Intravenous Single-dose Toxicity of Mountain Ginseng Pharmacopuncture in Sprague-Dawley Rats

Kwangho Lee¹, Seungho Sun², Junsang Yu³, Chungsan Lim¹, Kirok Kwon⁴*

¹Department of Acupuncture & Moxibustion Medicine, Sangji University College of Korean Medicine, Wonju, Korea
²Department of Sasang Constitutional Medicine Sangji University College of Korean Medicine, Wonju, Korea
³Department of Internal Medicine, Sangji University College of Korean Medicine, Wonju, Korea
⁴Research Center of the Korean Pharmacopuncture Institute, Seoul, Korea

Key Words
acupuncture, ginseng, intravenous toxicity, mountain ginseng pharmacopuncture, oriental medicine

Abstract

Objectives: Mountain ginseng pharmacopuncture (MGP) is an extract distilled from either mountain cultivated ginseng or mountain wild ginseng. This is the first intravenous injection of pharmacopuncture in Korea. The word intravenous does not discriminate between arteries, veins, and capillaries in Oriental Medicine, but only the vein is used for MGP. The aim of this study is to evaluate the intravenous injection toxicity of MGP through a single-dose test in Sprague-Dawley (SD) rats.

Methods: Male and female 6-week-old SD rats were injected intravenously with MGP (high dosage of 20 mL/kg or low dosage of 10 mL/kg). Normal saline was injected into the rats in the control group by using the same method. After the rats have treated, we conducted clinical observations, body-weight measurements and histological observations.

Results: In this study, no mortalities were observed in any of the experimental groups compared to the control group. The lethal dose for intravenous injection of MGP was found to be over 20 mL/kg in SD rats.

Conclusion: Considering that the dosage of MGP generally used each time in clinical practice is about 0.3 mL/kg, we concluded with confidence that MGP is safe pharmacopuncture.

1. Introduction

Mountain ginseng pharmacopuncture (MGP) is the first intravenous injection pharmacopuncture using an extract distilled from mountain cultivated ginseng or mountain wild ginseng in Korean traditional medicine [1].

Mountain wild ginseng refers to ginseng (Panax ginseng C. A. Meyer) of the Araliaceae family that is naturally grown in the mountain [2]. Based on the growth environment and the cultivation, commercial ginseng can be classified into three grades; cultivated ginseng, mountain cultivated wild ginseng and mountain wild ginseng. Cultivated ginseng is grown artificially on farms and account for the major quantity of ginseng in the current market. Mountain wild ginseng is slower in growth and more sensitive to environmental changes than cultivated ginseng, showing a preference for are-
as with fluctuating daily temperatures and less exposure to direct sunlight. These differences may result in a variation of the active compounds between cultivated ginseng and mountain wild ginseng. Mountain cultivated wild ginseng can be considered as mimicry mountain wild ginseng; it is seeded and grown in forests and mountains. It is widely accepted in both Korea and China that mountain wild ginseng produces more potent medicinal activity than mountain cultivated wild ginseng or cultivated ginseng. However, only a few studies have been conducted to compare the food components and the pharmacological activities between mountain wild ginseng and both cultivated and mountain cultivated ginseng [3, 4].

Because authenticity tests of mountain wild ginseng are very difficult and confusing, scientific criteria of authenticity are needed. Thus, an objective diagnostic method that used gene analysis to identify mountain ginseng (MG) was developed. Comparing gene activation between numerous MG samples and regular ginseng, genes only existing in MG, such as p-NRT2, p-rpoC1, p-psbB, and p-GAPDH, were found [3-6]. Because of the high-cost and authenticity issues associated with mountain wild ginseng, mountain cultivated ginseng which is similar to mountain wild ginseng according to the gene analysis, is used for pharmacopuncture [1].

Although continuous clinical studies have been performed to obtain more objective data on the efficacies of MG, safety tests are still necessary. The aims of this study were to evaluate the safety of MG, and to conduct single-dose toxicity tests of MG.

2. Materials and Methods

18 Sprague-Dawley (SD) rats of each gender were obtained from a specific pathogen-free facility (Biotoxtech, Oh Chang, Korea) at 5 weeks of age and were used after a week of quarantine and acclimatization. The animals were housed in a room maintained at 19.0 – 23.2℃ under a relative humidity of 33.0% – 59.5%. The room was illuminated with artificial lighting for 07:00 to 19:00 hours and had 10 – 15 air changes per hour. Three animals were housed in suspended stainless-steel wire-mesh cages and were allowed sterilized tap water and commercial rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Harlan Laboratories, Inc., U.S.A.). This study protocol was approved by the institutional Animal Care of Biotoxtech Co. (Oh Chang, Korea).

The process for the manufacturing MGP was performed in a pathogen-free facility (Korean Pharmacopuncture Institute, Seoul, Korea), as described previously [7]. MG of over 10 years which was cultivated from the seeds of mountain wild ginseng, was selected for the MGP. First, we washed MG with running water and dried it in the air-conditioned room. Second, dried MG was powdered in to fine particles (size are below 10 μm) and decocted for 2 hours in distilled water. Remnants were then removed, and the decoction was distilled before yielding the desired herbal acupuncture. Then the pharmacopuncture was filtered using 0.1 μm filter paper and was then kept in the container. Finally, the pharmacopuncture was sterilized before being used.

Healthy male and female rats, 5 of each sex were selected and assigned to 1 of 3 groups: control group (normal saline), low-dose group (10 mg/kg), and high-dose group (20 mg/kg). MGP was administered to the rats by intravenous injection into the caudal vein. The control group was administered an equivalent volume of normal saline (Lot nos. DAJ9074, DAJ9076).

All animals were observed for clinical signs at 30 minutes, 1 hour and 2 hours after the first injection and at 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after the second injection. Clinical signs were observed daily from the injection day to 14 day after the first injection. The body weight of each rat was measured at the initiation of treatment, and at 3 days, 7 days and 14 days after the injection.

The scheduled day of study termination, all surviving animals were anesthetized by CO₂ inhalation. Tissues were obtained from the following organs of all surviving animals and fixed with 10% neutral buffered formalin solution: brain, heart, liver, spleen, kidney, lung, spinal nerves. These tissues were routinely processed, embedded in paraffin and sectioned. The sections were stained with hematoxylin–eosin (H&E) dye for microscopic examination. All organs and tissues taken from all animals were examined microscopically.

Data on body weights were tested by using SAS (version 9.2, SAS Institute Inc, USA). The variance was checked by using the Bartlett test [8]. If the variance was homogeneous, the data were subjected to one-way analysis of variance (ANOVA). If significant changes were observed on the one-way ANOVA, the data were analyzed by using the multiple comparison procedure of Dunnett’s test [9] (P < 0.05, P < 0.01).

3. Results

No treatment-related mortalities occurred in SD rats treated with any dose of the MGP. Also, no clinical signs were observed in the male and the female SD rats of the experimental groups during the observation period. Hematuria was observed in 1 of the rats of control groups, but it was not caused by MPG. No significant changes in
weight were observed in the experimental groups compared to the control group (Fig. 1, 2).

No abnormal macroscopic appearances were observed on necropsy in either the control or the experimental groups. All experimental groups showed no abnormal signs caused by intravenous injection of MGP. In the control and the high-dosage groups, some changes were observed in the kidney, lung, liver and spleen, but those changes seemed to have occurred naturally or accidentally (Fig. 3-6).

4. Discussion

Blood vessels are consist of veins and arteries [10, 11]. MGP is administered intravenously, and intravenous injection is enlisted as a legal manipulation in Korean classification procedures in medicine and resource. For this...
reason, intravenous injection of MGP has been studied [1].
In a randomized controlled trial of MGP, no significant changes in blood pressure, hematology, biochemistry and urine analysis were reported [12]. However, immune protein, which acts against microbes and pathogenic organisms, vitamin D binding protein, which protects the lung at the time of inflammatory response, ras-related protein Ral-A, which controls phospholipid metabolism, cytoskeletal formation, and membrane traffic, and transferrin which balances the iron level in the body were reported to be increased significantly, after the administration of MGP, but antitrypsin, which is secreted with inflammatory responses in the lungs, were reduced [13, 14]. Also no effects on heart rate variability [15] and pulse-wave factors were revealed in middle-aged women [16].
There are many types of flavonoid compounds, such as quercetin, hesperidin and anthocyanidins in MGP, and these constituents were reported to have actions that improved liver functions [17, 18], and that produced anti-oxidant [19-23], anti-cancer [7, 24-30], anti-hypertensive [31], anti-diabetic [32], anti-obesity [33] and anti-depressant effects [34]. Thus, MGP has been used to treat progressive

**Figure 3** Kidney tissue from an intravenous single-dose toxicity study of Mountain ginseng pharmacopuncture in Sprague-Dawley Rats. The high-dose group (20 mg/kg) shows mild changes in the focal cortex basophilic tubule and focal cystic outer medulla tubular dilatation. But such changes are commonly observed in SD rats. Pathological change was detected by H&E staining. Lt, Control; Rt, Experimental group. × 200.

**Figure 4** Liver tissue from an intravenous single-dose toxicity study of Mountain ginseng pharmacopuncture in Sprague-Dawley rats. The high-dose group (20 mg/kg) and the control group show minimal changes of focal perivascular cell infiltration, extramedullary hematopoisis and microgranuloma. Pathological change was detected by H&E staining. Lt, Control; Rt, Experimental group. × 200.
disorders such as cancer [35-40] and amyotropic lateral sclerosis [41].

The present study was conducted to evaluate the safety of MGP, and the results of this study showed no mortalities and no significant changes in SD rats caused by intravenous injection of MGP. These results correspond with those of an earlier study which reported that MGP caused negligible toxicity [24]. Although the safety of MGP has been demonstrated through a single-dose toxicity test, still further study, like a 4-week repeated toxicity test, is needed. Based on the results of this study, we can conclude with certainly that the lethal dose of MGP is higher than 20 mL/kg in SD rats and that intravenous injection of MGP in SD rats is a safe treatment considering that the usual clinical dose of MGP is about 0.3 mL/kg.

5. Conclusion

The field of traditional korean medicine (TKM) has a long waited technology that uses the vessel as a treatment medium, as the vessel provides the quickest path of drug ab-

Figure 5  Brain tissue from an intravenous single-dose toxicity study of Mountain ginseng pharmacopuncture in Sprague-Dawley rats. There are unremarkable changes in the brain, and the heart of the control and the 20 mL/kg groups. Pathological change was detected by H&E staining. Lt, Control; Rt, Experimental group. × 200.

Figure 6  Spinal nerves tissue from an intravenous single-dose toxicity study of Mountain ginseng pharmacopuncture in Sprague-Dawley rats. There are unremarkable changes in the spinal nerves of the control and the 20 mL/kg groups. Pathological change was detected by H&E staining. Lt, Control; Rt, Experimental group. × 200.
sorption. A limitation on treating patients is the difficulty of oral administration of medicines, but pharmacopuncture, especially MGP has opened the door for immediate treatment benefits. As the result of this study, we can conclude with certainly that intravenous injection of MGP is safe.

Acknowledgements

The author has no financial interest related to the material of this manuscript.

Conflict of interest

The authors declare that there are no conflict of interest.

References

1. Korean Pharmacopuncture Institute. Pharmacopunctureology. Seoul: Elsevier Korea; 2012. p. 191-7.
2. Kwon KR, Wi JS, Kim SW. [Literature study of wild ginseng]. J Pharmacopunct. 2003;6(2):67-75. Korean.
3. Lee KH, Kwon KR, Kang WM, Jeon EY, Jang JH. Identification and analysis of the chloroplast rpoC1 gene differentially expressed in wild ginseng. J Pharmacopunct. 2012;15(2):20-3.
4. Han YJ, Kwon KR, Kang WM, Jeon EY, Jang JH. Identification and analysis of the novel pGAPDH-w gene differentially expressed in wild ginseng. J Pharmacopunct. 2013;16(1):30-6.
5. Kwon KR, Park WP, Kang WM, Jeon EY, Jang JH. Identification and analysis of differentially expressed genes in mountain cultivated ginseng and mountain wild ginseng. J Acupunct Meridian Stud. 2011;4(2):123-8.
6. Han YJ, Kwon KR, Kang WM, Jeon EY, Jang JH. Identification and expression analysis of chloroplast p-psbB gene differentially expressed in wild ginseng. J Pharmacopunct. 2012;15(1):18-22.
7. Kwon KR. Anticancer effect of mountain ginseng pharmacopuncture to the nude mouse of lung carcinoma induced by NCI-H460 human non-small cell lung cancer cells. J Pharmacopunct. 2010;13(1):5-14.
8. M.S Bartlett. Properties of sufficiency and statistical test. Pros. R. Soc. Lond. A. 1937;160:268-82.
9. C.W. Dunnett. New tables for multiple comparisons with control. Biometrics. 1964;20(3):482-91.
10. Lee JY, Cho BJ, Kwon KR, Kang DI. [Literary study of hyeolmaek(血脈)]. J Pharmacopunct. 2005;8(3):11-9. Korean.
11. Kang MS. [Review on hyolmaek(血脈) through hwangjenaegyeong youngchu]. J Korean Acupuncture & Moxibustion Society. 2010;27(6):111-4. Korean.
12. Kwon KR. [A clinical study on the effects of intravenous wild ginseng herbal acupuncture on the human body]. J Pharmacopunct. 2004;7(1):15-26. Korean.
13. Kwon KR, Kang TS, Lee SK. [Analysis of serum protein before and after intravenous injection of wild ginseng pharmacopuncture]. J Pharmacopunct. 2004;7(3):5-25. Korean.
14. Lee DH, Kwon KR. [Analysis of serum protein before and after intravenous injection of cultivated wild ginseng herbal acupuncture]. J Pharmacopunct. 2006;9(2):17-38. Korean.
15. Roh JD, Kim LH, Song BY, Yook TH. [The effects of distilled wild ginseng herbal acupuncture on the heart rate variability (HRV)]. J Pharmacopunct. 2008;11(1):55-69. Korean.
16. Park SW, Kim YS, Hwang WD, Kim GC. [Effect of pulse wave factors in middle aged women by mountain cultivated ginseng pharmacopuncture]. J Pharmacopunct. 2011;14(1):35-49. Korean.
17. Kim YJ, Park DI, Kwon KR. [Case report on the improvement of liver functions by mountain cultivated wild ginseng pharmacopuncture]. J Pharmacopunct. 2009;12(2):107-12. Korean.
18. Kim YJ, Park HS, Kwon KR, Kim HY. [Protective effects of cultivated ginseng, cultivated wild ginseng of korean and chinese against CCl4 and t-BHP induced acute hepatotoxicity ICR mice]. J Pharmacopunct. 2007;10(1):101-7. Korean.
19. Choi EJ, Lee JM, Won SH, Kwon KR. [Effects of cultivated wild ginseng pharmacopuncture on lowering lipid and oxidative capacity in biochemical and molecular biological study in obese rats]. J Pharmacopunct. 2006;9(1):5-20. Korean.
20. Ahn YM, Park HS, Kwon KR. [Anti-cancer and anti-oxidant efficacies of wild ginseng and cultivated wild ginseng of korea and china]. J Pharmacopunct. 2007;10(1):5-16. Korean.
21. Kim HS, Park HS, Kwon KR. [Protective effect of wild ginseng extract against t-BHP-induced oxidative damage in HepG2 cells using DNA chip]. J Pharmacopunct. 2007;10(1):121-35. Korean.
22. Jang HY, Park HS, Kwon KR, Rhim TJ. [A study on the comparison of antioxidant effects among wild ginseng, cultivated wild ginseng, and cultivated ginseng extracts]. J Pharmacopunct. 2008;11(3):67-78. Korean.
23. Rhim TJ, Jeong HS, Kim YJ, Kim DY, Han YJ, Kwon HY, et al. [A study on the comparison of antioxidant effects among cultivated ginseng, and cultivated wild ginseng extracts]. J Pharmacopunct. 2009;12(2):7-12. Korean.
24. Kwon KR, Cho AL, Lee SG. [The study on acute and subacute toxicity and anti-cancer effects of cultivated wild ginseng herbal acupuncture]. J Pharmacopunct. 2003;6(2):7-27. Korean.

25. Cho HC, Lee SG, Kwon KR. [An experimental study on apoptosis of cultivated wild ginseng distilled herbal acupuncture by concentration level]. J Pharmacopunct. 2004;7(2):5-17. Korean.

26. Kwon KR, Yoon HC, Kim HH. [Anti-cancer effects of cultivated wild ginseng herbal acupuncture in C57BL/6 mice injected with B16/F10 cells reproductive toxicity by doxorubicin]. J Korean Acupuncture & Moxibustion Society. 2006;23(1):105-20. Korean.

27. Lim SY, Lee SJ, Kwon KR. [Anti-cancer and promoting effects of cultivated wild ginseng herbal acupuncture on hepatic metastatic model using colon26-L5 carcinoma cells]. J Korean Acupuncture & Moxibustion Society. 2006;23(1):121-34. Korean.

28. Cho BJ, Kwon KR. [Effects of cultivated wild ginseng herbal acupuncture to the serum cytokine on hepatic metastatic model using colon26-L5 carcinoma cells]. J Pharmacopunct. 2006;9(1):127-37. Korean.

29. Min BI, Kim HH, Seo IB, Kwon KR. [Antitumor effects and protective effects against doxorubicin-induced testicular toxicity of cultivated wild ginseng extract in the B16/F10 melanoma-bearing C57BL/6 mice]. J Pharmacopunct. 2007;10(1):85-100. Korean.

30. Hwang JW, Oh JH, Yoo HS, Lee YW, Cho CK, Kwon KR, et al. Mountain ginseng extract exhibits anti-lung cancer activity by inhibiting the nuclear translocation of NF-κB. Am J Chin Med. 2012;40(1):187-202.

31. Hong MH, Lim HK, Park JE, Jun NJ, Lee YJ, Cho MJ, et al. [The antihypertensive and vasodilating effects of adventitious root extracts of wild ginseng]. J. Korean Soc. Appl. Biol. Chem. 2008;51(2):102-7. Korean.

32. Park WP, Kwon KR, Lee E. [Effects of distilled cultivated wild ginseng herbal acupuncture in rats with diabetes induced high fat diet]. J Pharmacopunct. 2005;8(2):97-108. Korean.

33. Kim BW, Kwon KR. [The effect of cultivated wild ginseng extract on preadipocyte proliferation]. J Pharmacopunct. 2007;10(3):29-36. Korean.

34. Kwon SO, Choi SM, Kim MH, Lee BB, Park MW, Lee HJ, et al. [Antidepressant effect of the subchronic administration of the methanolic extract of wild-ginseng and cultivated-ginseng in mice tail suspension test]. J Korean Acupuncture & Moxibustion Society. 2009;26(4):99-106. Korean.

35. Kwon KR, Park CW, Ra MS, Cho CK. Clinical observation of multiple metastatic cancer patient with hepatocellular carcinoma treated with cultivated wild ginseng herbal acupuncture. J Korean Acupuncture & Moxibustion Society. 2005;22(2):211-7.

36. BK Park, CK Cho, KR Kwon, HS Yoo. [A case report for stage ⅢB squamous cell lung carcinoma patient treated with mountain ginseng pharmacopuncture]. J Pharmacopunct. 2007;10(3):143-7. Korean.

37. Bang SH, Kwon KR, Yoo HS. [Two cases of non small cell lung cancer treated with intravenous cultivated wild ginseng pharmacopuncture]. J Pharmacopunct. 2008;11(2):13-9. Korean.

38. Kwon KR, Kim HD, Kim JS, Yoo HS, Cho CK. Case series of non-small cell lung cancer treated with mountain ginseng pharmacopuncture. J Acupunct Meridian Stud. 2011;4(1):61-8.

39. Lee JH, Kwon KR, Cho CK, Han SS, Yoo HS. Advanced cancer patients treated with cultivated wild ginseng pharmacopuncture. J Acupunct Meridian Stud. 2010;3(2):119-24.

40. Lee YH, Kim CW, Lee KH. [A case report of monitoring PSA level changes in two prostate cancer patients treated with mountain ginseng pharmacopuncture and sweet bee venom along with western anticancer therapy]. J Pharmacopunct. 2011;14(4):81-8. Korean.

41. Ryu YJ, Lee KH, Kwon KR, Lee YH, Sun SH, Lee SJ. [Mountain ginseng pharmacopuncture treatment on three amyotrophic lateral sclerosis patients]. J Pharmacopunct. 2010;13(4):119-28. Korean.