Comparative Effect of Gromwell (Lithospermum erythrorhizon) Extract and Borage Oil on Reversing Epidermal Hyperproliferation in Guinea Pigs

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To compare the systemic efficacy of borage oil (Borago officinalis: BO) and gromwell (Lithospermum erythrorhizon), two plant species of the Boraginaceae family, epidermal hyperproliferation was induced in guinea pigs by a hydrogenated coconut oil diet for 8 weeks. Subsequently, guinea pigs were fed diets of BO (group HBO), organic extract (group HGO), or water extract (group HGW) of gromwell for 2 weeks. In groups HGO and HGW, proliferation scores and the level of ceramides, the major lipid maintaining epidermal barrier, were similar with those in normal control group BO fed BO diet for 10 weeks. Despite accumulation of 15-hydroxyeicosatrienoic acid (15-HETrE), the potent anti-proliferative metabolite of γ-linolenic acid (GLA: major polyunsaturated fatty acid in BO), the reversal of epidermal hyperproliferation and the ceramide level of group HBO were less than those of groups HGO and HGW. Taken together, our data demonstrate that gromwell is more effective in reversing epidermal hyperproliferation with a marked increase in ceramides.

Key words: gromwell; borage oil; anti-proliferation; ceramides; epidermis

The epidermis provides an efficient barrier against water loss through the skin. The epidermal permeability barrier is composed of intercellular lipid lamellae with ceramides, cholesterol, and fatty acids in the stratum corneum, the outermost layer of the epidermis. The barrier disruption causes an increase in transepidermal water loss (TEWL) and epidermal hyperproliferation, both of which are observed in abnormal skin conditions such as atopic dermatitis and essential fatty acid deficiency (EFAD). The disrupted barrier function in epidermis leads to the clinical manifestation of dryness and epidermal hyperproliferation in patients with atopic dermatitis. In essential fatty acid deficiency, an elevation of TEWL has been shown to bear a correlation with the degree of epidermal hyperproliferation.

The epidermal hyperproliferation seen in abnormal skin conditions has prompted the therapeutic use of a variety of systemic and topical anti-proliferative agents such as steroids combined with ultraviolet light (UVB). Similarly, retinoids, both systemic and topical, have also been used to arrest the proliferative aspect of skin disease, but full control of epidermal hyperproliferation by these agents without attending side effects has been elusive. In this regard, treatment alternatives, including dietary management, are of particular interest.

As a dietary supplement, borage oil (BO) (Borago officinalis: Boraginaceae family) has been reported to exert clinical efficacy in a variety of skin diseases and in the suppression of proliferation and inflammation in the skin. The major constituent of BO underlying this clinical efficacy is believed to be oxidative metabolites of γ-linolenic acid (GLA: 18:3n-6), prostaglandin E1 (PGE1), and 15-hydroxyeicosatrienoic acid (15-HETrE), which have been reported to exert anti-proliferative and anti-inflammatory effects. With the beneficial effects of GLA, borage oil has become popular in Western countries, as it is in Asia. But Borago officinalis, the major plant species of the Boraginaceae family for borage oil, is cultivated mainly in Europe and North America. Hence borage oil is limited in Asian countries due to the high cost of imports. In contrast, gromwell (Lithospermum erythrorhizon), another plant species of the Boraginaceae family native to East Asia, has been used traditionally in remedies for abnormal skin conditions, such as burns.

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and inflammation, in oriental folk medicine.\textsuperscript{15,16} Based on intensive studies using a variety of laboratory methodologies, gromwell extracts exert anti-proliferative, antimicrobial, and anti-inflammatory activities,\textsuperscript{17,18} but most of these activities in gromwell have been reported based on \textit{in vitro} studies, and only limited information is available on the systemic effects of gromwell as a dietary supplement. Since anti-proliferative activity in gromwell has been also reported mostly \textit{in vitro} studies using various cancer cell lines,\textsuperscript{19,20} we were particularly interested in investigating whether dietary supplementation of gromwell exerts anti-proliferative activity \textit{in vivo}. In this study, we compared the dietary effects of borage oil and gromwell in reversing epidermal hyperproliferation in guinea pigs, induced by an essential fatty acid deficient diet.

**Materials and Methods**

\textbf{Preparation of gromwell extracts.} Gromwell (the dried root of \textit{Lithospermum erythrorhizon} Sieb et Zucc) (the 2003 product of Youngiu, Gyeongangsuk-do, Republic of Korea) was purchased from the Kyungdong Oriental Medicine Market in Seoul, Republic of Korea. Since bioactive components of gromwell have been identified mostly in organic extracts,\textsuperscript{17,21,22} and in contrast, decocted extracts of gromwell with boiling water are usually used as remedies in oriental folk medicine,\textsuperscript{15,16} both organic and decocted water extracts of gromwell were prepared. For the preparation of organic extract (GO), gromwell (100 g) was cut into small pieces and extracted with 500 ml of chlorof orm (CHCl\textsubscript{3}):methanol (MeOH) (2:1, v/v) and 100 ml of 0.1 M KCl at room temperature for 12 h with stirring. The water extract of gromwell (GW) was prepared by decocting small pieces of gromwell (100 g) with 500 ml of boiling distilled water for 3 h. The extraction procedures of GO and GW were repeated twice. Each of the combined organic and water extracts was filtered through Whatman no. 2 paper, and lyophilized to yield either 23.5 g of organic extracts or 15.0 g of water extracts of gromwell.

\textbf{Guinea pigs and diets.} Fourteen-d-old male Hartley guinea pigs were purchased from Samtako Laboratory (Osan, Republic of Korea) and assigned to two groups. One group was fed a diet (Table 1) containing 40 g/kg hydrogenated coconut oil (HCO) (Dyets Inc., Bethlehem, PA), supplemented with 20 g/kg triolein (Sigma Chemical, St Louis, MO) to induce essential fatty acid deficiency (EFAD), as described previously.\textsuperscript{4,23} The other group, which served as the normal control, was fed a diet containing 60 g/kg borage oil (Degussa BioActive, Champaign, IL) (group BO). The control group was pair-fed to the mean of the EFA deficient group and given water supplemented with 0.5 g/l ascorbic acid to serve as an antioxidant.\textsuperscript{4,23,24} Guinea pigs were housed individually in an air-conditioned room (22–24°C) with a relative humidity range of 55–60%.

<table>
<thead>
<tr>
<th>Table 1. Compositions of Experimental Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition\textsuperscript{1}</td>
</tr>
<tr>
<td>Hydrogenated coconut oil</td>
</tr>
<tr>
<td>Borage oil</td>
</tr>
<tr>
<td>Casein\textsuperscript{3}</td>
</tr>
<tr>
<td>Cornstarch\textsuperscript{4}</td>
</tr>
<tr>
<td>Sucrose\textsuperscript{5}</td>
</tr>
<tr>
<td>Cellulose\textsuperscript{6}</td>
</tr>
<tr>
<td>Mineral mix\textsuperscript{5}</td>
</tr>
<tr>
<td>Vitamin mix\textsuperscript{5}</td>
</tr>
<tr>
<td>Agar\textsuperscript{7}</td>
</tr>
<tr>
<td>dl-Methionine\textsuperscript{8}</td>
</tr>
<tr>
<td>Potassium Acetate\textsuperscript{9}</td>
</tr>
<tr>
<td>Magnesium Oxide\textsuperscript{10}</td>
</tr>
<tr>
<td>Zinc carbonate\textsuperscript{11}</td>
</tr>
<tr>
<td>Organic extract of Gromwell</td>
</tr>
<tr>
<td>Water extract of Gromwell</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Diet composition was authorized for a purified gel diet of guinea pigs.\textsuperscript{24}
\textsuperscript{2}Group BO: BO diet for 10 weeks, Group HCO: HCO diet for 10 weeks, Groups HBO, HGO, and HGW: HCO diet for 8 weeks followed by 2 weeks of feeding BO diet (group HBO), GO diet (group HGO), or GW diet (group HGW)
\textsuperscript{3}Kyungdong, Sunbooks, Seoul, Republic of Korea
\textsuperscript{4}Sigma, St. Louis, MO
\textsuperscript{5}Briggs chick salt mixture A, contained in (g/kg) mix: CaCO\textsubscript{3}, 250; Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2}, 233; CaSO\textsubscript{4}•2H\textsubscript{2}O, 0.3; ferric citrate•5H\textsubscript{2}O, 67; MgSO\textsubscript{4}•7H\textsubscript{2}O, 83.3; MnSO\textsubscript{4}•4H\textsubscript{2}O, 7.0; K\textsubscript{2}O, 0.7; K\textsubscript{2}HPO\textsubscript{4}, 150; NaCl, 146.7; Na\textsubscript{2}HPO\textsubscript{4}, 121.7; ZnCO\textsubscript{3}, 0.3; ICN Biomedicals, Aurora, OH
\textsuperscript{6}Vitamin mix provided the following (mg/kg) of the complete diet: \textit{d}-tocopherol, 134; L-ascorbic acid, 1,800; choline chloride, 30,000; n-calcium pantothenate, 120; inositol, 200; menadione, 90; niacin, 180; p-aminobenzoic acid, 200; pyridoxine HCl, 40; riboflavin, 40; thiamin HCl, 40; retinyl acetate, 10.8; biotin, 0.8; folic acid, 3.6; cyanocobalamin, 0.054; ICN Biomedicals, Aurora, OH
\textsuperscript{7}Beeton Dickinson, Sparks, MD

At the end of 8 weeks, the EFA deficient guinea pigs were divided into four groups. To reverse the EFA deficiency, group 1 was fed a diet containing 60 g/kg borage oil (group HBO). Groups 2 and 3 were fed diets containing 40 g/kg hydrogenated coconut oil and 20 g/kg triolein, supplemented with dried powders of either organic (group 2: HGO) or water (group 3: HGW) extracts of gromwell. Group 4, the EFA deficient control, was fed a diet containing 40 g/kg hydrogenated coconut oil and 20 g/kg triolein (group HCO). The compositions of experimental diets are shown in Table 1, and the fatty acid compositions of oils and gromwell extracts are shown in Table 2. The EFA-
deficient guinea pigs were fed the respective diets for 2 weeks to evaluate the efficacy of each dietary supplement on the reversal of epidermal hyperproliferation. Group BO, the normal control group, continued to be fed a basal diet containing 60 g/kg borage oil for 2 additional weeks. The food intakes and body weights of all groups were monitored 3 times per week over the 10-week feeding period. There were no significant differences in the food intakes of the five groups, but the body weights of group HCO were less than those of the other groups as evidence of EFAD (data not shown). The body weights and macroscopic appearances of groups BO, HBO, HGO, and HGW did not differ (data not shown). All guinea pigs were sacrificed by cervical dislocation, and epidermal strips were quickly removed by a castroviejo electrokeratome (customized by Dae Jong Machinery, Ilsan, Republic of Korea), as described previously.

Histological evaluation of epidermal proliferation. For morphological evaluation of the epidermis, 4 mm² biopsies were excised from the skin of groups BO, HCO, HBO, HGO, and HGW and placed in neutral buffered formalin. The specimens were fixed, stained with hematoxylin and eosin (H and E), sectioned to a thickness of 6 μm, and affixed to slides for viewing by light microscopy (200× magnification). The sections were evaluated for thickening of the epidermal layer (a characteristic feature of epidermal hyperproliferation) by two investigators, including a pathologist who was unaware of the experimental diets and groups. Proliferation scores ranging from 1 to 5 (1: low; 3: moderate; 5: high, 0.5 scale) were allotted to each section.

Epidermal DNA synthesis. Mitotic activities in the epidermis of each group were ascertained by [³H]thymidine incorporation. Four mm² epidermal biopsies were placed in 1× DMEM (Dulbecco’s Modified Eagle Medium) in which [³H]thymidine (3.7 MBq/l) had been dissolved. After incubation for 3 h at 37 °C, the epidermis was rapidly frozen in liquid N₂ to stop DNA synthesis. The epidermis was thawed, placed in 2 ml of 0.5 mol/l NaOH, and heated at 95 °C for 30 min to dissolve the tissue and release DNA. Aliquots were placed on cellulose filters (Millipore, Bedford, MA) previously treated with 10 g/l trichloroacetic acid. Filters with absorbed DNA were dried, and [³H]thymidine incorporated into DNA was measured by scintillation counting. Aliquots of solution after heating at 95 °C were dissolved in ethidium bromide (EtBr) buffer (10 μg/ml in 0.1 M tris–HCl, 0.1 M NaCl, pH 7.4). The quantitative fluorescence of the DNA-EtBr reaction was used to measure endogenous DNA levels in the samples using a fluorometer (Modulus Fluorometer, Turner Biosystems, Sunnyvale, CA). Specific [³H]thymidine incorporation was calculated as the amount of [³H]thymidine Bq/μg DNA.

Lipid analysis. Epidermal strips were homogenized with a polytron. Total lipids were extracted with chloroform (CHCl₃):methanol (MeOH) (2:1, v/v) and weighed, as reported previously. A portion of the crude epidermal homogenate was used to measure protein concentration by a modification of the Lowry method using bovine serum albumin as the standard.

The fatty acid compositions of total lipids were determined by gas chromatography after transmethylation in 6% methanolic HCl, as described previously. The gas chromatograph (Hewlett-Packard 5730A) was equipped with an SPB-225 fused silica capillary column (30 m × 0.25 mm × 0.15 μm; Supelco, Bellefonte, PA) with an oven temperature of 140 °C increasing 4 °C/min to 240 °C. Detection was performed with a flame ionization detector (FID).

Hydroxy fatty acids from the epidermal homogenate were extracted with ice-cold CHCl₃:MeOH (2:1, v/v) after acidification to pH 3.0. The profiles were determined by reverse-phase high performance liquid chromatography (RP-HPLC) using a Beckman 5 μm octadecyl silica (ODS 18) column (25 cm × 4.6 mm i.d.; Beckman, Fullerton, CA), which was run isocratically at a flow rate of 1.0 ml/min in a solvent system of 74% methanol and 26% H₂O containing 0.08% acetic acid. The hydroxy fatty acids separated were monitored at 237 nm and compared with authentic standards, as described in our previous report.

Ceramide quantitation. The total lipids extracted were fractionated into ceramides by high performance thin layer chromatography (HPTLC) on 0.20 mm silica gel 60-coated plates (Merks, Darmstadt, Germany), as described previously. Specifically, the samples applied to the plates with Linomat 5 auto sampler (CAMAG; Muttenz, Switzerland) were first developed to 1.0 cm and then to 3.5 cm in CHCl₃:MeOH:acetone (76:20:4, v/v/v). They were then developed to 7.5 cm in CHCl₃:acetone:MeOH (80:10:10, v/v/v) and finally developed to the top in CHCl₃:ethylacetate:ether:MeOH (76:20:6:2, v/v/v/v). Each stage of development was carried out with an automatic multiple development

### Table 2. Fatty Acid Compositions of Oils and Gromwell Extracts

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>HCO</th>
<th>BO</th>
<th>GO</th>
<th>GW</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:0</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10:0</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>51.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>17.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>8.8</td>
<td>8.7</td>
<td>11.4</td>
<td>9.2</td>
</tr>
<tr>
<td>18:0</td>
<td>7.1</td>
<td>3.6</td>
<td>8.3</td>
<td>7.1</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>16.6</td>
<td>27.1</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>36.2</td>
<td>46.0</td>
<td>47.3</td>
<td></td>
</tr>
<tr>
<td>18:3n-6</td>
<td>22.3</td>
<td>7.2</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>

1 Data were measured in g/100 g total fatty acids.
2 Only the major fatty acids are listed.
3 HCO, hydrogenated coconut oil; BO, borage oil; GO, organic extract of gromwell; GW, water extract of gromwell.

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**Fatty Acid Compositions of Oils and Gromwell Extracts**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>HCO</th>
<th>BO</th>
<th>GO</th>
<th>GW</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:0n-6</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>36.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:3n-6</td>
<td>22.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data were measured in g/100 g total fatty acids.**
(AMD) apparatus (CAMAG; Muttenz, Switzerland) after the plates were completely air-dried. The fraction containing ceramides that comigrated with respective standards were scanned at 420 nm with a TLC III scanner (CAMAG; Muttenz, Switzerland). The level of ceramides in each sample was quantified by calibration curves with the various concentrations of external standards of ceramides, and expressed as \( \mu g \) ceramides/\( \mu g \) protein.

**Statistical analysis.** Data are expressed as mean \( \pm \) SD. All data were analyzed by one-way ANOVA using the SAS statistical procedures (SAS 6.03, SAS Institute, Cary, NC)\(^{32}\) and the differences among the groups (BO, HCO, HBO, HGO, and HGW) were determined by Tukey’s multiple comparison test. Differences with \( p < 0.05 \) were considered significant.

**Results**

**Histological evaluation of epidermal proliferation and epidermal DNA synthesis**

Histological evaluation of the epidermis of group HCO demonstrated characteristic epidermal thickening (acanthosis) and hyperplasia as evidence of EFAD, as compared with that of control group BO (Fig. 1A and B). In the epidermis of groups HBO, HGO, and HGW, the hyperproliferative state was not apparent. On a quantitative basis, the proliferation score of group HBO was higher than that of normal control group BO, which reflected the feeding period for borage oil (10 weeks in group BO vs. 2 weeks in group HBO). The proliferation scores of groups HGO and HGW were significantly lower than that of group HBO, but were similar to that of group BO.

The results of histological evaluation were further confirmed by \( ^{3}H \)thymidine incorporation studies (Fig. 2). The hyperproliferative epidermis of group HCO highly incorporated \( ^{3}H \)thymidine as compared to the control epidermis of group BO, which suggests that epidermal hyperproliferation was associated with an increase in DNA synthesis. The DNA synthesis in the

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**Fig. 1.** Effect of Gromwell Extracts and Borage Oil on Epidermal Proliferation in Guinea Pigs.

A, Histological appearance of epidermal proliferation in control guinea pigs fed a borage oil (BO) (a) diet for 10 weeks, and EFA-deficient guinea pigs fed a hydrogenated coconut oil (HCO) diet for 10 weeks (b) or 8 weeks followed by 2 weeks of feeding with diets containing borage oil (HBO) (c), organic extract of gromwell (HGO) (d), or water extract of gromwell (HGW) (e). Arrows indicate that the bottom layer of epidermal and epidermal proliferation is correlated with epidermal thickness from the arrow to the top. Note acanthosis (epidermal thickening) in the epidermis of group HCO. B, Scores of epidermal proliferation in guinea pigs fed different diets as indicated in A. Values are means \( \pm \) SD (\( n = 10 \)). Means with different letters differ, \( p < 0.05 \).

**Fig. 2.** \( ^{3}H \)Thymidine Incorporation into Epidermal DNA of Control Guinea Pigs Fed a Borage Oil (BO) Diet for 10 Weeks, and EFA-Deficient Guinea Pigs Fed a Hydrogenated Coconut Oil (HCO) Diet for 10 Weeks or for 8 Weeks Followed by 2 Weeks of Feeding with Diets Containing Borage Oil (HBO), Organic Extract of Gromwell (HGO), or Water Extract of Gromwell (HGW).

Values are means \( \pm \) SD (\( n = 10 \)). Means with different letters differ, \( p < 0.05 \).
epidermis of group HBO was higher than of group BO, which is consistent with the histological evaluation. In groups HGO and HGW, DNA synthesis was suppressed more efficiently than in group BO, irrespective to the degree of reversed hyperproliferation in histological evaluation, as shown in Fig. 1.

**Total lipid and fatty acid analysis**

Total lipid in groups BO, HCO, HBO, HGO, and HGW did not differ, and this accounted for about 8.2–8.9% of the epidermis of guinea pig (dry weight) (data not shown). But fatty acid analysis of epidermal total lipids (Table 3) from the epidermis of group HCO revealed an accumulation of eicosatrienoic acid (20:3n-9), an abnormal fatty acid generated from oleic acid (18:1n-9) during the development of EFAD.4,23) In parallel, the level of linoleic acid (LA: 18:2n-6), the major polyunsaturated fatty acid (PUFA) available for enzymatic oxygenation,23) was notably diminished. In groups of BO, HBO, HGO, and HGW, no eicosatrienoic acid (20:3n-9) was detected.

In groups BO and HBO, significant levels of γ-linolenic acid (GLA: 18:3n-6) and dihomo-γ-linolenic acid (DGLA: 20:3n-6), an elongase product of GLA, were incorporated into epidermal lipids. But the levels of these two PUFAs and LA incorporated into the epidermal total lipids of group HBO were less than those of group BO (p < 0.05), which reflected the feeding period for borage oil (10 weeks in group BO vs. 2 weeks in group HBO). Notably, small but significant levels of GLA and DGLA were incorporated into the epidermal lipids of groups HGO and HGW, reflecting the GLA content of GO and GW in Table 2. Although GO and GW contained 46.0–47.3 g/100 g of LA (Table 2), the level of LA incorporated into the epidermal total lipids of groups HGO and HGW was not higher than that of group HCO. The levels of AA in the epidermis of groups BO, HBO, HGO, and HGW were not significantly different.

**Hydroxy fatty acid analysis**

The biological efficacy of polyunsaturated fatty acid in reversing epidermal hyperproliferation is largely dependent on lipoxygenase metabolites (hydroxy fatty acids).33,34) Analysis of epidermal hydroxy fatty acids (Fig. 3) revealed that the contents of 13-hydroxyoctadecadienoic acid (13-HODE) (13-lipoxygenase metabolite of LA) and 15-HETE in the epidermis of groups BO, HBO, and HCO reflected the level of LA and DGLA incorporated into epidermal total lipids, as shown in Table 3. The most abundant hydroxy fatty acid, 13-HODE, was derived from the most abundant PUFA, LA,4,23) in these three groups in the order BO > HBO > HCO (Fig. 3A). 15HETE was detected only in

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**Table 3.** Polynsaturated Fatty Acid (PUFA) Composition of Total Lipids in the Epidermis of Guinea Pigs

<table>
<thead>
<tr>
<th>PUFA2</th>
<th>BO</th>
<th>HCO</th>
<th>HBO</th>
<th>HGO</th>
<th>HGW</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2</td>
<td>22.4 ± 3.5*</td>
<td>12.9 ± 1.7c</td>
<td>16.6 ± 1.9b</td>
<td>8.7 ± 3.4d</td>
<td>10.1 ± 2.5c</td>
</tr>
<tr>
<td>18:3</td>
<td>3.9 ± 0.6a</td>
<td>0.1 ± 0.1d</td>
<td>2.2 ± 0.8b</td>
<td>1.3 ± 0.3c</td>
<td>1.0 ± 0.2c</td>
</tr>
<tr>
<td>20:3</td>
<td>2.2 ± 0.3a</td>
<td>nd</td>
<td>1.2 ± 0.5b</td>
<td>0.3 ± 0.2c</td>
<td>0.4 ± 0.2c</td>
</tr>
<tr>
<td>20:4</td>
<td>1.3 ± 0.4</td>
<td>0.4 ± 0.3</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

1Values are mean ± SD, n = 10. Means with different letters in the same rows differ, p < 0.05, nd, not detected.
2Only major polyunsaturated fatty acids (PUFA) that are available for enzymatic oxygenation and eicosatrienoic acid [20:3(n-9)] only generated in EFAD are listed.
3Group BO, BO diet for 10 weeks; Group HCO, HCO diet for 10 weeks; Groups HBO, HGO, and HGW, HCO diet for 8 weeks followed by 2 weeks of feeding BO diet (group HBO), GO diet (group HGO), or GW diet (group HGW)

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**Fig. 3.** Altered Endogenous Levels of 13-HODE (A) and 15-HETE (B) in the Epidermis of Control Guinea Pigs Fed a Borage Oil (BO) Diet for 10 Weeks, and EFA-Deficient Guinea Pigs Fed a Hydrogenated Coconut Oil (HCO) Diet for 10 Weeks or for 8 Weeks Followed by 2 Weeks of Feeding with Diets Containing Borage Oil (HBO), Organic Extract of Gromwell (HGO), or Water Extract of Gromwell (HGW).

Values are means ± SD (n = 10). Means with different letters differ, p < 0.05, n.d., not detected.
groups BO and HBO (Fig. 3B). The level of 15-HETrE in the epidermis of group BO was almost two-fold that of group HBO. Although groups HGO and HGW reversed the hyperproliferative state of the epidermis in EFA-deficient guinea pigs (Figs. 1 and 2), the level of 13-HODE in groups HGO and HGW was not different from that in group HCO. Furthermore, 15-HETrE was not detected in the epidermis of these two groups, confirming that although GLA was detected, the level of DGLA (the major substrate of 15-HETrE) in the epidermis of groups HGO and HGW was not high enough to change the level of 15-HETrE (Table 3, Fig. 3B). As detected in low levels, 15-hydroxyeicosatetraenoic acid (15-HETE), a 15-lipoxygenase metabolite of arachidonic acid known to be moderately proinflammatory,35 was not significantly different among the five groups (data not shown). No other lipoxygenase metabolites of AA, 12-HETE or 5-HETE, were detected in any of the groups.

Ceramide quantitation

In the epidermis, ceramides combined with cholesterol and free fatty acids, form the extracellular lamellar membrane structure of the epidermal barrier.1 Depletion of ceramides has been suggested to be an etiological factor for epidermal barrier disruption, an increase in transepidermal water loss (TEWL), and epidermal hyperproliferation.1–3,5 Quantiﬁcation of ceramides (Fig. 4) revealed that the level of ceramide synthesis in the epidermis was inversely correlated with epidermal hyperproliferation (Figs. 1 and 2). The level of ceramides was signiﬁcantly diminished in the hyperproliferative epidermis of HCO. In the reversed epidermis of groups HBO, HGO, and HGW, the level of ceramides was increased in all three groups in the order HGO > HGW > HBO, but the ceramide level of group HBO was signiﬁcantly less than that of normal control group BO.

Discussion

Reductions in body weight and epidermal hyperproliferation were established in guinea pigs fed a hydrogenated coconut oil diet (group HCO). Since signiﬁcant reduction in body weight in group HCO as evidence of EFA deﬁciency has been reported extensively in many previous reports,1,2,29,36 and the body weights and macroscopic appearances of groups BO, HBO, HGO, and HGW did not differ, in this study we focused on comparing the dietary effect of borage oil and gromwell on reversing epidermal hyperproliferation, which is also evidence of EFA.4,23 After feeding of borage oil (seed oil of Borago ofﬁcinalis, the major plant species of the Boraginaceae family) for 2 weeks, epidermal hyperproliferation disappeared in group HBO, the proliferation score (HCO: 5.6, HBO: 3.9, 69.6% of HCO) and thymidine incorporation into the epidermis (HCO: 411.7 cpm/μg DNA, HBO: 245.3 cpm/μg DNA, 59.6% of HCO) of group HBO being less than those of group HCO (Figs. 1 and 2). A similar anti-proliferative effect was observed in groups HGO and HGW. In preliminary dose-ﬁnding experiments with gromwell, no dietary effect was found at the 1% level, while 5% and 10% of gromwell reversed EFA deﬁciency in a non-dose-dependent manner, and hence 5% was used in the current study. In fact, dietary supplementation of either organic or water extracts from 5% gromwell for 2 weeks was more effective at reversing the hydrogenated coconut oil diet-induced epidermal hyperproliferation, since the proliferation scores of groups HGO and HGW were lower than those of group HBO (HBO: 3.9, HGO: 0.9, HGW: 1.5) (Fig. 2). Furthermore, thymidine incorporation into the epidermis of groups HGO and HGW was signiﬁcantly less than that of group HBO, as well as of normal control group BO, which was fed a borage oil diet for 10 weeks (BO: 194.7, HBO: 245.3, HGO: 143.8, HGW: 104.2) ([3H]thymidine cpm/μg DNA), demonstrating the novel observation that dietary supplementation of gromwell is capable of reversing the histological and biochemical symptoms of epidermal hyperproliferation.

The biological efficacy of borage oil in reversing epidermal hyperproliferation is largely explained by the relationship between the incorporation of linoleic acid (LA) (18:2n-6) and γ-linolenic acid (GLA) (18:3n-6), two major polyunsaturated fatty acids (PUFAs) of borage oil, into epidermal lipids, and the epidermal level of 15-lipoxygenase metabolites (hydroxyl fatty acids).33,34,37 In fact, the 15-lipoxygenase metabolite of LA, 13-hydroxyoctadecadienoic acid (13-HODE), appears to be necessary in regulating the proliferative rate of epidermal keratinocytes.37 Although not a major PUFA in epidermis, GLA is promptly elongated into dihomo-γ-linolenic acid (DGLA, 20:3n-6), followed by the 15-lipoxygenase enzyme into 15-hydroxyeicosatrienoic acid (15-HETrE) in the epidermis,36,38 which has biopotency superior to 13-HODE in anti-proliferation.39
Coinciding with the levels of LA, GLA, and DGLA incorporated into total lipids (Table 3), the levels of 15-lipoxygenase metabolites, 13-HODE, and 15-HETE in groups BO, HBO, and HCO were in the order BO > HBO > HCO, which reflects the levels of these PUFAs in the oils (borage oil vs. hydrogenated coconut oil), or the feeding period (10 weeks feeding of borage oil in group BO vs. 2 weeks feeding of borage oil in group HBO). 13-HODE and 15-HETE both have biopotency in anti-proliferation; thus the reversal of epidermal hyperproliferation in group HBO (Figs. 1, 2 and 4). When coupled with lower levels of LA and DGLA and of 15-lipoxygenase metabolites, 13-HODE and 15-HETE (not detected), in the epidermis of groups HGO and HGW than of group HBO (Table 3, Fig. 3), these results suggest that the reversal of epidermal hyperproliferation (Figs. 1 and 2) is to be attributed to an increase in ceramide synthesis rather than the increased level of PUFAs or their 15-lipoxygenase metabolites in the epidermis. These results agree with previous studies of lipid abnormalities in psoriasis, which reported that assessment of the lesional epidermis of psoriasis patients revealed no alteration in the levels of LA or other fatty acids as compared with the non-lesional epidermis of the same patients. On the other hand, the ceramide level was significantly reduced in the lesional epidermis of psoriasis patients, and a positive correlation was observed between the percentage reduction of ceramide synthesis and the clinical severity of the psoriasis. As well as being a structural component maintaining the integrity of the epidermal barrier, ceramides function as intracellular mediators of anti-proliferation and apoptosis. In fact, ceramides appear to modulate the activity of several direct targets, including ceramide-activated protein kinase (CAPK), ceramide-activated protein phosphatase (CAPP), and protein kinase C-ζ (PKCζ). These then couple the action of ceramides to intracellular responses, such as activation of pJNK (c-Jun-N-terminal kinase), inhibition of ERK, activation of NF-kB, activation of the retinoblastoma gene product (Rb), and regulation of c-Myc expression, ultimately inducing anti-proliferation and apoptosis.

Gromwell referred to as zicao in China, shikon in Japan, and jacho in Korea, has been used as an herbal medicine for the treatment of wounds and inflammation and the healing burns, and has also been used as a colorant in cosmetics, fabrics, and foods in diverse cultures. Additionally, it has been used for the production of traditional liquor (Jindo hongju) in Korea. As bioactive components, napthaquinones such as shikonin and its derivatives have been characterized mostly in organic extracts of gromwell, which have anti-proliferative activities in various cancer cells with several mechanisms of action, such as inhibition of DNA topoisomerase, induction of apoptosis by caspase-3 activation, and anti-proliferative activity with down-regulation of activated ERK and activation of pJNK. But in our study, both the organic extract and the water extract of gromwell showed anti-proliferative activity, which suggests that well-characterized bioactive components such as napthaquinones or fatty acids might be extracted.
with water as well as with organic solvents. On the other hand, glycans such as lithosperms A, B, and C have been identified in water extract of gromwell, which has been reported to have hypoglycemic activity in mice. Concerning the dermatological activities of carbohydrates, it has been found that levels of skin hydration and epidermal lipid are inversely correlated with the fasting plasma glucose level in patients with diabetes mellitus. Glycans such as lithosperms A, B, and C have also been reported to have hypoglycemic activity in mice. Concerning the dermatological activities of carbohydrates, it has been found that levels of skin hydration and epidermal lipid are inversely correlated with the fasting plasma glucose level in patients with diabetes mellitus. Glycans such as lithosperms A, B, and C have also been reported to have hypoglycemic activity in mice.

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