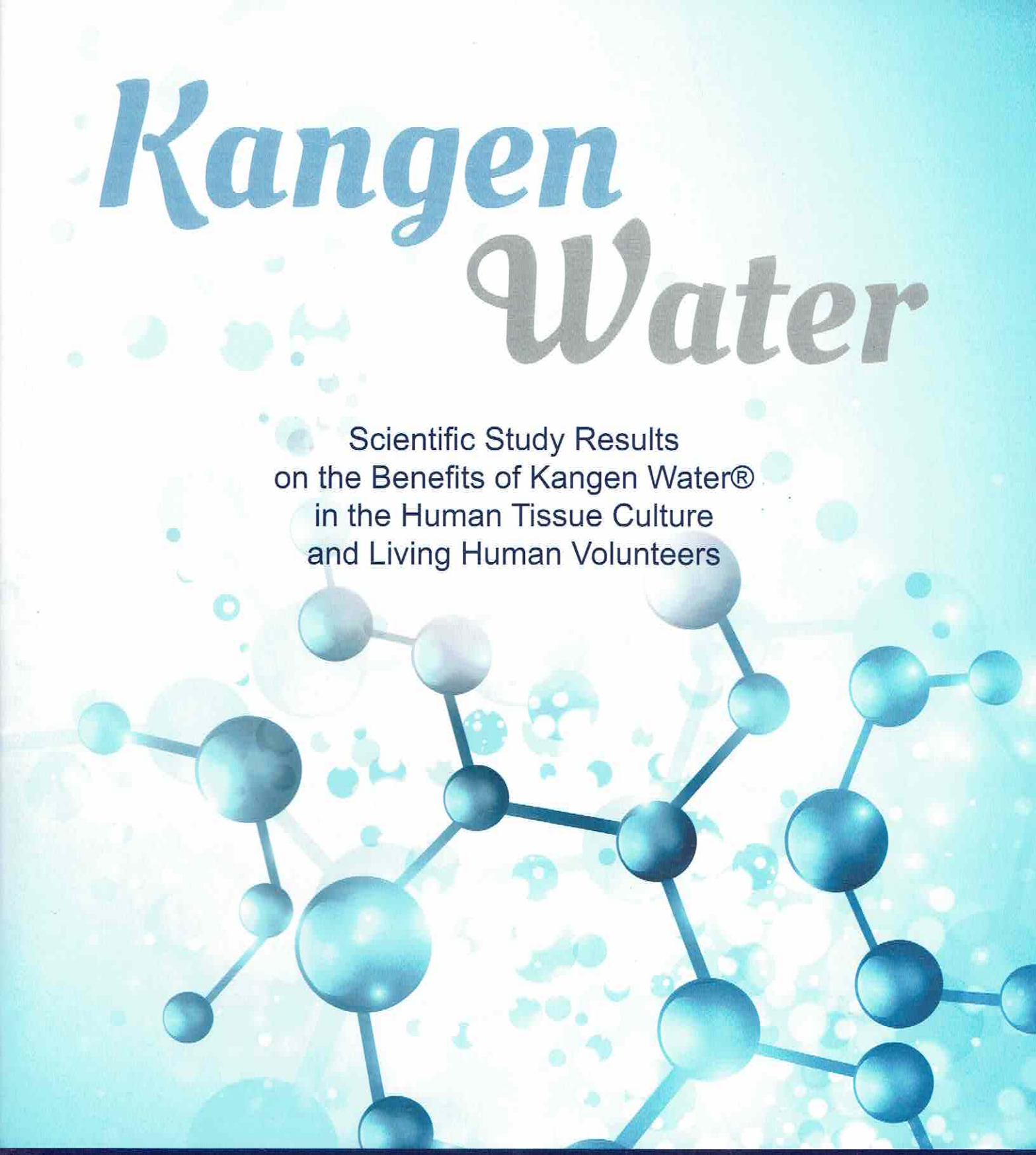
Kangen Water

Scientific Study Results
on the Benefits of Kangen Water®
in the Human Tissue Culture
and Living Human Volunteers



A Report Prepared by

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Kangen Water

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Introduction

My first encounter with alkaline ionized water was by chance and I give full credit to a patient (and later friend) of mine. Knowing how sceptical I am, he parked a water ionizing machine by my kitchen sink and hooked it up. I was not the least bit interested in the thing but he thought I'd like the water the machine produced and so, at his suggestion, I took my first drink of ionized water.

Bear in mind that, like most people living in the US, I was not really used to drinking water. I was struck by how good the water tasted and how easy it seemed to go down. As a doctor and a scientist, I needed to know more and in the fall of 2007 I bought my first water ionizing machine.

I did some research about the machine and ionized water overall and then made a video describing my experience with drinking the water. The video received a modest amount of attention and it found its way to some people who distributed Enagic Water Ionization Machines. One thing led to another and in the spring of 2008 I was asked to become the medical advisor of Enagic USA.

Enagic is a Japanese company that has had great success manufacturing and selling water ionizing machines in Japan. They developed a network of distributors with a patented compensation plan and in Japan the machine is a legitimate medical device and widely in use. Not so in the US. Their water is branded as Kangen Water®.

The issue with perception of alkaline ionized water in the States is caused by many contributing factors. First of all, there are US government regulations that only allow certain claims to be made on anything ingested. Enagic decided to refer to its product as a water that was alkaline, and had antioxidant properties. They presented that the water was micro clustered which provided great drinkability as well as promoting a healthy lifestyle and superior hydration. All of this is within federal guidelines, and the fact that many studies suggest that the majority of Americans are chronically dehydrated (due to drinking acidic soft drinks or expensive bottled water which is also quite acidic) made for a good, within policy, marketing strategy.

However, the information coming from Enagic distributors at times was a bit sketchy. The relationship between the Enagic company and its distributors is that the distributors are independent of the company. With independence regarding advertising, a few distributors went overboard about the results of drinking ionized water. Claims were made of miraculous cures and efficacy on just about every disease known to man.

The great Nobel Laureate Otto Heinrich Warburg was quoted from his work about the metabolism of cancer cells. "Cancer cannot live in an alkaline environment" was proselytized in numerous distributor meetings, drawing inferences that Dr. Warburg never said. The health benefits of the water were extrapolated from hundreds of studies in the literature on invertebrate and other animal models, which have no relevance to the human condition.

And the stories didn't end. When the effects of molecular hydrogen became known in medical literature, suddenly a craze began that claimed it was the hydrogen that provided all of the benefits of ionized alkaline water. The fact that one can clearly see hydrogen bubbles in the electrolyzed water was given as proof not just of the presence of hydrogen, but also of efficacy. Every human study on molecular hydrogen always concluded with a cautionary note that the health benefits of molecular hydrogen were still being elucidated but this warning got conveniently left out. The fact that studies on molecular hydrogen were considered to be a work in process did not deter overzealous distributors from drawing totally inappropriate conclusions.

This collection of hyperbole and half-truths went a long way towards discrediting the value of ionized water. As there are great benefits to drinking and using ionized water in our daily lives, I found myself giving talks throughout the country, trying to separate fact from fiction and use reasonable deductions from the few available studies to prove the worth of the water.

In order to prove efficacy of Kangen Water®, studies need to show that a structure function relationship exists. The water needs to effect functions within the human body in a favourable manner. If there is a claim of antioxidant effect about the water, a measurable effect within the blood or plasma of the person drinking the water must be demonstrated.

In the past the tests for antioxidant activity (ORAC) have failed to show such an effect, however this was most likely due to the test methodology. Using a different methodology was the answer. Our first tests in living human red cells was positive for an antioxidant effect and the beginning of other more sophisticated studies which evolved from that.

Study Goals

Studies had already shown that the pH of highly acidotic animals could be altered favourably by ionized alkaline water. But what was needed were structured tests to see if the same alterations occurred in live human beings. And so the idea for the trials detailed in this study was born.

I went to work defining testing parameters as well as getting the study funded. The best way to really study the efficacy of the water in living human beings was to test the effect of water in actual human volunteers. There were difficulties in working out the methodology including determining the antioxidant capacity of a colourless liquid. This was solved when I found a laboratory in Oregon, which had a proprietary assay for antioxidant capacity using human red cells in tissue culture. I felt that this methodology should be able to show the antioxidant properties of the water. It was clear from the start that Enagic was the right choice for funding the test. However, because Enagic is a privately held company (with one owner) it was difficult to get access to decision makers who could provide financial support for research studies. I was, after some time, able to get an appointment with Mr. Oshiro, the owner of the company. He agreed to provide the money for testing the product for antioxidant capacity.

With both testing protocols defined and funding in place, we were ready to get started. My close friend (and very successful Enagic distributor) Fred Brown accompanied me to Klamath Falls, Oregon, to the lab I had found with the proprietary tests for antioxidant capacity. We met with Dr. Gitte Jensen, Director of the Natural Immune System Laboratory and we developed a strategic plan. We were able to define study plans and protocols for Enagic USA sponsored research trials on the efficacy of Kangen Water® as relevant to human health.

At last, there was finally a chance to provide the public with solid scientific evidence about ionized water.

The plan was to do the tissue culture studies first and then do a human trial with healthy volunteers to see how the water affects the response of the body to exercise-induced inflammation and recovery. So we started with the tissue cultures.

HYDRATION

Ionized water found in

nature's glaciers

can now be enjoyed

by family and friends

through the process of

Electrolysing

regular tap water!

Research Results

The first study in tissue culture showed the following:

- **Kangen Water® has antioxidant properties which are measurable against plain tap water with a pH of 8.5**

- **There was a significant suppression of the inflammatory response by neutrophils with Kangen Water®**

- **An additional testing of the water showed that in the presence of glucose, the mitochondria of the cell up regulate their activity. In other words, the engine of the cell runs better with Kangen™ Water.**

On a side note, before the results that we were so anxiously looking for appeared, there was the inevitable encounter with Murphy's Law. After the initial batch of testing, the numbers looked very odd. It appeared that Klamath Falls tap water was as good as Kangen Water® in antioxidant efficacy! For reasons that are still unclear, the Oxidation Reduction Potential produced by the machine we installed registered only in the low -200s. Our good friend Mr. Fukuda came to the rescue and 2 new machines were installed. The new machines produced the expected ORP of -600 which solved the problem and saved the day. Had we f ailed to produce a positive result, the further testing would never have been done and my credibility would be zero.

With the favourable results of testing of the water and tissue culture, Mr. Oshiro authorized the expenditure for a clinical trial of Kangen Water®. The trial was titled "Sports Recovery and Inflammation Management with Alkaline Ionized Water". This trial would require 12-13 human volunteers (for statistical significance), and was registered with the National Institute of Health.

In this trial, the response of the body to stress and recovery from stress was studied against plain tap water. The details of the results are shown in the studies that follow. Because of the complexity of doing medical research in human volunteers, the trial took longer than anticipated. Each clinical trial needs to be approved by an independent medical board and has to meet all ethical standards for human trials described by the NIH. All participants need to be properly screened for health issues and fitness to do the necessary exercises and so recruitment takes time. However, when the results came in I submit that it was worth the time and effort to make sure everything was done right.

NIS LABS
CONTRACT RESEARCH LABORATORY

It is clear the Kangen Water® has properties that cause the human body to respond more favourably to stress, specifically the following:

- Production of more red cells to increase oxygen-carrying capacity
- Production of neutrophils to modulate and reduce inflammatory response

Each participant of the study also provided serum and plasma samples that are being kept frozen so that future testing for specific cytokines related to inflammation and other factors can be done. I believe that with this clinical trail, Kangen Water® has established a solid foundation for the veracity of its claims of alkalinity and antioxidant capacity in the living human body, and further work will reveal even more benefits of the water.

I believe that Kangen Water® is a unique and highly effective source of water, promoting health and wellbeing. The results of the studies so far show the uniqueness of alkaline ionized water when compared to any other standard drinking water and I am looking forward to continuing our research in the dynamic field.

Enagic

ENAGIC SD 501 Platinum

Tests

3 tests were done on the Alkaline Ionized Water:

119-001 - Testing of antioxidant capacity and cellular protection in vitro.
119-002 - Effect on cellular viability and energy production.
119-004 - Sports recovery and inflammation management

Here are a few relevant terms.

pH
pH stands for "potential hydrogen". It indicates the level of hydrogen in a substance and is measured on a scale. Proper body pH is important for good health.

ORP
ORP stands for Oxidation-Reduction Potential. Oxidation is what turns an apple brown after it is cut, or causes metal to rust. The measurement of a liquid's oxidizing potential is measured in +ORP. Most tap water in the US is between +200 to +600 mV.
Anti-oxidants slow or inhibit the process of oxidation. Antioxidants are measure in -ORP. AIW with a high pH has a -ORP, making it an antioxidant. In some of the tests below, the ORP for the AIW tested is -800 mV.

In vitro
While the general public is used to the term in vitro to mean somehow related to pregnancy, in scientific testing it means the tests are done on microorganisms, cells, or biological molecules. Tests 119-001 and 119-002 were done in vitro, while test 119-004 was done on humans.

119-001-September, 2015

Test 119-001. Testing of antioxidant capacity and cellular protection in vitro.

Test 119-001 was mainly about learning about the properties of Alkaline Ionized Water (AIW) and how best to test them.

This test focused on 2 things. One was to identify how to handle AIW and what dose range should be used when working with human cell cultures. The other was to actually document antioxidant and anti-inflammatory properties of AIW.

As far as the antioxidant properties, the test results showed that the direct antioxidant capacity of AIW is mild, but reproducible. However, the testing of cellular antioxidant protection did not show protection of cells by AIW above that of tap water. Because the testing protocols included a strong induction of free radical stress, it was suggested by the laboratory that a modified protocol might allow the tests to show cellular protection from free radical stress in a future project.

As far as the anti-inflammatory properties, the exposure of inflammatory cells to AIW reduced the inflammatory production of free radicals by the cells. Further testing is advised in this area, both in cell models and in human clinical trials.

Antioxidant tests had a bit of a rough start. The first 3 tests were unusable, as the water being produced by the installed Kangen machine was not able to generate the correct pH level. A new machine was used and test results showed that the direct antioxidant capacity of AIW is mild, but real and reproducible. In addition, the production of free radicals by inflammatory cells exposed to AIW was reduced.

2 additional tests were recommended after 119-001 was completed. One, 119-002, focused on another round of in vitro testing but this time including AIW's effects on protection of mitochondrial function. The other, 119-004, addressed AIW and its possible role in accelerated recovery from exercise-induced inflammation.

119-002-December, 2015

Test 119-002. Alkaline ionized water: Effect on cellular viability and energy production.

Test 119-002 was focused on testing the effects of AIW on cell viability/mitochondrial metabolic function in cell cultures.

The testing used two different cell cultures:

1) Short-lived polymorph nuclear (PMN) cells cultured for 4 hours. The PMN cells are shorter-lived, and can perform almost immediate, aggressive inflammatory reactions, either as part of anti-bacterial immune defence reactions or as part of chronic inflammation.

2) Peripheral blood mononuclear cells (PBMC) cultured for 4 and 24 hours. The PBMC cells are longer-lived and include immune cells such as Natural Killer cells, lymphocytes, and monocytes, responsible for antigen-presentation, immuno globulin and cytokine production, and support of long-term immune memory

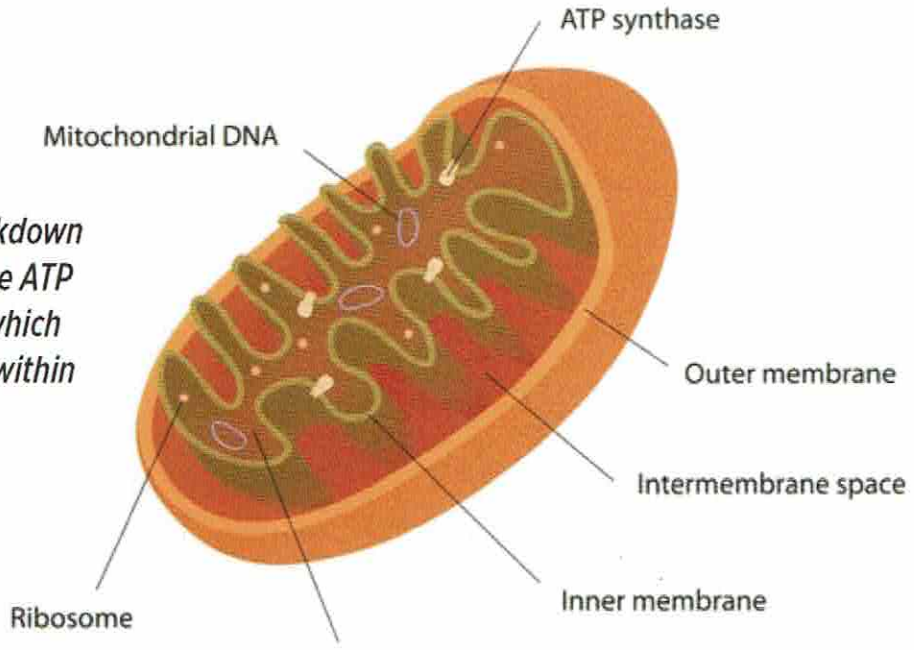
- **In 4 hour PMN cell cultures, AIW treatment led to an increase in metabolism de pending upon th dose and using the medium containing higher levels of D glucose.**
- **In 4 hour PBMC cultures, AIW had no effect on cellular viability/metabolism.**
- **In 24 hour PBMC cultures, AIW as well as tap water slightly reduced viability/metabolism in the presence of the medium containing lower/moderate levels of D glucose.**

This data suggest that the physiological effects of consuming AIW could be at least in part mediated through positive effects on mitochondria.

Once again, it was advised that further testing be done to document the properties of AIW in additional immune/inflammatory cell models, as well as in human clinical pilot studies.

Mitochondria

are responsible for the breakdown of sugar molecules to release ATP (adenosine triphosphate), which is used to transport energy within the cell for metabolism



119-004-August, 2016

Test 119-004.-Sports recovery and inflammation management

Test 119-004 was concerned with evaluating the effects of consuming alkaline ionized water (AIW) on reduction of inflammation. This was a human clinical study.

A randomized, controlled, cross-over study design was used that involved 13 people.
During the run-in period, each person was asked to track their water consumption. Some participants consumed tap water first and some consumed Kangen™ Water first.

Test subjects were not required to be in a fasting state however, they were instructed to abstain from strenuous exercise in the 8 hours before each clinic visit. Study participants were asked to follow similar routines prior to each visit, and to take notes about their meal that was prior to the first visit so they could have a similar meal before the next visit.

If they were having a meal before the visit, they were asked to eat a bland meal at least one hour prior to arrival, and to not eat, use any nicotine-containing products, consume caffeine or supplements for 1 hour before the clinic visit. Study participants were encouraged to drink water.

At the visits involving an exercise challenge, an initial blood draw was done to compare biomarkers after 1 week of consuming either AIW or tap water as well as baseline for evaluation of exercise-induced inflammation/ resolution. In addition, finger sticks were performed to measure lactate levels in the blood before and after the exercise.

The study was designed around the following outcome measures:

- Primary outcome measure: Changes in numbers of blood cells (as measured by a Complete Blood Count (CBC) with differential count)

- Secondary outcome measure: Change in plasma lactate (as measured by a lactate meter and finger sticks); - Tertiary outcome measure: Subjective reporting of changes to energy levels, soreness, and overall wellbeing.

Each exercise challenge consisted of a short burst of high-intensity interval training (HIIT), where 20 seconds of exercise are followed by 20 seconds of rest. For the safety of the study participants, and for the maximum muscular challenge to induce lactate build-up, they were instructed to keep a high intensity of each work-out during each exercise challenge.

The exercise challenge involved 5 rounds, where one round included:

Push-ups 20 seconds - Rest 20 seconds - Sit-ups 20 seconds - Rest 20 seconds - Air squats 20 seconds - Rest 20 seconds

During each rest, the pulse was tracked, and used to calculate average and max heart rate. There were no statistically significance differences in average or maximum heart rates, or VO2max, after AIW consumption wher compared to tap water.

At the beginning of each study visit, questionnaires were completed to evaluate subjective feedback on vitality and wellbeing. The study coordinator also tracked what medications and other supplements were being consumed.

At each visit involving an exercise session, the study participant went through an additional questionnaire, both immediately after exercise and at 1 hour after exercise.

The questions included:

- **Physical energy level**
  - During workout
  - Immediately after workout
  - One hour after workout
- **Mental energy level during workout**
  - Immediately after workout
  - One hour after workout
- **Post-exercise soreness**
- **Overall pain**

Additional questions addressed general well-being, physical and mental energy levels, mental focus, and sleep quality.

Overall, the results pointed to health benefits related to the following core findings:

- Increased energy (oxygen delivery, subjective sense of energy, increased alertness and relaxation, reduced restlessness)
- Increased readiness for repair (increased platelet counts, reduced soreness)
- Reduced levels of inflammatory cells

These effects contributed to an increased state of wellness.

The main finding in this study is that consumption of A|W supported physiological response to the exercise challenge better than tap water. In particular, the objective measures pertaining to the statistically significant increases in red blood cell haemoglobin and haematocrit translate to an increase in the blood's oxygen-carrying capacity.

The second finding was reduced levels of inflammatory cells in the blood after consuming A|W, compared to tap water. For some types of inflammatory cells, the reduction associated with A|W consumption was statistically significant when compared to tap water.

The combination of the increase in the blood's oxygen-carrying capacity and the reduced number of inflammatory cells in the blood after A|W consumption may possibly provide an underlying explanation for the reduced soreness after the exercise challenge.

There was a mild trend to a faster return to normal plasma lactate levels after the exercise challenge when A|W had been consumed before the challenge.

The result presented in this report confirm that consuming A|W has measurable effects on various bio-markers and the subjective sense of wellbeing.

The data are highly interesting, and it should be considered that these results be core data in a manuscript.

The initial study design included testing of serum for inflammatory markers and immune - modulating cytokines, as well as testing of plasma for markers for oxidative stress. For budget reasons, these tests were not included in the core clinical study; however, both serum and plasma have been banked frozen at -80oC, such that these tests could be considered once we saw the outcome of the clinical study.

Summary and Conclusions

Testing Kangen Water® for antioxidant activity compared to ordinary water showed the Kangen Water® had greater antioxidant capacity in living human tissue cultured in cells. In addition, the testing showed a reduction in the inflammatory response when exposed to inflammatory cells. Cellular metabolism and energy production in Neutrophil cells was enhanced (improved) significantly in the presence of glucose. To put it simply, Kangen Water® improved the function of the cellular engine.

The testing of Kangen Water® in sports recovery and inflammation revealed an increase in red cell production with Kangen Water® resulting in increased oxygen delivery and carbon dioxide removal. There was also a reduction in the release of inflammatory cells, which reduces exercise stress related to inflammation. Increased production of platelets with the consumption of Kangen Water® was also observed and suggests an improved readiness for stress-induced damage repair. From a subjective point of view, the study participants drinking the water showed a statistically significant improvement in recovery from the exercises and an improved sense of wellbeing.

Plasma and serum saved from all participants in the study will be analysed in a future study for a variety of cytokines and oxidative stress makers to further broaden the knowledge based on the efficacy of Kangen Water®. These initial studies sponsored by Enagic clearly demonstrate that the water is unique and certainly has a role in promoting good health.

Kangen Water®

CHANGE YOUR WATER...
CHANGE YOUR LIFE!

NIS LABS
Natural Immune Systems Inc

September 30, 2015.

Report for:

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Medical Advisor

Enagic USA, Inc.

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Torrance, CA 90503-2419

119-001. Alkaline ionized water: Testing of antioxidant capacity and cellular protection in vitro.

Joni Lee Beaman

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Date: Sept. 30, 2015.

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NIS LABS
Natural Immune Systems Inc

December 23, 2015.

Report for:

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Report 119-002. Alkaline ionized water: Effect on cellular viability and energy production.

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August 17, 2016.

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Report 119-004. Alkaline ionized water: Sports recovery and inflammation management.

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December 23, 2014.

Strategic Planning Document For:

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Strategic Research Plan for Alkaline Ionized Water.

Strategic Research Plan for the Alkaline Ionized Water

Purpose

To plan a focused series of research projects in order to build a solid and credible scientific portfolio for the alkaline ionised water product. The building of this portfolio is important for demonstrating sincere efforts to document that the product is effective, both to clients and to regulatory agencies such as the Federal Trade Commission and the Food and Drug Administration.

The immediate goal is to project a clear path, with meaningful first steps in place and initiated, as soon as possible.

the long-term goal is to have a robust data volume to support marketing claims, including the credibility of having several peer-reviewed scientific publications, each addressing different aspects of the effects of the consuming the alkaline ionised water.

This document outlines the path and the proposed projects to achieve these goals.

Background

Enagic is the leader in selling machines for home and industrial use for alkaline ionised water (AIW).

Much research has been done in Japan, but no systematic research has been done in USA on the biological properties of the water.

Enagic USA needs to initiate the process of creating a solid scientific portfolio on in vitro and clinical research results.

A level SD 501 machine (Enagic USA) has been installed at NIS Labs. Alkaline ionised water (AIW) will be tested in a series of bioassays for antioxidant and anti-inflammatory effects, and regulation of cellular behaviour and activation processes.

In parallel, over the coming 4-6 months, we will design a select series of clinical studies, starting with a small feasibility study to explore the effects of AIW in exercise-induced inflammation in healthy adults, as a model for regulating inflammation in the absence of disease.

Planning a Sequence of Interconnected Projects

The following plan includes steps where each step has independent value for marketing, while also helping plan subsequent steps in more detail, for an efficient long term plan.

1. Proof of concept: Optimal handling of the AIW in vitro and in cell cultures: Does AIW have anti-oxidant and the anti-inflammatory properties of relevance for biological systems?

2. Solidify the support of a healthy inflammation response: Document key biological inflammatory responses in vitro in the presence verses absence of AIW, pertaining both to the induction and the resolution of an inflammatory insult;

3. Document that these biological effects in vitro are effective for protecting cellular viability and mitochondrial function;

4. Clinical pilot study: Document effects on baseline and exercise-induced inflammation and its resolution.

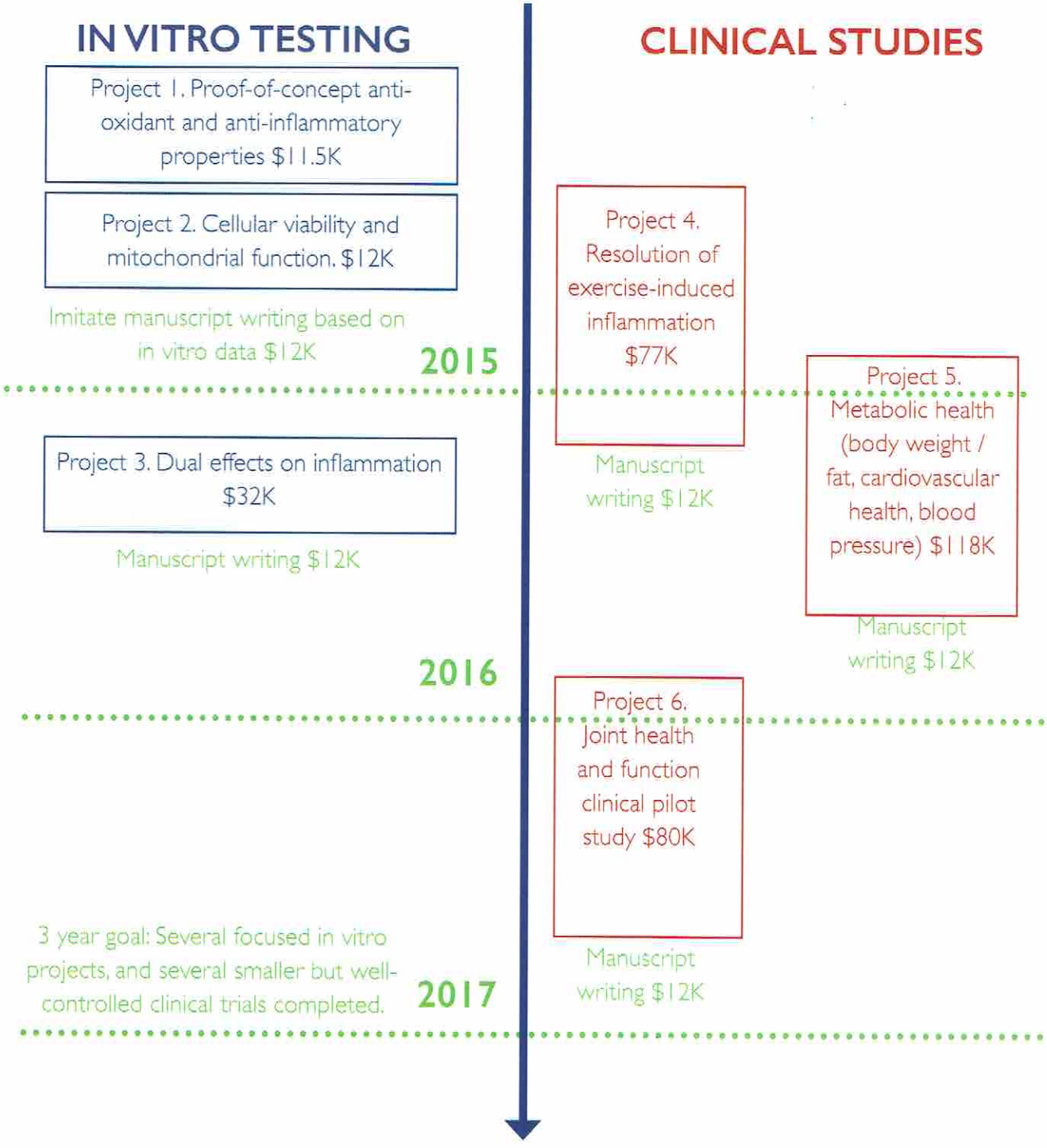
5. Metabolic health, through broad support of cardiovascular and digestive/metabolic health, aiming for healthy weight, lipid profile and blood pressure.

6. Joint health: this is intended as a smaller study, to document pain relief and improved joint function, using NIS Labs' capacity for joint health studies using digital inclinometry.

Workflow Projections for 2014 - 2016

The work outlined here is shown as projected, in phases over the coming 3 years. If priorities change, this workflow will be adjusted. During this work flow, as suitable data accumulate, manuscript writing on completed work can happen in parallel to on-going studies.

Approximate budgets are indicated to help planning, however, please note that the clinical studies are extremely lose estimates only; these studies will be planned i more detail over the coming 6 months.



Published Medical Results

1. Biochem Biophys Res Commun. 1997 May 8;234(1):269-74.
Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage.

Shirahata S(1), Kabayama S, Nakano M, Miura T, Kusumoto K, Gotoh M, Hayashi H, Otsubo K, Morisawa S, Katakura Y.

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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 O.-2 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. Reduce water suppresses single-strand breakage of DNA b active oxygen species produced by the Cu(II)-catalysed oxidation of ascorbic acid in a dose-dependent manner, suggesting that reduced water can scavenge not only O2.- and H2O2, but also 1O2 and .OH.

DOI: 10.1006/bbrc.1997.6622

PMID: 9169001 [Indexed for MEDLINE]

1. Oxid Med Cell Longev. 2014;2014:869121. doi: 10.1155/2014/869121. Epub 2014 Oct 14.

Electrochemically reduced water protects neural cells from oxidative damage.

Kashiwagi T(1), Yan H(2), Hamasaki T(2), Kinjo T(2), Nakamichi N(1), Teruya K(3), Kabayama S(4), Shirahata S(3).

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Aging-related neurodegenerative disorders are closely associated with mitochondrial dysfunction and oxidative stresses and their incidence tends to increase with aging. Brain is the most vulnerable to reactive species generated by a higher rate of oxygen consumption and glucose utilization compared to other organs.

Electrochemically reduced water (ERW) was demonstrated to scavenge reactive oxygen species (ROS) in several cell types. In the present study, the protective effect of ERW against hydrogen peroxide (H2O2) and nitric oxide (NO) was investigated in several rodent neuronal cell lines and primary cells. ERW was found to significantly suppress H2O2 (50-200 μM) induced PC12 and SFME cell deaths. ERW scavenged intracellular ROS and exhibited a protective effect against neuronal network damage caused by 200 μM H2O2 in N1E-115 cells. ERW significantly suppressed NO-induced cytotoxicity in PC12 cells despite the fact that it did not have the ability to scavenge intracellular NO. ERW significantly suppressed both glutamate induced Ca(2+) influx and the resulting cytotoxicity in primary cells.

These results collectively demonstrated for the first time that ERW protects several types of neuronal cells by scavenging ROS because of the presence of hydrogen and platinum nanoparticles dissolved in ERW.

DOI: 10.1155/2014/869121

PMCID: PMC4212634

PMID: 25383141 [Indexed for MEDLINE]

1. Biol Res. 2013;46(2):147-52. doi: 10.4067/S0716-97602013000200005.

Electrolyzed-reduced water increases resistance to oxidative stress, fertility and lifespan via insulin/IGF-1-like signa in C. elegans.

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Electrolyzed-reduced water (ERW) scavenges reactive oxygen species and is a powerful anti-oxidant. A positive correlation between oxidative stress and aging has been proved in many model organisms. In Caenorhabditis elegans, many long-lived mutants showed reduced fertility as a trade off against longevity phenotype. We aimed to study the effect of ERW on oxidative stress, fertility and lifespan of C. elegans. We also investigated the genetic pathway involved in the effect of ERW on resistance to oxidative stress and lifespan. We compared lifespan and fertility of worms in media prepared with distilled water and ERW.

ERW significantly extended lifespan and increased the number of progeny produced.

Then the effect of ERW on resistance to oxidative stress and lifespan of long-lived mutants was determined. ERW increased resistance to oxidative stress and lifespan of eat-2, a genetic model of dietary restriction, but had no effect on those of age-1, which is involved in insulin/insulin-like growth factor (IGF)-1-like signal. In addition, knockdown of daf-16, the downstream mediator of insulin/IGF-1-like signal, completely prevented the effect of ERW on lifespan.

These findings suggest that ERW can extend lifespan without accompanying reduced fertility and modulate resistance to oxidative stress and lifespan via insulin/IGF-1-like signal in C. elegans.

DOI: 10.4067/S0716-97602013000200005

PMID: 23959012 [Indexed for MEDLINE]

1. PLoS One. 2017 Feb 9;12(2):e0171192. doi: 10.1371/journal.pone.0171192. eCollection 2017.

Electrochemically reduced water exerts superior reactive oxygen species scavenging activity in HT1080 cells than the equivalent level of hydrogen-dissolved water.

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Electrochemically reduced water (ERW) is produced near a cathode during electrolysis and exhibits an alkaline pH, contains richly dissolved hydrogen, and contains a small amount of platinum nanoparticles. ERW has reactive oxygen species (ROS)-scavenging activity and recent studies demonstrated that hydrogen-dissolved water exhibits ROS-scavenging activity. Thus, the antioxidative capacity of ERW is postulated to be dependent on the presence of hydrogen levels; however, there is no report verifying the role of dissolved hydrogen in ERW. In this report, we clarify whether the responsive factor for antioxidative activity in ERW is dissolved hydrogen. The intracellular ROS scavenging activity of ERW and hydrogen-dissolved water was tested by both fluorescent stain method and immuno spin trapping assay. We confirm that ERW possessed electrolysis intensity-dependent intracellular ROS-scavenging activity, and ERW exerts significantly superior ROS-scavenging activity in HT1080 cells than the equivalent level of hydrogen-dissolved water. ERW retained its ROS-scavenging activity after removal of dissolved hydrogen, but lost its activity when autoclaved. An oxygen radical absorbance capacity assay, the 2,2-diphenyl-1-picrylhydrazyl assay and chemiluminescence assay could not detect radical-scavenging activity in both ERW and hydrogen-dissolved water. These results indicate that ERW contains electrolysis-dependent hydrogen and an additional antioxidative factor predicted to be platinum nanoparticle.

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1. Nephrol Dial Transplant. 2010 Aug;25(8):2730-7. doi: 10.1093/ndt/gfq082. Epub 2010 Feb 26.
Electrolysed-reduced water dialysate improves T-cell damage in end-stage renal disease patients with chronic haemodialysis.
Huang KC(1), Hsu SP, Yang CC, Ou-Yang P, Lee KT, Morisawa S, Otsubo K, Chien CT. Author information:
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BACKGROUND: T-cell damage by increased oxidative stress in end-stage renal disease (ESRD) patients undergoing chronic haemodialysis (HD) led to the increased T-cell apoptosis and the alteration of surface markers and Th1/Th2 ratio in CD4(+) T lymphocytes. Antioxidant electrolysed-reduced water (ERW) was used as the dialysate in ESRD patients undergoing chronic HD to test for improve oxidative stress-related T-cell apoptosis, alterations of surface markers and intracellular cytokine profile.

METHODS: We evaluated apoptosis formation by annexin V, CD25-related surface markers, and cytokine ratio of Th1/Th2 in CD4(+) T lymphocytes and Tc1/Tc2 in CD8(+) T lymphocytes of 42 ESRD patients haemodialysed with ERW for 1 year. RESULTS: In comparison to 12 healthy individuals, the ESRD patients had more T-cell apoptosis and less CD3(+), CD4(+) and CD8(+) T cells and CD25/CD69/CD94/CD3(+) phenotypes at baseline. Lower intracellular IL-2 and IFN-gamma levels in the Th1/CD4(+) and Tc1/CD8(+) cells and higher intracellular IL-4, IL-6 and IL-10 levels in the Th2/CD4(+) and Tc2/CD8(+) cells were also noted in the ESRD patients. After a 1-year ERW treatment, the patients had a decrease in T-cell apoptosis and increases in CD3(+), CD4(+) and CD8(+) cell numbers and CD25/CD69/CD94/CD3(+) phenotypes in the T cells. The intracellular IL-2 and IFN-gamma levels in the Th1/Tc1 cells significantly ($P < 0.05$) increased and the intracellular IL-4, IL-6 and IL-10 levels in the Th2/Tc2 cells decreased.

Furthermore, the Th1/Th2 and Tc1/Tc2 cytokine ratios were improved toward a normal status.

CONCLUSION: One-year ERW treatment effectively ameliorated T-cell apoptosis, altered CD25-related surface markers and intracellular cytokine profile in the HD patients.

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1. Biol Pharm Bull. 2008 Jan;31(1):19-26.
Inhibitory effect of electrolyzed reduced water on tumor angiogenesis.

Ye J(1), Li Y, Hamasaki T, Nakamichi N, Komatsu T, Kashiwagi T, Teruya K, Nishikawa R, Kawahara T, Osada K, Toh K, Abe M, Tian H, Kabayama S, Otsubo K, Morisawa S, Katakura Y, Shirahata S.

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Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis. Tumor cells are exposed to higher oxidative stress compared to normal cells. Numerous reports have demonstrated that the intracellular redox (oxidation/reduction) state is closely associated with the pattern of VEGF expression. Electrolyzed reduced water (ERW) produced near the cathode during the electrolysis of water scavenged intracellular H(2)O(2) and decreased the release of H(2)O(2) from a human lung adenocarcinoma cell line, A549, and down-regulated both VEGF transcription and protein secretion in a time-dependent manner. To investigate the signal transduction pathway involved in regulating VEGF expression, mitogen-activated kinase (MAPK) specific inhibitors, SB203580 (p38 MAPK inhibitor), PD98059 (ERK1/2 inhibitor) and JNKi (c-Jun N-terminal protein kinase inhibitor) were applied. The results showed that only PD98059 blocks VEGF expression, suggesting an important role for ERK1/2 in regulating VEGF expression in A549 cells. As well, ERW inhibited the activation of extracellular signal-regulated kinase (ERK) in a time-dependent manner. Co-culture experiments to analyze in vitro tubule formation assay revealed that A549 cell-derived conditioned medium significantly stimulated the formation of vascular tubules in all analyzed parameters; tubule total area, tubule junction, number of tubules, and total tubule length. ERW counteracted the effect of A549 cell-conditioned medium and decreased total tube length ($p<0.01$). The present study demonstrated that ERW down-regulated VEGF gene transcription and protein secretion through inactivation of ERK.

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1. Biol Pharm Bull. 2007 Feb;30(2):234-6.
Preservative effect of electrolyzed reduced water on pancreatic beta-cell mass in diabetic db/db mice.
Kim MJ(1), Jung KH, Uhm YK, Leem KH, Kim HK.

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(1)Department of Obesity Management, Graduate School of Obesity Science, Dongduk Women's University, Seoul, South Korea. mijakim@dongduck.ac.jp Oxidative stress is produced under diabetic conditions and involved in progression of pancreatic beta-cell dysfunction. Both an increase in reactive oxygen free radical species (ROS) and a decrease in the antioxidant defense mechanism lead to the increase in oxidative stress in diabetes. Electrolyzed reduced water (ERW) with ROS scavenging ability may have a potential effect on diabetic animals, a model for high oxidative stress. Therefore, the present study examined the possible anti-diabetic effect of ERW in genetically diabetic mouse strain C57BL/6J-db/db (db/db). ERW with ROS scavenging ability reduced the blood glucose concentration, increased blood insulin level, improved glucose tolerance and preserved beta-cell mass in db/db mice. The present data suggest that ERW may protect beta-cell damage and would be useful for antidiabetic agent.

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