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EFFECTS OF DEEP-SEA WATER ON CARDIAC ABNORMALITY IN HIGH-CHOLESTEROL DIETARY MICE

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ABSTRACT

Our recent studies indicated that electrodialysed deep-sea water (ED-DSW) revealed the cardiovascular health effects, such as hindrances of the dietary-induced evaluation of total cholesterol (TC), triglyceride (TG) and nonhigh-density-lipoprotein cholesterol (HDL-C), as well as improvement of HDL-C/non-HDL-C ratio and blood pressure. In the current study, we further revealed the beneficial effects of ED-DSW on high-cholesterol dietary mice by reducing abnormal cardiac architecture, apoptosis and enhancing insulin-like growth factor-1 receptor (IGF-1R) cardiac survival signaling compared with those mice that were treated with distilled water, reverse osmosis (RO)-DSW or 10% (v/v) dilution with ddH2O (10% DSW). These findings further clarified the possible mechanisms of cardiac protective effects of ED-DSW and might suggest ED-DSW as an ingredient of cardiovascular health food in some niche markets.

PRACTICAL APPLICATIONS

Deep-sea water (DSW) has been applied on several fields including aquaculture, agriculture, food processing, cosmetics and medicine. Recently, we have indicated that high-cholesterol dietary mice drinking electrodialysed DSW (ED-DSW) showed decreased serum lipids, blood pressure and improving blood cholesterol profile. In this study, we further demonstrated the beneficial effects of ED-DSW on high-cholesterol dietary mice by reducing abnormal cardiac architecture, apoptosis in left ventricle and increasing cardiac survival signaling, such as insulin-like growth factor-1 receptor, phosphoinositide-3-kinases and p-AKT/AKT ratio. Altogether, these findings indicated that ED-DSW revealed the cardiac protective effects and might be suggested as an ingredient of cardiovascular health food in a variety of niche markets.

INTRODUCTION

Cardiovascular disease (CVD) is associated with hypercholestrolmia and considered as a crucial issue of public health (Kromhout *et al*. 1995; Rosamond *et al*. 2008). Evidences have indicated that excess dietary cholesterol is responsible for the hypercholesterolemia and has been recognized as the significant risk factor to cause cardiac injury or diseases (Kinscherf *et al*. 2003). Indeed, various studies have indicated that hypercholesterolemia reduced endomyocardial coronary flow reserve, capillary density and induced capillary endothelial cell apoptosis, coronary atherosclerosis and myocardial fibrosis (Theilmeier *et al*. 2002; Kinscherf *et al*. 2003). Deep-sea water (DSW), defined as the seawater at depths between about 500 and 1,000 m (Broecker *et al*. 1985), is clean and rich in minerals, such as calcium (Ca), magnesium (Mg) and potassium (K) (Fogg and Thake 1987; Toyota and Nakashima 1998). As we know, the DSW has been used in a therapy of atopic dermatitis for a long time (Adachi *et al*. 1998; Hataguchi *et al*. 2005). Recently, DSW has been also used for thalassotherapy (Tsuchiya *et al*. 2004; Hataguchi *et al*. 2005). However, the roles of DSW in the therapies remain poorly understood.

Apoptosis is responsible for the loss of cardiomyocytes in cardiomyopathy and regarded as a predictor of adverse outcomes in subjects with CVD or heart failure (Haunstetter and Izumo 1998; Narula *et al*. 1999; Lee *et al*. 2006). Both Fas-dependent and mitochondrial-dependent apoptotic pathways are considered as major pathways directly to cause cardiac apoptosis (Narula *et al*. 1999; Fujio *et al*. 2000; Athanasiou *et al*. 2007). In contrast, activation of insulin-like growth factor-1 (IGF-1) is demonstrated to be beneficial to improve cardiac functions. Several studies have indicated that IGF-1 plays a crucial role in protection of cardiac myocytes and low IGF-1 levels are associated with high risk for myocardial infarction and heart failure (Ren *et al*. 1999; Bishopric *et al*. 2001). Besides, various cardiac risk factors are also important in development of cardiac disorders such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Cardiac ANP and BNP levels are increased in myocardial infarction of animal models (Cameron *et al*. 2004), heart failure (Luchner *et al*. 1998), hypertrophy (Kawakami *et al*. 1996) and also in human cardiac diseases (Saito *et al*. 1989). Because of abounding inorganic materials and a better Mg^{2+}/Ca^{2+} ratio, DSW has been applied in health food and medical use. However, limited scientific evidence to these benefits on cardiac protection was available.

The application of DSW has been applied on several fields including aquaculture, agriculture, food processing, cosmetics and medicine (Nomura 1995; Nakasone and Akeda 2000; Tsuchiya *et al*. 2004). Recently, we have indicated that ED-DSW decreased serum lipids, blood pressure and improved blood cholesterol profile (Chang *et al*. 2010). To further clarify the protective mechanism of DSW, the objective of the present study was to evaluate and compare the effects of different treated DSWs on improvement of cardiac apoptosis and survival signaling.

MATERIALS AND METHODS

Animal, Diets and Drinking Water

Twenty-four male ICR mice at 7 weeks of age were purchased from the animal center, National Taiwan University (Taipei, Taiwan) and housed in an animal room at 22+2C with a 12/12 h light-dark cycle under supervision of the Institutional Animal Care and Use Committee at Chung Shan Medical University. These mice were fed standard chow diets and distilled water for one week. After the acclimation period, all mice were fed with AIN-76A diet containing 1% cholesterol (TestDiet® Division, PMI® Nutrition International Purina Mills LLC., Richmond, IN). Meanwhile, mice were randomly divided into four groups and received distilled water (NDW), DSW treated by reverse osmosis (RO-DSW), DSW treated by electrodialysis (ED-DSW) and 10% (v/v) diluted DSW with ddH_2O (10% DSW), respectively. Original DSW was picked up from seawater below 700 m in the outer sea of Hua-Lien County, Taiwan at the same time. Briefly, RO-DSW is obtained via a self-assemble system, which constitutes four main parts, pretreatment, high-pressure pump, membrane assembly and post-treatment. The membrane assembly includes ultra-filtration membrane (HUF-125-50, Hydrosep Inc., Burbank, CA), nano-filtration membrane (DK4040F, DESALTM GE Osmonics, Bryan, TX) and two reverse-osmosis membranes (HS-SW2-4040F, Hydrosep Inc., and CPA2-4040, Hydranautics Inc., Santa Ana, CA). ED DSW is obtained via an electrodialysis system (Micro Acilyzer S3, Astom Co., Tokyo, Japan). The driving forces for RO and ED-DSW are pressure and electric potential, respectively. Large amounts of DSW and treated DSWs (RO and ED-DSW) were offered by Energy and Environment Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan, that had been sterilized in a pasteurization process (80C, 60 s) and immediately stored in sterilized bottles at −20C until feeding mice. The mineral contents in each drinking water were analyzed by using an inductively optical emission spectrometer (JY ULTIMA 2000, Horiba, Les Ulis, France). The major minerals, Na, Ca, Mg and K, as well as hardness of each different drinking water, were described in our recent publication (Chang *et al*. 2010). All mice were fed diets and assigned drinking water *ad libitum* for 8 weeks.

Terminal Transferase dUTP Nick End Labeling Assay

Left ventricular (LV) tissues of hearts from different groups of mice were embedded into OCT compound (Tissue-Tek,

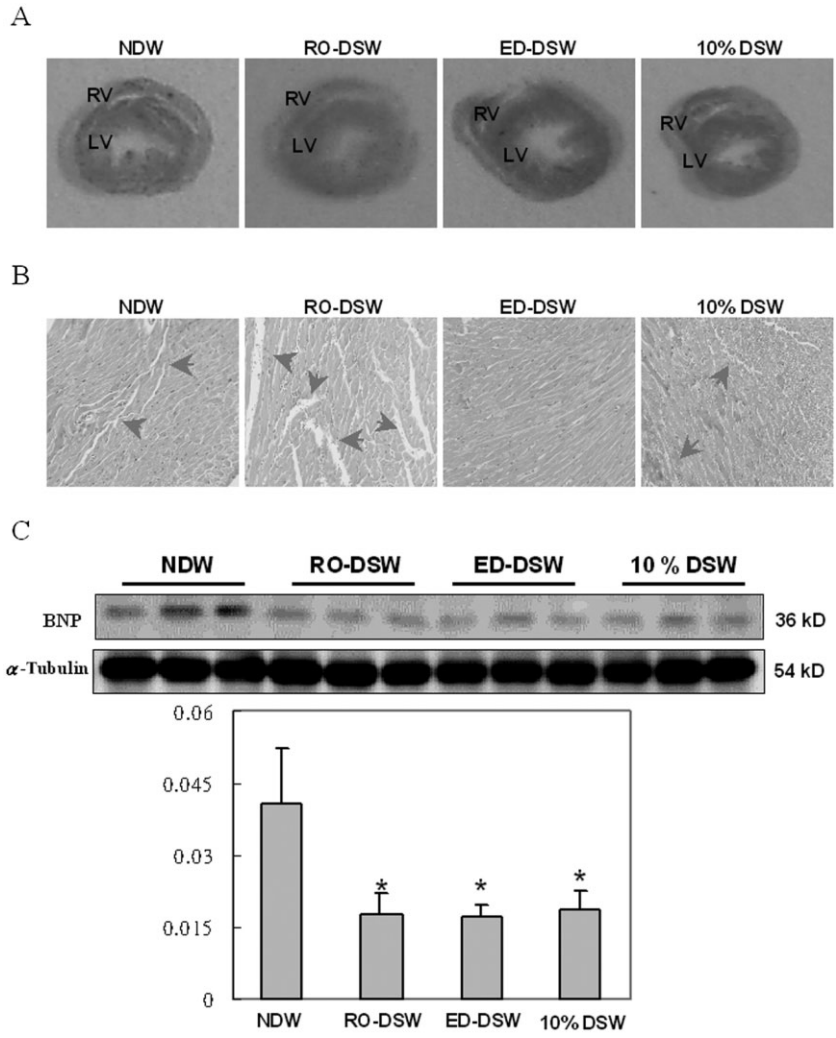

FIG. 1. EFFECTS OF DEEP-SEA WATER ON CARDIAC ARCHITECTURE AND BNP EXPRESSION
Hematoxylin and eosin staining of (A) cardiac transverse and (B) left ventricle sections in different groups of mice. (C) The protein product of BNP in left ventricle of heart from different groups of mice. Bars represent the relative protein quantification of BNP on the basis of α-tubulin.
* indicates significant difference compared with the NDW group.
BNP, brain natriuretic peptide; NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water.

Miles Inc., Elkhart, IN) and snap frozen in liquid nitrogen. The frozen tissue blocks were sectioned at 5 μm and fixed in 4% paraformaldehyde (Sigma, Saint Louis, MO) in 0.1 M phosphate-buffered saline (PBS), pH 7.4 for 20 min at RT. After washing for 30 min with 0.1 M PBS, the tissue sections were incubated with 3% H_2O_2 in methanol for 10 min at room temperature. TUNEL reaction mixture was freshly prepared according to the manufacture's instruction (Roche Applied Science, Inc., Nutley, NJ) and a total volume of 100 μL terminal deoxytransferase reaction mixture was incubated with the tissue sections for 1 h at RT in the dark. The tissue sections were then rinsed with 0.1 M PBS containing 4′,6-diamidino-2-phenylindole (DAPI) and observed with a fluorescence microscope. The number and percentage of TUNEL-positive cells was counted and determined by counting 1×10^3 cardiac cells from five random selected fields. All measurements were performed by at least three independent animals in a blinded manner.

Western Blotting

The PRO-PREP™ protein extraction solution containing 1 mM phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluoride, 1 mM ethylenediaminetetraacetic acid, 1 uM pepstatin A, 1 uM leupeptin, 0.1 uM aprotinin (iNtRON Biotechnology Co., Seoul, Korea) was used for protein extraction. The left ventricular tissues of hearts from different groups of mice were analyzed for immunoblotting and similar results were observed in the same group. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 12.5% acrylamide gel, was performed. Protein samples were denatured for 5 min in boiling water with sample buffer (pH 6.8, 0.0625 M Tris–HCl buffer, containing 2.3% SDS, 5% 2-mercaptoethanol and 10% glycerol). Samples applied to the gel were run at 100–150 V for 1.5 h and electrophoretically transferred to nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). The membrane was then soaked in

TABLE 1. PHYSIOLOGICAL CHANGE OF LEFT VENTRICLES

	NDW (N = 6)	RO-DSW (N = 6)	ED-DSW (N = 6)	10% DSW (N = 6)
Diameter of LV (mm)	4.10 ± 0.27	4.53 ± 0.15	3.53 ± 0.15*	4.13 ± 0.15
Thickness of LV (mm)	1.13 ± 0.15	1.60 ± 0.10*	0.70 ± 0.10*	1.17 ± 0.15
Thickness/diameter (mm)	0.28 ± 0.02	0.35 ± 0.03*	0.20 ± 0.03*	0.28 ± 0.03

Values are mean ± standard deviation.
* Indicates $P < 0.05$, compared with NDW.
LV, left ventricle.

PBS with 5% nonfat dry milk for 30 min at room temperature to saturate irrelevant protein binding sites. Antibodies against BNP, tumor necrosis factors (TNF)-α, Fas, cytochrome c, t-BH3 interacting domain death agonist (Bid), cleaved caspase-9, cleaved caspase-3, Bcl-2 homologous antagonist/killer (Bak), B-cell lymphoma-extra large (Bcl-X_L), phosphorylated-Bcl-2-associated death promoter(p-Bad), Bad, IGF-1R, phosphoinositide-3-kinases (PI3K), p-AKT, AKT andα-tubulin (Upstates, Charlottesville, VA; Santa Cruz Biotechnology, Santa Cruz, CA) were diluted in PBS with 2.5% BSA and incubated for 1.5 h with gentle agitation at room temperature. The membranes were washed twice with PBS-Tween for 1 h and secondary antibody conjugated with horseradish peroxidase (HRP) was added. Pierce's Supersignal West Dura HRP Detection Kit (Pierce Biotechnology Inc., Rockford, IL) was used to detect antigen–antibody complexes. The blots were also scanned and quantified by densitometry (Appraise, Beckman-Coulter, Brea, CA).

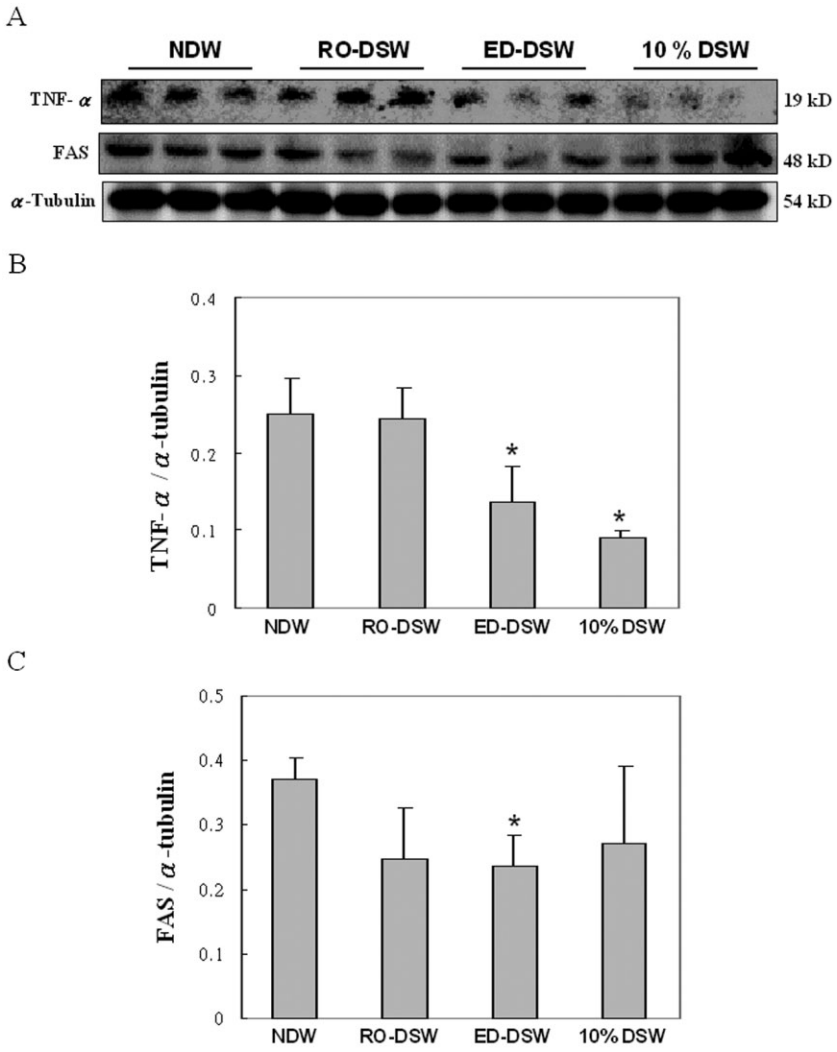

FIG. 2. EFFECTS OF DEEP-SEA WATER ON CARDIAC TNF-A AND FAS EXPRESSION
The protein products of (A) TNF-α (19 kDa) and Fas (48 kDa) in left ventricle of heart from different groups of mice. Alpha-tubulin (54 kDa) served as an internal control. Bars represent the relative protein quantification of (B) TNF-α and (C) Fas on the basis of α-tubulin.
* indicates significant difference compared with the NDW group.
NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water; TNF-α, tumor necrosis factor alpha.

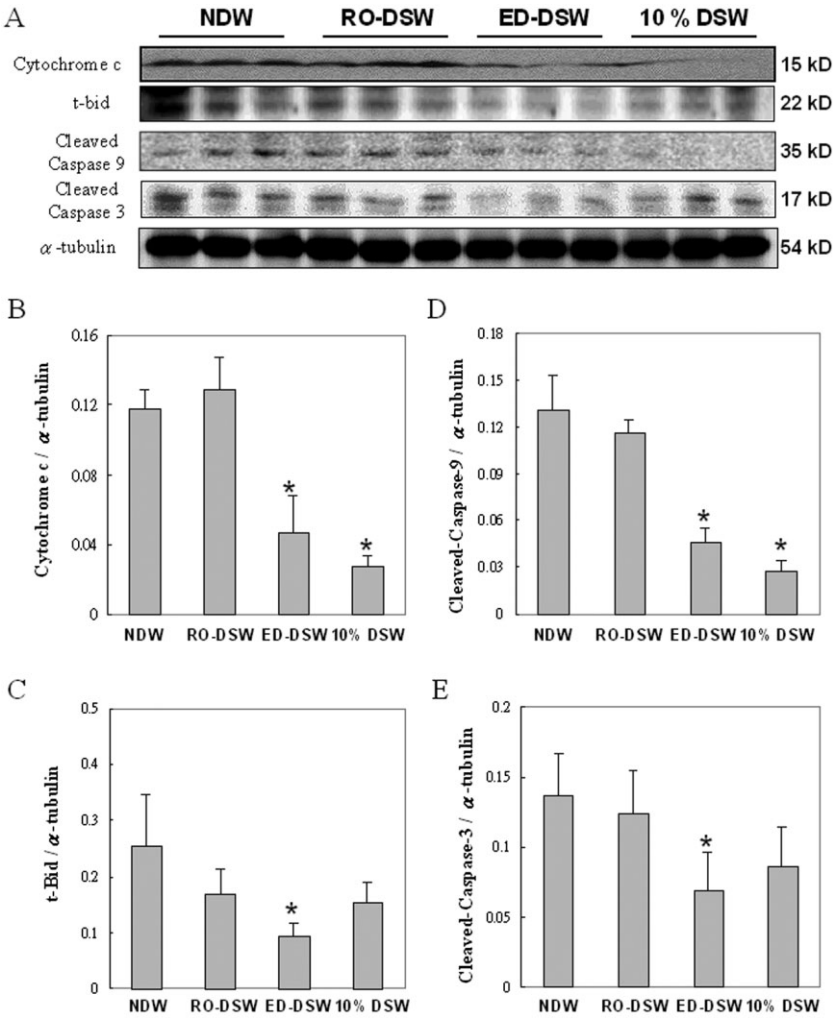

FIG. 3. EFFECTS OF DEEP-SEA WATER ON CARDIAC MITOCHONDRIAL- DEPENDENT APOPTOTIC SIGNALING
The protein products of (A) Cytochrome C (15 kDa), t-Bid (22 kDa), activate caspase-9 (35 kDa) and activate caspase-3 (17 kDa) in left ventricle of heart from different groups of mice. Alpha-tubulin (54 kDa) served as an internal control. Bars represent the relative protein quantification of (B) Cytochrome C (C) t-Bid (D) cleaved caspase-9 and (E) cleaved caspase-3 on the basis of α-tubulin.
* indicates significant difference compared with the NDW group.
NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water; TNF-α, tumor necrosis factor alpha.

Hematoxylin–Eosin (HE) and Sudan III Staining

The left ventricular tissues of hearts from different groups of mice were embedded into OCT compound (Tissue-Tek, Miles Inc., Elkhart, IN) and snap frozen in liquid nitrogen. For Sudan III staining, the frozen sections were sectioned at 5 μm and socked in 50% ethanol before immersed in the dark in the Sudan III solution in 70% ethanol for 20 min. The sections were then washed with 50% ethanol and immersed in Hematoxyl solution for 3 min as the negative staining. Photomicrographs were obtained using Zeiss Axiophot microscopes. (Carl Zeiss AG, Oberkochen, Germany)

Statistical Analysis

The experiment was conducted using a completely random design (Steel and Torrie 1980). Data were analyzed using analysis of variance (SAS Institute, Inc., Cary, NC; 2002). A significant difference was used at 0.05-probability level and differences between treatments were tested using the least significant difference test (Freud and Wilson 1997). All statistical analyses of data were performed using SAS.

RESULTS

Changes of Cardiac Characters and Architectures in Left Ventricle of Mice from Four Different Drinking Water Groups

To investigate the variation of myocardial architecture in hearts of mice from the four different drinking water groups, we performed the histopathological analysis by hematoxylin and eosin staining. Figure 1A revealed cardiac transverse sections with H–E staining. The thickness of LV (mm) and ratio of thickness to diameter were significantly

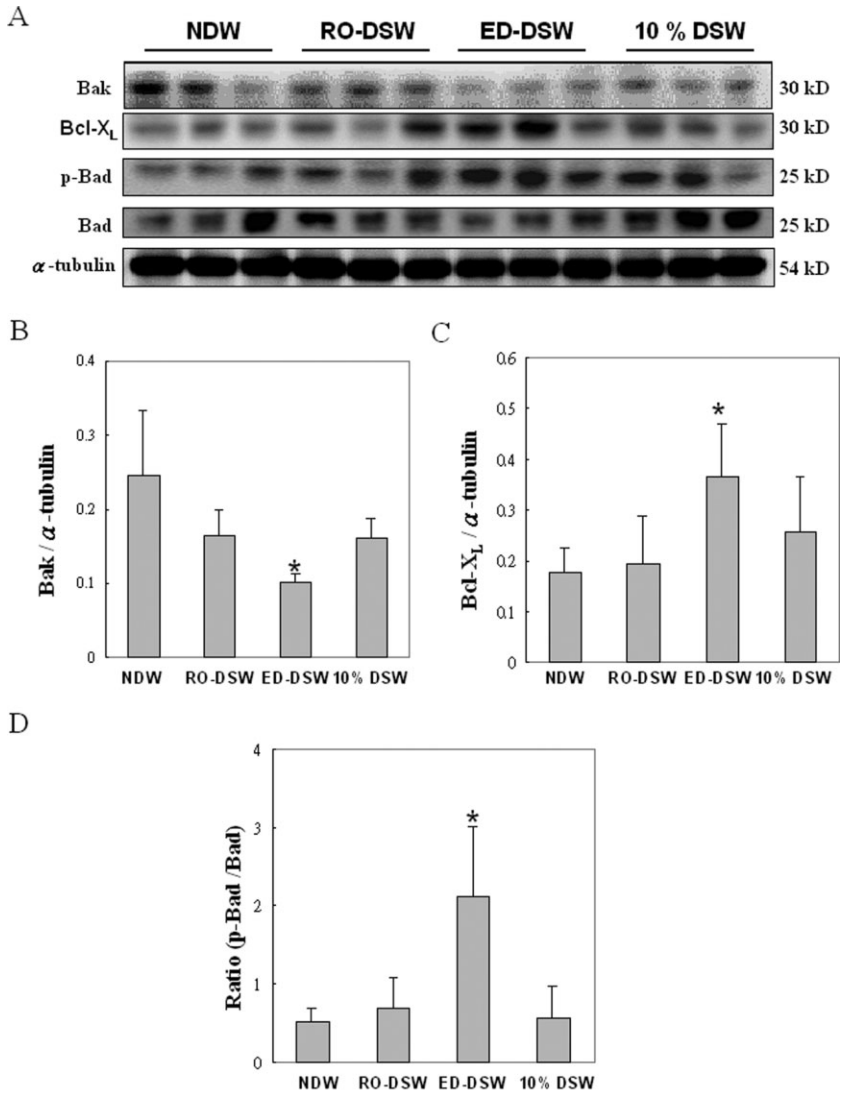

FIG. 4. EFFECTS OF DEEP-SEA WATER ON CARDIAC BAK, BCL-X_L, PBAD AND BAD EXPRESSION

The protein products of (A) Bak (30 kDa), Bcl-X_L (30 kDa), p-Bad (25 kDa) and Bad (25 kDa) in left ventricle of heart from different groups of mice. Alpha-tubulin (54 kDa) served as an internal control. Bars represent the relative protein quantification of (B) Bak (C) Bcl-X_L, on the basis of α-tubulin and (D) ratio of p-Bad to Bad.

* indicates significant difference compared with the NDW group.

NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water.

increased in hearts of mice from the RO-DSW group compared to the NDW group. In contrast, significantly decreased LV diameter, LV thickness and ratio of thickness to diameter were detected in hearts of mice from the ED-DSW group compared to the NDW group (Table 1). Additionally, we found that the ventricular myocardium in the RO-DSW group showed more abnormal architecture compared with the NDW group, which revealed cardiomyocyte disarray and the increased interstitial space. In contrast, less abnormal architecture in the ED-DSW and 10% DSW groups was observed compared with the NDW group (Fig. 1B). However, significantly decreased BNP protein was detected in left ventricle of mice from the RO-DSW, ED-DSW, 10% DSW compared to the NDW group (Fig. 1C).

Changes of Fas- and Mitochondrial-Dependent Apoptotic Components in Left Ventricle of Mice from Four Different Drinking Water Groups

To study the effects of different drinking water on Fas-dependent apoptosis in hearts of mice, Western blotting was performed. The protein products of TNF-alpha were significantly decreased in left ventricle of mice from the ED-DSW and 10% DSW groups compared to the NDW group (Fig. 2A,B). Meanwhile, the significantly decreased Fas level was observed in left ventricle of mice from the ED-DSW group compared to the NDW group (Fig. 2C). To investigate the variation of mitochondrial-dependent apoptotic components in cardiac tissue of mice, the protein products of

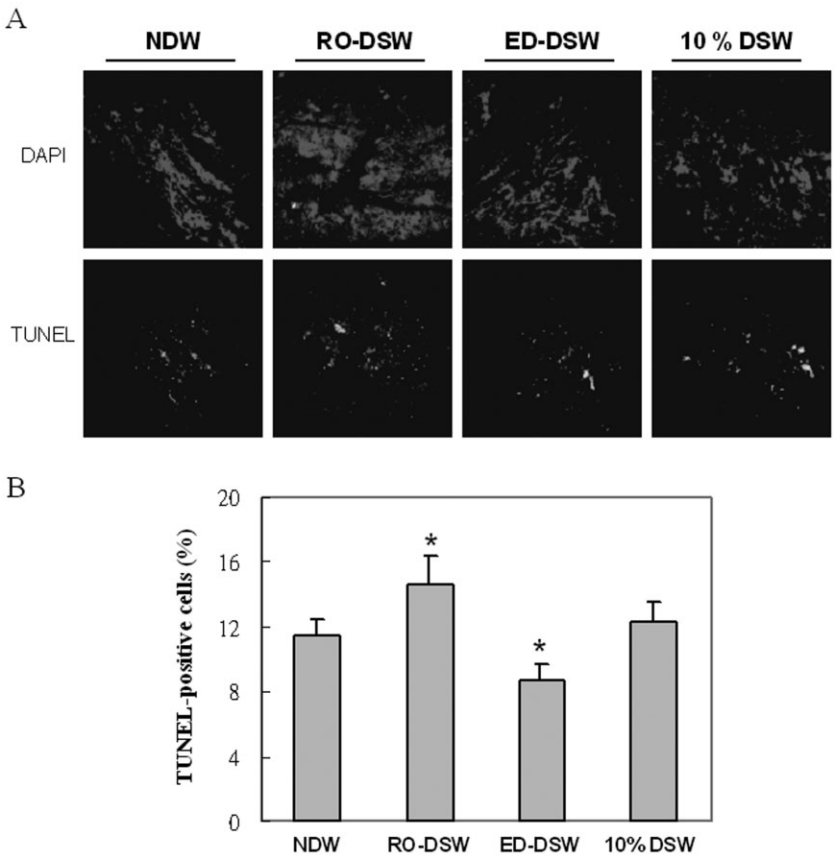

FIG. 5. EFFECTS OF DEEP-SEA WATER ON CARDIAC APOPTOSIS
(A) The representative stained apoptotic cells of cardiac sections with TUNEL assay in left ventricle of heart from different groups of mice. (B) The percentages of apoptotic cells were calculated. The images of myocardial architecture were magnified 100 times. Bars present the percentage of TUNEL-positive cells relative to total cells (10 mice × 10 scope field count in each group) and indicate mean values ± standard deviation.
* and # indicate significant difference compared to the NDW group.
NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water.

cytochrome-c, t-Bid, cleaved caspase-9 and cleaved caspase-3 were examined with Western blotting (Fig. 3). Significantly decreased cytochrome c, cleaved caspase-9, t-Bid and cleaved caspase-3 were detected in left ventricle of mice from the ED-DSW group compared to the NDW group (Fig. 3B–E). Meanwhile, significantly decreased cytochrome c and cleaved caspase-9 were observed in left ventricle of mice from the 10% DSW group compared with the NDW group (Fig. 3B,D).

Changes of Apoptosis Related Proteins and Cardiac Apoptotic Cells in Left Ventricle of Mice from Four Different Drinking Water Groups

To further investigate the influence of different drinking water on expression of anti-apoptotic proteins in hearts of mice, Bak, Bcl-X_L, phosphorylated Bad (p-Bad) and Bad were examined (Fig. 4A). Significantly decreased Bak, an apoptosis-related protein, was detected in left ventricle of mice from the ED-DSW group compared with those from the NDW group (Fig. 4B). In contrast, significantly increased anti-apoptotic proteins, including Bcl-X_L and ratio of p-Bad to Bad, were observed in left ventricle of mice from the ED-DSW group compared to those from the NDW group (Fig. 4C,D). Additionally, we further investigated the cardiac apoptotic cells in left ventricle of mice with TUNEL assay (Fig. 5A). Significantly increased TUNEL-positive cardiac cells were detected in left ventricle of mice from the RO-DSW group compared to those from the NDW group. In contrast, significantly decreased TUNEL-positive cardiac cells were detected in left ventricle of mice from the ED-DSW group compared to those from the NDW group (Fig. 5B).

Change of Cardiac Survival Signaling Components in Left Ventricle of Mice from Four Different Drinking Water Groups

To further investigate the variation of cardiac survival signaling components in hearts of mice from four different drinking water groups, the protein levels of IFG-1R, PI3K, p-AKT and AKT were examined (Fig. 6A). The protein products of IGF-1R and PI3K were significantly increased in left ventricle of mice from the RO-DSW, ED-DSW and 10% DSW groups compared with those from the NDW group, respectively (Fig. 6B,C). Additionally, significantly increased ratio of p-Akt to Akt was observed in left ventricle of mice from the

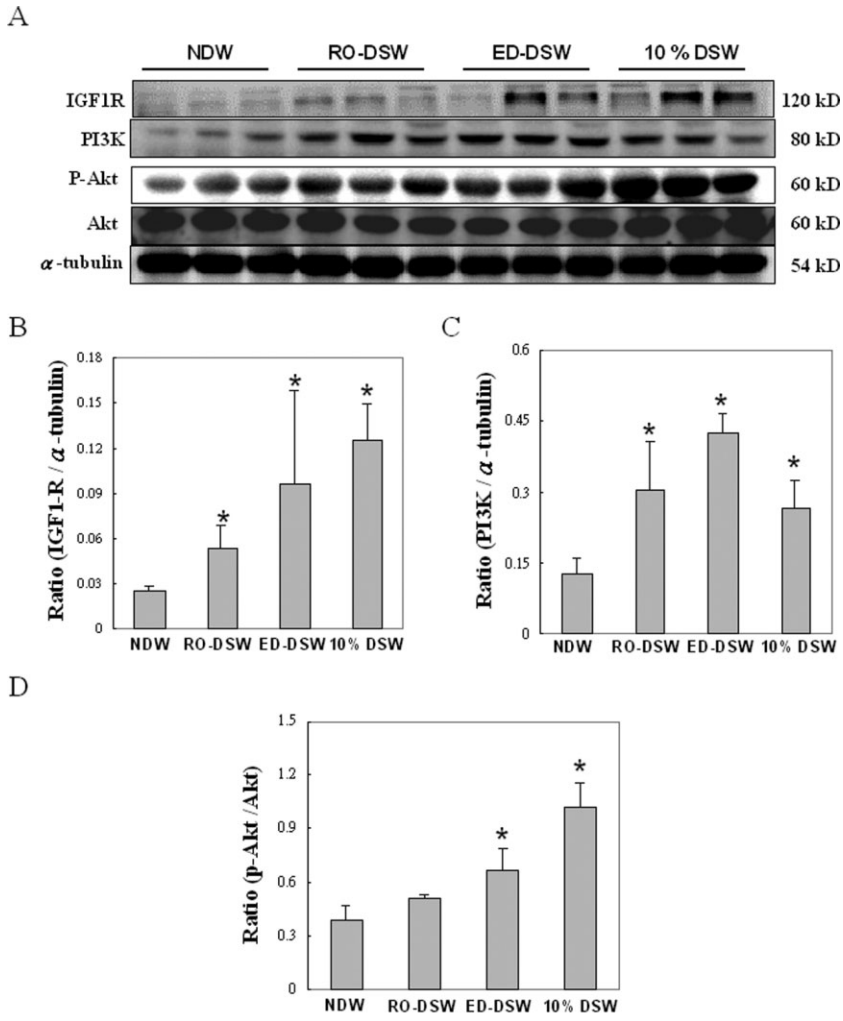

FIG. 6. EFFECTS OF DEEP-SEA WATER ON IGF-1R CARDIAC SURVIVAL SIGNALING
The protein products of (A) IGFI-R (120 kDa), PI3K (80 kDa), p-AKT (60 kDa) and Akt (60 kDa) in left ventricle of heart from different groups of mice. Alpha-tubulin (54 kDa) served as an internal control. Bars represent the relative protein quantification of (B) IGFI-R (C) PI3K, on the basis of α-tubulin and (D) ratio of p-Akt to Akt.
*indicates significant difference compared with the NDW group.
NDW, group that received distilled water; RO-DSW, DSW treated by reverse osmosis; ED-DSW, DSW treated by electrodialysis; 10% DSW, 10% (v/v) diluted DSW with ddH_2O; DSW, deep-sea water; IGF-1R, insulin-like growth factor-1 receptor; PI3K, phosphoinositide-3-kinase.

ED-DSW and 10% DSW groups compared to those from the NDW group, respectively (Fig. 6D).

DISCUSSION

Dietary fats and cholesterol have been strongly associated with a variety of heart diseases (Woodside and Kromhout 2005; Ghafoorunissa 2009). Although previous studies have conducted to investigate the pharmacological activities of different treated DSWs (Yoshioka *et al*. 2003; Miyamura *et al*. 2004), few researches of DSW on cardiac apoptosis and survival signaling were available. In the current study, we further reported that less abnormal cardiac architecture significantly decreased cardiac apoptosis were detected in mice from the ED-DSW group compared to the NDW group, as well as the significantly decreased TUNEL-positive cells and increased $Bcl\text{-}X_L$ and p-Bad/Bad ratio in left ventricle of mice from the ED-DSW group compared to the NDW group. Additionally, significantly increased IGF-IR cardiac survival signaling was observed in left ventricle of mice from the ED-DSW group compared to the NDW group.

The relationship between mineral contents of drinking water and cardiovascular mortality was firstly proposed (Schroeder 1960), however, the precise mechanisms are still unclear. Various studies have demonstrated that appropriate administrations of calcium (Ca) and magnesium (Mg) can reduce cardiovascular risk factors. A previous study has indicated that Ca supplementation causes beneficial changes in circulating lipids in normal older women (Reid *et al*. 2002). Calcium was also reported on its hypolipidemic effects. LDL-C/HDL-C in patients with mild to moderate hypercholesterolemia can be improved by consuming 1.2-gram calcium carbonate (CaCO4) daily for 6 weeks (Bell *et al*. 1992). Injections of Mg sulfate and ethylenediamine tetraacetic acid have a definite prophylactic effect on atherogenesis in cholesterol-fed rabbits, and may have some therapeutic value in the

regression phase (Evans *et al*. 2001). Another study also reported that dietary Mg prevents the progress of atherosclerosis in cholesterol-fed rabbits by inhibiting accumulation in the aortic wall (Ouchi *et al*. 1990). In the current study, we demonstrated that ED-DSW reveals significant benefits by reducing cardiac abnormal architecture, apoptosis and inducing IGF-IR survival signaling, whereas other DSW administrations revealed only partial effects of cardioprotective effects. It is possible that the proportion of Mg2+ and Ca2+ in ED-DSW increases the intracellular Mg2+ levels in vascular smooth muscle cells and further does not increase blood pressure when mice consume high-cholesterol diets. However, an examination of serum mineral contents affected by drinking DSW is necessary in further experiments.

Many studies have indicated a strong association among high cholesterol diet, heart diseases and cardiac apoptosis (Haunstetter and Izumo 1998; Narula *et al*. 1999; Woodside and Kromhout 2005; Lee *et al*. 2006; Ghafoorunissa 2009). A variety of cardiac risk factors, such as ANP and BNP and apoptotic signaling components, such as Fas-dependent and mitochondrial related molecules, are important in developing cardiac disorders. In contrast, activation of IGF-1 is demonstrated to be beneficial to improve cardiac functions. The IGF-1 is a peptide hormone in most tissues and plays crucial roles in many biological processes mediated by the IGF-1 receptor, including the regulation of protein turnover, potent mitogenic and cell differentiation (Laviola *et al*. 2007). Previous study has demonstrated that IGF-1 is essential in the proliferative capacity of ventricular myocytes during postnatal development (Cheng *et al*. 1995). Another study also indicated that IGF-1 acts as a vascular protective factor and is beneficial in the treatment of chronic heart failure (Abbas *et al*. 2008). Therefore, increased expression of IGF-1R-mediated signaling is considered beneficial on cardiovascular disorders. In the current study, we revealed significantly decreased apoptotic components, such as Fas, cytochrome c, cleaved caspase-9, t-Bid and cleaved caspase-3, and increased levels of IGF-1R survival signaling components, such as PI3K and ratio of p-Akt/Akt, in left ventricle of mice administrated with ED-DSW compared with other mice that were treated with other DSWs and suggested its function on cardiac survival.

CONCLUSIONS AND IMPLICATIONS

ED-DSW showed the best benefit on cardiovascular health compared with other treated DSWs. Since the application of DSW is blooming in many countries such as the United States, Norway and Japan (Nakasone and Akeda 2000), it warrants further exploiting as a basis of preventive agent based on its benefits might be suggested as an ingredient of cardiovascular health food in some niche markets.

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