Introduction

Burn injuries, among all injuries, may be the worst disability because of the formation of hypertrophic scar and contracture after the healing of deep burns. Scar contracture and uncontrollable proliferation of scar tissue are subsequent to destruction and loss of dermis and contribute to the formation of scars and keloids.

For burn therapy, the most conventional treatment uses skin grafts. These grafts can compensate the tissue loss at multiple levels acting as occlusive dressings and provide both skin replacements and stimuli for healing. Problems associated with grafts

**The Effects of Gamisipjeon-tang on the Skin Regeneration of Deep Second Degree Burns in Mice**

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Objective: This study aimed to ascertain the curative effects of Gamisipjeon-tang (GST) used for wound healing on the skin regeneration of deep second degree burns in mice.

Material & Methods: In vitro, the IκB kinase (IKK) mRNA expression, inducible nitric oxide synthase (iNOS) mRNA expression, and cyclooxygenase-2 (COX-2) mRNA expression in the GST concentration from 1 ng/ml to 10 ng/ml were measured. In vivo, the mice were divided into four groups: the normal group, the BE group (burn-elicited group, control group), the DC group (Duoderm CGF-treated group after burn elicitation), and the GST group (Gamisipjeon-tang treated group after burn elicitation). To determine the anti-inflammatory effects, nuclear factor (NF)-κB p65, iNOS, COX-2 positive reaction were measured by immunohistochemistry. To estimate the skin regenerative effects, change of burn area, 5-bromo-2′-deoxyuridine (BrdU), and fibroblast growth factor (FGF) positive reaction were analyzed.

Results: In vitro, the iNOS, IKK, COX-2 mRNA expression decreased according to the increase of GST concentration. The significant decrease of COX-2, iNOS, NF-κB positive reaction were the highest in the GST group, followed by the DC group and the BE group (p<0.05). The diameter of burn area was significantly decreased in the GST group as compared to that in the DC and BE group (p<0.05). The BrdU and FGF positive reaction increased more significantly in the GST group than in the DC group, and more significantly in the DC group than in the BE group on the 3rd and 7th day after burn (p<0.05). FGF positive reaction increased in the BE and DC group, whereas it decreased significantly in the GST group on the 14th day (p<0.05). The BrdU positive reaction increased in the BE group, whereas it decreased significantly in the DC and GST group on the 14th day (p<0.05).

Conclusions: This study shows that GST could decrease the inflammatory response and accelerate the skin regeneration as compared to the duoderm CGF in mice with deep second degree burns.

Key Words: deep second degree burns, Gamisipjeon-tang (Jiaweishiquan-tang), duoderm CGF, iNOS, NF-κB, FGF, BrdU,
as scars and keloids, have prompted the research toward an alternative that would be more widely available with properties close to those of natural skin. Research in this field resulted in the use of bio-synthetic materials engineered as living skin replacements\(^4\). Recently, dermal substitution and burn healing have become one of the most exciting research areas in biomaterial sciences. Duoderm is a kind of bio-synthetic hydrocolloid dressing. It makes the wound moist, so that the wound heals through autolysis about the sphacelus\(^5\). Although there have been many recent advances in this field, commercially available products and the biological materials currently described in experimental studies are not fully satisfying for the treatment of severely burned patients\(^4,6\).

In the other parts of burn therapy, there have been various investigations about the curative effects of emitted-qi therapy, cold water therapy\(^7,8\), low level laser therapy\(^9-14\), and external treatments with herbal medicine and arachidonate-rich oil extracted from mortierella alpina ulmus dressing, mixture of ear shell ash and sesame oil, lithospermi radix and gardeniae fructus extracts\(^15-19\). Most of these reports explained only the mild effects and didn’t explain the curative effects through the comparative study with conventional therapy.

In oriental medicine, there are a few studies about burns. These studies\(^20-24\) include an experimental study about external preparation and case reports of second degree and the third degree burn patients treated with oral herbal medicine, acupuncture, and the external treatment with herbal medicine. However, most of these studies didn’t investigate the curative effects for each treatment such as oral herbal medicine, acupuncture, and external treatment with herbal medicine. Most of these studies didn’t undertake a comparative study with conventional therapy. Traditional oriental herbal medicine, Gamisipjeon-tang also called Jiaweishiquan-tang, was used for wound healing through assisting energy, draining pus, and helping regeneration of skin\(^25\). In the clinical studies\(^20,26\), this medicine was used for wound healing in burns and atopic dermatitis.

The aim of this study is firstly to investigate the anti-inflammatory effects and skin regenerative effects of Gamisipjeon-tang (GST), and secondly to compare the effects of internal herbal medicine with those of duoderm CGF as a synthetic wound dressing.

### Experiment

#### 1. Materials

1) Animals

Six weeks old male BALB/c mice were purchased from the Orient (Seongnam, Korea). Animals were adjusted to the aseptic environment for 2 weeks before the experiment, and mice weighing 20 g were used for experiments. The mice were classified into the normal group (n=10), the burn elicited group (control group, BE, n=30), the duoderm CGF-treated group after burn elicitation (DC, n=30), and the Gamisipjeon-tang treated group after burn elicitation (GST, n=30). The BE, DC, and GST groups were divided into 3rd (n=10), 7th (n=10), and 14th (n=10) day groups after burn elicitation. The animals in the BE, DC, and GST groups were anesthetized with sodium pentobarbital (50 mg·kg\(^{-1}\) IP). After carefully shaving off the hair from the dorsal surfaces, the dorsal area (11 mm in diameter) was exposed to 100 C water for 10 s in all burn groups.

2) Reagents

MTT solution (2 mg·mL\(^{-1}\) [3-(4, 5-dimethylthiazol-2-y)-2, 5-diphenyl tetrazolium bromide]), 10% fetal bovine serum (FBS), lipopolysaccharide (LPS), TRIzol reagent, Dulbecco’s modified Eagle’s medium (DMEM), and 5-bromo-2’-deoxyuridine (BrdU) were purchased from Sigma (St. Louis, MO, USA). Penicillin (1,000 unit/mL) and streptomycin (1,000 μg/mL) were used from Gibsco (Grand Island, NY,
USA).

Rabbit anti-mouse inducible nitric oxide synthase (iNOS, 1:250), anti goat anti fibroblast growth factor (FGF, 1:100) antigen, nuclear factor (NF)-κB p65 (1:250), normal goat serum, biotinylated goat anti-mouse Ig G, mouse anti-mouse iNOS (1:200), and rabbit anti-mouse cyclooxygenase-2 (COX-2, 1:50) were from Santa cruz Biotec (Santa cruz, CA, USA). Mouse anti-mouse BrdU (1:50) was obtained from Amersham International (Bucks, UK).

3) Cell culture

RAW 264.7 cells were purchased from Korean Cell Line Bank (KCLB, Korea). These cells were maintained at subconfluence in a 95% air, 5% CO₂ humidified atmosphere at 37°C. Cells were cultured in DMEM supplied with 10% FBS and antibiotics (100 unit/mL penicillin, 100 μg/mL streptomycin).

4) Preparation of duoderm CGF wound dressing

The duoderm CGF (Convatec, USA, 11 mm in diameter) was applied on the burned skin of mice in the DC group during the experiment.

5) Preparation of GST

GST, which contains eighteen species of herbal-medicine (Table 1), was purchased from Dongguk University International Hospital (Ilsan, Korea). The 2 packs (138 g) of GST (69 g) were prepared by decocting the dried herbs with 500 mL boiling distilled water for approximately 2 hr. After filtering, it was concentrated in 50 mL by vacuum evaporation using the rotary evaporator. This GST extract (2.5 mg/kg/day, p.o.) was orally administered to mice in the GST group. GST extract used in vitro was dried with a freeze dryer after concentration under decompression. It was used in concentration from 0.5 mg/mL to 2.0 mg/mL as a result of MTT assay.

Table 1. Contents of Gamisipjeon-tang (GST)

<table>
<thead>
<tr>
<th>Pharmacognostic nomenclature</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astragali Radix</td>
<td>6.0</td>
</tr>
<tr>
<td>Rehmanniae Radix Preparat</td>
<td>4.0</td>
</tr>
<tr>
<td>Angelicae gigantis Radix</td>
<td>4.0</td>
</tr>
<tr>
<td>Cnidii Rhizoma</td>
<td>4.0</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>4.0</td>
</tr>
<tr>
<td>Poria</td>
<td>4.0</td>
</tr>
<tr>
<td>Paeoniae Radix Alba</td>
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</tr>
<tr>
<td>Atractylodis macrocephalae Rhizoma</td>
<td>4.0</td>
</tr>
<tr>
<td>Citri Pericarpium</td>
<td>4.0</td>
</tr>
<tr>
<td>Linderae Radix</td>
<td>4.0</td>
</tr>
<tr>
<td>Schizandrae Fructus</td>
<td>4.0</td>
</tr>
<tr>
<td>Cinnamomi Ramulus</td>
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<tr>
<td>Glycyrrhizae Radix</td>
<td>4.0</td>
</tr>
<tr>
<td>Lonicerae Flos</td>
<td>4.0</td>
</tr>
<tr>
<td>Forsythiae Fructus</td>
<td>4.0</td>
</tr>
<tr>
<td>Zingiberis Rhizoma Recens</td>
<td>3.0</td>
</tr>
<tr>
<td>Jujubae Fructus</td>
<td>2.0</td>
</tr>
<tr>
<td>Angelicae dahuricae Radix</td>
<td>2.0</td>
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<tr>
<td>Total amount</td>
<td>69.0</td>
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2. Method

1) Effects on the inhibition of inflammatory gene related to NF-κB activation

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was performed to investigate the influence of iNOS, COX-2 mRNA expression, and IκB kinase (IKK) by GST. RAW 264.7 macrophages (5 × 10^5 cells/well) were cultured in 6-well plate. After 12 hr, the cells were treated by LPS for 2 hr to induce the NF-κB activation, managed with 0.5 μg/ml, 1.0 μg/ml, 1.5 μg/ml, 2.0 μg/ml concentration of GST, and incubated at 37°C for an additional 24 hr. Total cellular RNA was extracted using TRIzol reagent and quantified with fluorometer (introgen, USA). cDNA synthesis was performed with RT-PCR kit (Promega, Madison, USA). PCR was performed to measure the IKK, iNOS, and COX-2 primer (Table 2). PCR products were separated on 1-2% agarose gels with electrophoresis, and intensity of bands was quantified by image analysis with Optimas 5.2 (Optima, USA).

2) Effects on the skin regeneration in the burned mice

1) Measurement of the burn area

The burn area (11 mm in diameter) was measured with the caliper (Mitutoyo, JAPAN) on the 3rd, 7th, and 14th days after burn elicitation.

2) Measurement of the anti-inflammatory effects

Changes of inflammatory gene expression in the dermis

The immunohistochemical staining was accomplished using the NF-κB p65 (1:500) to examine the changes of NF-κB distribution and then performed with mouse anti-mouse iNOS (1:200) and rabbit anti-mouse COX-2 (1:50) to investigate the distribution of iNOS and COX-2 in the tissues.

3) Regenerative effects in the burned skin

FGF activation

Immunohistochemical staining using anti goat anti FGF (1:100) antibody was performed to examine the changes of epidermal cell proliferation by FGF activation in burned skin.

Epidermal cell proliferation

Mice were treated by intraperitoneal injection with BrdU 50 μg/kg and then burned skin was extirpated after 1, 3, and 5 hr. Samples were applied in 2N HCl solution at 4°C, 37°C for 20 min for DNA-denaturation. After treating with 0.1 M borate buffer solution, normal goat serum containing 0.01% proteinase K was added for 1 hr, applied with mouse anti-mouse BrdU (1:50) at 4°C in the incubation chamber for 72 hr. Afterwards the immunohistochemical staining was carried out with an identical process.

3) Statistical analysis & Image analysis

Table 2. The Primer of IKK, COX-2, iNOS, and β-actin mRNA

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequences</th>
<th>Product (bp)</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>IKK</td>
<td>5’ CCA CCC AGT TCC ACA AGT CT 3’</td>
<td>380</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5’ CCT CCA CTG CGA ATA GCT TC 3’</td>
<td>527</td>
<td>30</td>
</tr>
<tr>
<td>iNOS</td>
<td>5’-AGACTGAGATTTGGCTGTCCTCC-3’</td>
<td>624</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5’-AGAAGTGAGGTACATCTGGAGCC-3’</td>
<td>840</td>
<td>35</td>
</tr>
<tr>
<td>COX-2</td>
<td>5’-TCTCCAACACTCTCTAC-3’</td>
<td>840</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5’-GCACGATCGTCTCGTTGACT-3’</td>
<td>840</td>
<td>35</td>
</tr>
<tr>
<td>β-actin</td>
<td>5’-GGAGAGAGATCGTGCCACCCACACAC-3’</td>
<td>840</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5’-CCTGCTTGCTGATCCACATCGTG-3’</td>
<td>840</td>
<td>35</td>
</tr>
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</table>

Abbreviation : IKK, IκB kinase; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.
All values are expressed as the means ± standard deviation (S.D.). The data of burn area, BrdU and FGF positive reaction were analyzed with repeated measured analysis of the variance (ANOVA). The other data were analyzed with one-way ANOVA. As a post hoc test, Tukey HSD and Tamhane was used (p<0.05) to delineate the significant differences between groups. Probability values of less than 0.05 were considered to be statistically significant. Statistical analysis performed using SPSS 13.0.

Images were analysed by Optima 5.2 (Optima Co., USA).

Results

1. Inhibition of NF-κB activation–intricated cytokine by GST in vitro

1) Inhibition of IKK mRNA expression

The IKK mRNA expression increased in the RAW 264.7 macrophages by LPS stimulation. However, IKK mRNA expression decreased in the concentration after GST treatment and the decrement was 1.5% in the 0.5 mg/mL, 6% in the 1 mg/mL, 12% in the 1.5 mg/mL, and 30% in the 2 mg/mL concentration (Fig. 1).

2) Inhibition of iNOS mRNA expression

The increment of iNOS mRNA expression was observed in the RAW 264.7 macrophages by LPS stimulation. However, the decrement was observed in the concentration after GST treatment and it was 5.3% in the 0.5 mg/mL, 22% in the 1 mg/mL, 36% in the 1.5 mg/mL, and 63% in the 2 mg/mL concentration (Fig. 1).

3) Inhibition of COX-2 mRNA expression

The COX-2 mRNA expression by LPS stimulation increased in the RAW 264.7 macrophages. However, it decreased in the concentration after GST treatment and decrement was 12% in the 0.5 mg/mL, 20% in the 1 mg/mL, 31% in the 1.5 mg/mL, 55% in the 2 mg/mL (Fig. 1).

2. Effects of skin regeneration in the burned mice

1) Measurement of the burn area

The burn area was significantly decreased as time went by. It decreased by 8.19% in the DC group, by 12.10% in the GST group on the 3rd day, by 20.57% in the DC group, by 31.18% in the GST group.

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Fig. 1. In vitro test for inhibition of NF-κB activation–intricated cytokine by GST.

A. Inhibition of IKK, iNOS and COX-2 mRNA expression.

The RAW 264.7 macrophages were treated with LPS for 2 hr prior to the addition of indicated concentration (0.5 ~ 2 mg/mL) of GST, and the cells were further incubated for 24 hours. The LPS-induced IKK, iNOS, and COX-2 mRNA expression were dose-dependently decreased in GST-treated RAW 264.7 macrophages.

B. Image analysis of relative intensity for IKK (I), iNOS (II), and COX-2 (III) mRNA expression.

Abbreviation: LPS, lipopolysaccharide; IKK, IκB kinase; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.
group on the 7th day, by 20.93% in the DC group, and by 40.59% in the GST group on the 14th day as compared to the BE group (Table 3, Fig. 2).

2) Measurement of anti-inflammatory effects

① Changes of NF-κB positive response

NF-κB p65 positive response was strongly appeared in the nucleus and cytoplasm surrounding the nuclear membrane, and was observed the decrement in the

DC and GST groups as compared to the BE group. It increased by 493% in the BE group as compared to the normal group, whereas it decreased by 33% in the DC group, by 56% in the GST group as compared to the BE group (Table 4, Fig. 3).

② Changes of iNOS positive response

iNOS positive response was strongly shown in the cytoplasm surrounding the nuclear membrane, and

Statistical tests by repeated measured ANOVA was used.

Abbreviation : NOR, No treated mice; BE, Burn elicited mice; DC, duoderm CGF-treated mice after burn elicitation; GST, Gamisipjeon-tang treated mice after burn elicitation; 3rd, on the 3th day after burn elicitation; 7th, on the 7th day after burn elicitation; 14th, on the 14th day after burn elicitation.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>NOR</th>
<th>BE</th>
<th>DC</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>0</td>
<td>10.50 ± 0.18</td>
<td>9.64 ± 0.11</td>
<td>9.23 ± 0.10</td>
</tr>
<tr>
<td>7th</td>
<td>0</td>
<td>9.43 ± 0.91³</td>
<td>7.49 ± 0.08³</td>
<td>6.49 ± 0.11³</td>
</tr>
<tr>
<td>14th</td>
<td>0</td>
<td>4.73 ± 0.10³</td>
<td>3.74 ± 0.09³</td>
<td>2.81 ± 0.09³</td>
</tr>
</tbody>
</table>

P<0.05; significant differences among the BE, DC, GST group.

³; significant differences compared to the 3rd group.

Fig. 2. The curative effects of GST for burn caused skin damage (x4).

A. morphology on the 3rd day after burn elicitation.
B. morphology on the 7th day after burn elicitation.
C. morphology on the 14th day after burn elicitation.

The size of burn caused damages in DC and GST groups was decreased than that in the BE group as time went by and these decrease had probability (p<0.05).

Abbreviation : NOR, No treated mice; BE, Burn elicited mice; DC, duoderm CGF-treated mice after burn elicitation; GST, Gamisipjeon-tang treated mice after burn elicitation.
The Effects of *Gamisipjeon-tang* on the Skin Regeneration of Deep Second Degree Burns in Mice

observed the decrease in the DC and GST groups as compared to the BE group. It increased by 639% in the BE group as compared to the normal group, whereas it decreased by 44% in the DC group, by 54% in the GST group as compared to the BE group (Table 4, Fig. 3).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>NOR</th>
<th>BE</th>
<th>DC</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB p65</td>
<td>551 ± 11</td>
<td>3,266 ± 69</td>
<td>2,193 ± 64</td>
<td>1,441 ± 75</td>
</tr>
<tr>
<td>iNOS</td>
<td>452 ± 10</td>
<td>3,339 ± 63</td>
<td>1,860 ± 41</td>
<td>1,530 ± 36</td>
</tr>
<tr>
<td>COX-2</td>
<td>358 ± 9</td>
<td>5,256 ± 76</td>
<td>1,666 ± 66</td>
<td>1,032 ± 45</td>
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</tbody>
</table>

(image analysis for 200,000 particles / range of intensity : 80-130) Statistical tests by one way ANOVA was used. As a result of post hoc test, the significant differences were measured among all the groups. Abbreviation : NF-κB p65, nuclear factor-κB p65. Other abbreviation is same as Table 3.

Changes of COX-2 positive response

COX-2 positive response was strongly presented in the cytoplasm and observed the decrease in the DC and GST groups as compared to the BE group. It increased by 1,368% in the BE group as compared to the normal group, whereas it decreased by 68% in

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**Fig. 3.** The anti-inflammatory effects of GST through regulation of inflammatory response on the 7th day after burn elicitation.

A. Suppression of NF-κB p65 activation.

The NF-κB p65 positive reacted cell (arrow) in GST-treated mice remarkably decreased (NF-κB p65 immunohistochemistry, ×400).

B. Inhibition of iNOS production.

The iNOS positive reacted cell (arrow) in GST-treated mice remarkably decreased (iNOS immunohistochemistry, ×400).

C. Inhibition of COX-2 production.

The COX-2 positive reacted cell (arrow) in GST-treated mice remarkably decreased (COX-2 immunohistochemistry, ×400).

Abbreviation : NF-κB p65, nuclear factor-κB p65; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.

Other abbreviation is same as Fig. 2.
the DC group, by 80% in the GST group as compared to the BE group (Table 4, Fig. 3).

3) Regenerative effects in the burned skin
   (1) FGF activation

   The FGF positive reaction of epidermal basal layer was appeared strongly in the cytoplasm. The larger increase was observed in the BE group than in the normal group as the time goes, 174% than in the normal group on the 3rd day, 29% on the 7th day than on the 3rd day, 395% on the 14th day than on the 7th day.

   However, the different changes were shown in the DC and GST group as compared to the BE group. The larger increase was shown 94% on the 3rd day, 181% on the 7th day in the DC group than in the

\[
\begin{array}{cccc}
\text{Days} & \text{NOR} & \text{BE} & \text{DC} & \text{GST} \\
3rd & 1,807 \pm 55 & 3,512 \pm 74 & 3,608 \pm 90 & \\
7th & 659 \pm 12 & 2,336 \pm 54^1 & 6,571 \pm 80^1 & 8,111 \pm 82^1 \\
14th & 11,552 \pm 153^3 & 7,085 \pm 89^3 & 4,198 \pm 52^3 \\
\end{array}
\]

(image analysis for 200,000 particles / range of intensity : 80-130)

Statistical tests by repeated measured ANOVA was used.
\(^1\); significant differences among the BE, DC, GST group.
\(^3\); significant differences compared to the 3rd group.
Abbreviation : FGF, fibroblast growth factor
Other abbreviation is same as Table 3.

The epidermal regeneration of GST through regulation of fibroblast growth factor (FGF) activation after Burn elicitation (FGF Immunohistochemistry, ×40D).

A. morphology on the 3rd day after burn elicitation.
The FGF positive reacted cell (arrow) in GST-treated mice remarkably increased.
B. morphology on the 7th day after burn elicitation.
The FGF positive reacted cell (arrow) in GST-treated mice remarkably increased.
C. morphology on the 14th day after burn elicitation.
The FGF positive reacted cell (arrow) in GST-treated mice remarkably decreased.
Other abbreviation is same as Fig. 2.
The Effects of Gamisipjeon-tang on the Skin Regeneration of Deep Second Degree Burns in Mice

The decrease was shown by 38% in the DC group as compared to the BE group on the 14th day. The larger increase was observed by 100% on 3rd day, by 247% on the 7th day in the GST group as compared to the BE group. The decrease was observed by 64% in the GST group as compared to the BE group on the 14th day (Table 5, Fig. 4).

Epidermal cell proliferation

The BrdU positive reaction in the epidermal basal layer was strongly shown in the nucleus and increased in the BE group as compared to in the normal group as time went by. The BrdU positive reaction increased 33% larger in the BE group than in the normal group on the 3rd day, 84% larger on the 7th day than on the 3rd day and 213% larger on the 14th day as compared to the normal group (Table 5, Fig. 4).

Table 6. The Image Analysis of Epidermal Proliferation (BrdU) in GST-treated Mice after Burn Elicitation

<table>
<thead>
<tr>
<th>Days</th>
<th>NOR</th>
<th>BE†</th>
<th>DC‡</th>
<th>GST§</th>
</tr>
</thead>
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<tr>
<td>3rd</td>
<td>1,357 ± 60</td>
<td>1,661 ± 71</td>
<td>3,796 ± 25</td>
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<tr>
<td>7th</td>
<td>2,505 ± 72†</td>
<td>7,025 ± 78‡</td>
<td>11,103 ± 206§</td>
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<tr>
<td>14th</td>
<td>7,825 ± 85§</td>
<td>6,752 ± 65‡</td>
<td>1,897 ± 44‡</td>
<td></td>
</tr>
</tbody>
</table>

(image analysis for 200,000 particles / range of intensity : 80-130)

Statistical tests by repeated measured ANOVA was used.
†; significant differences among the BE, DC, GST group.
‡; significant differences compared to the 3rd group.
Abbreviation : BrdU, 5-bromo-2'-deoxyuridine.
Other abbreviation is same as Table 3.

Fig. 5. The epidermal regeneration of GST through regulation of proliferation after burn elicitation (5-bromo-2'-deoxyuridine (BrdU) immunohistochemistry, ×1000).
A. morphology on the 3rd day after burn elicitation.
B. morphology on the 7th day after burn elicitation.
C. morphology on the 14th day after burn elicitation.
The BrdU positive reaction (arrow) in GST-treated mice remarkably increased.
Other abbreviation is same as Fig. 2.
the 14th day than on the 7th day.

However, the BrdU positive reaction of DC and GST group was different from that of BE group. That of DC group was 22% larger on the 3rd day, 181% larger on 7th day and 13% smaller on the 14th day than that of BE group. That of GST group was 178% larger on the 3rd day, 344% larger on 7th day and 76% smaller on the 14th day than that of BE group (Table 6, Fig. 5).

Discussion and Conclusion

The conventional burn therapy has generally included wound cleansing with bland soap, 0.05% chlorhexidine, normal saline, nitrofurazone mesh gauze, vaseline gauze, and a topical agent such as silver sulfadiazine cream, mafenide, or gentamicin cream. Recently, synthetic wound dressing such as silicone gel sheet, aquacell Ag, and hydrocolloid dressing, and surgery such as escharotomy, tangential excision, and skin grafting were used to compensate for the previous therapy by moisturizing scarred areas, removing the exudate, preventing scar tissue from growing, and decreasing the treatment period.

Universally used silver sulfadiazine in burn patients was manufactured by Fox in 1968. Cho et al. reported that it caused cytotoxicity to the epidermis and dermis. It may prevent the inflammation, but it may also damage the dermal cell and delay initial therapy. Therefore silver sulfadiazine was not used in this study.

Winter said that the wetting surface preserve the growth factor and promotes the movement of epithelial cell, so that it will accelerate dermal regeneration. Based on this theory, many synthetic dressings have been developed. Hydrocolloid dressing absorbs the exudate and gradually becomes a gel state, so this gel state makes the burn region moist. This wet state preserves the burn region, provides suitable warmth, and protects from physical stimulation. Duoderm is a kind of hydrocolloid dressing, and duoderm CGF is specially used for most exudate.

In oriental medicine, there are a few studies about burns. Most of these studies are case reports that include oral herbal medicine, acupuncture, aqua-acupuncture, and the external application of herbal medicine. In superficial second degree burns, acupuncture treatment was effective; however, in deep second and third degree burns, other oriental treatments were combined. In these studies, the effects of each treatments were not investigated. The experimental study regarding external application measured the effects of yolk sac oil compared to the those of Mebo ointment and ziyungao. This experiment was studied merely through histological finding, which did not study the mechanism in detail.

In burn wound healing, there are two stages. The first stage involves the inflammatory phase, and the second stage is the new tissue formation phase. During the inflammatory phase, infiltrating neutrophils aid in the removal of foreign agents in the burn area. It was found that polysaccharides could accelerate the infiltration of inflammatory cells, consequently accelerating wound cleaning. When the new tissue formation phase occurs, fibroplasia begins by forming granulation tissue within the wound space.

The objective of this study was to examine the anti-inflammatory effects and skin regenerative effects of GST in mice with deep second degree burns and to compare the curative effects of GST with those of duoderm CGF.

LPS as endotoxin, stimulates the production of inflammatory mediators such as nitric oxide (NO), TNF-α, interleukins, prostanoids, and leukotrienes. NO, prostaglandins, and inflammatory cytokines are the major targets for the treatment of inflammatory disorders. The iNOS, which is induced by either bacterial LPS of TNF-α and IFN-γ in macrophages, produces NO. Expression of iNOS is closely related with the up-regulation of NF-κB, whose sites...
have been identified in the promoter region of iNOS gene\(^4^0\). Nitric oxide synthase (NOS) plays a major role in regulating vascular tone, neurotransmission, killing of microorganisms, tumor cells, and other homeostatic mechanisms\(^4^1\). High levels of NO have been reported in the inflammation and the suppression of iNOS parallels the inhibition of NO production\(^4^2\).

COX, a key enzyme required for the synthesis of prostaglandins, exists as two different isoforms, COX-1 and -2. COX-2 catalyzes the inducible production of prostaglandins, which clearly represents an important step in the inflammatory process.\(^4^3\). In the previous report, the pro-inflammatory cytokines modulated the expression of COX-2 through NF-κB, which is implicated in gene expression.\(^4^4\)

NF-κB is composed mainly of two proteins, p50 and p65. In unstimulated cells, NF-κB is bound to the inhibitory protein IκB in the cytoplasm. In response to several stimuli such as viral and bacterial infections, IκBs are phosphorylated by IKKs, ubiquitinated, and proteolytically degraded thereby allowing NF-κB to translocate to the nucleus and inducing transcription of pro-inflammatory genes.\(^4^7\) NF-κB up-regulates the expression of pro-inflammatory genes such as acute phase proteins, cellular adhesive molecules (CAMs), inducible nitric oxide synthase (iNOS), interleukins, proteases, and the intensity of the inflammatory activity.\(^4^8,4^9\) NF-κB is an oxidative stress response transcription factor, and activated NF-κB can be blocked by antioxidants.\(^5^0\) In vitro, iNOS, COX-2, and IKK positive reaction decreased GST concentration-dependently. In vivo, iNOS, COX-2, and IKK as inflammatory factors were investigated in the GST, duoderm treated mice and only burn-elicited mice. The positive reactions were lowest in the GST-treated mice, followed by the duoderm CGF-treated mice and only burn-elicited mice. These data could be predicted that GST was more effective than duoderm CGF in the anti-inflammatory response.

Gospodarowicz et al.\(^5^1\) isolated a protein from bovine pituitary gland that markedly promoted growth of fibroblasts. This protein was named fibroblast growth factor (FGF). Bohlen et al.\(^5^2\) elucidated the presence of two types of FGF protein with different isoelectric points, basic and acidic sides, which were designated as basic (basic FGF) and acidic (acidic FGF). The FGF family of growth factors is comprised of seven members that have a variety of growth and differentiation activities.\(^5^3\) These factors bind heparin with relatively high affinity and are thus termed “heparin binding growth factors”\(^5^4\). The FGFs are structurally related proteins that play important roles in cell proliferation, differentiation, and migration, as well as in disease processes such as tumor angiogenesis.\(^5^5\) FGF-1 and -2 are potent mitogens and differentiation factors for mesoderm derived cells, which cause increases in vitro invasiveness and in vivo metastasis of cancer cells.\(^5^3\) FGF may play a pivotal role in cutaneous wound healing by activating local macrophages with the effects continuing up to the remodeling stage for several weeks after the initial injury. Faster wound healing is highly expected to prevent severe systemic damage or sequela such as invasive wound infection and sepsis.\(^5^6\) bFGF is used for the treatment of intractable skin ulcers by accelerating the proliferation of fibroblasts, the regeneration of the blood network and finally the granulation.\(^5^7\) FGF promoted growth of fibroblast, and increased more in the GST-treated mice than in the duoderm CGF-treated mice and only burn-elicited mice on the 3rd, and 7th days. However, on the 14th day, it was decreased in the GST-treated mice, whereas it increased in the duoderm CGF-treated mice and only burn-elicited mice on the 14th day. These results could explain that GST-treated mice had more rapid skin regenerative effects than duoderm treated mice and only burn-elicited mice.

BrdU, analog of thymidine, is incorporated into DNA during the S-phase of the cell cycle and is a useful alternative to labeling proliferation cells with tritiated thymidine.\(^5^8\) In previous study, the hyperplasia
of epidermis in the hairless mice irradiated with UVB. The level of BrdU expression in epidermis dramatically increased compared with un-irradiated control\textsuperscript{59}. BrdU labeling the cell proliferation was increased more rapidly in the GST-treated mice than in the duoderm CGF-treated mice and only burn-elicited mice on the 3rd, and 7th days. However, on the 14th day it was more quickly decreased in the GST-treated mice than in the duoderm CGF, whereas it was increased in the BE group. These results could explain that GST-treated mice take a rapid process in the skin regeneration as compared to the duoderm CGF-treated mice and only burn-elicited mice with referring the other data.

In vitro, the inflammatory response decreased GST concentration-dependently. According to the result in vitro, our study in vivo has processed on the two point of view, firstly the inflammatory response as iNOS, COX-2, and NF-κB positive reaction and secondly the skin regenerative factor as change of the burn area, FGF, BrdU positive reaction after burns. GST could be explained to have more rapid effects than the duoderm CGF in the anti-inflammatory response and the skin regeneration.

In oriental medicine, GST is known to have wound healing effects and that we want to investigate the effects of skin regeneration as compared to the conventional therapy such as synthetic dressings. Recently other than the oral herbal medicine\textsuperscript{20-22}, the acupuncture\textsuperscript{24}, external herbal medicine therapy\textsuperscript{25} were known to be effective in the previous study. More various studies would need to be accomplished for the effective therapy in burns.

In conclusion, these findings could indicate that GST has the anti-inflammatory effects and skin regenerative effects, and this is significantly more effective than duoderm CGF.

References

12. Rho MH, Yong JH. The effects of the low power
The Effects of Gamisipjeon-tang on the Skin Regeneration of Deep Second Degree Burns in Mice


42. Raghav SK, Gupta B, Shrivastava A, Das HR. Inhibition of lipopolysaccharide-inducible nitric oxide synthase and IL-1β through suppression of NF-κB activation by 3-(1’-1’-dimethyl-allyl)-6-hydroxy-7-methoxy-coumarin isolated from Ruta graveolens L. European Journal of Pharmacology. 2007;560:69-80.


46. So HS, Park RK, Oh HM, Pae HO, Lee JH, Chai KY, Ahung SY, Chung HT. The methanol extract of Spiraea prunifolia var. simpliciflora root inhibits the generation of nitric oxide and superoxide in RAW 264.7 cells. J. Ethnopharmacol. 1999;68:209-17.


