Hydrogen-rich electrolyzed warm water represses wrinkle formation against UVA ray together with type-I collagen production and oxidative-stress diminishment in fibroblasts and cell-injury prevention in keratinocytes

Shinya Kato [a,1], Yasukazu Saitoh [a,2], Keizou Iwai [b], Nobuhiko Miwa [a,*,3]

[a] *Laboratory of Cell-Death Control BioTechnology, Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Nanatsuka 562, Shobara, Hiroshima 727-0023, Japan*
[b] *The Chugoku Electric Manufacturing Co. Inc., Oozu 4-4-32, Minami-ku, Hiroshima 732-8564, Japan*

ARTICLE INFO

Article history:
Received 19 May 2011
Received in revised form 16 September 2011
Accepted 28 September 2011
Available online xxxx

Keywords:
Hydrogen water
UV-A
Type-I collagen
Oxidative stress
Hydrogen water bathing
Wrinkle repression

ABSTRACT

Hydrogen-rich electrolyzed warm water (HW) was prepared at 41 °C and exhibited dissolved hydrogen (DH) of 1.13 ppm and an oxidation–reduction potential (ORP) of −741 mV in contrast to below 0.01 ppm and +184 mV for regular warm water (RW). Fibroblasts OUMS-36 and keratinocytes HaCaT were used to examine effects of HW against UVA-ray irradiation. Type-I collagen was synthesized 1.85- to 2.03-fold more abundantly by HW application for 3–5 days than RW in OUMS-36 fibroblasts, and localized preferentially around the nuclei as shown by immunostain. HW application significantly prevented cell death and DNA damages such as nuclear condensation and fragmentation in UVA-irradiated HaCaT keratinocytes as estimated by WST-1 and Hoechst 33342 assays. HW significantly suppressed UVA-induced generation of intracellular superoxide anion radicals in both the cell lines according to NBT assay. Wrinkle repression was clinically assessed using a HW-bathing. Six Japanese subjects were enrolled in a trial of HW-bathing (DH, 0.2–0.4 ppm) every day for 3 months. HW-bathing significantly improved wrinkle in four subjects on the back of neck on 90th day as compared to 0 day. Thus, HW may serve as daily skin care to repress UVA-induced skin damages by ROS-scavenging and promotion of type-I collagen synthesis in dermis.

Hydrogen water intake via tube-feeding for patients with pressure ulcer and its reconstructive effects on normal human skin cells *in vitro*

Qiang Li[1], Shinya Kato[2], Daigo Matsuoka[3], Hiroshi Tanaka[1] and Nobuhiko Miwa[1*]

Abstract

Background: Pressure ulcer (PU) is common in immobile elderly patients, and there are some research works to investigate a preventive and curative method, but not to find sufficient effectiveness. The aim of this study is to clarify the clinical effectiveness on wound healing in patients with PU by hydrogen-dissolved water (HW) intake via tube-feeding (TF). Furthermore, normal human dermal fibroblasts OUMS-36 and normal human epidermis-derived cell line HaCaT keratinocytes were examined *in vitro* to explore the mechanisms relating to whether hydrogen plays a role in wound-healing at the cellular level.

Methods: Twenty-two severely hospitalized elderly Japanese patients with PU were recruited in the present study, and their ages ranged from 71.0 to 101.0 (86.7 ± 8.2) years old, 12 male and 10 female patients, all suffering from eating disorder and bedridden syndrome as the secondary results of various underlying diseases. All patients received routine care treatments for PU in combination with HW intake via TF for 600 mL per day, in place of partial moisture replenishment. On the other hand, HW was prepared with a hydrogen-bubbling apparatus which produces HW with 0.8-1.3 ppm of dissolved hydrogen concentration (DH) and −602 mV to −583 mV of oxidation-reduction potential (ORP), in contrast to reversed osmotic ultra-pure water (RW), as the reference, with DH of < 0.018 ppm and ORP of +184 mV for use in the *in vitro* experimental research. In *in vitro* experiments, OUMS-36 fibroblasts and HaCaT keratinocytes were respectively cultured in medium prepared with HW and/or RW. Immunostain was used for detecting type-I collagen reconstruction in OUMS-36 cells. And intracellular reactive oxygen species (ROS) were quantified by NBT assay, and cell viability of HaCaT cells was examined by WST-1 assay, respectively.

Results: Twenty-two patients were retrospectively divided into an effective group (EG, n = 12) and a less effective group (LG, n = 10) according to the outcomes of endpoint evaluation and the healing criteria. PU hospitalized days in EG were significantly shorter than in LG (113.3 days vs. 155.4 days, $p < 0.05$), and the shortening rate was approximately 28.1%. Either in EG or in LG, the reducing changes (EG: 91.4%; LG: 48.6%) of wound size represented statistically significant difference versus before HW intake ($p < 0.05$, $p < 0.001$). The *in vitro* data demonstrate that intracellular ROS as quantified by NBT assay was diminished by HW, but not by RW, in ultraviolet-A (UVA)-irradiated HaCaT cells. Nuclear condensation and fragmentation had occurred for UVA-irradiated HaCaT cells in RW, but scarcely occurred in HW as demonstrated by Hoechst 33342 staining. Besides, under UVA-irradiation, either the mitochondrial reducing ability of HaCaT cells or the type-I collagen construction in OUMS-36 cells deteriorated in RW-prepared culture medium, but was retained in HW-prepared culture medium as shown by WST-1 assay or immunostain, respectively.

(Continued on next page)

* Correspondence: miwa@butsuryo.ac.jp
[1]Department of Radiological Technology, Faculty of Health Sciences, Butsuryo College of Osaka, Otorikitamachi 3-33, Nishi-ku, Sakai, Osaka 593-8328, Japan
Full list of author information is available at the end of the article

Research Article

The Drinking Effect of Hydrogen Water on Atopic Dermatitis Induced by *Dermatophagoides farinae* Allergen in NC/Nga Mice

Rosa Mistica C. Ignacio,[1] Hyun-Suk Kwak,[2] Young-Uk Yun,[2] Ma. Easter Joy V. Sajo,[1] Yang-Suk Yoon,[1] Cheol-Su Kim,[3] Soo-Ki Kim,[3] and Kyu-Jae Lee[1]

[1]Department of Environmental Medical Biology, Wonju College of Medicine, Yonsei University, Wonju, Gangwon 220-701, Republic of Korea
[2]ECO Solution Team, Digital Media and Communications R&D Center, Samsung Electronics Co., Ltd., Suwon, Gyeonggi 443-742, Republic of Korea
[3]Department of Microbiology, Wonju College of Medicine, Yonsei University, Wonju, Gangwon 220-701, Republic of Korea

Abstract

Hydrogen water (HW) produced by electrolysis of water has characteristics of extremely low oxidation-reduction potential (ORP) value and high dissolved hydrogen (DH). It has been proved to have various beneficial effects including antioxidant and anti-inflammatory effects; however, HW effect on atopic dermatitis (AD), an inflammatory skin disorder, is poorly documented. In the present study, we examined the immunological effect of drinking HW on *Dermatophagoides farinae*-induced AD-like skin in NC/Nga mice. Mice were administered with HW and purified water (PW) for 25 days. We evaluated the serum concentration of pro-inflammatory (TNF-α), Th1 (IFN-γ, IL-2, and IL-12p70), Th2 (IL-4, IL-5, and IL-10), and cytokine expressed by both subsets (GM-CSF) to assess their possible relationship to the severity of AD. The serum levels of cytokines such as IL-10, TNF-α, IL-12p70, and GM-CSF of mice administered with HW was significantly reduced as compared to PW group. The results suggest that HW affects allergic contact dermatitis through modulation of Th1 and Th2 responses in NC/Nga mice. This is the first note on the drinking effect of HW on AD, clinically implying a promising potential remedy for treatment of AD.

Positive Effects of Hydrogen Water on 2,4-Dinitrochlorobenzene-Induced Atopic Dermatitis in NC/Nga Mice

Yang-Suk Yoon,[a,#] Ma. Easter Joy Villarosa Sajo,[a,#] Rosa Mistica Coles Ignacio,[a] Soo-Ki Kim,[b] Cheol-Su Kim,[b] and Kyu-Jae Lee*[,a,c]

[a] *Department of Environmental Medical Biology, Wonju College of Medicine, Yonsei University;* [b] *Department of Microbiology, Wonju College of Medicine, Yonsei University; Wonju, Gangwon 220–701, Republic of Korea; and* [c] *Institute for Poverty Alleviation and International Development, Yonsei University; Wonju, Gangwon 220–710, Republic of Korea.*

Atopic dermatitis (AD) is a chronically relapsing, pruritic, eczematous skin disorder accompanying allergic inflammation. AD is triggered by oxidative stress and immune imbalance. In the present study, we investigated the effect of drinking hydrogen water (HW) on 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis in NC/Nga mice and found that HW ameliorated DNCB-induced AD-like clinical symptoms. In line with this, the level of reactive oxygen species in the HW group was significantly inhibited compared with that in the purified water (PW) group. In parallel, HW enhanced glutathione peroxidase activity in DNCB-induced AD as compared with the PW group. Accordingly, the levels of thymus and activation-regulated chemokine and cytokines were significantly decreased in the HW group compared with the PW group. Notably, the levels of Th_2 cytokine, interleukin-5 (IL-5), and proinflammatory cytokines such as tumor necrosis factor-α and IL-6 in HW-fed mice were significantly lower than in control and PW-fed mice. The total serum immunoglobulin E level was also markedly reduced in the HW group. The collective results indicate that HW suppresses DNCB-induced AD in NC/Nga mice *via* redox balance and immune modulation and could be a safe clinical fluid treatment for AD.