Deep Sea Minerals Prolong Life Span of Streptozotocin-Induced Diabetic Rats by Compensatory Augmentation of the IGF-I-Survival Signaling and Inhibition of Apoptosis

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ABSTRACT: Consumption of deep sea minerals (DSM), such as magnesium, calcium, and potassium, is known to reduce hypercholesterolemia-induced myocardial hypertrophy and cardiac-apoptosis and provide protection against cardiovascular diseases. Heart diseases develop as a lethal complication among diabetic patients usually due to hyperglycemia-induced cardiac-apoptosis that causes severe cardiac-damages, heart failure, and reduced life expectancy. In this study, we investigated the potential of DSM and its related cardio-protection to increase the life expectancy in diabetic rats. In this study, a heart fail-
ure rat model was developed by using streptozotocin (65 mg kg⁻¹) IP injection. Different doses of DSM-1× (37 mg kg⁻¹ day⁻¹), 2× (74 mg kg⁻¹ day⁻¹) and 3× (111 mg kg⁻¹ day⁻¹), were administered to the rats through gavages for 4 weeks. The positive effects of DSM on the survival rate of diabetes rats were determined with respect to the corresponding effects of MgSO₄. Further, to understand the mechanism by which DSM enhances the survival of diabetic rats, their potential to regulate cardiac-apoptosis and control cardiac-dysfunction were examined. Echocardiogram, tissue staining, TUNEL assay, and Western blotting assay were used to investigate modulations in the myocardial contractile function and related signaling protein expression. The results showed that DSM regulate apoptosis and complement the cardiomyocyte proliferation by enhancing survival mechanisms. Moreover DSM significantly reduced the mortality rate and enhanced the survival rate of diabetic rats. Experimental results show that DSM administration can be an effective strategy to improve the life expectancy of diabetic subjects by improving cardiac-cell proliferation and by controlling cardiac-apoptosis and associated cardiac-dysfunction. © 2014 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2014.

**Keywords:** deep sea minerals; diabetes mellitus; cardiac-dysfunction; IGFI; cardiac-apoptosis

**INTRODUCTION**

Diabetes mellitus (DM) is one of the most common chronic diseases which is characterized by chronic hyperglycemia and is caused due to defects in insulin secretion and insulin action (DeFronzo et al., 1992). Cardiac-dysfunction and associated complications are the major causes of morbidity and mortality associated with diabetes (Huang and Lee, 2012). Estimates show that the risk for cardiovascular diseases increases by five folds among DM patients (Manson et al., 1991; Haffner et al., 2002). Statistics from World Health Organization indicate that the global annual incidence of cardiovascular disease remains high and is one of the common causes of human death. As DM is associated with various complications including renal; neuronal; cardiovascular and digestive dysfunctions, the mortality rates in DM subjects are generally high compared to the normal population. Death rates are up to three-folds higher in DM population and heart diseases are found to be the principle cause of the higher death rates therefore DM patients are known to have higher risk of death, lower survival, and lower life expectancy (Sasaki, 1994; Gu et al., 1998; Lee et al., 1998; Brun et al., 2000; Saydah et al., 2002; Romon et al., 2008).

Hyperglycemia is the key initiator of cardiac-dysfunction associated with DM by activation and destroying the regulation of several metabolic pathways (Seferovic Mitrovic et al., 2012). Modulation in the levels of insulin-like growth factor I (IGFI) is one of the critical conditions observed during the pathogenesis of DM (Thrailkill, 2000). Abnormal levels of IGFI subsequently cause changes in the IGFI signaling pathway and drastically alter the survival responses in cardiac tissues. Defects in the levels of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) that are important constituents of insulin and IGFI-receptor (IGFI-R) signaling affects cardiac cell survival and elevates susceptibility to apoptosis (Liu et al., 1996; Ren et al., 1999; Sun et al., 2000; Hong et al., 2001; Vincent and Feldman, 2002). Therefore impairment in the IGFI signaling, to a significant extend, leads to cardiac-apoptosis in diabetic animals and humans (Matsui and Davidoff, 2007). The mitochondria are the prime site of action for apoptosis-regulating proteins of B-cell lymphoma-2 (Bcl-2) family, such as Bcl-2 and Bcl-2 antagonist of cell death (Bad) (Bishoprpic et al., 2001). Maintaining the proportion in the levels of pro-apoptotic and pro-survival members of Bcl-2, by interacting with and neutralizing each other, regulates apoptosis (Behr et al., 2001). Defects in the relative balance of these factors affect the mitochondrial membrane potential and cause cytochrome c release (Kubasiak et al., 2002). However, the role of IGFI and the Bcl-2 family associated pro-survival mechanisms in diabetes related heart diseases are not much clear.

To improve the life expectancy of DM patients various strategies, including blood glucose control and insulin regulation, are considered as a treatment for DM related complications. Controlling the cardiac-apoptosis and promoting the cell survival are two other important strategies to be considered while treating DM related cardiac-dysfunction. Various alternative medicines with such potential are been extensively studied and reported (Broderick et al., 2005; Khong et al., 2011; Chang et al., 2013; Shida et al., 2013; Zhang et al., 2013).

Sea water below 200 m from sea level is deprived of exposure to sunlight and therefore it has little or no surface pathogens and is rich in mineral contents known as deep sea minerals (DSM). Deep-sea water (DSW) and their constituent DSM are effectively been applied on several fronts such as in aquaculture, agriculture, food processing, cosmetics and medicine. In a recent research, high-cholesterol dietary mice consuming electrodialyzed DSW showed decreasing serum lipid levels, blood pressure and improving blood cholesterol profile (Chen et al., 2013; Sheu et al., 2013b). DSW contains a variety of trace elements and is rich in magnesium, calcium and potassium (Chen et al., 2013). These metal elements are known to cause beneficial changes in the circulating lipids and are also known to prevent cholesterol accumulation in the aortic wall (Ouchi et al., 1990).
Therefore, DSM can also exhibit cardio-protection against various cardiovascular diseases. Here we report evidences for the cardio-protective effects of DSM obtained from deep seas adjacent to Hualien, Taiwan (ROC). The primary composition of this seawater mineral concentrate was ionic magnesium ($\text{Mg}^{2+}$), which was $24,960 \text{ mg L}^{-1}$ (Sheu et al., 2013a). The study reveals the positive effects of DSM to enhance longevity of DM rats by enhancing cardiac function. The underlying molecular mechanism of cardio-protection includes suppression of pro-apoptotic proteins and enhancement of IGFI mediated survival proteins (IGFI, p-PI3K, and p-Akt) and pro-survival proteins of Bcl-2 family (p-Bad, Bcl-2, and Bcl-xL) in streptozotocin (STZ)-induced DM rats. The results show that DSM administration is potentially an effective means to enhance life expectancy in DM subjects with cardiac complications.

**MATERIAL AND METHODS**

**Deep Ocean Minerals**

DSM (LC-90K Do-Minerals) from depths below 662 m from the surface of the outer sea around Hualien County, Taiwan (ROC) was collected and supplied by Taiwan Yes Deep Ocean Water.

**Animal Studies**

The 8-week-old male Sprague Dawley rats were purchased from BioLASCO Taiwan (Taipei, Taiwan, ROC) and were randomly divided into six groups. Group I rats were the control ones ($n = 10$), group II were STZ induced DM rats ($n = 10$), group III were STZ induced rats treated with MgSO$_4$, group IV, V, and VI were STZ induced rats treated respectively with 1, 2, and 3 of DSM ($n = 10$). After allowing the rats to remain fasting for 24 h, DM was induced by giving a single intraperitoneal injection (IP) of STZ (65 mg kg$^{-1}$ body weight) dissolved in 10 mM sodium citrate, pH 7.0. Rats in each experimental group was fed with different multiples (1, 2, and 3) of 37 mg DSM/kg/day or an aqueous solution of MgSO$_4$ (111 mg kg$^{-1}$ day$^{-1}$) through gavage administration for a period of 4 weeks and the blood glucose levels were checked on a weekly basis. All the rats were fed with normal feed (Lab Diet 5001; PMI Nutrition International, Brentwood, MO). All protocols were reviewed and approved by the Institutional Review Board (IRB), and the animal care and use committee of the China Medical

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Untreated</th>
<th>MgSO$_4$</th>
<th>1× DSM$^a$</th>
<th>2× DSM$^a$</th>
<th>3× DSM$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>W0</td>
<td>112.67 ± 13.85</td>
<td>492.8 ± 92.39</td>
<td>422.33 ± 80.36</td>
<td>445.4 ± 92.8</td>
<td>412 ± 100.3</td>
<td>410.5 ± 69.35</td>
</tr>
<tr>
<td>W1</td>
<td>103.67 ± 11.67</td>
<td>558.8 ± 59.56</td>
<td>494.8 ± 67.18</td>
<td>565.25 ± 66.14</td>
<td>526.89 ± 60.29</td>
<td>556.25 ± 62.69</td>
</tr>
<tr>
<td>W2</td>
<td>111.67 ± 6.12</td>
<td>586.5 ± 33.07</td>
<td>482.2 ± 67.13</td>
<td>509.88 ± 59.73</td>
<td>558.44 ± 73.45</td>
<td>549.57 ± 67.3</td>
</tr>
<tr>
<td>W3</td>
<td>102.17 ± 14.20</td>
<td>582.8 ± 33.23</td>
<td>465.75 ± 105.6</td>
<td>470.13 ± 79.16</td>
<td>506.44 ± 90.22</td>
<td>536.83 ± 70.83</td>
</tr>
<tr>
<td>W4</td>
<td>112.75 ± 6.43</td>
<td>580.13 ± 43.02</td>
<td>441.75 ± 27.35</td>
<td>500 ± 53.74</td>
<td>461.86 ± 59.29</td>
<td>463 ± 148.66</td>
</tr>
</tbody>
</table>

$^a$DSW: deep sea water.

Table I. Effect of DSM on blood glucose

![Fig. 1.](image-url)
University, Taichung, Republic of China, and the study was conducted in accordance with the principles of laboratory animal care (Health, 1984).

**Histological Assay**

The rat hearts were excised, soaked in formalin, sequentially dehydrated in 100, 95, and 75% alcohol and embedded in paraffin wax. The paraffin-embedded tissue blocks were sliced into 0.2-μm-thick sections using microtome and deparaffinized by immersion in xylene. The slices were stained with hematoxylin and eosin (H&E), rinsed with water, and dehydrated using 100, 95, and 75% alcohol and rinsed twice in xylene. Photomicrographs were obtained using a Zeiss Axioskop microscope (Carl Zeiss Microscopy, Thornwood, NY).

**DAPI Staining and TUNEL Assay**

The deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) assay was performed treating the tissue sections with proteinase K, and incubated with permeabilization solution followed by blocking buffer; intermittent washing was done twice with PBS. The sections were then incubated at 37°C in terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP for 60 min by using an apoptosis detection kit (Roche Applied Science, Indianapolis, IN) according to manufacturer’s instruction. Under florescence (excitation wavelength of 460 nm and detection in the range of 515–565 nm) TUNEL-positive nuclei (fragmented DNA) were illuminated in bright green. To visualize the nuclei, the tissue sections were stained with 0.1 μg mL⁻¹ 4, 6-diamidino-2-phenylindole (DAPI), and photographed at 454 nm UV light using a Zeiss Axioskop microscope.

**Tissue Protein Extraction**

Cardiac tissue extracts were obtained by homogenizing the left ventricles of the rat hearts in lysis buffer (100 mg mL⁻¹). The homogenates were then centrifuged at 12,000 × g for 40 min. The supernatants were collected and stored at −80°C for further experiments.

**Western Blot Analysis**

Protein concentrations in the tissue extracts were determined by the Lowry’s protein assay method. Protein samples were separated by 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) with a constant supply of 75 V. The proteins were then transferred to Polyvinylidene fluoride (PVDF, GE Healthcare Life Sciences, Pittsburgh, PA) membranes by supplying 50 V current for 3 h. The membranes were washed in 3% bovine serum albumin (BSA) in TBS buffer and then incubation with specific primary antibodies (Santa

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**Table II. Effect of DSM on cardiac features**

<table>
<thead>
<tr>
<th>Cardiac Features</th>
<th>Control</th>
<th>Untreated</th>
<th>MgSO₄</th>
<th>1× DSW*</th>
<th>2× DSW*</th>
<th>3× DSW*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional shortening (FS), %</td>
<td>41.98 ± 1.40</td>
<td>31.36 ± 1.28</td>
<td>39.11 ± 1.68</td>
<td>34.85 ± 0.96</td>
<td>±39.91 ± 0.85</td>
<td>34.77 ± 1.08</td>
</tr>
<tr>
<td>Ejection fraction (EF), %</td>
<td>77.92 ± 1.56</td>
<td>64.39 ± 1.80</td>
<td>74.74 ± 1.94</td>
<td>69.44 ± 1.31</td>
<td>75.83 ± 0.96</td>
<td>69.73 ± 1.46</td>
</tr>
</tbody>
</table>

*aDSW: deep sea water.*

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**Fig. 2.** Effect of Deep sea minerals on the survival of diabetes mellitus rats. The results show that deep sea minerals (DSM) reduce the mortality rate (A) and enhance the life expectancy by improving the survival rates (B) of diabetes mellitus rats (n = 10).
Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase-labeled secondary antibodies were used for detection, and pictures were taken with Fujifilm LAS-3000 (GE Healthcare Life Sciences).

**Echocardiography**

M-mode echocardiographic examinations were performed using a 6–15 MHz linear transducer (15–6 L) via a parasternal long axis approach. Left ventricular M-mode measurements at the level of the papillary muscles included left ventricular internal end-diastolic dimensions (LVIDd), left ventricular internal end-systolic dimensions (LVIDs), interventricular septum (IVS), posterior wall thicknesses (LVPW), ejection fraction (EF) and fractional shortening (FS). EF% was calculated by (end-diastolic volume (EDV)-End-systolic volume (ESV)/EDV\(^3\)) \times 100, and FS% was calculated by \([ (LVIDd - LVIDs)/LVIDd] \times 100\).

**Statistical Analysis**

The results shown are the means ±SD of three independent experiments. Statistical analysis was performed by one-way analysis of variants. For paired samples, Student’s \(t\) test was applied.

**RESULTS**

**Effect of DSM on Blood Glucose of STZ-Induced Rats**

Periodical blood tests performed in STZ administered rats revealed that STZ causes defects in the glucose metabolism. The fast blood glucose level was found to increase drastically after 24 h of STZ induction, clearly indicating the emerging DM condition. In treatment groups, the blood glucose levels further increased during the first week of treatment, the levels however reduced in the following weeks. In the DSM treatment groups, the blood glucose level that increased in the first 2 weeks of treatment decreased considerably in the following 2 weeks. However, the 2× DSM treatment was comparatively more effective among other treatment groups (Table I). The MgSO\(_4\) treatment also showed a similar trend.

**Effect of STZ in Cardiac Features**

Echocardiography performed on the test rats revealed that the FS% and EF% decreased considerably and the values were alarming in comparison with the control groups revealing defects in the cardiac function (Fig. 1). Whereas, in the treatment groups the FS% and EF% were better, and particularly in the 2× DSM treatment groups the values were significantly higher than the DM groups indicating a strong protective effect provided by the DSM treatment. Treatment with MgSO\(_4\) also increased the FS% and EF% in the STZ induced DM rat hearts (Fig. 1, Table II).

**Mortality and Survival Rate of DM Rats**

STZ induced diabetics greatly affected the survival of tested rats and treatment with different concentrations of DSM significantly increased the survival in the STZ induced rats. The mortality rates in the diabetic rat group were found to be significantly high (63%) when compared with the healthy control.
The mortality rate remained low in the groups with 1, 2, or 3 of DSM (respectively 22%; 11%; 33%, n = 9) (Fig. 2). In the group that was fed with MgSO₄, the mortality rate was relatively low (17%, n = 6).

Heart Biopsy

Hematoxylin and eosin staining of the heart tissue sections of STZ-induced DM rats showed the appearance of cardiomyocytes in a disordered arrangement with increased interstitial space. However, the arrangement of cardiomyocytes in the treatment group rats were more ordered and the heart sections of 2× DSM groups in particular, was similar to the control group normal rats and were better than the MgSO₄ treatment groups (Fig. 3).

Nucleic Acid Stain

The nuclei of cardiomyocytes were stained in blue with DAPI, and specific DNA fragments caused by apoptosis process were stained in green color after TUNEL assay. Fluorescent microscopic studies revealed cleaved nuclear DNA in the heart section of STZ-induced DM rats. Treatment with DSM reduces the number of apoptotic nuclei (red arrow) to a level comparable to that of control rats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Effect of DSM on Apoptosis Pathway

To determine the effects of DSM on apoptosis machinery the expression levels of apoptosis related proteins in the rat
heart were determined by western blot analysis. The results show a significant increase in the expression of the extrinsic apoptosis related fas-Associated protein with Death Domain (FADD) and the fas receptor. The expression of the respective downstream proteins such as the caspase-8 and caspase-3 were also found to increase in the DM rats (Fig. 5). The levels of the intrinsic apoptosis proteins such as BH3 interacting-domain death agonist (Bid), Bad, Bcl-2 homologous antagonist killer (Bak) and cytochrome C (Cyt-C) were also found to increase in the DM rats. However the DM effects on the apoptosis proteins were found to be eliminated in the treatment group rats (Fig. 6).

**Effect of DSM on Survival**

To determine the effects of DSM on apoptosis the expression levels of apoptosis related proteins in the rat hearts were determined by western blot analysis. The results show
that STZ induced diabetes activated IGF-1/PI3K/Akt expressions that are associated with cell survival pathway and the DSM treatment complemented the PI3K/Akt mediated survival by promoting the survival pathway proteins further. But MgSO₄ treatment was found to surprisingly suppress the DM induced survival proteins to the levels comparable to that in the control group rat hearts (Fig. 7).

**DISCUSSION**

Recent studies on DSW show that DSM has a great potential for pharmaceutical applications. They are found to be effective against high fat diet related cardiac abnormalities. DSW has been reported to stimulate both osteoblastogenesis and osteoclastogenesis and provide recovery from osteoporosis (Liu et al., 2013). They are also found to be active in modulating blood pressure by exhibiting hypolipidemic effects (Sheu et al., 2013b). DSW also show promising evidences of providing cardio-protective effects.

**DSM Enhances the Longevity of DM Rats**

The STZ induced DM-related adverse changes in the rat hearts and the cardio-protection provided by DSM to increase the life expectancy of STZ induced DM rats were studied in detail. The STZ-induced diabetes rats are known to be excellent model for DM studies as they exhibit many of the pathophysiological conditions observed in humans, such as hypoinsulinemia, hyperglycemia, cardiac hypertrophy, cardiomyopathy and heart failure (De Angelis et al., 2002; Shiomi et al., 2003; Cheng et al., 2013). STZ induced DM severely affected the survival of the experimental rats within 4 weeks of induction, indicating the severity of DM related complications. The complications associated with DM are generally fatal and therefore life expectancy of DM subjects is generally low (Sasaki, 1994; Gu et al., 1998; Lee et al., 1998; Brun et al., 2000; Saydah et al., 2002; Romon et al., 2008). Whereas, in the treatment groups the mortality rates were significantly low during the 4 week treatment period indicating the life extending effects provided by DSM.

**DSM Reduces Blood Glucose But Do Not Effectively Control Hyperglycemia**

Normal blood glucose level in healthy rats and in healthy human is <100 mg dL⁻¹, (Kawamori et al., 2004). In this study, the blood glucose level of STZ-induced DM rats increased to 580 mg dL⁻¹ (almost five times higher than control group rats) within 4 weeks of induction indicating

**Fig. 6.** Protein expression analysis of intrinsic apoptosis pathway proteins by western blotting. Levels of Bid, Bad, Bak and cyt-C proteins increased in streptozotocin (STZ)-induced diabetes mellitus rats whereas treatment with 1× or 2× or 3× of DSM reduced the expression of these proteins. Quantifiable representation on expression levels of Bid (B), Bad (C) and cyt-C (D) were made by normalizing their expression with that of α-tubulin internal control. * = P < 0.05 and ** = P < 0.01 when compared with control group, # = P < 0.05 and ### = P < 0.01 when compared with diabetes group.
the development of hyperglycemic conditions. Although various concentrations of DSM treatment significantly reduced the blood glucose level, the blood glucose was not regulated to its normal level. Therefore, the treatments did not restore or directly complement the impaired function of beta cells that were destroyed by STZ (Szkudelski, 2001).

**DSM Treatment Protects the Normal Cardiac Function**

In a healthy human the EF% value is >55 and the FS% is >30 whereas, in a normal heart of a healthy rat, the EF% is usually above 70 and the FS% is above 40, reduction in the

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**Fig. 7.** Protein expression analysis of survival pathway proteins by western blotting. Levels of IGF-1, PI3K, p-Akt, Bcl-2, and Bcl-xL proteins increased in streptozotocin (STZ)-induced diabetes mellitus rats and when treated with $1 \times$ or $2 \times$ or $3 \times$ of deep sea minerals (DSM) the expression levels increased further. Quantifiable representation on expression levels of IGF-1 (B), Bcl-2 (C), p-Akt (D) and Bcl-xL were made by normalizing their expression with that of α-tubulin internal control. $^* = P < 0.05$, $^{**} = P < 0.01$ and $^{***} = P < 0.001$ when compared with control group.
respective values is an indication of heart dysfunction. Therefore, the decrease in the values of EF% to 64 and FS% to 31 reveals the onset of cardiac dysfunction in STZ-induced DM rats. Whereas in the treatment groups the effects of STZ were significantly inhibited showing an ameliorating effect of DSM on DM conditions and DM related cardiac defects.

DM is a strong risk factor for the development of heart failure in humans and it increases the apoptosis of cardiomyocytes by 85-folds, causing a reduction in the heart weight. The adverse effects also include progressive deterioration of the left ventricular function and the loss of cardiomyocytes either through apoptosis or necrosis (Kuo et al., 2009). The TUNEL assay on the STZ induced DM rat hearts show that cardiac dysfunction was also accompanied by increased apoptosis of cardiomyocytes. However, cardiac apoptosis in DM rats that were treated with 2x and 3x DSM was significantly reduced when observed after 4 weeks of treatment. The reduction in the level of apoptosis and the corresponding improvement in the heart function reveal that DSM treatment provides cardio-protection by suppressing hyperglycemia associated cardiac apoptosis.

**DSM Establishes Cardio-Protection by Suppressing Apoptosis and Enhancing Survival Mechanism**

To find the molecular mechanism behind cardiac dysfunction in DM and the appropriate protection provided by DSM treatment, the expression levels of apoptosis and survival pathway proteins were determined. Two major cellular pathways are known to attribute to the induction and progression of apoptosis in response to different types of stimuli (Green, 1998). The events in the intrinsic pathway involve the disruption of mitochondrial membrane potential and thereby release the cytochrome c that in turn triggers caspase 9. The extrinsic pathway proceeds by activation of the death receptors such as the Fas receptor, recruitment of the adaptor molecule FADD and activation of caspase 8. The members of the Bcl-2 family of proteins-Bcl-2 and the Bax are respectively anti-apoptotic and pro-apoptotic factors. In our previous reports, Fas/FADD pathway mediated activation of caspase-8 cleavage and apoptosis of cardiomyocytes were found to be the major causes of heart dysfunction (Lee et al., 2008, 2013; Huang et al., 2012b). The results indicate that
the proteins of the intrinsic and the extrinsic apoptosis pathway that were elevated in STZ-induced rats were regulated when treated with DSM. The results show that DSM treatment in DM rats inhibited both the Fas/FADD-mediated apoptosis pathway and prevented cardiomyopathy.

Increased expression levels of caspase-3 in the cardiac tissue, as determined by Western blotting analysis, reveals the possible involvement of caspase-3 in the DM related cardiomyocyte apoptosis. Caspase-3 modulates both mitochondria-dependent and Fas-death-receptor-dependent apoptotic pathways and it is therefore an important molecular marker of apoptosis (Huang et al., 2012a). The DM mediated caspase-3 activation was also characterized by a relative increase in the levels of Fas, FADD, and cleaved caspase-8 that was accompanied by an elevation in the levels of pro-apoptotic proteins such as Bid, Bad, BaK and cytoplasmic Cyt-C. However DSM treatment significantly reduced the DM related effects on the apoptotic proteins.

IGF-I has been known to improve myocardial function and prevent heart failure (Cheng et al., 2013). The IGF-I mediated signaling pathway is also known to mediate the suppression of apoptosis in cardiac cells and in variety of other cells. The IGF-I associated proteins such as the p-PI3k, p-Akt, Bcl-2, and Bcl-xL were found to be enhanced in the STZ-induced DM rat hearts, which could be possibly the result of a basal survival mechanism activated to counter the elevated cardiac apoptosis (Huynh et al., 2010; Troncoso et al., 2014). The results also show that DSM treatment further enhances the IGF-I mediated survival mechanism of DM rat hearts. However, MgSO₄ treatment did not show any enhancement in the survival proteins but in contrary it inhibited the elevated basal survival mechanism in the DM rats. This suggests that one or more factors in the DSM, other than MgSO₄, are involved in elevating the survival mechanism in the DM cardiomyocytes. Hence DSM treatment has more potentials than the MgSO₄ treatment in improving cardiac health. DSM treatment induced enhancement in the survival pathway also correlated with the decrease in the mortality rates of the treated DM rats. Therefore DSM enhances the life expectancy of DM rats by playing a complementary role in enhancing the survival mechanism of rat heart and by simultaneous inhibition of apoptosis (Fig. 8).

CONCLUSION

Our findings reveal that the effects of DM in cardiac tissue include alterations in cardiac morphology, cell survival and death. These modulations form the basis of the possible molecular mechanisms behind the DM induced cardiac events and mortality. As, cardiomyocyte apoptosis is a potential end-stage condition, the protective effect conferred by DSM treatment can be considered as a significant phenomenon. In conclusion, our results emphasize that DSM administration regulates hyperglycemia related apoptosis to a reasonable threshold by enhancing survival of the heart cells and suppressing apoptosis. Therefore further test on DSM can be effectively carried out to find the possibilities for its successful pharmaceutical applications.

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animals used in testing, research and training. Fed Regist 49: 29350–29351.


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