

# The wound healing and anti-inflammatory effects of *Panax ginseng* C.A Meyer

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## Abstract

Ginseng has been used as miraculous panacea since ancient times in oriental countries. In spite of voluminous work, ginseng still remains mysterious herb, but its value is becoming more recognized in the pharmaceutical and cosmetic fields.

In this study, we investigated the effect of *Panax ginseng* on wound healing using two experimental methods. First, we studied the effect of ginseng on artificial wound of cultured human keratinocyte monolayer. Individual components from ginseng (ginsenoside Rb2, Rc, Re, Rg1, and panasenoside) and ginseng extrats were examined. Of them, compared with control, ginsenoside Rb2 and Rg1 needed much shorter time to recover original appearance of momolayer. Second, we investigated the effect of ginseng on acute injury on dorsal skin of hairless mice. We here observed that ginseng has prominent effect than Madecasol(asiaticoside), a well known wound healing agent. These results were deduced that ginseng promoted wound healing in the wound region due to its stimulation of biosynthesis of various endogeneous materials that have relation to wound healing. Furthermore, we conformed that ginsenoside Rg1 exhibited anti-inflammatory activity on rat paw edema induced by carageenan.

These results suggest that *Panax ginseng* C.A Meyer can be used in the cosmetics in that its wound healing and anti-inflammatory effects.

## Introduction

Recently, people have become more aware of and interested in ginseng, the mysterious herb of oriental medicine. Ginseng, especially korean ginseng, has a long history and tradition. It has been used since ancient times in the orient as a miraculous panacea for all kinds of illness and desease.

For half a century, many research workers have tried to identify a scientific basis for the wide use of ginseng. Many components of ginseng was isolated by advance of chromatographic technique and development of analytical instruments. Tanaka et al. isolated saponins from ginseng and determined its chemical structure(1), and Shibata named it ginsenoside(2). Ginseng saponins, the major components of ginseng, are dammarane type of triterpene glycosides different from saponins of other plant origin. Therefore, the study of ginseng has been focused on saponins.

Breckhman and Dardymov insisted that ginseng is a kind of adoptogen which increases nonspecific resistance of an organism to a variety of untoward influences(3). A review of all the actions of ginseng indicates that it has the ability to prolong the normal functions of the body and its organs beyond the normal span of life and to prevent the senility and debility that accompany old age.

Ginseng is used not only in medicines, but also in cosmetics all over the world now. Although there is no actual proof of its value, ginseng has been used as a remedy for the promotion of cellular function, or as a prophylaxis against biological aging of skin. Our present study concerned the effect of ginseng on wound healing to provide scientific basis of cosmetic application of ginseng.

Wound healing proceeds with inflammation and granulation tissue formation, followed by extracellular matrix deposition and remodeling. The process involves the interactions of keratinocytes, fibroblasts, and inflammatory cells that have migrated to the wound site(4). Recent studies suggested that several growth factors released from these cells are involved in the healing process and that application of exogeneous growth factors promotes wound healing in vivo(5). Several natural products(*centella asiatica*, *aloe vera* etc.)have been known to promote wound healing. In the present study, the effect of ginseng on wound healing was tested by observing cell migration rate in artificial wound region of human epidermal keratinocyte monolayer, using indivisual saponins purified from ginseng. For *in vivo* study, we used hairless mouse. We here investigated the effect of ginseng on acute injury on dorsal skin of hairless mice. Our another purpose in this study is to conform whether ginseng has anti-inflammatory activity. For this we used rat paw edema model induced by carrageenan.

## **Materials and Methods**

### ***Preparation of ginsenoside Rb2***

Roots of *Panax ginseng* purchased from Kyong-dong market, in Seoul, Korea was pulverized and extracted with 70% alcohol at boiling temperature. The extract was evaporated and dissolved in water, followed by solvent extraction with water-saturated buthanol. The buthanol fraction was loaded in silicagel column and eluted with chloroform:methanol:water=65:30:4 to obtain ginsenoside Rb2 fraction. The Rb2 fraction was reloaded in silicagel column and eluted with water-saturated buthanol and recrystallized in EtOH-BuOH solution to obtain ginsenoside Rb2.

### ***Preparation of ginsenoside Rg1***

The buthanol fraction obtained above was loaded in C-18 column and eluted with 25% MeOH to obtain ginsenoside Rg1 fraction. The Rg1 fraction was recrystallized in EtOH-MeOH solution to obtain pure ginsenoide Rg1.

### ***In Vitro experiment of the effect of Panax ginseng on wound healing.***

The human epidermal keratinocytes were separated from neonatal foreskin(6). The keratinocytes were added to the keratinocyte growth media purchased from Life Technologies at a concentration of  $5.7 \times 10^4$ /ml and the mixture was pipetted into 6 well plate for culture(3ml/well). And the keratinocytes was cultured in the incubator until ready for experiment. When the plate reached 90% confluency, vertical cleavage was made on keratinocyte monolayer by plastic implement having 1mm of width and 0.5cm of length. The media was then immediately exchanged into new media containing 0.1mg/ml of test materials.(ginsenoside Rb2, ginsenoside Rg1). After 5,25,25,35hrs respectively, the condition of cells of wound region was observed under the microscope compared with control.

### ***In vivo experiment of the effect of Panax ginseng on wound healing***

About 2cm length of artificial wound was made on the dorsal skin of hairless mice. The wound was sutured, followed by topical application of 10ul of test materials(10mg/ml) on the wound area everyday for 6 days. During experimental period, the wound area was observed by naked eyes, and after 6 days evaluated by double blind test. For comparison of the effect of *Panax ginseng* we used Madecasol(asiaticoside)which is a well-known wound healing agent.

### ***Measurement of anti-inflammatory activity of Panax ginseng***

The initial hind paw volume of male sprague Dawley rats was determined volumetrically using Ugo-Basile Plethysmometer. A 1% solution of carrageenan in saline(0.1ml/rat) was injected subcutaneously into the right hind paw 1h after the test substances had been administered intraperitoneally. The control group received the vehicle. Paw volumes were measured 5hrs after carrageenan had been injected, and the volume of the edema was determined(7). The results were expressed as percentage hind paw swelling, as compared with the initial hind paw volume. Indomethacin was used as a standard drug.

## **Results**

### ***The effect of Panax ginseng on the recovery of artificial vertical cleavage of cultured human epidermal keratinocyte monolayer.***

We measured relative rates that the vertical cleavage of cultured keratinocyte monolayer was recovered to the initial state as the passage of time, after test material had been treated. Ginsenoside Rb2 and Rg1 showed significant effect to recover the cleavage. After 25hrs ginseng treated cells showed perfect migration to the wound region, and the trace of cleavage was not observed. In contrast, not treated cells rarely migrated and cleavage was still remained after 35hrs.

**The effect of *Panax ginseng* on the acute injury on the dorsal skin of hairless mice.**

As shown in table 1, both ginsenoside Rb2 and Rg1 showed prominent effect on the wound healing than Madecasol(asiaticoside), a standard drug.

Samples	Percentage of healing (n=6)
Ginsenoside Rg1	75
Ginsenoside Rb2	80
Madecasol	50
Saline	36

Table 1. The effect of *Panax ginseng* on the acute injury on the dorsal skin of hairless mice. Samples were treated 10ul(10mg/ml) every day for 6 days.

**The anti-inflammatory activity of *Panax ginseng***

Ginsenoside Rg1(50mg/kg) showed a significant inhibitory activity on carrageenan induced rat paw edema, as shown in fig.1.

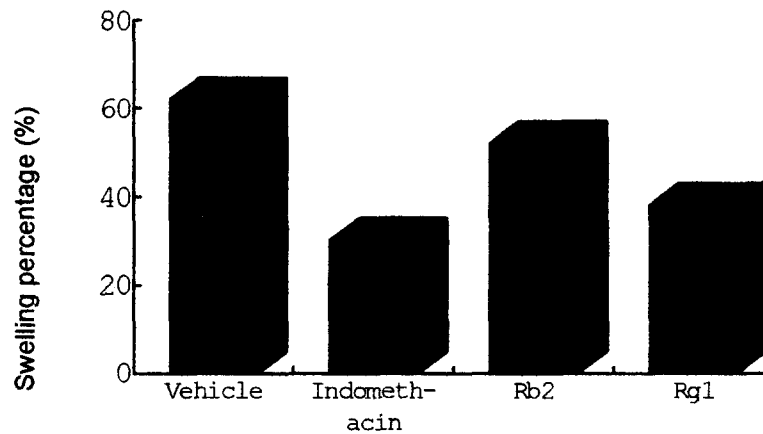


Fig.1 The effect of saponins and indomethacin on the rat paw edema induced by carrageenan. Rb2 and Rg1: 50mg/kg I.P administration, indomethacin: 30mg/kg I.P administration(n=6)

## **Discussion**

Above results clearly demonstrated that Panax ginseng stimulates wound healing. Cutaneous wound repair provides a complex, highly regulated series of biological events in which we can study the role of proteoglycans, glycosaminoglycans, collagenase, fibronectin, and several growth factors in cellular migration, proliferation, differentiation, and matrix synthesis and assembly(8). Collagenase has been known to break the collagen linkage between cells, to facilitate migration of cells. And fibronectin helps cell adhesion. Through the experiment using cultured keratinocyte monolayer we clarified that ginseng promoted cell migration and adhesion in the wound region. Therefore, it was deduced that ginseng promoted biosynthesis of, or activated endogenous substances (fibronectin, collagenase etc.) which stimulate the cellular migration.

Hiroshi Tanaka et al. reported that Panax ginseng increased the content of GAGs (glycosaminoglycans) in the cultured human skin fibroblast(9). GAGs have been known to play a vital role in wound healing process. This is considered as another reason why ginseng shows the effect of wound healing.

We also confirmed that ginsenoside Rg1 has the anti-inflammatory activity (Fig.1). The standard drug, indomethacin (30mg/kg) showed more potent inhibition than that of Rg1. Although the mechanism of anti-inflammation of Rg1 is unclear, it is interesting in light of the fact that roots of ginseng have been employed for the management of peripheral circulatory disorders. A further study to identify the mechanism of anti-inflammatory activity of ginsenoside Rg1 is proceeding now.

Our findings are meaningful in the aspect that we found out pure compound from ginseng that has wound healing and anti-inflammatory effects. Finally, we suggest that our findings have potentially important implications for the cosmetic use of Panax ginseng in skin protective products.

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