Technical Note

Effect of Korean Red Ginseng through comparative analysis of cardiac gene expression in db/db mice

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ABSTRACT

Korean Red Ginseng (KRG) is an herbal oriental medicine known to alleviate cardiovascular dysfunction. To analyse the expression of diabetic cardiac complication-associated genes in db/db mice, we studied the cardiac gene expression following KRG treatment. In result, a total of 585 genes were found to be changed in db/db mice. Among the changed expression, 245 genes were up-regulated, and 340 genes were down-regulated. In addition, the changed gene expressions were ameliorated by KRG. In conclusion, KRG may be possible to normalize cardiac gene expressions in db/db mice.

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The prevalence of diabetes mellitus (DM) at all ages was estimated at 2.8% in 2000 and 4.4% in 2030. DM could lead to an increase in diabetic cardiovascular complications (DCC), becoming global public health threats [1,2]. However, there are no confirmed remedies for DCC. Traditionally, Korean Red Ginseng (KRG, Panax ginseng Meyer) is used as a principal herbal medicine in Far East Asia. The major biological functions of KRG are known to be helpful in cardiovascular diseases because of its pharmacological activities such as anti-inflammatory, antioxidant, and ameliorative effects on blood flow and cardiac function [3–7]. Yet, there is no a sure proof showing the benefits of cardiovascular effect of KRG in type 2 diabetic db/db mice. Considering these facts, we determined to study the expression of DCC-associated genes in db/db mice. Further, we assessed the reversal of this expression following KRG treatment.

In the present study, the systematic analysis of hemodynamics and gene expression profiling was performed in the heart of type II diabetic db/db mice before and after KRG treatment. Six-week-old, male, db/db mice (BKS.Cg-Dock7m +/+ Lepr(db/J) strain) and db/+ mice were purchased from Orient, Korea. The blood glucose levels in db/db mice were significantly higher than in db/+ mice (596.3 ± 85.4 and 253.7 ± 8.4, respectively). After acclimatization, animals were divided randomly into 5 groups (n = 9): normal control group (N/C), 200 mg/kg KRG alone group (200KRG-alone), KRG-un-treated db/db group (db/db), 100 mg/kg KRG-treated db/db group (db/db-100KRG), and 200 mg/kg KRG-treated db/db group (db/db-200KRG). All animals were housed under standard temperature (22 ± 1°C), humidity (55 ± 5%), and light conditions (12 h light/dark cycle). Administration of KRG was conducted by feeding the chow mixed with KRG for 16 weeks. The dose of KRG was selected following a preliminary experiment administrated at various doses. KRG powder was purchased from the Korea Ginseng Corporation (Daejeon, Korea). At a two-week interval for a total of 16 weeks, biochemical and hemodynamic study were performed. At the end of the experimentation, microarray gene expression profiling was analyzed on the extracted RNA samples (Fig. 1). The Principles of Laboratory Animal Care were followed according to the Guidelines for Institutional Animal Care and Use Committees (IACUC) of Jeonbuk National University (Jeonju, Korea). For all studies, data are expressed as mean ± standard error of mean. Comparison between the groups was analyzed by Student’s t-tests and one-way analysis of variance. Significance was statistically considered at p < 0.05.

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Above all, we assessed the cardiovascular function of KRG by hemodynamic data such as heart rate (HR), left ventricle peak systolic pressure (LVSP), maximal rates of developed left ventricular pressure (+dP/dt\text{max}), minimal rates of developed left ventricular pressure (−dP/dt\text{max}), fractional shortening (FS) and ejection fraction (EF). We found that HR revealed no significant difference in

![Fig. 1. Experimental protocol. All animals divided randomly into 5 groups (n = 9, respectively): N/C, 200 mg/kg KRG, db/db, db/db + 100 mg/kg KRG and db/db + 200 mg/kg KRG. At a two-week interval for a total of 16 weeks, biochemical and hemodynamic study were performed. At the end of the experimentation, microarray gene expression profiling was analyzed on the extracted RNA samples. N/C, normal control; KRG, Korean Red Ginseng.](image1)

![Fig. 2. The effect of KRG on echocardiographic evaluation to assess the cardiovascular function by hemodynamic data such as (A) heart rate, (B) peak systolic pressure, (C) +dP/dt\text{max} and (D) −dP/dt\text{max}. Data are presented as means ± SE; *p < 0.05, **p < 0.01 compared N/C; *p < 0.05, **p < 0.01 compared with db/db control. N/C, normal control; +dP/dt\text{max}, maximal rates of developed left ventricular pressure; −dP/dt\text{max}, minimal rates of developed left ventricular pressure; KRG, Korean Red Ginseng; SE, standard error.](image2)
each group (Fig. 2A). Functional parameters such as LVSP, +dP/dt\textsubscript{max} and −dP/dt\textsubscript{max} were increased in \textit{db/db} group after 10 weeks compared with in N/C groups. Whereas, \textit{db/db}+100KRG group reduced the level of LVSP and −dP/dt\textsubscript{max}, after 16 and 12 weeks, respectively. Similarly \textit{db/db}+200KRG group reduced the level of LVSP, +dP/dt\textsubscript{max} and −dP/dt\textsubscript{max}, after 12, 10, and 8 weeks, respectively (Fig. 2B).

Currently, it was well known that FS and EF are the most precious function index for clinical application [8]. As revealed in Fig. 3A, there are no remarkable differences in FS among each groups until 6 weeks. But \textit{db/db} group decreased the level of FS since 8 weeks. 200KRG-alone group was shown no changes, which indicating that KRG does not show any side effects. The FS value was 47.6 ± 4.69%, 49.6 ± 4.01%, and 51.7 ± 3.71% in 8 weeks; 47.7 ± 4.02%, 49.7 ± 4.22%, and 49.4 ± 4.15 in 10 weeks; 46.9 ± 3.69%, 49.9 ± 3.98%, and 51.7 ± 3.98% in 12 weeks; 45.4 ± 4.02%, 48.5 ± 3.18%, and 49.9 ± 3.87% in 14 weeks; 43.6 ± 4.81%, 47.1 ± 4.03%, and 47.1 ± 3.99% in 16 weeks, for \textit{db/db}, \textit{db/db}+100KRG and \textit{db/db}+200KRG, respectively (Fig. 3A). Similarly, as revealed in Fig. 3B no significant differences were observed in EF among each group until 6 weeks. However, \textit{db/db} group decreased the average level of EF since 8 weeks. 200KRG-alone group was also shown no changes. The EF value were 89.1 ± 4.2%, 87.6 ± 3.4%, and 91.6 ± 2.9% in 8 weeks; 82.6 ± 4.7%, 83.2 ± 3.7%, and 90.6 ± 2.8% in 10 weeks; 82.7 ± 3.9%, 82.9 ± 3.9%, and 87.6 ± 2.7% in 12 weeks; 78.5 ± 3.2%, 81.4 ± 3.2%, and 85.6 ± 3.1% in 14 weeks; 79.4 ± 4.3%, 81.6 ± 3.0%, and 85.9 ± 3.4% in 16 weeks, for \textit{db/db}, \textit{db/db}+100KRG and \textit{db/db}+200KRG, respectively. After all, administration of KRG inhibited the decreases of EF and FS levels. These facts propose that KRG has a potential to reduce the cardiac ventricular dysfunction in \textit{db/db} mice.

Meanwhile, the cardiac troponin T (cTnT) levels and myeloperoxidase (MPO) activity of all groups were shown in Fig. 3C and D. Regarding cTnT activity, 200KRG-alone group did not show significant changes compared with the N/C group. The cTnT levels in \textit{db/db} group were increased after 16 weeks (0.57 ± 0.0221 mg/l) compared with those in N/C groups (0.54 ± 0.0270 mg/l). While those in \textit{db/db}+200KRG group decreased (0.52 ± 0.0310 mg/l) compared with those in \textit{db/db} group (Fig. 3C). In an assay of neutrophil infiltration, the MPO activity in \textit{db/db} group was increased (6.9 ± 0.32 nmol/mg for 12 weeks and 7.1 ± 0.57 nmol/mg for 16 weeks) compared with that in the N/C (5.9 ± 0.21 nmol/l for 12 weeks and 6.17 ± 0.22 nmol/l for 16 weeks) and 200KRG-alone group (6.26 ± 0.22 nmol/mg for 12 weeks and 6.31 ± 0.20 nmol/mg for 16 weeks). While that in \textit{db/db}+200KRG group led to decrease (6.2 ± 0.54 nmol/mg for 12 weeks and 6.4 ± 0.51 nmol/mg for 16 weeks) compared with that in \textit{db/db} group (Fig. 3D).

For investigation of global gene expression, total RNAs extracted from mouse cardiac tissue were analyzed using the whole mice genome microarray. We performed hierarchical clustering to get a rough estimate of the number of changed genes of \textit{db/db} group, \textit{db/db}+100KRG group and \textit{db/db}+200KRG group as compared with N/C. In Fig. 4, gene expression levels of N/C are shown in black color as
baselines. Red color indicates gene overexpression and green color indicates gene underexpression compared to N/C. A total of 585 genes were differentially changed in the \( \text{db/db} \) group: 245 genes showed 2-fold upregulated expression and 340 genes showed 2-fold downregulated expression. In the \( \text{db/db} + 100\text{KRG} \) group, a total of 578 genes were differentially changed: 224 genes were upregulated and 354 genes were downregulated. In the \( \text{db/db} + 200\text{KRG} \) group, a total of 557 genes were differentially changed: 287 genes were upregulated and 270 genes were downregulated (Fig. 4A). Hierarchical clustering of apoptosis, inflammation and stress response genes were shown in Fig. 4D, respectively. We observed a relieved pattern of the number of differentially expressed genes (DEGs) in the \( \text{db/db} \) group by KRG treatment. Also, DEGs were divided into the functions: cell apoptosis, behavior, adhesion, differentiation, migration, proliferation, growth, homeostasis, immune response, inflammation, lipid metabolism, stress response,

| Gene symbol | Description | Fold change (\( \text{db/db} \)) | Fold change (\( \text{db/db} + 100\text{KRG} \)) | Fold change (\( \text{db/db} + 200\text{KRG} \)) |
|-------------|-------------|-------------------------------|--------------------------------|--------------------------------|
| Bcl3        | B-cell leukemia/lymphoma 3 | 2.1668                        | 1.4606                          | 1.1840                          |
| Cideb       | Cell death-inducing DNA fragmentation factor, alpha subunit-like effector B | 2.0333                        | 1.9911                          | 1.9025                          |
| Gadd45b     | Growth arrest and DNA-damage-inducible 45 beta | 2.2262                        | 1.4778                          | 1.4152                          |
| Myc         | Myelocytomatosis oncogene | 3.0172                        | 2.5677                          | 1.9027                          |
| Prune2      | Prune homolog 2 (Drosophila) | 3.8508                        | 3.1494                          | 2.7030                          |
| Thbs1       | Thrombospondin 1 | 10.4962                        | 9.6363                          | 6.9609                          |
| Sfpr2       | Secreted frizzled-related protein 2 | 0.3653                        | 0.4420                          | 0.5760                          |
| Sna2        | Snail homolog 2 (Drosophila) | 0.4028                        | 0.4867                          | 0.6122                          |

\( \text{db/db} \): KRG-untreated \( \text{db/db} \) group; \( \text{db/db} + 100\text{KRG} \): 100 mg/kg KRG-treated \( \text{db/db} \) group; \( \text{db/db} + 200\text{KRG} \): 200 mg/kg KRG-treated \( \text{db/db} \) group.
Table 2
List of genes related with inflammation showing reversed expression by the administration of KRG in db/db mice

| Gene symbol | Description | Fold change (db/db) | Fold change (db/db + 100KRG) | Fold change (db/db + 200KRG) |
|-------------|-------------|---------------------|----------------------------|----------------------------|
| C5ar1       | Complement component 5a receptor 1 | 2.1100               | 1.8013                        | 1.2296                     |
| Ccl12       | Chemokine (C–C motif) ligand 12 | 2.0020               | 1.9699                        | 0.5957                     |
| Cxcl1       | Chemokine (C-X-C motif) ligand 1 | 5.1132               | 4.2344                        | 4.65223                    |
| Slc27a1     | Solute carrier family 27 (fatty acid transporter), member 1 | 6.7424               | 5.9982                        | 2.5752                     |
| Ugt1a1; -1a2, -1a5, -1a6a; -1a6b, -1a7c, -1a9, -1a10 | UDP glucuronosyltransferase 1 family, polypeptide A1, A2, A5, A6A, A6B, A7C, A9, A10 | 3.2937               | 2.1530                        | 1.5849                     |
| Pfkfb1      | 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1 | 2.1084               | 1.1797                        | 1.1569                     |
| Fmod        | Fibromodulin | 3.1993               | 1.4630                        | 1.4492                     |

Gene expression was ameliorated by the administration of KRG in db/db mice. The list of genes is as follows: inflammation-related genes such as C5ar1, Ccl12, Cxcl1, Slc27a1, Pfkfb1 and Fmod; and stress response-related genes such as Ugt1a1, Pfkfb1 and Fmod. The expression levels of these genes in db/db mice were upregulated or downregulated as compared to those from N/C, while they were alleviated in KRG-treated groups. It indicates that the changed gene expressions were ameliorated by the administration of KRG in dose-dependent fashion.

In conclusion, we revealed KRG may normalize gene expressions caused by DCC in type 2 diabetic mice model. These data showed that KRG plays a role in various biological pathways in DCC and that some of genes associated with these pathways are sufficiently responsive to KRG. That means KRG have therapeutic effects on DCC by adjusting the expression levels of genes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.06.001.

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