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Short Communication

Korean Red Ginseng modulates immune function by upregulating $CD4^+CD8^+$ T cells and NK cell activities on porcine

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ABSTRACT

In the present study, we investigated whether treatment with KRG improve the parameters of immune activity such as the cytotoxicity, populations of $CD4^+$ $CD8^+$T cell, $CD3^-CD172^-CD8^+$ NK cell and $CD172^+$ monocyte as well as natural cytotoxicity receptors such as Nkp46, Nkp44, Nkp30. In results, KRG significantly increased these immune activities. These results indicate that KRG has distinct immune-enhancing effects by increasing the roles of T cells and NK cell in porcine.

Korean Red Ginseng (KRG) has been widely used as medicine and for tonifying in Korea, China, and Japan. Recently, numerous studies have suggested that KRG show antiinflammatory, antioxidant, and anticancer effects [1,2]. However, pharmacological effects of KRG, its immunostimulatory ability on porcine is poorly understood. The pig (*Sus scrofa domesticus*) is an omnivorous, monogastric species with many advantages to serve as an animal model for human diseases. There are very high similarities to humans in physiological functions of the immune system, e.g., the presence of tonsils, which are absent in rodents. The porcine immune system resembles man for more than 80% of analyzed parameters in contrast to the mouse with only about 10% [3]. In addition, the pig can easily be bred, and there are less emotional problems to use them as experimental animals than dogs or monkeys. Meanwhile, there are many markers available to characterize immune cells. Therefore, the present study aimed to newly investigate the immunostimulatory functions of KRG in terms of signaling molecules, cytokines, and immune cells such as T and NK cells on porcine.

KRG was supplied by Korea Ginseng Corporation (KGC, Daejeon, Korea). The contents of ginsenosides in KRG analyzed by HPLC-evaporative light scattering detector (ELSD) method [4] were mainly composed of ginsenoside Rb1, 8.27 mg/g; Rc, 3.90 mg/g; Rg1, 2.01 mg/g; Re, 2.58 mg/g; Rg2S, 1.35 mg/g; Rh1, 0.95 mg/g; Rd, 1.09 mg/g; and other minor ginsenosides. KRG was dissolved in tap water to dose of 3 and 6 g. Other chemicals were purchased from Sigma (St. Louis, MO, USA). KRG was dissolved in autoclaved distilled water and administrated by per oral. The total twenty-four male Yorkshire piglets weighing 15 ± 1.5 kg were purchased from a farm (Kimje, Jellabukdo, Korea) and used in present study. Animals were housed in colony cages at an ambient temperature of 22 ± 2°C and humidity of 45 ± 10°C with alternating 12 h light/dark cycles. For study, animals were divided into four groups in each group (n = 6, total two times). In normal control, animals were received in tap water daily for 14 days. In shame group, animals were orally administered with 0.2 % (vol/vol) starch orally once daily for 14 days. In the 3 and 6 g KRG groups, animals were treated with 3 and 6 g/day KRG (gastric gavages, respectively) for 14 days. (Fig. 1A). To analysis, blood from individual pigs were collected into tubes containing ethylenediaminetetra-acetic acid (Becton Dickinson, Parsippany, NJ). Then whole blood samples were centrifuged at 600 × g for 15 min at room temperature, after which the supernatant for cytokines analysis was carefully collected and stored at −70°C. The different types of cytokines (IL-2, IL-4, IL-6 and IL-10) were measured using enzyme-linked assay and performed according to the instructions. Also, to isolate peripheral blood

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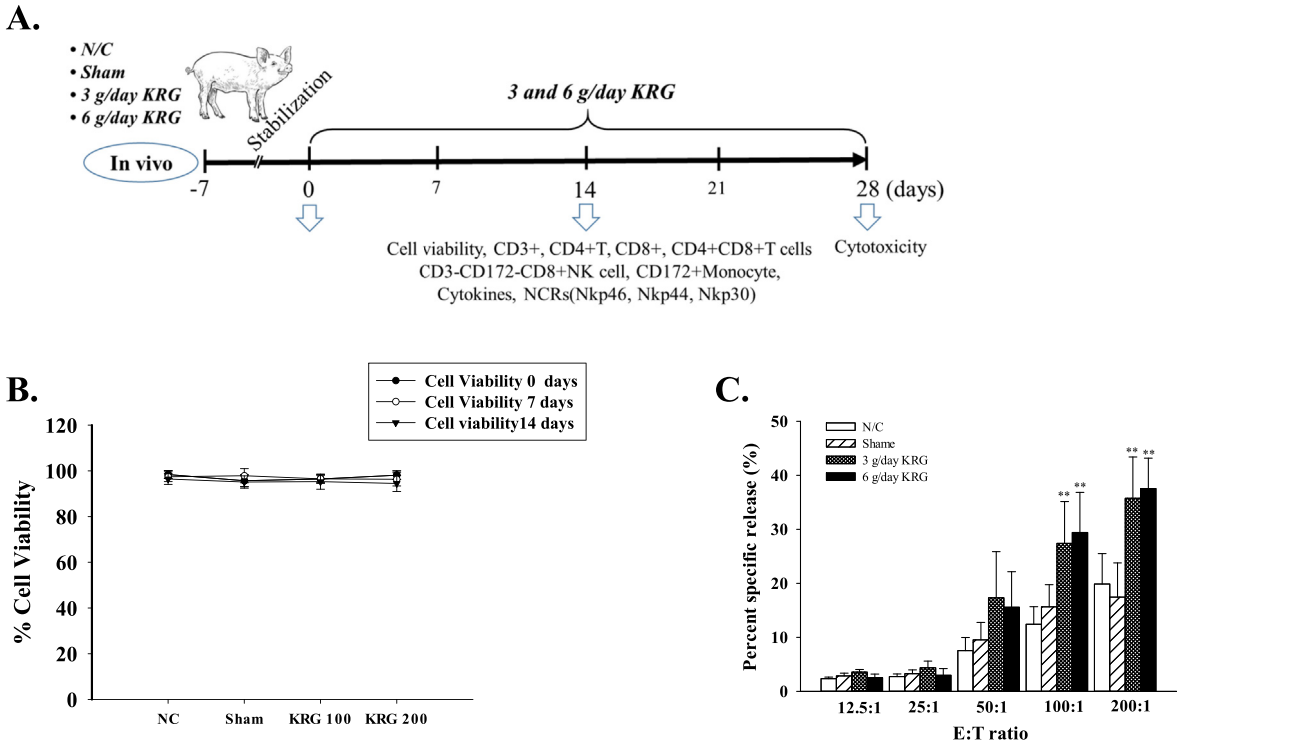

Fig. 1. Schematic diagram for the experiment, male porcine were treated with 3 and 6 g/day KRG and immune parameters were analyzed after 14 and 28 days (A). Cell viability after exposure to KRG at concentrations ranging from 100 and 200 μg/ml of 0, 7 and 14 days was evalulated (B). Cytotoxic activity was analyzed in each time-point after KRG treatment (C).

mononuclear cells (PBMC), whole blood was loaded on Ficoll-Paque (GE healthcare lifesciences, Sweden) following the previous method [5]. After centrifuging whole blood at 600 × g for 20 min, buffy coat was harvested and washed with PBS. PBMC was washed and prepared for measuring the immune cell activities. Also, for analyzing of cytotoxicity, porcine splenocytes were isolated following the previous method [6]. All statistics were calculated using SigmaPlot for Windows version 12.0 (Systat Software, Inc., IL, USA). Data were subjected to one-way analysis of variance (ANOVA) and statistical significance was considered at $P < 0.05$. In results, KRG extract did not elicit any cytotoxicity at any time periods of exposure at the concentrations up to 100 and 200 μg/ml for the time periods of 0, 7, and 14 days. Namely, it significantly didn't affect the viability of splenocytess in a KRG dose-dependent manner (Fig. 1B). Based on these findings, animal experiment was performed using the concentrations of 3 and 6 g/day KRG extract. Because if 6 g KRG is administered to 20 kg porcine, it corresponds to 300 mg/kg, which corresponds to the concentration commonly administered in animal experiments using KRG [7]. The cytotoxic property of effector cells, splenocytes, against target cells, Yac-1 cells, was analyzed. As seen in Fig. 1C, the ratios of effector cells and target cells were 12.5:1 to 200:1. A significant increase in cytotoxic function in response to treatment of KRG was observed 28 days later. In low ratio of effector cell (12.5:1, 25:1 and 50:1), cytotoxic changes were significantly difference ($P > 0.05$). However, high ratio of effector cell (100:1 and 200:1), cytotoxic alteration show significant difference ($P < 0.05$) (Fig. 1C). These results show that KRG treatment could be more cytotoxic activities against target cells than no treated group. And, level of Th1 cytokine (IL2), and Th2 cytokines (IL-4, IL-6 and IL-10) were increased after treatment of 3 and 6 g/day KRG. Namely, Th1 and Th2 cytokine productions in KRG groups were significantly higher in animals than in control group. (Fig. 2) Namely, both Th1 and Th2 cytokines were

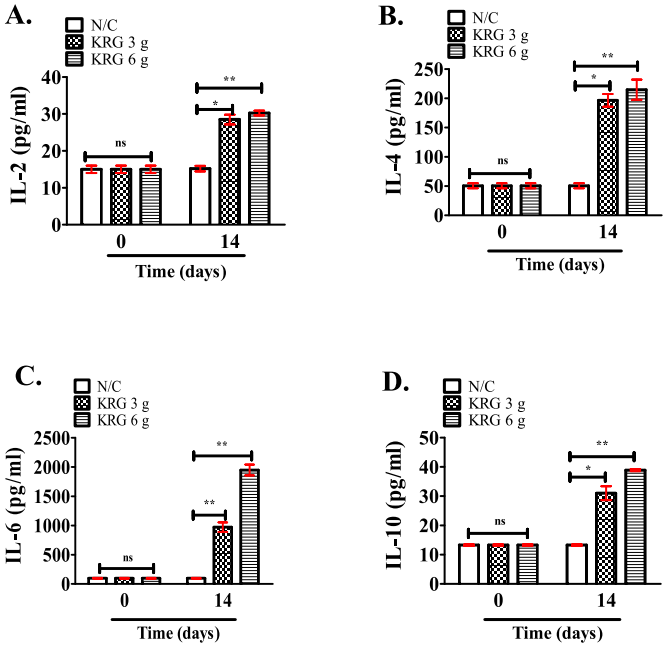

Fig. 2. Effect of KRG on the serum levels of the cytokines by ELISA. The secretion of IL-2, IL-4, IL-6 and IL-10 by lymphocytes, as compared with the control group were significantly increased (*$P < 0.05$, **$P < 0.01$).

significantly increased when treatment of KRG, especially the Th2 cytokines increased by larger margin, which indicating specific Th2 immune responses were stimulated when porcines were administrated with KRG. Therefore, when KRG was administered, immune responses may result from the production of Th1 and Th2

cytokines. And, to study whether KRG had a beneficial effect on the maintenance of T cells, NK cell, and monocyte, PBMC of the pig were assayed by flow cytometry. The results showed that KRG treatment significantly increased CD4+T cell (**P < 0.01; Fig. 3A), CD8+T cell (*P < 0.05, **P < 0.01; Fig. 3B), CD4+CD8+T cell (**P < 0.01; Fig. 3C), CD3-CD172-CD8+NK cell (**P < 0.01; Fig. 3D), and CD172+monocyte (*P < 0.05, **P < 0.01; Fig. 3E) in the compared with those in the normal control group. Namely, compared with the normal group, the KRG-treated groups had a significantly higher ratio of these immune cells. These results indicated that KRG had a beneficial effect on these immune cells function in porcine. Because ginseng may induce NK cell cytotoxicity against tumor cells by a secretion of granzyme B in animal model [8], we examined whether the treatment of KRG increased granzyme B activity. Fig. 4 show that KRG treatment have significantly increased the expression of granzyme B than normal group (*P < 0.05, **P < 0.01, Fig. 4A and B(a)). We have plan to further study for confirmation the KRG treatment will correlated with Nk cells surface marker expression as showed of NK activating receptors whether the increase in cytolytic activity [9]. This was done by cytofluometry using MoAb specific for NKp46, NKp44 and NKp30. As a result, our data showed that NKp30 and NKp46 were significantly more abundant than normal group (*P < 0.05, **P < 0.01) (Fig. 4B(b), (d)). Only, the expression of NKp44 was significantly increased in 6 g KRG group (*P < 0.05 **P < 0.01), whereas there is no difference in 3 g KRG group after 14 days (P > 0.05) (Fig. 4B(c)). Taken together, these results indicate that KRG increases porcine immune activityes by cytokines production. These findings are valuable, but further study is necessary to elucidate the effect in KRG exhibiting these effects.

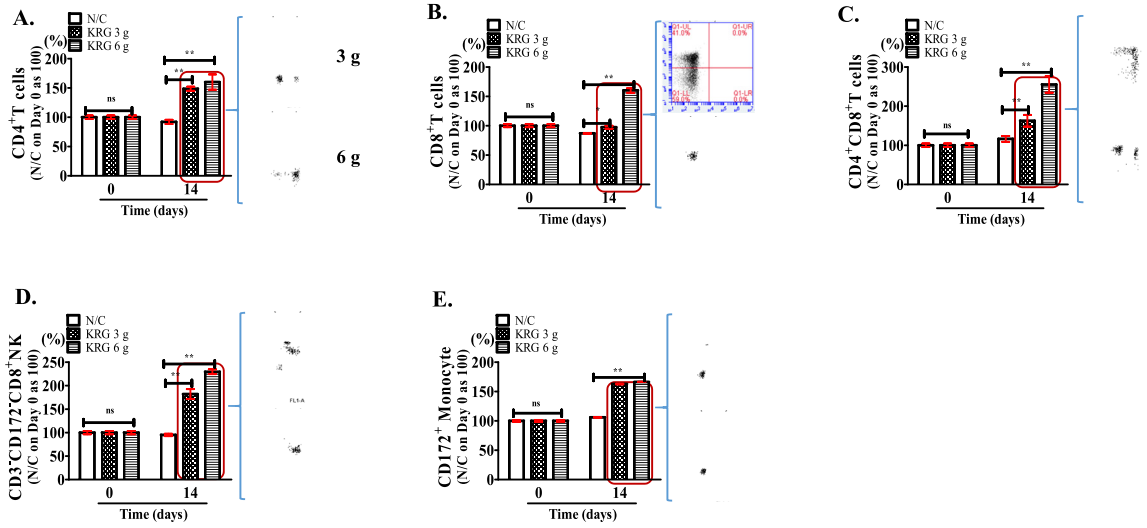

Fig. 3. Effect of KRG on the activities for T cells, NK cell, and monocyte. KRG treatment significantly increased CD4+T cell (A), CD8+T cell (B), CD4+CD8+T cell (C), CD3-CD172-CD8+NK cell (D), and CD172+monocyte (E) (*P < 0.05, **P < 0.01, respectively).

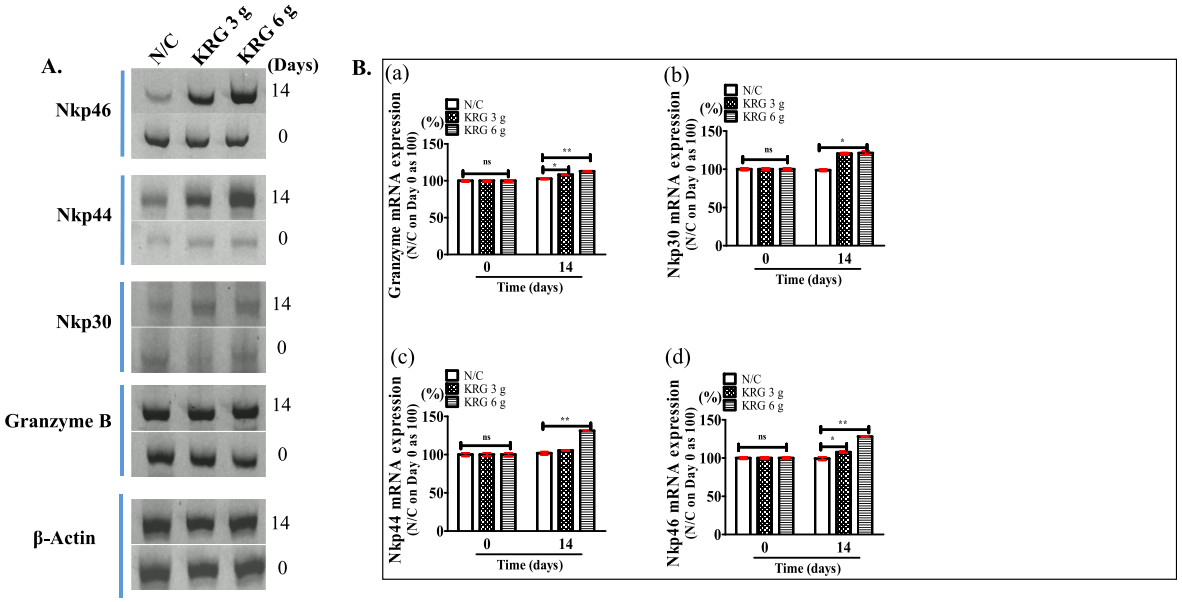

Fig. 4. Effect of KRG on the expression of granzyme B and production of natural cytotoxicity receptors. The levels of granzyme B, NKp30, NKp44 and NKp46 were significantly more abundant than normal group, excepting Nkp44 in 3 g/day KRG group after 14 days (*P < 0.05, **P < 0.01).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Xiaoguang C, Hongyan L, Xiaohong L, Zhaodi F, Yan L, Lihua T, Rui H. Cancer chemopreventive and therapeutic activities of red ginseng. J Ethnopharmacol 1998;60:71–8.

[2] Kim JH, Hahm DH, Yang DC, Kim JH, Lee HJ, Shim I. Effect of crude saponin of Korean Red Ginseng on high-fat diet-induced obesity in the rat. J Pharmacol Sci 2005;97:124–31.

[3] Dawson HA. Comparative assessment of the pig, mouse and human genomes: structural and functional analysis of genes involved in immunity an inflammation. McAnultiy PA,Dawjan Canderup ADWC. In: Hassintgs KL, editor. The minipig in biomedical research. Boca Raton: CRC Press; 2011. p. 664.

[4] Kwon SW, Han SB, Park IH, Kim JM, Park MK, Park JH. Liquid chromatographic determination of less polar ginsenosides in processed ginseng. J Chromatogr A 2001;921:335–9.

[5] Kim H, Jang M, Kim Y, Choi J, Jeon J, Kim J, Hwang YI, Kang JS, Lee WJ. Red ginseng and vitamin C increase immune cell activity and decrease lung inflammation induced by influenza A virus/H1N1 infection. J Pharm Pharmacol 2016 Mar;68(3):406–20.

[6] Wang Y, Yuan L, Cui X, Xu W, Fang S, Li Z, Lu M, Wu Y, Ma X, Chi X, et al. Ginseng stem-leaf saponins in combination with selenium promote the immune response in neonatal mice with maternal antibody. Vaccines (Basel) 2020;8(4):755. Dec 11.

[7] Kim S, Lee Y, Cho J. Korean red ginseng extract exhibits neuroprotective effects through inhibition of apoptotic cell death. Biol Pharm Bull. 2014;37(6):938–46.

[8] Sun Y, Guo M, Feng Y, Zheng H, Lei P, Ma X, Han X, Guan H, Hou D. Effect of ginseng polysaccharides on NK cell cytotoxicity in immunosuppressed mice. Exp Ther Med 2016 Dec;12(6):3773–7.

[9] Tipsmark CK, Jørgensen C, Brande-Lavridsen N, Engelund M, Olesen JH, Madsen SS. Effects of prolactin and cortisol on natural killer (NK) cell surface expression and function of human natural cytotoxicity receptors (NKp46, NKp44 and NKp30). Gen Comp Endocrinol 2009;163(3):270–7. Sep. 15.