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Research note

Fermented ginseng, GBCK25, ameliorates hemodynamic function on experimentally induced myocardial injury

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ABSTRACT

In the present study, we investigated whether treatment with GBCK25 facilitated the recovery of hemodynamic parameters, left ventricle systolic pressure, left ventricular developed pressure, and electrocardiographic changes. GBCK25 significantly prevented the decrease in hemodynamic parameters and ameliorated the electrocardiographic abnormality. These results indicate that GBCK25 has distinct cardioprotective effects in rat heart.

Ginseng root, as an herbal medicine, has been widely used in the Orient for thousands of years [1,2]. In the present study, the protective effect of ginseng fermented with new strain *Saccharomyces servazzii* GB-07 and pectinase enzyme (GBCK25) on the ischemia–reperfusion (I/R) injury in an isolated rat heart was evaluated [3–5].

GBCK25 was kindly obtained by General Bio Co., Ltd. (Generalbio Co, Jeollabuk-do, Korea) using a standardized process. Namely, the procedure was conducted using complex fermentation combined with new strain *Saccharomyces servazzii* GB-07 and pectinase enzyme for 5 d to convert general ginsenoside into Compound K. The contents of ginsenosides in GBCK25 were composed of ginsenoside Rg1, 26.74 mg/g; Re, 62.15 mg/g; Rh1(s) + Rg2(s), 14.93 mg/g; Rb1, 3.22 mg/g; Rc, 4.38 mg/g; Ra1, 5.67 mg/g; Rb2, 26.76 mg/g; Rb3, 3.83 mg/g; Rd, 14.61 mg/g; Rg3(s), 6.23 mg/g; CK, 23.22 mg/g; and other minor components. GBCK25 was dissolved in a modified Krebs-Henseleit (KH) buffer which consisted of 120.0mM NaCl, 1.2mM $MgSO_4$, 4.8mM KCl, 1.2mM KH_2PO_4, 25mM $NaHCO_3$, 11.0mM glucose, and 25mM $CaCl_2$. In this study, the reagents were purchased from Sigma (St. Louis, MO, USA) and were of analytical grade.

The study was conducted using 35 male Sprague-Dawley rats weighing 200 ± 20 g. All the animals were obtained from SLC Inc. (Shizuoka, Japan). The Principles of Laboratory Animal Care were followed in accordance with the "Guideline for Institutional Animal Care and Use Committees" of Chonbuk National University (Jeonju, Korea).The Sprague-Dawley rat hearts were perfused for a total of 180 min. This perfusal consisted of a pre-ischemia period (i.e., equilibration for 30 min, followed by 60 min ischemia, and 120 min reperfusion at 37°C (Fig. 1). The hearts were divided into five experimental groups ($n = 7$, each group). In the normal control (N/C) group, hearts were perfused with KH buffer without ischemia. In GBCK25 control, hearts were perfused with buffer for 30 min, and perfusion was followed for 60 min plus 120 min without ischemia. In the I/R group, hearts were perfused with buffer for 30 min, followed by 60 min ischemia and 120 min reperfusion. In the 200 mg/kg and 400 mg/kg GBCK25 groups, hearts were perfused with buffer for 30 min, followed by 60 min of ischemia and 120 min reperfusion, respectively (Fig. 1). After pretreatment with 200 mg/kg and 400 mg/kg GBCK25 for 7 d, the rats were anesthetized with 25–30 mg of pentobarbital intraperitoneally. Hearts were excised and were immersed in 4°C solution to prevent myocardial damage. After the hearts were stabilized for 30 min, ischemia was induced for 60 min. The hemodynamic data such as perfusion pressure, coronary flow, aortic flow, and cardiac output and

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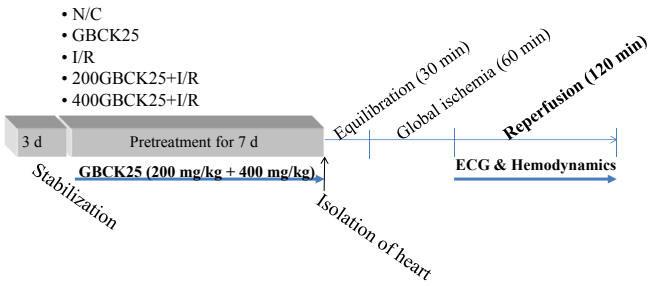

Fig. 1. All experimental groups began with a 30-min perfusion period to allow for stabilization. Then, the hearts were divided into the normal control (N/C) group, the GBCK25 alone treated group (GBCK25), the 60 min ischemia and 120 min reperfusion group (I/R control), the 60 min ischemia and 120 min reperfusion, which received administration of 200 mg/kg GBCK25 before ischemia induction (200GBCK25+I/R), and the 60 min ischemia and 120 min reperfusion, which received administration of 400 mg/kg GBCK25 before ischemia induction (400GBCK25+I/R). ECG, electrocardiogram; I/R, ischemia–reperfusion.

electrocardiogram (ECG) parameters were studied. The developed maximal rates of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were recorded after 120 min reperfusion. Both $+dP/dt_{max}$ and $-dP/dt_{max}$ were studied as indices of cardiac contractility. All statistics were calculated using SigmaPlot for Windows version 12.0 (Systat Software, Inc., USA). For all studies, significance was statistically considered at $p < 0.05$.

The effect of GBCK25 on the hemodynamics was assessed by measuring cardiac function including coronary flow, aortic flow, and cardiac output. These parameters were substantially decreased by I/R induction to an average of 66.37 ± 4.92%, 69.51 ± 4.65%, and 65.04 ± 3.27% compared to an N/C group as 100%, respectively. However, pretreatment with GBCK25 (200 mg/kg and 400 mg/kg) increased coronary flow, aortic flow, and cardiac output to an average of 72.68 ± 4.79%, 73.24 ± 5.02%, and 72.86 ± 6.39%, respectively, using 200 mg/kg GBCK25, and to an average of 82.42 ± 5.31%, 81.37 ± 4.17%, and 82.43 ± 5.21%, respectively, using 400 mg/kg GBCK25 (Fig. 2). Furthermore, I/R induction significantly decreased average left ventricle systolic pressure (LVSP) values; 69.6 ± 3.2% (baseline), 68.7 ± 4.1% (30 min), 66.5 ± 4.6% (60 min), 66.4% (90 min), and 63.2 ± 3.5% (120 min) compared to the N/C group as 100 %, respectively.

In contrast, pretreatment with 200 mg/kg GBCK25 significantly increased LVSP values; 75.7 ± 3.3% (baseline), 74.2 ± 3.6% (30 min), 74.3 ± 3.2% (60 min), 73.2 ± 3.1% (90 min), and 73.1 ± 3.5% (120 min) compared to the N/C group as 100 %, respectively. Pretreatment with 400 mg/kg GBCK25 significantly increased LVSP values; 82.4 ± 4.1% (baseline), 84.5 ± 4.7% (30 min), 83.7 ± 3.9% (60 min), 81.9 ± 3.6% (90 min), and 82.7 ± 3.7% (120 min) compared to the N/C group as 100%, respectively (Fig. 3). Likewise, I/R induction resulted in a significant fall in average $+dP/dt_{max}$ values to 52.9 ± 3.75% for 120 min, whereas pretreatment with GBCK25 significantly increased the average $+dP/dt_{max}$ values to 63.69 ± 4.74% in 200 mg/kg GBCK25, and 84.63 ± 4.55% in 400 mg/kg GBCK25 for 120 min, respectively (Fig. 4A). Under the same conditions, the average $-dP/dt_{max}$ values were 62.75 ± 3.63% compared to the N/C group as 100% in the I/R group. However, GBCK25 significantly increased $-dP/dt_{max}$ values to an average of 69.65 ± 3.52% in 200 mg/kg GBCK25, and 78.47 ± 4.71% in 400 mg/kg GBCK25 for 120 min, respectively (Fig. 4B). As seen in Figs. 2–4,

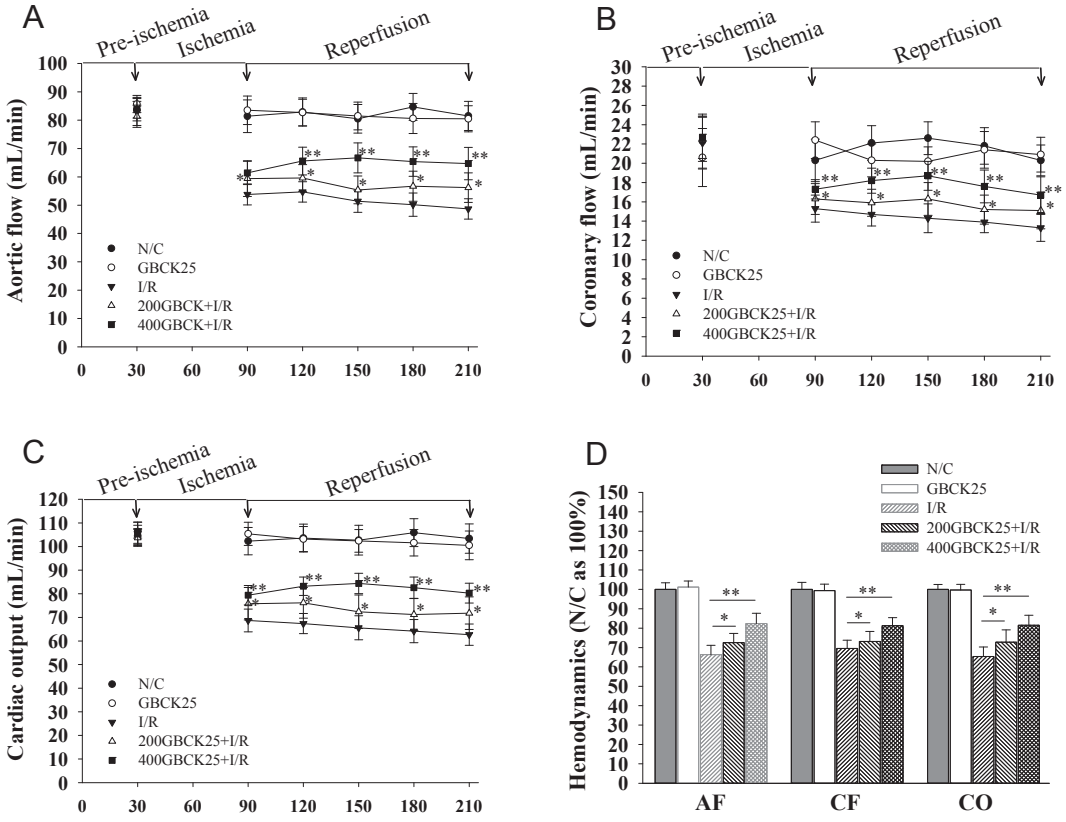

Fig. 2. Effects of 200 mg/kg and 400 mg/kg GBCK25 between (A) aortic flow, (B) coronary flow, (C) cardiac output, and (D) average percent for 120 min reperfusion on hemodynamics. Each histogram represents the mean ± SD ($n = 7$). * $p < 0.05$ compared with I/R group, respectively. ** $p < 0.01$ compared with I/R group, respectively. I/R, ischemia–reperfusion; N/C, normal control group.

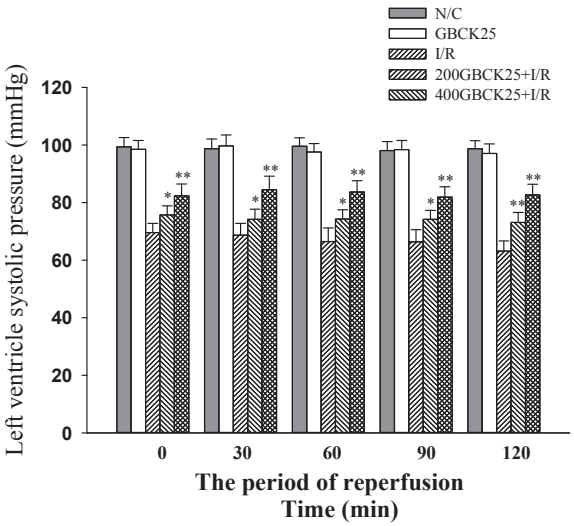

Fig. 3. Effects of 200 mg/kg and 400 mg/kg GBCK25 on left ventricle systolic pressure left ventricle systolic pressure (LVSP). These hemodynamic parameters were estimated at 30 min intervals throughout the 120-min reperfusion period. Results were representative of nine independent experiments. Values are expressed as mean ± SD. * $p < 0.05$. ** $p < 0.01$. I/R, ischemia–reperfusion; N/C, normal control group.

there was no difference between hemodynamics such as LVSP and $\pm dP/dt_{max}$ between the N/C and the GBCK25 groups. These results suggest that GBCK25 itself did not influence cardiac hemodynamic function in the experiments.

In the ECG study, the normal group showed a normal ECG. No significant differences on conduction intervals for the GBCK25 group and the N/C group were observed as seen in Fig. 5. However, when studying ECG parameters after 30 min ischemia and 120 min reperfusion, the QRS interval tended to be significantly delayed compared to the N/C group (Fig. 5). In I/R control, an average of QRS values was 138.24 ± 5.22% for 120 min compared to the N/C group (an average N/C value as 100). As shown in Fig. 5, animals in the I/R group also produced the pathological R amplitude, showing transmural cardiac infarction. However, the QRS interval significantly shortened in the 200 mg/kg and 400 mg/kg GBCK25-treated groups compared to the I/R group for 120 min I/R induction. Average values of QRS intervals were 130.71 ± 4.95% for the 200 mg/kg GBCK25-treated group and 122.16 ± 4.73% for the 400 mg/kg GBCK25-treated group compared to the I/R control group for 120 min (Fig. 5). As shown in Fig. 5B, the 400 mg/kg GBCK25-treated group was more effective than the 200 mg/kg GBCK25-treated group ($p < 0.5$, $p < 0.01$). The results indicated that treatment with 400 mg/kg GBCK25 is more effective than with 200 mg/kg GBCK25 in the preservation of atrioventricular conduction. In addition, in the GBCK25 control group, QT interval alteration was similar to the N/C group, as seen in Fig. 5C. Moreover, I/R induction produced significant delayed QT interval compared to N/C animals. In the I/R control group, an average QT value is 136.37 ± 5.22 % for 120 min when compared to the N/C group (an average N/C value as 100%). For the 200 mg/kg and 400 mg/kg GBCK25-treated groups, QT interval significantly shortened compared to the I/R group. Namely, the average values of QT interval for 120 min were 129.18 ± 4.52% for the 200 mg/kg GBCK25-treated group and 123.51 ± 3.73% for the 400 mg/kg GBCK25-treated group. As shown in Fig. 5, the GBCK15 control group had no significant effectiveness in the QT interval study for total 180 min I/R periods ($p > 0.5$). The results indicated that treatment with GBCK25 can be effective in the preservation of repolarization. Also, it is known that heart rate interval is studied with the R to R wave (RR) [6].

In the present study, a normal RR interval was obtained, similar to the N/C group. However, in the I/R group, the RR interval tended to be significantly delayed compared to the N/C group. Compared to the N/C group (N/C value as 100%), the I/R group had an RR interval of 123.66 ± 3.71% for 120 min reperfusion. These values were changed when 200 mg/kg and 400 mg/kg GBCK25 were used as shown in Fig. 5D. Namely, the RR intervals for 200 mg/kg and 400 mg/kg GBCK25-treated groups were 118.22 ± 2.95% and 110.61 ± 2.89%, respectively. These data were significantly shorter than the I/R control for 120 min reperfusion.

These results indicated that treatment with 200 mg/kg and 400 mg/kg GBCK25 can be effective in the preservation of heart rate in rats (Fig. 5D). Therefore, the present study provides a preliminary possibility for the application of GBCK25. However, further studies need to be carried out to give assurance that these results can be used for humans. In the point of the safety and efficacy of GBCK25, prospective further studies should be considered.

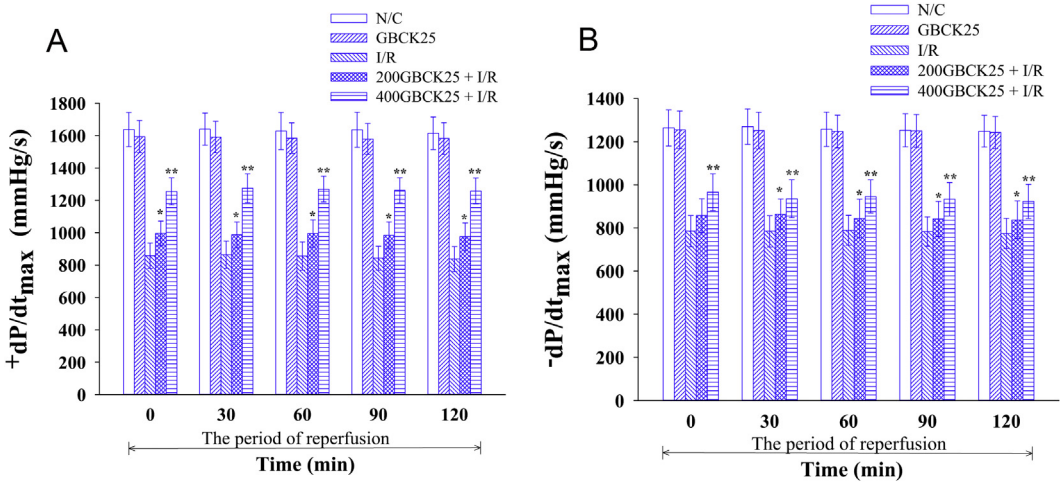

Fig. 4. Effects of 200 mg/kg and 400 mg/kg GBCK25 on (A) $+dP/dt_{max}$ and (B) $-dP/dt_{max}$. These hemodynamic parameters were estimated at 30-min intervals throughout the 120-min reperfusion period. Results were representative of nine independent experiments. Values are expressed as mean ± SD. * $p < 0.05$ compared with I/R. ** $p < 0.01$ compared with I/R. $+dP/dt_{max}$, maximal rate of contraction; $-dP/dt_{max}$, maximal rate of relaxation; I/R, ischemia–reperfusion; N/C, normal control group.

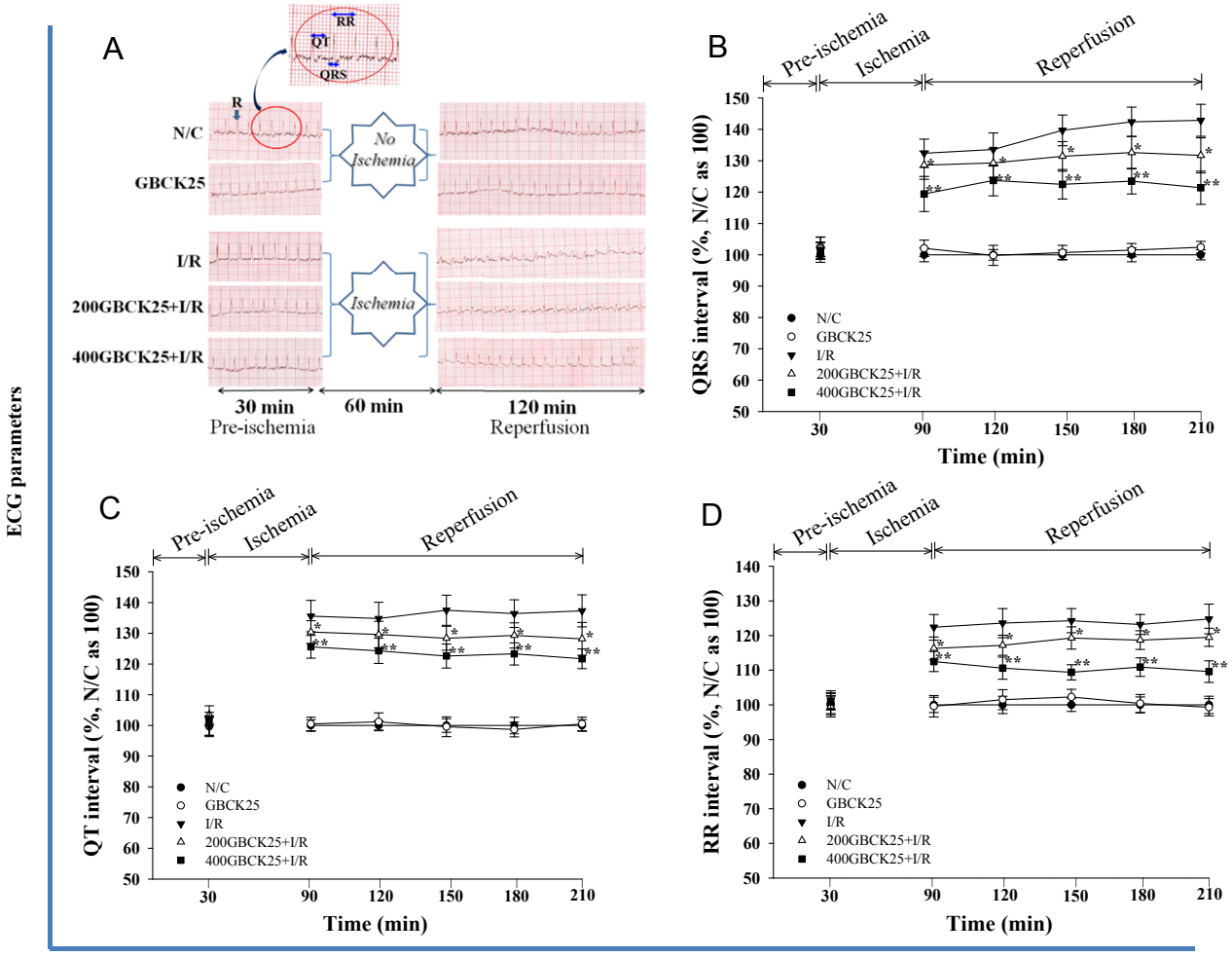

Fig. 5. Effects of 200 mg/kg and 400 mg/kg GBCK25 on representative electrocardiogram tracings. (A) Enlarged ECG patterns such as QRS complex, QT, and RR intervals are shown (shown in circle area on ECG of N/C lane). These pictures were representative ECG patterns in each group. Effects of 200 mg/kg and 400 mg/kg GBCK25 on (B) QRS, (C) QT, and (D) RR intervals are shown. Values are expressed as mean ± SD for eight independent experiments in each group. * $p < 0.05$ compared with I/R. ** $p < 0.01$ compared with I/R. ECG, electrocardiogram; I/R, ischemia–reperfusion; N/C, normal control group.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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