O appears to be related to the changes in free radicals and antioxidant enzyme levels. [Supported in part by Zanix Co. and Mr. Waterman Co., Tokyo, Japan].

Summary:

1. Lifelong intake of both reduced (pH 9.0) and hyper-reduced (pH 10.0) water caused no harm to mice compared to tap water.
2. Survival is increased significantly by hyper-reduced water in one strain (MRL/lpr).

3. Slight increased life span in the other strain (NZBxNZW F1).
 3. Reduced and hyper-reduced water appears to increase T cell numbers, and decrease B cells.
 4. Both reduced and hyper-reduced water appears to increase antioxidant mRNA levels.
 5. New clinical and animal studies are needed to confirm above results.
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3. Physiological effects of alkaline ionized water: Effects on metabolites produced by intestinal fermentation

by Takashi Hayakawa, Chiko Tushiya, Hisanori Onoda, Hisayo Ohkouchi, Haru~to Tsuge (Gifu University, Faculty of Engineering, Dept. of Food Science)

We have found that long-term ingestion of alkaline ionized water (AIW) reduces cecal fermentation in rats that were given highly fermentable commercial diet (MF: Oriental Yeast Co., Ltd.). In this experiment, rats were fed MF and test water (tap water, AIW with pH at 9 and 10) for about 3 months. Feces were collected on the 57th day, and the rats were dissected on the 88th day. The amount of ammonium in fresh feces and cecal contents as well as cecal free-glucose tended to drop down for the AIW group. In most cases, the amount of free-amino acids in cecal contents did not differ significantly except for cysteine (decreased in AIW with pH at 10) and isoleucine (increased in AIW with pH at 10).

Purpose of tests

Alkaline ionized water electrolyzers have been approved for manufacturing in 1965 by the Ministry of Health and Welfare as medical equipment to produce medical substances. Alkaline ionized water (AIW) produced by this equipment is known to be effective against gastrointestinal fermentation, chronic diarrhea, indigestion and hyperchylia as well as for controlling gastric acid. *1 This is mainly based on efficacy of the official calcium hydroxide. *2 By giving AIW to rats for a comparatively long time under the condition of extremely high level of intestinal fermentation, we have demonstrated that AIW intake is effective for inhibition of intestinal fermentation when its level is high based on some test results where AIW worked against cecal hypertrophy and for reduction in the amount of short-chain fatty acid that is the main product of fermentation.*3 We have reported that this is caused by the synergy between calcium level generally contained in AIW (about 50 ppm) and the value of pH, and that frequency of detecting some anaerobic bacteria tends to be higher in alkaline ionized water groups than the other, although the bacteria count in the intestine does not have significant difference. Based on these results, we made a judgment that effect of taking AIW supports part of inhibition mechanism against abnormal intestinal fermentation, which is one of the claims of efficacy that have been attributed to alkaline ionized water electrolyzers. *4 On the other hand, under the dietary condition of low intestinal fermentation, AIW uptake does not seem to inhibit fermentation that leads us to believe that effect of AIW uptake is characteristic of hyper-fermentation state. Metabolites produced by intestinal fermentation include indole and skatole in addition to organic acids such as short-chain fatty acid and lactic acid as well as toxic metabolites such as ammonium, phenol and p-cresol. We do not know how AIW uptake would affect the production of these materials. In this experiment, we have tested on ammonium production as explained in the following sections.

Testing methods

Four-week-old male Wistar/ST Clean rats were purchased from Japan SLC Co., Ltd. and were divided into 3 groups of 8 each after preliminary breeding. AIW of pH 9 and 10 was produced by an electrolyzer Mineone ROYAL NDX3 1 OH by Omco Co., Ltd. This model produces AIW by electrolyzing water with calcium lactate

added. On the last day of testing, the rats were dissected under Nembutal anesthesia to take blood from the heart by a heparin-treated syringe. As to their organs, the small intestines, cecum and colon plus rectum were taken out from each of them. The cecum was weighed and cleaned with physiological saline after its contents were removed, and the tissue weight was measured after wiping out moisture. Part of cecal contents was measured its pH, and the rest was used to assay ammonium concentration. The amount of ammonium contained in fresh feces and cecal contents was measured by the Nessler method after collecting it in the extracted samples using Conway's micro-diffusion container. Fecal free-glucose was assayed by the oxygen method after extraction by hot water. Analysis of free amino acids contained in cecal contents was conducted by the Waters PicoTag amino acid analysis system.

Test results and analyses

No difference was found in the rats' weight gain, water and feed intake and feeding efficiency, nor was any particular distinction in appearance identified. The length of the small intestines and colon plus rectum tended to decline in AIW groups. PH value of cecal contents was higher and the amount of fecal free-glucose tended to be lower in AIW groups than the control group. Since there was no difference in fecal discharge itself, the amount of free-glucose discharged per day was at a low level. The amount of discharged free-glucose in feces is greater when intestinal fermentation is more intensive, which indicates that intestinal fermentation is more inhibited in AIW groups than the control group. Ammonium concentration in cecal contents tends to drop down in AIW groups (Fig. 1). This trend was most distinctive in case of fresh feces of one of AIW groups with pH 10 (Fig.2) AIW uptake was found to be inhibitory against ammonium production. In order to study dynamics of amino acids in large intestines, we examined free amino acids in the cecal contents to find out that cysteine level is low in AIW groups whereas isoleucine level is high in one of AIW groups with pH 10, although no significant difference was identified for other amino acids.

Bibliography

1. "Verification of Alkaline Ionized Water" by Life Water Institute, Metamor Publishing Co., 1994, p.46
- *2. "Official Pharmaceutical Guidelines of Japan, Vol. IT' by Japan Public Documents Association, Hirokawa Publshin Co., 1996
- *3. "Science and Technology of Functional Water" (part) by Takashi Hayakawa, Haruhito Tsuge, edited by Water Scienll cc Institute, 1999, pp.109-116
- *4. 'Tasics and Effective Use of Alkaline Ionized Water" by Takashi Hayakawa, Haruhito Tsuge, edited by Tetsuji Hc kudou, 25th General Assembly of Japan Medical Congress 'Tunctional Water in Medical Treatment", Administratio~ Offices, 1999, pp. 10- 11

4. Reduced Water for Prevention of Diseases

Part paper delivered by Dr. Shirahata to the Functional Water Symposium in Tokyo by Dr. Sanetaka Shirahata, Graduate school of Genetic Resources Technology, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.

It has long been established that reactive oxygen species (ROS) cause many types of damage to bio-molecules and cellular structures that, in turn result in the development of a variety of pathologic states such as diabetes, cancer and aging. Reduced water is defined as anti-oxidative water produced by reduction of water.

Electrolyzed reduced water (ERW) has been demonstrated to be hydrogen-rich water and can scavenge ROS in vitro (Shirahata et al., 1997). The reduction of proton in water to active hydrogen (atomic hydrogen, hydrogen

radical) that can scavenge ROS is very easily caused by a weak current, compared to oxidation of hydroxyl ion to oxygen molecule. Activation of water by magnetic field, collision, minerals etc. will also produce reduced water containing active hydrogen and/or hydrogen molecule.

Several natural waters such as Hita Tenryosui water drawn from deep underground in Hita city in Japan, Nordenau water in Germany and Tlacote water in Mexico are known to alleviate various diseases. We have developed a sensitive method by which we can detect active hydrogen existing in reduced water, and have demonstrated that not only ERW but also natural reduced waters described above contain active hydrogen and scavenge ROS in cultured cells. ROS is known to cause reduction of glucose uptake by inhibiting the insulin-signaling pathway in cultured cells. Reduced water scavenged intracellular ROS and stimulated glucose uptake in the presence or absence of insulin in both rat L6 skeletal muscle cells and mouse 3T3/L1 adipocytes. This insulin-like activity of reduced water was inhibited by wortmannin that is specific inhibitor of PI-3 kinase, a key molecule in insulin signaling pathways.

Reduced water protected insulin-responsive cells from sugar toxicity and improved the damaged sugar tolerance of type 2 diabetes model mice, suggesting that reduced water may improve insulin-independent diabetes mellitus.

Cancer cells are generally exposed to high oxidative stress. Reduced water cause impaired tumor phenotypes of human cancer cells, such as reduced growth rate, morphological changes, reduced colony formation ability in soft agar, passage number-dependent telomere shortening, reduced binding abilities of telomere binding proteins and suppressed metastasis.

Reduced water suppressed the growth of cancer cells transplanted into mice, demonstrating their anticancer effects in vivo. Reduced water will be applicable to not only medicine but also food industries, agriculture, and manufacturing industries.

[Also see above for Dr. Sanetaka Shirahata paper "***Electrolyzed reduced water scavengers active oxygen species and protects DNA from oxidative damage***"]

5. Clinical Improvements Obtained From The Intake Of Reduced Water

Extracts from the ***Presentation At The Eighth Annual International Symposium On Man And His Environment in Health And Disease*** on February 24th 1990, at The Grand Kempinski Hotel, Dallas, Texas, USA by **Dr. H. Hayashi, M.D. and Dr. M Kawamura, M.D.**, on: **THE CONCEPT OF PREHEPATIC MEDICINES**

Since the introduction of alkaline ionic water in our clinic in 1985, we have had the following interesting clinical experiences in the use of this type of water. By the use of alkaline ionic water for drinking and the preparation of meals for our in-patients, we have noticed :

- Declines in blood sugar levels in diabetic patients.
- Improvements in peripheral circulation in diabetic gangrene.
- Declines in uric acid levels in patients with gout.
- Improvements in liver function exams in hepatic disorders.
- Improvements in gastro-duodenal ulcer and prevention of their recurrences.

- Improvements in hypertension and hypotension.
- Improvements in allergic disorders such as asthma, urticaria, rhinitis and atopic dermatitis.
- Improvements in persistent diarrhea which occurred after gastrectomy.
- Quicker improvements in post operative bowel paralysis.
- Improvements in serum bilirubin levels in new born babies.

Confirming clinical improvements, we have always observed changes of stools of the patients, with the colour of their feces changing from black-brown colour to a brighter yellow-brown one, and the odour of their feces becoming almost negligible.

The number of patients complaining of constipation also decreased markedly. The change of stool findings strongly suggests that alkaline ionic water intake can decrease the production of putrefied or pathogenic metabolites.

Devices to produce reduced water were introduced into our clinic in May 1985. Based on the clinical experiences obtained in the past 15 years, it can be said that introduction of electrolyzed-reduced water for drinking and cooking purpose for in-patients should be the very prerequisite in our daily medical practices. Any dietary recipe cannot be a scientific one if property of water taken by the patients is not taken into consideration.

The Ministry of Health and Welfare in Japan announced in 1965 that the intake of reduced water is effective for restoration of intestinal flora metabolism.

6. Effects of alkaline ionized water on formation & maintenance of osseous tissues

by Rei Takahashi Zhenhua Zhang Yoshinori Itokawa

(Kyoto University Graduate School of Medicine, Dept. of Pathology and Tumor Biology, Fukui Prefectural University)

Effects of calcium alkaline ionized water on formation and maintenance of osseous tissues in rats were examined. In the absence of calcium in the diet, no apparent calcification was observed with only osteoid formation being prominent. Striking differences were found among groups that were given diets with 30% and 60% calcium. Rats raised by calcium ionized water showed the least osteogenetic disturbance. Tibiae and humeri are more susceptible to calcium deficiency than femora. These results may indicate that calcium in drinking water effectively supplements osteogenesis in case of dietary calcium deficiency. The mechanism involved in osteoid formation such as absorption rate of calcium from the intestine and effects of calcium alkaline ionized drinking water on maintaining bone structure in the process of aging or under the condition of calcium deficiency is investigated.

Osteoporosis that has lately drawn public attention is defined as "conditions of bone brittleness caused by reduction in the amount of bone frames and deterioration of osseous microstructure." Abnormal calcium metabolism has been considered to be one of the factors to contribute to this problem, which in turn is caused by insufficient calcium take in, reduction in enteral absorption rate of calcium and increase in the amount of calcium in urinal discharge. Under normal conditions, bones absorb old bones by regular metabolism through osteoid formation to maintain their strength and function as supporting structure. It is getting clear that remodeling of bones at the tissue level goes through the process of activation, reabsorption,

reversal, matrix synthesis and mineralization. Another important function of bones is storing minerals especially by coordinating with intestines and kidneys to control calcium concentration in the blood. When something happens to this osteo metabolism, it results in abnormal morphological changes. Our analyses have been focusing mostly on the changes in the amount of bones to examine effects of calcium alkaline ionized water on the reaction system of osteo metabolism and its efficiency. This time, however, we studied it further from the standpoint of histology. In other words, we conducted comparative studies on morphological and kinetic changes of osteogenesis by testing alkaline ionized water, tap water and solution of lactate on rats. Three-week-old male Wistar rats were divided into 12 groups by conditions of feed and drinking water. Feeds were prepared with 0%, 30%, 60% and 100% of normal amount of calcium and were given freely. Three types of drinking water, tap water (city water, about 6ppm of Ca), calcium lactate solution (Ca=40ppm) and alkaline ionized water (Ca =40ppm, pH=9, produced by an electrolyzer NDX 4 LMC by Omco OMC Co., Ltd.) were also given freely. Rats' weight, amount of drinking water and feed as well as the content of Ca in drinking water were assayed every day. On the 19th and 25th days of testing, tetracycline hydrochloride was added to the feed for 48 hours so as to bring its concentration to 30mg/kg. On the 30th day, blood samples were taken under Nembutal anesthesia, and tibiae, humeri and femora were taken out to make non decalcified samples. Their conditions of osteoid formation and rotation were observed using Villanueva bone stain and Villanueva goldner stain.

Three groups that were given different types of drinking water and the same amount of Ca in the feed were compared to find out no significant difference in the rate of weight gain and intakes of feed and drinking water. Alkaline ionized water group had significantly greater amount of tibiae and humeri with higher concentration of calcium in the bones.

The group of 0% calcium in the feed saw drastic increase in the amount of osteoid. There was not much difference by types of drinking water. Almost no tetracycline was taken into tibiae and humeri, although a small amount was identified in femora. As a result, osteogenesis went as far as osteoid formation, but it was likely that decalcification has not happened yet, or most of newly formed bones were absorbed.

As to the groups of 30% and 60% calcium in the feed, increase in the area of tetracycline take in was more identifiable with higher clarity in descending order of alkaline ionized water, calcium lactate solution and tap water groups. Especially in case of tap water group, irregularity among the areas of tetracycline take in was distinctive. The group of 100% calcium in the feed saw some improvements in osteogenesis in descending order of alkaline ionized water, calcium lactate solution and tap water. In any case, bone formation seemed to be in good condition at near normal level.

Alkaline ionized water was regarded to be effective for improvements of osteogenesis under the conditions of insufficient calcium in the feed. Also, the extent of dysosteogenesis differed by the region. That is, tibiae and humeri tend to have more significant dysosteogenesis than femora.

In addition, there is a possibility that osteo metabolism varies depending on enteral absorption rate of calcium, adjustment of discharge from kidneys and functional adjustment of accessory thyroid in the presence of alkaline ionized water. We are now studying its impact on calcium concentration in the blood. We are also examining whether it is possible to deter bone deterioration by testing on fast aging mouse models.

7. Selective stimulation of the growth of anaerobic micro-flora in the human intestinal tract by electrolyzed reducing water.

Med Hypotheses. 2005;64(3):543-6. **Vorobjeva NV** .

Department of Physiology of Microorganisms, Biology Faculty, Lomonosov Moscow State University, 119992 Moscow, Russia. nvvorobjeva@mail.ru 96-99% of the "friendly" or residential micro-flora of intestinal tract of humans consists of strict anaerobes and only 1-4% of aerobes. Many diseases of the intestine are due to a disturbance in the balance of the microorganisms inhabiting the gut. The treatment of such diseases involves the restoration of the quantity and/or balance of residential micro-flora in the intestinal tract. It is known that aerobes and anaerobes grow at different oxidation-reduction potentials (ORP). The former require positive E(h) values up to +400 mV. Anaerobes do not grow unless the E(h) value is negative between -300 and -400 mV. In this work, it is suggested that prerequisite for the recovery and maintenance of obligatory anaerobic micro-flora in the intestinal tract is a negative ORP value of the intestinal milieu. Electrolyzed reducing water with E(h) values between 0 and -300 mV produced in electrolysis devices possesses this property. Drinking such water favours the growth of residential micro-flora in the gut. A sufficient array of data confirms this idea. However, most researchers explain the mechanism of its action by an antioxidant properties destined to detox the oxidants in the gut and other host tissues. Evidence is presented in favour of the hypothesis that the primary target for electrolyzed reducing water is the residential micro-flora in the gut.

8. Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage.

Biochem Biophys Res Commun. 1997 May 8;234(1):269-74.

Shirahata S , Kabayama S , Nakano M , Miura T , Kusumoto K , Gotoh M , Hayashi H , Otsubo K , Morisawa S , Katakura Y .

Institute of Cellular Regulation Technology, Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka, Japan. sirahata@grt.kyushu-u.ac.jp

Active oxygen species or free radicals are considered to cause extensive oxidative damage to biological macromolecules, which brings about a variety of diseases as well as aging. The ideal scavenger for active oxygen should be 'active hydrogen'. 'Active hydrogen' can be produced in reduced water near the cathode during electrolysis of water. Reduced water exhibits high pH, low dissolved oxygen (DO), extremely high dissolved molecular hydrogen (DH), and extremely negative redox potential (RP) values. Strongly electrolyzed-reduced water, as well as ascorbic acid, (+)-catechin and tannic acid, completely scavenged O₂⁻ produced by the hypoxanthine-xanthine oxidase (HX-XOD) system in sodium phosphate buffer (pH 7.0). The superoxide dismutase (SOD)-like activity of reduced water is stable at 4 degrees C for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving, but was lost by opened autoclaving or by closed autoclaving in the presence of tungsten trioxide which efficiently adsorbs active atomic hydrogen. Water bubbled with hydrogen gas exhibited low DO, extremely high DH and extremely low RP values, as does reduced water, but it has no SOD-like activity. These results suggest that the SOD-like activity of reduced water is not due to the dissolved molecular hydrogen but due to the dissolved atomic hydrogen (active hydrogen). Although SOD accumulated H₂O₂ when added to the HX-XOD system, reduced water decreased the amount of H₂O₂ produced by XOD. Reduced water, as well as catalase and ascorbic acid, could directly scavenge H₂O₂. Reduced water suppresses single-strand breakage of DNA by active oxygen species produced by the Cu(II)-catalyzed oxidation of ascorbic

acid in a dose-dependent manner, suggesting that reduced water can scavenge not only O_2^- and H_2O_2 , but also $1O_2$ and $\cdot OH$.

9. The mechanism of the enhanced antioxidant effects against superoxide anion radicals of reduced water produced by electrolysis.

Biophys Chem. 2004 Jan 1;107(1):71-82.

Hanaoka K , Sun D , Lawrence R , Kamitani Y , Fernandes G .

Bio-REDOX Laboratory Inc. 1187-4, Oaza-Ueda, Ueda-shi, Nagano-ken 386-0001, Japan.

hanak@rapid.ocn.ne.jp

We reported that reduced water produced by electrolysis enhanced the antioxidant effects of proton donors such as ascorbic acid (AsA) in a previous paper. We also demonstrated that reduced water produced by electrolysis of 2 mM NaCl solutions did not show antioxidant effects by itself. We reasoned that the enhancement of antioxidant effects may be due to the increase of the ionic product of water as solvent. The ionic product of water (pK_w) was estimated by measurements of pH and by a neutralization titration method. As an indicator of oxidative damage, Reactive Oxygen Species- (ROS) mediated DNA strand breaks were measured by the conversion of supercoiled ϕ X-174 RF I double-strand DNA to open and linear forms. Reduced water had a tendency to suppress single-strand breakage of DNA induced by reactive oxygen species produced by $H_2O_2/Cu(II)$ and $HQ/Cu(II)$ systems. The enhancement of superoxide anion radical dismutation activity can be explained by changes in the ionic product of water in the reduced water.

10. Protective mechanism of reduced water against alloxan-induced pancreatic beta-cell damage: Scavenging effect against reactive oxygen species

Authors: Li Y.1; Nishimura T.1; Teruya K.1; Maki T.1; Komatsu T.1; Hamasaki T.1; Kashiwagi T.1; Kabayama S.2; Shim S-Y.1; Katakura Y.1; Osada K.1; Kawahara T.1; Otsubo K.2; Morisawa S.2; Ishii Y.3; Gadek Z.4; Shirahata S.5

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Abstract: Reactive oxygen species (ROS) cause irreversible damage to biological macromolecules, resulting in many diseases. Reduced water (RW) such as hydrogen-rich electrolyzed reduced water and natural reduced waters like Hita Tenryosui water in Japan and Nordenau water in Germany that are known to improve various diseases, could protect a hamster pancreatic beta cell line, HIT-T15 from alloxan-induced cell damage. Alloxan, a diabetogenic compound, is used to induce type 1 diabetes mellitus in animals. Its diabetogenic effect is exerted via the production of ROS. Alloxan-treated HIT-T15 cells exhibited lowered viability, increased intracellular ROS levels, elevated cytosolic free Ca^{2+} concentration, DNA fragmentation, decreased intracellular ATP levels and lowering of glucose-stimulated release of insulin. RW completely prevented the generation of alloxan-induced ROS, increase of cytosolic Ca^{2+} concentration, decrease of intracellular ATP level, and lowering of glucose-stimulated insulin release, and strongly blocked DNA fragmentation, partially

suppressing the lowering of viability of alloxan-treated cells. Intracellular ATP levels and glucose-stimulated insulin secretion were increased by RW to 2–3.5 times and 2–4 times, respectively, suggesting that RW enhances the glucose-sensitivity and glucose response of beta-cells. The protective activity of RW was stable at 4 °C for over a month, but was lost by autoclaving. These results suggest that RW protects pancreatic beta-cells from alloxan-induced cell damage by preventing alloxan-derived ROS generation. RW may be useful in preventing alloxan-induced type 1-diabetes mellitus.

11. Electrolyzed hydrogen-saturated water for drinking use elicits an anti-oxidative effect: a feeding test with rats.

Biosci Biotechnol Biochem. 2005 Oct;69(10):1985-7

Yanagihara T, Arai K, Miyamae K, Sato B, Shudo T, Yamada M, Aoyama M.

MiZ Co., Ltd., Fujisawa-shi, Kanagawa, Japan.

A new type of electrolyzed hydrogen-saturated (EHS) water was produced using a water-electrolyzing device equipped with a special cation exchanger. Use of the EHS water for drinking in a feeding test with rats elicited an anti-oxidative effect. After intra-peritoneal injection of 2,2-azobis-amidinopropane dihydrochloride, urinary secretion of 8-hydroxydeoxyguanosine and hepatic formation of peroxidized lipid were significantly lessened in rats which had received the EHS water for one week. These results suggest the possibility that this drinking water shows an effect in reduction of oxidative stress in the body.