Animal Cell Technology: From Target to Market
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Anti-Oxidative Water Improves Diabetes

Abstract

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Anti-Oxidative Water Improves Diabetes

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Abstract. Electrolyzed reduced water and natural waters such as Hita Tenryosui water in Japan, Nordenau water in Germany and Tracote water in Mexico, which are known to improve various diseases, were all anti- oxidative waters which could scavenge intracellular reactive oxygen species. The anti-oxidative waters stimulated not only the glucose uptake of rat myotube L6 cells, but also the secretion of insulin from a pancreatic beta cell line HIT-T15. The anti-oxidative waters improved the damage in the sugar tolerance test of type 2 diabetes model mice (*db/db* mice). A clinical investigation demonstrated that Nordenau water could significantly improve the diabetes mellitus.

1. Introduction

Reactive oxygen species (ROS) such as $^1O_2$, O_2^-, H_2O_2 and $\cdot OH$ are known to cause various diseases including diabetes mellitus. Electrolyzed reduced water (ERW) produced near cathode during electrolysis of water scavenged ROS and protected DNA from oxid-ative damage *in vitro* (1). Hayashi proposed a water regulation theory that hydrogen-rich

active damage in vivo (1). Kajiyama proposed a water regulation theory that hydrogen rich water can scavenge ROS (2) and reported that the daily intake of ERW improved diabetes mellitus (3). ERW and natural reduced water such as Hita Tenryosui water stimulated glucose uptake of rat L6 myotubes (4). There are several natural waters drawn from deep underground such as Nordenau water in Germany and Tracote water in Mexico as well as Hita Tenryosui water in Japan, which are called as miracle water because of curing power against various diseases. Here we demonstrate that ERW and the natural waters are all anti-oxidative water and scavenge ROS in cultured cells. The anti-oxidative waters stimulated not only the glucose uptake into insulin-responsive muscle cells but also the insulin secretion from beta cells of pancreas. The anti-oxidative water improved the damage in the sugar-tolerance test of diabetes model mice and also improved the symptoms of human diabetes.

2. Materials and Methods

Waters. ERW was prepared by electrolysis of activated charcoal treated tap water or ultra pure water containing 0.01% NaCl using an electrolyzing device (TI-8000, Nihon Trim Co., Ltd., Osaka). The pH and ORP of ERW were 10.5 to 11.5 and −650 to −850 mV, respectively. ERW was used after neutralized with HCl. Hita Tenryosui water was supplied by Hita Tenryosui Co. Ltd., Hita, Japan. Nordenau water was given by Mr. Theo Tommes, Nordenau, Germany. Tracote water was supplied by Nihon TV Co. Ltd. Mineral waters A and B were those commercially sold in Japan.

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Measurement of intracellular ROS scavenging effects. L6 cells derived from rat skeletal muscle were differentiated into insulin responsive myotubes by cultivation in 2% FBS/DME medium for 7 days (4). Intracellular redox state of L6 cells cultured in medium prepared with each waters was measured after 72 hour cultivation by a confocal laser microscopy using a fluorescent dye, 2',7' - dichlorofuluoroescein diacetate (DCFH-DA) as a probe (5).

Measurement of insulin secretion from beta cells of pancreas. HIT-T15 cell line derived from Syrian hamster pancreatic beta cells were cultured in Ham' F12 medium supplemented with 10% dialyzed horse serum and 2.5% FBS. The cells (1×10^4 cells/ml) were inoculated into a 6 well microplate. After 3 days, the medium was changed to medium containing each water and the cultivation was done in the presence of 10 mM glucose for 72 hours. Insulin was measured by a insulin EIA kit (Amarsham Pharmacia).

Animal experiments using type 2 diabetes model mice. Type 2 diabetes model mice (C57BL/KsJ·Db^{+}/Db^{+}) were obtained from Nihon Crea Co. Ltd. The mice, which have a defect in the reptin receptor gene, cannot control appetite and suffer from diabetes via obesity. Blood sugar level was measured using a dexistar Z sensor equipped with a enzyme electrode (Bayer-Sankyo, Tokyo). Sugar tolerance test was performed by intraperitoneal injection of glucose solution (1 mg glucose in physiological salt solution/30 g weight). Blood sugar lever was determined at 0, 30 and 60 minutes after the injection (6).

Clinical analysis on the effect of Nordenau water against diabetes. Changes in the relevant test parameters of 139 diabetes patients were examined. The average age of the test persons (72 female, 67 male) was 68.4 years; the average duration of their stay in Nordenau was 4 days for group 1 (79 patients = 56.8%), 5 or more days for group 2 (60 patients = 43.2%) taking tow tunnel walks of 30 minutes each and consuming 2 litres of Nordenau water daily. The patients were being looked after by us diagnostically within the scope of what is known as 'course control' and they gave us permission to use their diagnostic parameters for scientific purposes, provided we guaranteed their anonymity. We tested the diabetes-relevant test parameters (blood sugar, HbA1c, chol., trigl., HDL and LDL) twice under the same conditions i.e. on their arrival and on their departure at the same time of day. During the period of observation neither their regular medication nor their diet instructions was altered. The tested persons were particularly reminded to continue the medication prescribed by their general practitioners, to stick to their individual diet plans and not to alter any of their behavioural patterns. The statistical interpretation of the data contains: (a) The descriptive statistics of the whole group and of both subgroups. (b) A pairing-directed T-Test. (c) A proportional evaluation of the entire group and of both subgroups. (d) Variance- and co-variance analyses. The allowance for error probability was set at 5%.

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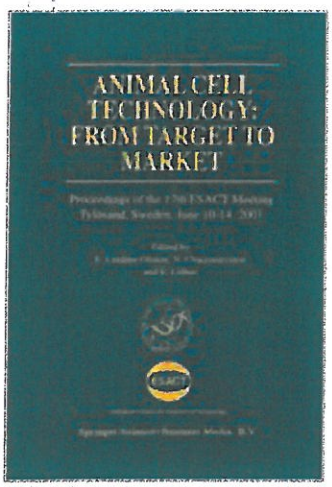

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