Preservative Effect of Electrolyzed Reduced Water on Pancreatic β-Cell Mass in Diabetic *db/db* Mice

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Oxidative stress is produced under diabetic conditions and involved in progression of pancreatic β-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 β-cell mass in *db/db* mice. The present data suggest that ERW may protects β-cell damage and would be useful for antidiabetic agent.

Key words   electrolyzed reduced water; diabetic mice; blood glucose; insulin; glucose tolerance; pancreatic β-cell mass

All oxidative reactions are a continuous source of potentially cytotoxic reactive oxygen species (ROS), which play an important role in living systems both through their beneficial and detrimental effects.[1] Under physiological conditions, ROS are fully inactivated by an elaborated cellular and extracellular antioxidant defense system.[2] However, under certain conditions increased generation of ROS and/or reduction of the antioxidant capacity leads to enhanced ROS activity and oxidative stress.

Hyperglycemia, the primary clinical manifestation of diabetes, is the most responsible factor for the development of various chronic diabetic complications.[1] The chronic presence of high glucose levels enhances the production of ROS from protein glycation and glucose autoxidation.[3] Diabetes also disturbs natural antioxidant defense systems.[4] Previous studies have shown that antioxidants such as quercetin, ascorbate and β-carotene resulted in an improvement of the antioxidant status in diabetic rats.[5,6]

During the progression of type 2 diabetes, the development of both insulin resistance and α- and β-cell dysfunctions appear to be the basic metabolic abnormalities leading to the long term disease.[7] Once hyperglycemia becomes apparent, β-cell function progressively deteriorates: glucose-induced insulin secretion becomes further impaired and degranulation of β-cells becomes evident, often accompanied by a decrease in the number of β-cells.[8] The maintenance of β-cell mass is a dynamic process, undergoing both increases and decreases to maintain glycemia within a narrow physiological range.[9] The majority of patients with obesity causing insulin resistance are not diabetic, as their capacity for β-cell compensation is maintained but, 15—20% of these individuals become diabetic, when the β-cells lose their compensatory ability.[9] Therefore, one approach to preventing and treating diabetes could be through the enhancement of β-cell mass.

Electrolysis of aqueous NaCl or KCl solutions by diaphragm-type electrolyzing devices produces oxidized and reduced water at the anodic and the cathodic side, respectively. Electrolyzed reduced water (ERW) exhibits high pH (pH 10—12), low dissolved oxygen (1.3—3.5 mg/l), high dissolved hydrogen (0.3—0.6 mg/l), and significant negative redox potential (-400—-900 mV). The ideal scavenger for ROS is "reactive hydrogen". Since oxidative damage has been implicated in the etiology of diabetic complication, ERW, a potent ROS scavenger, may have a therapeutic role in diabetic mellitus. Therefore, the present study examined the beneficial effect of ERW on β-cell damage and glycemic control in diabetic mouse strain of C57BL/6J-*db/db* (*db/db*) which exhibits many of the metabolic disturbances of human type 2 diabetes including hyperglycemia, obesity, and insulin resistance.[10]

MATERIALS AND METHODS

Materials   The ERW was produced by AK-3000 (Nexus, Korea). This water was produced by the electrolysis of water from a municipal water system. The ERW used in this study has the following physical properties: pH 10.24±0.03 and an oxidative-reduction potential of -400.2 ± 17.3 mV.

Animals   Genetically diabetic male *db/db* (C57BL/6J *db/db*) and their non-diabetic heterozygous littermates (*db/$-$*) at 3 weeks of age were purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A.). Mice were housed under temperature (20—26 °C) and light (12-h light/dark cycle) controlled conditions. All animals had free access to standard rodent pellet food (NIH #31M, Samtako, Korea), except when fasted before experiments. The study has been carried out along the "Principles of laboratory animal care" (WHO, 1985), and the "guidelines of the Animal Care and Use Committee" of the Kyunghee University. The *db/db* (D) and non-diabetic control mice (N) were each randomly divided into 2 groups ($n=8$); tap water (DC, NC) or ERW (DE, NE) group.

Blood Glucose and Insulin Measurements   At the end of 4 weeks of experimental period, mice were fasted overnight and injected with glucose (1 g/kg, i.p.). Blood samples were collected from the tail vein at various time points

(0—120 min) after glucose loading, and blood glucose levels were measured by One touch Basic glucose measurement system (Life Scan Inc., U.S.A.). Mice were killed by decapitation immediately after 120 min blood sample was taken and blood samples were taken from the cervical wound. Plasma glucose and insulin concentrations were determined with commercially available kits (Sigma Co., U.S.A.).

Immunohistochemical Staining of Pancreatic Islet Cell Pancreatic islet cell mass is a major determinant of the insulin secretory capacity. The four groups were used for histological studies. Pancreases were removed, and then fixed with 10% formalin. Immunohistochemical staining of β-cells using an anti-insulin antibody (Dako, Santa Barbara, CA, U.S.A.) was performed[11] with 5 μm sections of formalin fixed and paraffin embedded pancreas.

Statistical Analysis Results were presented as mean± S.E.M., and the data were analyzed by ANOVA followed by Tukey HSD's *post-hoc* test.

RESULTS AND DISCUSSION

No differences in food intake, water intake, and body weight were observed by ERW consumption in *db/db* and non-diabetic control mice (data not shown). Blood glucose levels of diabetic mice (DC) were significantly higher than non-diabetic control mice (NC), and ERW consumption significantly lowered (41%) the blood glucose levels in hyperglycemic *db/db* mice (DE) without any effect in non-diabetic control mice (NE, Table 1). Plasma insulin levels of diabetic mice were more than 2-fold higher than control mice indicating insulin resistance in type 2 diabetes. Interestingly, ERW administration also increased insulin level in diabetic mice without any effect in control mice, suggesting elevated insulin release in diabetic mice. This increased insulin levels in diabetic mice resulted from increased pancreatic β-cell mass (Fig. 2).

Intraperitoneal glucose tolerance test revealed that glucose tolerance in diabetic *db/db* mice exhibited significantly higher glucose level during all time points determined (Fig. 1). After glucose loading, the increase in serum glucose concentrations in diabetic mice was very slow, while normal mice exhibited sharp increase in glucose level with peak concentration at 15 min, indicating delayed glucose homeostasis in *db/db* mice. ERW administration ameliorated glucose tolerance in diabetic *db/db* mice without any affect in control mice.

Significant histological differences were noted between *db/db* mice and their non-diabetic littermates. When β-cells were stained with anti-insulin antibodies, weak staining of β-cells were observed in *db/db* mice (Fig. 2c) compared with control mice (Fig. 2a). ERW supplemented *db/db* mice exhibited strong staining (Fig. 2d), as seen in the lean littermates (Fig. 2b).

This study clearly demonstrated that treatment with ERW ameliorates hyperglycemia and improves glucose handling capacity in obese diabetic *db/db* mice. In addition, immunohistochemical examination revealed that treatment with ERW can prevent loss of β-cell mass resulting in increase of insulin secretory capacity.

Recently, pancreatic β-cells emerged as a target of oxidative stress-mediated tissue damage. Because of the relatively

Table 1. Fasting Blood Glucose and Insulin Levels

	NC	NE	DC	DE
Blood glucose (mg/dl)	129.0 ± 6.8^a	125.4 ± 3.9^a	490.1 ± 32.4^b	287.9 ± 34.5^c
Blood insulin (ng/ml)	0.71 ± 0.02^a	0.68 ± 0.01^a	1.55 ± 0.09^b	5.72 ± 0.49^c

Values are means±S.E.M. ($n=8$); means in same raw with different superscripts are significantly different ($p<0.05$).
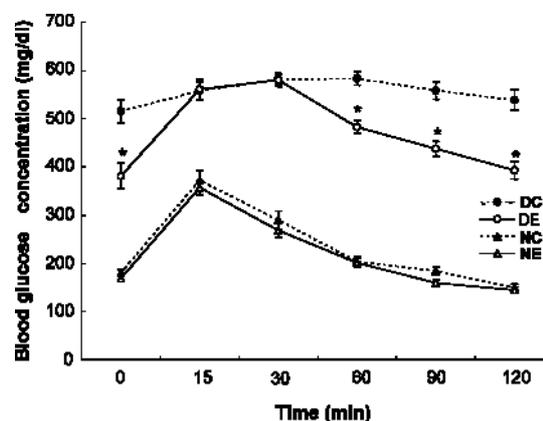

Fig. 1. Effect of Electrolyzed Reduced Water (ERW) on Glucose Tolerance in Genetically Diabetic *db/db* Mice

Normal *db/−* mice fed tap water (NC); normal *db/−* mice fed ERW (NE); *db/db* mice fed tap water (DC); *db/db* mice fed ERW (DE). Data are expressed as means±S.E.M.
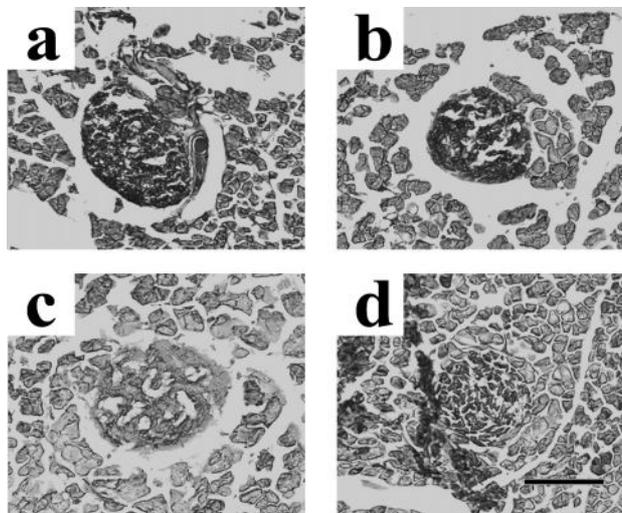

Fig. 2. Images of Pancreases Immunohistochemical Staining for Insulin

Pancreases from non-diabetic control (a), non-diabetic treated with ERW (b), diabetic *db/db* control (c), or ERW treated *db/db* mice (d) were immunostained with anti-insulin antibody. (c) shows weak staining of β-cells with anti-insulin antibodies in *db/db* mice compared with non-diabetic control (a). *db/db* mice consumed ERW exhibited strong staining (d), as seen in control (a). The bar indicates 100 μm.

low expression of antioxidant enzymes,[12] pancreatic β-cells may be rather sensitive to ROS attack when they are exposed to oxidative stress. Chronic hyperglycemia is not only a marker of poor glycemic control in diabetes but is itself a cause of impairment of both insulin secretion and biosynthesis: prolonged exposure of pancreatic β-cells to high glucose levels is known to cause β-cell dysfunction, called glucose toxicity.[13] Such damaged β-cells often display extensive degranulation, and are clinically associated with the development of diabetes in some model animals for type 2 dia-

betes.[14] Thus, it is likely that oxidative stress plays a major role in β-cell deterioration in type 2 diabetes. The restoration of glucose induced secretory capacity after ERW consumption is thought to be due to elimination of glucose toxicity resulting in protection from the toxic effects of ROS. Indeed, ERW consumption increased β-cell mass (Fig. 2) and insulin level (Table 1). Tanaka *et al.*[15] reported that chronic hyperglycemia impairs β-cell function at the level of insulin synthesis as well as insulin secretion, and all of these adverse consequences can be prevented by antioxidants. ERW, containing active atomic hydrogen with high reducing ability which can contribute to ROS scavenging activity,[16] could reduce oxidative stress in pancreatic islets and loss of β-cell mass was eventually prevented. Therefore, ERW administration improved islet β-cell function resulting in increased release of circulating insulin and improved insulin sensitivity, and thus, ameliorate hyperglycemia and delay the development of diabetes in these diabetic mice model.

To our knowledge, this is the first report showing protective effect of ERW on β-cell mass in diabetic animal models.

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