Appl Biol Chem (2017) 60(1):95–99
DOI 10.1007/s13765-016-0240-1

Online ISSN 2468-0842
Print ISSN 2468-0834

NOTE

Anti-diabetic effect of magnesium salt extracts from deep-sea water in C57BLKS/J-db/db mice

Min Hee Hwang[1] · Dong Gun Lee[1] · Eun Byeol Go[1] · Miju Cho[1] · Young Sig Park[2] · Namhyun Chung[1]

Received: 22 November 2016 / Accepted: 4 December 2016 / Published online: 21 January 2017

Abstract Recently, the incidence of diabetes has increased steadily worldwide. Type 2 diabetes constitutes about 90% of all cases of diabetes, and it is associated with many complications. Currently, the drugs for therapy of type 2 diabetes are considerably limited owing to disadvantages such as side effects and high rate of secondary failure. To overcome these problems, it is necessary to develop effective therapeutic agents from safe natural products. Deep-sea water (DSW) is abundant in minerals such as potassium (K), calcium (Ca), and magnesium (Mg). Mg supplements are known for their usefulness in the treatment of type 2 diabetes. In this study, we examined the anti-diabetic effect of Mg salt extracts from DSW in a diabetic mouse model. We observed that the groups treated with Mg salt extract showed better response toward fasting blood glucose level and oral glucose tolerance test when compared to the positive control. Overall, the Mg salt extract improved the symptoms of impaired fasting glucose and glucose tolerance, suggesting that it can be used as an effective agent for the treatment of type 2 diabetes mellitus.

Keywords Anti-diabetic · Deep-sea water · Diabetes mellitus · Magnesium salt extract

Introduction

The incidence of diabetes has been increasing steadily worldwide, and diabetes has become a serious health problem (Kim 2011). Insulin resistance and impaired insulin secretion by the pancreatic beta cells result in diabetes mellitus. There are of types diabetes mellitus: type 1 is insulin-dependent diabetes mellitus (IDDM) and type 2 is non-insulin-dependent diabetes mellitus (NIDDM; Dey et al. 2003). The prevalence of diabetes mellitus in the United States and remaining countries is approximately 6 and 3%, respectively (Xie et al. 2003). The global prevalence of diabetes mellitus for all ages is expected to increase to 4.4% in 2030 (Grinter and Simko 2013).

NIDDM accounts for approximately 90% of all cases of diabetes. It is a chronic metabolic disease as it involves disturbances in the lipid and carbohydrate metabolism. NIDDM induces hyperinsulinemia, dyslipidemia, and postprandial hyperglycemia (Lei et al. 2007). The main symptoms of diabetes are thirst, polyuria, fatigue, and weight loss (Johansen and Gregersen 1977). The most serious complications associated with diabetes are diabetic ketoacidosis and non-ketotic hyperosmolar state. These complications can induce unconsciousness or coma, and the lack of proper treatment can lead to death (Berger and Keller 1992). Chronic diabetes mellitus is associated with various characteristic complications such as retinopathy, which can lead to amblyopia and blindness, nephropathy, which can cause kidney failure and increase the risk of ulceration, Charcot joints, amputations resulting from nerve damage in the feet, and autonomic dysfunction due to

Min Hee Hwang, Dong Gun Lee, and Eun Byeol Go contributed equally to this study.

✉ Young Sig Park
pysku@korea.ac.kr

✉ Namhyun Chung
nchung@korea.ac.kr

[1] Department of Biosystems Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

[2] Functional Food Research Center, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

abnormal sympathetic and parasympathetic nerves. In addition, patients with diabetes have a high risk of cardiovascular, cerebrovascular, and peripheral vascular diseases (Alberti et al. 1998). Therefore, the management of these diseases is very important in patients with diabetes. Currently, the drugs used for the treatment of type 2 diabetes are considerably limited owing to disadvantages such as side effects and high rate of secondary failure (Xie et al. 2003). To overcome these problems, it is necessary to develop effective therapeutic agents from safe natural products.

Deep-sea water (DSW) refers to seawater at depths of 100–500 m below the sea surface. Compared to the surface seawater, DSW is cleaner, more hygienic, with high content of minerals such as potassium (K), calcium (Ca), and magnesium (Mg) (Hwang et al. 2016). DSW salts, such as solar and bamboo salts possessing these characteristics, have been used in various fields. For example, DSW has been used for drinking purposes, fish production and processing, agricultural production, and it finds applications in pharmaceutical, cosmetic, and health industries (Takahashi 2002). However, further researches are required to increase the utilization and value of DSW-related products. Mg supplements are known to be useful for the treatment of type 2 diabetes. Mg exerts beneficial effects in improving glycemic control and preventing the development of chronic complications (Lima et al. 1998). DSW contains Mg in abundance together with other useful minerals. Therefore, DSW could be developed as a useful resource for the treatment of type 2 diabetes. In the present study, we examined the anti-diabetic effect of Mg salt extracted from DSW in vivo.

Materials and methods

Animal experiments

Nine-week-old male C57BLKS/J-db/db (with diabetes) and C57BLKS/J-db/m+ (without diabetes) mice were obtained from Shizuoka Laboratory Center (Seoul, Korea) and acclimated for 2 weeks. The animals were housed in facilities at the Laboratory Animal Research Center, College of Life Sciences and Biotechnology at Korea University. The animals were maintained at 25 ± 2 °C and 60 ± 10% relative humidity in 12-h light/dark cycle. This research was approved by the Institutional Animal Care and Use Committee (IACUC) of Korea University. The Mg salt extracts were provided by Korea Research Institute of Ships and Ocean Engineering (Daejeon, Korea; Hwang et al. 2016). The solution of Mg salt extracts was prepared by dissolving the extracts in distilled water by ultrasonication for 30 min. The experimental groups were administered 3, 135, 405, and 675 mg/kg of Mg salt extracts from DSW once daily for 6 weeks.

Fasting blood glucose level

The blood glucose concentration was monitored weekly for 6 weeks. Before the experiment, the animals were fasted for 8 h. The experimental groups were divided into six groups: negative control group (without diabetes), positive control group (with diabetes), and four diabetic groups treated with Mg salt extracts from DSW. The fasting blood glucose levels were measured in blood samples obtained from the tail vein with a glucose meter (AGM-4100 GlucoDr., All Medicus Co., Anyang, Korea). To obtain reliable data, all test measurements were conducted five times (Hwang et al. 2009).

Oral glucose tolerance test

The oral glucose tolerance test was conducted at 6 week after the administration of Mg salt extract. The experimental groups were divided into six groups as described above. After fasting for 16 h, the mice were administered 2 g/kg of glucose dissolved in phosphate-buffered saline. The blood glucose level was measured from the tail vein blood at 0, 30, 60, 90, 120, 150, and 180 min after glucose administration using the above-mentioned glucose meter (Kim et al. 2008).

Statistical analysis

The differences between the positive control and treatment groups were determined by an independent sample *t* test.

Results

Changes in body weight

The body weight was measured once a week during the experiment period. The body weight change during 6 weeks of treatment with Mg salt extract is presented in Table 1. The changes in body weight were not statistically significant in all the experimental groups. The body weight of the positive control increased continuously while that of the Mg salt-treated groups showed a general tendency toward reduction after an initial increase. The maximum change was in the range 41.83–42.93 g until 3 weeks.

Effect of Mg salt extract on fasting blood glucose levels

The fasting blood glucose levels were measured once a week in tail vein blood after 8 h of fasting throughout the experimental period of Mg salt extract administration. The changes in fasting blood glucose levels are shown in

Table 1 Body weight changes in mice during administration of Mg salt extracts from DSW

	0 week	1 week	2 week	3 week	4 week	5 week	6 week
Negative control	22.83 ± 0.62	21.78 ± 1.30	21.72 ± 1.52	21.28 ± 1.63	20.64 ± 1.44	21.19 ± 1.71	21.34 ± 1.42
Positive control	41.08 ± 1.42	42.40 ± 1.44	42.95 ± 1.61	43.11 ± 1.90	43.23 ± 2.41	43.86 ± 2.62	43.80 ± 2.39
Mg 3 mg/kg	41.07 ± 1.98	42.40 ± 3.52	41.88 ± 3.17	40.92 ± 4.80	39.93 ± 3.51	40.29 ± 4.73	39.65 ± 6.37
Mg 135 mg/kg	41.06 ± 1.53	41.69 ± 1.66	42.48 ± 1.32	42.93 ± 1.54	42.70 ± 1.33	42.55 ± 1.72	41.44 ± 1.53
Mg 405 mg/kg	41.08 ± 1.69	42.21 ± 1.53	42.67 ± 2.10	42.65 ± 2.23	41.55 ± 3.54	39.57 ± 3.95	39.66 ± 4.89
Mg 675 mg/kg	41.09 ± 2.03	41.57 ± 2.68	41.61 ± 2.82	41.83 ± 3.03	41.41 ± 3.58	39.74 ± 3.39	37.62 ± 3.71

Data indicate mean ± SD, Unit: g

Fig. 1A. Compared to the positive control group, all treatment groups had a higher mean blood glucose level until 3 weeks. Then the mean glucose level began to decrease, and it became lower than that of the positive control. The average blood glucose levels during 6 weeks of Mg salt extract administration was in the order of positive control > Mg 3 > Mg 135 > Mg 405 > Mg 675 mg/kg. The blood glucose level after treatment with Mg 675 mg/kg was statistically significant (327.10 ± 100.45 mg/dL, $p < 0.01$) when compared to that of the positive control (Fig. 1B).

Effect of Mg salt extracts on oral glucose tolerance test

The oral glucose tolerance test was performed to examine the effect of Mg salt extract on glucose regulation in type 2 diabetes. Various concentrations of Mg salt extracts from DSW were administered for 6 weeks to C57BLKS/J-db/db mice, a model of type 2 diabetes. The change in blood glucose levels up to 120 min is shown in Fig. 2, where the blood glucose level at 0 min was set to 100. All groups treated with Mg salt extract showed a gradual improvement in impaired glucose tolerance (IGT) with time compared to the positive control (Fig. 2A). When compared to the positive control, all groups treated with Mg salt extract had a statistically significant decrease in the values of oral glucose tolerance test. However, the extent of change was not dependent on Mg concentration (Fig. 2B).

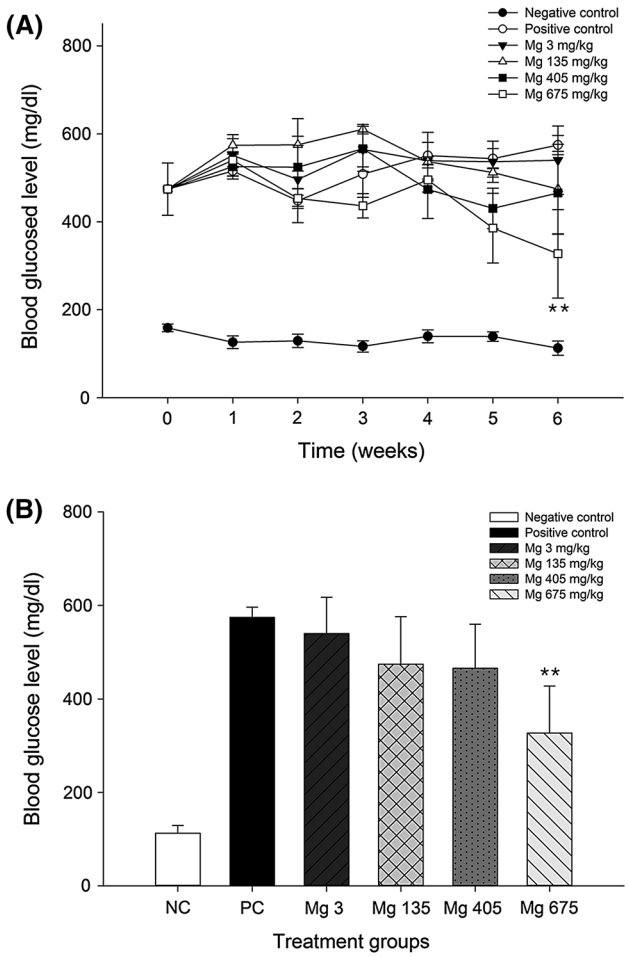

Fig. 1 Effect of Mg salt extract on fasting blood glucose levels. The mice were treated with Mg salt extracts from deep-sea water for 6 weeks. Change in fasting blood glucose levels for 6 weeks (**A**); comparison of fasting blood glucose levels on week 6 (**B**). Data are represented as mean ± SD ($n = 4$). $^{**}p < 0.01$ versus positive control group (Student's t test)

Discussion

In this study, we investigated the anti-diabetic effect of Mg salt extracts from DSW. The body weight changes were not significant in all the experimental groups. However, the average body weight of the treated groups showed a tendency to increase 41.83–42.93 g during the first half of the treatment period. Thereafter, the average body weight of the treated groups tended to decrease slowly. Previous studies have shown that the improvement of insulin sensitivity in type 2 diabetes was associated with body weight decrease owing to increased storage ability of glycogen in the muscles (DeFronzo and Ferrannini 1991; Friedman

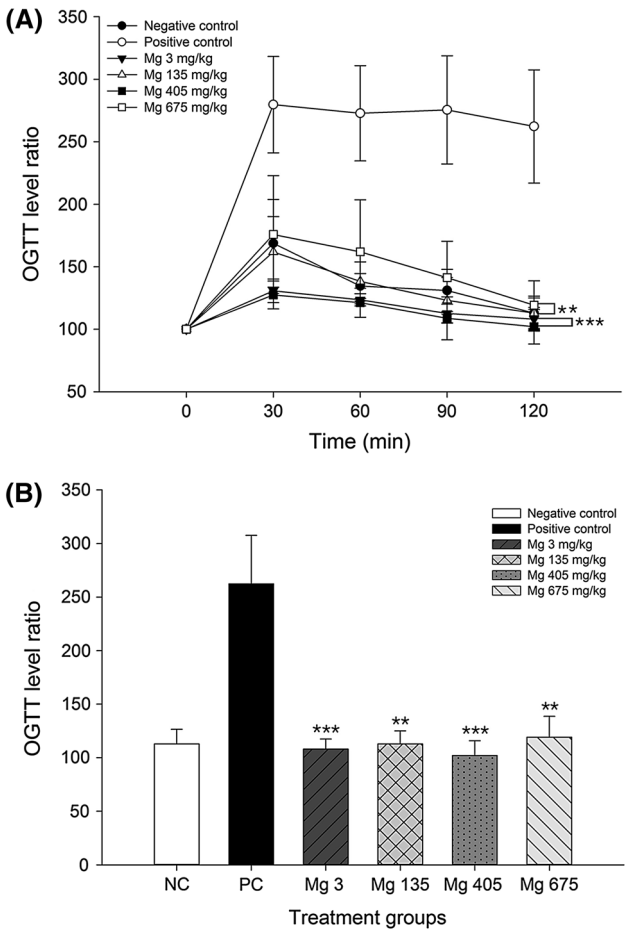

Fig. 2 Effect of Mg salt extract on oral glucose tolerance test. The blood glucose level at 0 min was set to 100. Variation in glucose levels in oral glucose tolerance test (OGTT) after treatment with Mg salt extracts from DSW at 0 min in mice (**A**); comparison of OGTT values 120 min after treatment with glucose (**B**). Data are represented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$ versus positive control group (Student's *t* test)

et al. 1992). Therefore, additional experiments are needed to confirm long-term body weight changes.

The fasting blood glucose level is an indicator of impaired fasting glucose (IFG). When the blood glucose is above a certain level, insulin stimulates glycogen synthesis, and glucose gets stored in the liver as glycogen. High fasting blood glucose level indicates that the liver produced excess glucose to compensate the decline in blood glucose level in the fasting state. This suggests that the liver has a weakened ability to regulate glucose metabolism owing to insulin deficiency or decrease in insulin sensitivity (Gerstein et al. 2006; Jiang and Zhang 2003). The fasting blood glucose levels showed a tendency toward gradual decrease in all groups treated with Mg salt extract (Fig. 1). In addition, the fasting blood glucose levels tended to decrease with an increase in Mg salt extract concentration. Therefore, Mg salt extract might have an ability to improve the IFG condition.

The oral glucose tolerance test is an indicator of IGT. IGT is caused by impairment in insulin function, which suggests the presence of insulin resistance or defects in the pancreatic beta cells that secrete insulin (Abdul-Ghani et al. 2006; Tuomilehto et al. 2001). In this study, after glucose (2 mg/kg) treatment, when compared to the positive control, the Mg salt extract-treated groups showed a noticeable decrease in blood glucose levels. Thus, the values of oral glucose tolerance test were statistically significant in Mg 3, 135, 405, and 675 mg/kg ($p < 0.001$, <0.01, <0.001, and <0.01, respectively) groups compared to those in the control. Therefore, it can be suggested that Mg salt extract might have a potential to improve IGT condition. Mg salt extracts might render an increased potential to improve the IGT and IFG conditions positively. In conclusion, Mg salt extract could be considered as an effective agent for the treatment of type 2 diabetes.

Acknowledgments This research was a part of the project titled 'Evaluation of Food Safety on Deep Sea Water Mineral Extract,' funded by the Ministry of Oceans and Fisheries, Korea.

References

Abdul-Ghani MA, Tripathy D, DeFronzo RA (2006) Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care 29:1130–1139

Alberti KG, Zimmet PZ, Consultation W (1998) Definition, diagnosis and classification of diabetes mellitus and its complications part 1: diagnosis and classification of diabetes mellitus—provisional report of a WHO consultation. Diabet Med 15:539–553

Berger W, Keller U (1992) Treatment of diabetic ketoacidosis and non-ketotic hyperosmolar diabetic coma. Baillieres Clin Endocrinol 6:1–22

DeFronzo RA, Ferrannini E (1991) Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194

Dey L, Xie JT, Wang A, Wu J, Maleckar SA, Yuan CS (2003) Anti-hyperglycemic effects of ginseng: comparison between root and berry. Phytomedicine 10:600–605

Friedman JE, Dohm GL, Leggett-Frazier N, Elton CW, Tapscott EB, Pories WP, Caro JF (1992) Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. Effect on muscle glucose transport and glucose transporter GLUT4. J Clin Investig 89:701–705

Gerstein HC, Yusuf S, Holman RR, Bosch J, Anand S, Avezum A, Bjdaj A, Chiasson J, Conget I, Dagenais G, Davis M, Diaz R, Dinccag N, Enjalbert M, Escalante A, Fodor G, Hanefeld M, Hedner T, Jolly K, Keltai M, Laakso M (2006) Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. Lancet 368:1096–1105

Ginter E, Simko V (2013) Global prevalence and future of diabetes mellitus. Adv Exp Med Biol 771:35–41

Hwang HS, Kim HA, Lee SH, Yun JW (2009) Anti-obesity and antidiabetic effects of deep sea water on ob/ob mice. Mar Biotechnol 11:531–539

Hwang MH, Cho MJ, Lee DG, Go EB, Park YS, Chung N (2016) Single- and repeated-dose oral toxicity tests of deep sea water mineral extracts in ICR mice. J Biol Chem 59:1–5

Jiang GQ, Zhang BB (2003) Glucagon and regulation of glucose metabolism. Am J Physiol-Endocrinol Metab 284:E671–E678

Johansen K, Gregersen G (1977) A family with dominantly inherited mild juvenile diabetes. Acta Med Scand 201:567–570

Kim DJ (2011) The epidemiology of diabetes in Korea. Diabetes Metab J 35:303–308

Kim DJ, Jeong YJ, Kwon JH, Moon KD, Kim HJ, Jeon SM, Lee MK, Park YB, Choi MS (2008) Beneficial effect of chungkukjang on regulating blood glucose and pancreatic beta-cell functions in C75BL/KsJ-db/db mice. J Med Food 11:215–223

Lei H, Ma X, Wu WT (2007) Anti-diabetic effect of an alpha-glucan from fruit body of maitake (*Grifola frondosa*) on KK-Ay mice. J Pharm Pharmacol 59:575–582

Lima MDL, Cruz T, Pousada JC, Rodrigues LE, Barbosa K, Cangucu V (1998) The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. Diabetes Care 21:682–686

Takahashi M (2002) Future source, take up from sea. Academy Press, Seoul

Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M, Aunola S, Cepaitis Z, Moltchanov V, Hakumaki M, Mannelin M (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344:1343–1350

Xie JT, Wang AB, Mehendale S, Wu J, Aung HH, Dey L, Qiu SX, Yuan CS (2003) Anti-diabetic effects of *Gymnema yunnanense* extract. Pharmacol Res 47:323–329