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Actions of Korean Ginseng and Benzoyl Peroxide on Inflammation Relevant to Acne*

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Abstract□ The intradermal injection of Propionibacterium acnes (ATCC 6919) into the ears of female Sprague-Dawley rats produced a chronic inflammation with the formation of acneiform lesions. Inflammation was characterized by more than four times of ear thickness and 2.8 times of ear weight at day 21. Histologically, massive infiltration of neutrophils, macrophage and lymphocytes, hyperplastic epidermis, comedones containing keratin mass and inflammatory materials were observed. Both ginseng sapoin and extract from Korean red ginseng significantly reduced the ear thickness and their effects were similar to that of benzoyl peroxide. Ginseng samples and benzoyl peroxide modified lipid constituents of P. acnes-injected rat ear tissues. Even though no marked histological changes in inflammatory lesions were observed in ginseng-treated ear tissues, Korean red ginseng showed a possibility of reduce in the risk of acne development.

Keywords□ Korean ginseng, benzoyl peroxide, inflammation, acne.

Introduction

Ginseng is a particularly interesting and valuable plant for topical application because of its mysterious effects on biological systems including skin[1-3], non-irritating[4,5] and water soluble property. From the ancient times, ginseng has been used as a part of oriental prescription for some skin disorders[6]. Even though the mechanisms of action for treatment have not been clarified, ginseng is still favorably used for cutaneous disorders as a part of oriental medicine. Acne belongs to these kinds of skin diseases. There were a few studies on ginseng and skin disorders. In 1954, 10% ginseng alcohol extract cured eczema with itching but the effect was not consistent[7]. In 1971, cold cream containing ginseng root controlled eczema and wrinkles[8]. In 1980's, anti-inflammatory effect of ginseng saponin ointment[9] and the effect of ginseng powder and saponin on *Candida albicans*[10] were reported.

Acne is a disease that uniquely affects man[11]. A widely accepted hypothesis concerning the pathogenesis of acne states that irritation due to free fatty acids in the follicle leads to an inflammatory response, including hyperkeratinization of the epithelial cells lining the wall of the follicle, and thus ultimately to the acne lesions[12]. Even though the lack of an exact duplicate of acne in the animal kingdom has seriously hindered research progress, there are several existing models of acne; rabbit ear comedogenesis[13], the Mexican hairless dog[14], and human back skin occlusion[15]. Whereas all of these models are characterized by the formation of comedones like acne in humans, none approximates the chronic inflammation seen in human inflammatory acne. De Young *et al.*[16] developed Propionibacterium acnes-induced rat ear inflammation model as a simple system of chronic inflammation with relevant to human inflammatory acne. Recently, the ef-

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fect of ginseng on rabbit ear comedogenesis was studied and it was demonstrated that *Panax ginseng* extracts might have effects on keratinization process of the skin by decreasing the sizes of comedones and loosening the desquamating horny cells in comedones[17]. In our previous study[18], ginseng saponin prevented experimental hyperkeratosis in guinea pig skin by controlling enzyme activities involved in epidermal cellular metabolism and reducing abnormally accumulated lipids in epidermis.

Present study was designed to determine the effect of Korean ginseng on Propionibacterium acnes-induced rat ear inflammation by measuring ear thickness and ear weight, analysis of lipid constituents of ear tissues as well as histological examination. Their effects were compared to that of benzoyl peroxide which has been effectively used for acne treatment.

Materials and Methods

Materials

Gram staining kit (Accustain), thimerosal, benzoyl peroxide, squalene, triolein, oleic acid, cholesterol, phosphatidyl choline, hematoxylin, eosin and glutaraldehyde were obtained from Sigma Chemical Co. (St. Louis, MO). Paraplast tissue embedding medium was purchased from Monoject Co. (St. Louis, MO). Propioni bacterium acnes (ATCC 6919) was obtained in a lyophilized state from American Type Culture Collection (Rockville, MD). Brain heart infusion media and peptone from Difco Co. (Detroit, MI), and anaerobic jar and gas pak from BBL Microbiology Systems, Beckton Dikinson Co. (Cockeysville, MD) and silica gel 60F 254 plate (0.2 mm) from Merck Chemical Co. (Darmstadt, Germany) were purchased. Triglyceride test kit from Iatron Laboratories, Inc. (Tokyo, Japan) and non-esterified fatty acid test kit from Nissui Pharmaceutical Co. (Tokyo, Japan) were used. Ginseng saponin was prepared from powdered Korean red ginseng by the procedure described by Ando *et al.*[19]. Water extract of Korean red ginseng was obtained from Korea Ginseng Factory, Korea Tobacco & Ginseng Coorporation.

Rats

Female Sprague-Dawley rats, 100-120 g, were obtained from the animal breeding room of the Korea Ginseng & Tobacco Research Institute and were allowed free access to food and water throughout the experimental period.

Bacteria sample

Propionibacterium acnes (ATCC 6919) was purchased and grown on brain heart infusion media, pH 7.4 at 25°C. Bacteria from post log phase cultures was harvested, heat-killed (95°C, 5 min) and lyophilized, prior to injection.

Injection of bacteria

Bacteria was diluted to a final concentration of 7 mg/ml in physiologic saline containing 0.01% thimerosal as a preservative. Saline control also contained 0.01% thermerosal. With 28-gauge needle, bacteria was injected intradermally in 20 μl aliquots (140 μg) in the central, ventral portion of the right ears of rats.

Sample treatment

ginseng saponin, water extract from Korean red ginseng and benzoyl peroxide were dissolved in 90% acetone, 50% acetone and 100% acetone, respectively. Samples were applied topically to the right ear in a total volume of 40 μl (20 μl to each side of ear) immediately after P. acnes injection. Ginseng samples (0.4 mg) and benzoyl peroxide (4 mg) were applied daily and bacteria (140 μg) or saline (20 μl) was injected every other day during 21 days.

Ear thickness and ear punch weight

Ear thickness was measured at day 21 using a micrometer (Mitutoyo MFG. Co., Japan). After rats were sacrificed, ear punches were weighed (1.2 cm diameter punch / animal). Ears of 8 rats were measured and punched for each group.

Lipid extraction, seperation and determination of lipid classes

Ear punch tissue (6 rats / group) was minced

Table 1. The effect of ginseng and benzoyl peroxide on *P. acnes*-induced ear thickness

	Ear thickness (mm)	% Saline
Saline	0.8800±0.046	100
Bacteria (B)	3.6500±0.225	414
B+ Saponin	2.5967±0.185*	295
B+BP	2.4575±1.020*	279
B+ Extract	2.7363±0.190*	311

Ginseng samples (0.4 mg) and benzoyl peroxide (BP; 4 mg) were topical applied daily for 21 days. *P. acnes* (140 μg) was intradermally injected every other day during experimental period. Data are expressed as mean±SE of 8 rats. An asterisk indicates a value significantly different from bacteria value by Student's t-test, *; $p<0.005$.

Table 2. The effect of ginseng and benzoyl peroxide on *P. acnes*-induced ear weight

	Wt/punch (g/1.2 cmD.)	% Saline
Saline	0.0954±0.0027	100
Bacteria (B)	0.2267±0.0352	280
B+ Saponin	0.2137±0.0178	224
B+BP	0.2085±0.0092	219
B+ Extract	0.1964±0.0186	206

Ginseng samples (0.4 mg) and benzoyl peroxide (BP; 4 mg) were topical applied daily for 21 days. *P. acnes* (140 μg) was intradermally injected every other day during experimental periods. Data are expressed as mean±SE of 8 rats.

with scissors (<1 mm^3), extracted with chloroform/methanol (2:1), dried under nitrogen gas and stored at −20°C until use. The determination of the lipid classes was accomplished by thin-layer chromatography (TLC) seperation[20]. Exactly 100 μg of each lipid extract was applied to a thin-layer plate, 20×20 cm, coated with a 0.2 mm thick layer of silicagel G that had been cleaned by development with chloroform/methanol (2:1). The chromatogram was first developed with hexane/diethylether/acetic acid (80:20:2) to 6-cm level and then with hexane/diethylether (95:5) to 10-cm level; furthermore, relatively polar lipids on the plate were developed to 10-cm level with hexane. After drying, the chromatogram was sprayed with 30% aqueous sulfuric acid and charred by heating at 105°C for 20 min in a convective oven. After cooling, the charred chromatogram was quantitated by an absorbance reflection mode, 530 nm wavelength, and a zigzag scanning on a photo densitometer (CS-910, Shimadzu, Koyto) equipped with an integrator (DR-2, Shimadzu). The amount of each lipid class was calculated as percentage of total lipid by measurement of the densitometric area for each spot; the area sizes were proportional to the weights of the respective constituents in the lipid mixture. For spectrophotometric analysis of triglyceride and free fatty acid, evaporated extract was redissolved in 20 μl and 50 μl of methanol for triglyceride (μg/mg lipid) and free fatty acid (nEq/mg lipid).

Histological examination

Rat ear tissue was fixed in 10% neutral buffered formalin and dehydrated with a graded series of alcohols before embedding in paraffin. Sections, 5 μ in thickness, were stained with hematoxylin and eosin and viewed under the light microscope. Microphotographs were taken with a Nikon Labphot Microscope (Tokyo, Japan).

Results and Discussion

Ear thickness and ear punch weight

After 10 time-injection with 140 μg of P. acnes during 21 days, ears were more than 4 times thicker and 2.8 times heavier than saline-injected controls at the site of injection (Table 1, 2). Ear thickness data was similar to the result reported by De Young *et al.*[16]. They found that ear thickness was a characteristic factor of inflammation relevant to acne and caused by massive infiltration of neutrophils, macrophages and lymphocytes, inter-and intracellular edema and hyperplastic cords of epithelium in follicle overlying the inflammed area. Perisho *et al.*[21] reported that hyperplastic follicular epithelium impaired barrier function which could favor bacterial growth in the follicle by increasing the availability of water. These papers support heavier ear tissues by bacteria injection even though the value was not significantly different from saline control. Both ginseng saponin and extract from Korean red ginseng effectively reduced the ear thickness ($P<0.005$) and their effects were similar to that of

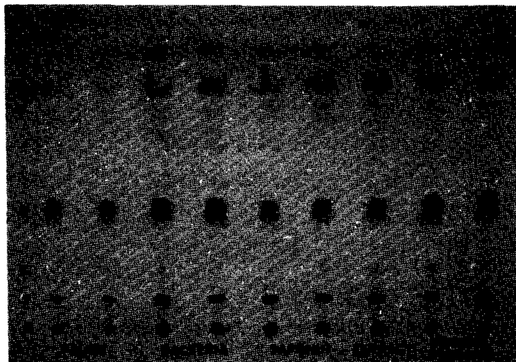

Fig. 1. Thin-layer chromatogram showing seperation of tissue lipids of rat ears. A, squalene; B, cholesterol esters; C, wax esters; D, triglyceride; E, free fatty acids; F, cholesterol; G, phospholipid.

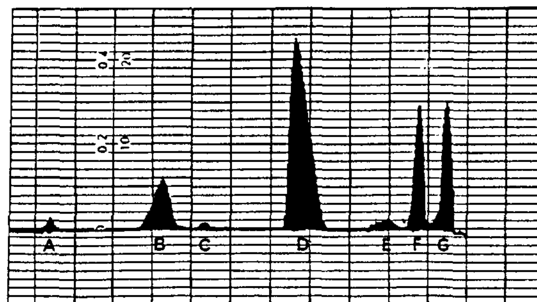

Fig. 2. Densitomertric scanning pattern of the charred thin-layer chromatogram of the tissue lipid of rat ear. A, squalene; B, cholesterol ester; C, wax ester; D, triglyceride; E, free fatty acids; F, cholesterol; G, phospholipid.

benzoyl peroxide. However, ear weights of bacteria-injected sites were not significantly reduced by topical application of ginseng samples or benzoyl peroxide.

Lipid constituents of rat ear tissues

The major factors involved in the pathogenesis of acne are sebum production, follicular bacterial microflora, follicular epithelial differentiation and inflammation[11]. Two possible mechanisms by which inflammation may be produced in acne are (a) release of free fatty acids by bacterial lipases from triglycerides in sebaceous material and (b) local destruction of cells and attraction of phagocytes by activation of the complement system by P. acnes antigen[22]. Free fatty acids produced may act as tissue irritants and promote the growth of P. acnes. They appear to be chemotactic to neutrophils and macrophages and may be cytotoxic to these cells[11]. Although the significance of free fatty acids in the generation of inflammation has been questioned recently, it is unquestionably agreed that effective antibiotic theraphy results in reduction in levels of P. acnes and free fatty acids. Therefore, we compared changes in lipid composition of rat ear tissues after sample treatment on bacteria-injected sites. As shown in Fig. 1, 2 tissue lipids were seperated into seven classes; squalene, cholesterol esters, wax esters, triglyceride, free fatty acids, cholesterol and phospholipid. Densitometric scanning pattern of the charred thin-layer chromatogram of bacteria-injected tissue showed larger peaks in almost all classes of lipids than those of saline-injected tissues. In comparison of total lipid contents by summation of densitometric area for each spot, saline group had 62.5 ± 3.8 and bacteria group had 92.8 ± 28.6. This demonstrated that total lipids increased by bacteria injection, which may be due to infiltration of foreign cells such as macrophages, neutrophils and lymphocytes and hyperplastic sebaceous follicles. Table 3 showed that each value in each class was significantly different between bacteria group and saline group except for cholesterol at $p = 0.005$. In only bacteria-injected group, free fatty acid (2.3 time of saline value), squalene (1.3 time), and phospholipid (1.4 time) increased while wax esters (0.58 time), triglyceride (0.75 time) and cholesterol esters (0.78 time) decreased as compared to saline-injected animals. Treatment of ginseng samples or benzoyl peroxide on bacteria injected tissues reduced free fatty acid to 69-75% of bacteria value. Since bacteria injection increased free fatty acids, the ratio of free fatty acids (FFA)/ triglyceride(TG) were compared in all groups by two ways; (1) densitometric areas of charred chromatogram of TLC, (%/%) and (2) spectrophotometric analysis of 1 mg of tissue lipid, (nEq/μg) (Fig. 3). Bacteria injection significantly increased the ratio of FFA/TG and treatment of ginseng samples or benzoyl peroxide reduced the

Table 3. The biochemical composition of tissu lipids on rat ears

	Squalene (%)	Cholesterol esters (%)	Wax esters (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)	Phospholipid (%)	Total lipid (%)
Saline	3.2±0.22	9.2±0.46	2.4±0.24	52.4±1.02	6.0±0.06	13.6±0.25	13.2±1.20	100
Bacteria (B)	4.2±0.17	7.2±0.41	1.4±0.10	39.4±1.67	13.8±0.46	15.7±1.16	18.3±1.01	100
B+Saponin	3.6±0.54	7.4±0.54	1.8±0.33	44.1±1.51*	10.4±0.30**	14.0±0.34	18.7±1.56	100
B+Extract	3.6±0.22	6.6±0.87	1.6±0.34	40.1±1.89	10.3±0.47**	16.6±0.91	20.9±1.48	100
B+Benzoyl Peroxide	4.1±0.76	8.1±1.35	1.7±0.40	41.7±1.60	9.5±0.16**	17.3±0.98	17.6±1.06	100

Ginseng samples (0.4 mg) and benzoyl peroxide (4 mg) were topically applied. Saline or bacteria(140μg) was intradermally injected. Lipid extract from rat ear punch was separated by TLC and measured by the densitometric area from charred chromatogram. Data are expressed as mean±SE of 6 rats. Asterisk indicates values significantly different from bacteria value;*: $p<0.05$.** : $p<0.005$ All values of bacteria group were significantly different from saline group except for cholesterol at $p=0.0005$.

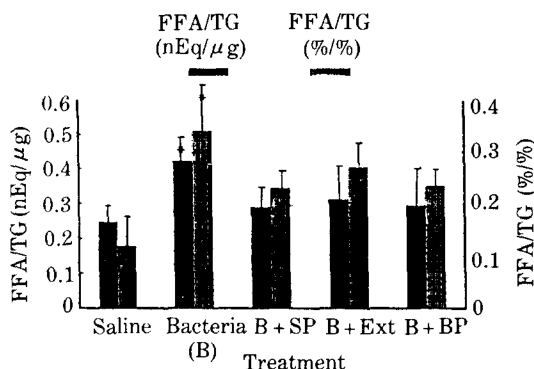

Fig. 3. Comparison of the ratios of free fatty acids/triglyceride from tissue lipids of rat ears. Ratios of FFA/TG were calculated from two ways: 1) densitometric areas of charred chromatogram of TLC, (%/%) and 2) spectrophotometric analysis of 1 mg of tissue lipid extract, (nEq/μg). An asterisk indicates a value significantly different from saline value by Student's t-test, *; $p<0.05$.

ratio of FFA/TG to the ratio in saline group.

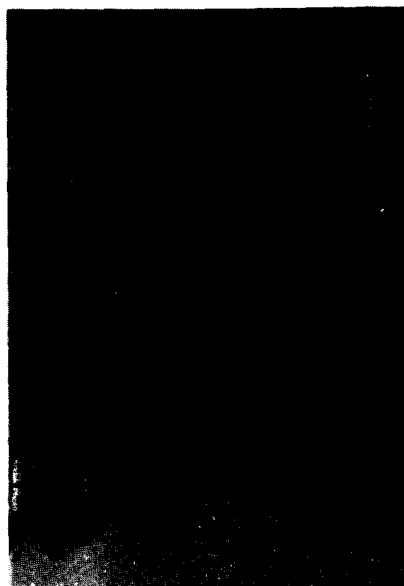

Fig. 4. Immediately after injection of P. acnes. Arrows indicate bacteria deposition as a baspophilic mass. E, epidermis; D. dermis; C, cartilage of rat ear (×100).

Histological examination

When animals were sacrificed immediately after injection (Fig. 4), deposited P. acnes (arrows) could be readily identified as a basophilic mass. Epidermis (E), dermis (D) and cartilage (C) of rat ear were clearly shown. Fig. 5 shows saline-injected ear at day 21. Ten times of injection with saline caused slight inflammation (arrows) at injected site. At day 21, bacteria injected tissues exherted hyperplastic epidermis (E), massive infiltration of neutrophils, macrophages and lymphocytes and comedones (CM) containing keratin mass and necrotic inflammatory materials. Adipocytes(A) were appeared in the dermis(D). The dramatic change was hyperplastic pilosebaceous follicle (arrows) (Fig. 6). In Fig. 7, encapsulating cords(EC) of epithelium overlying the

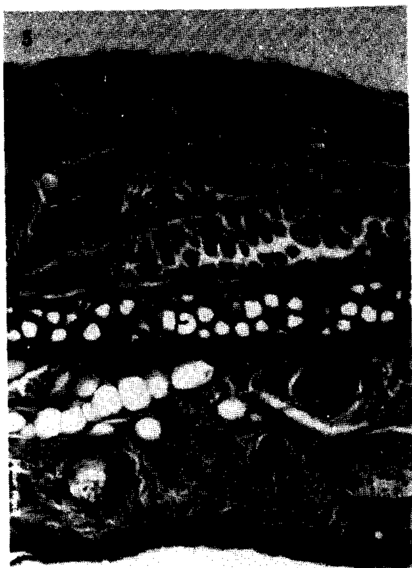

Fig. 5. Saline-injected ear tissue at day 21. Slight inflammation (arrows) at the injected site was shown (×100).

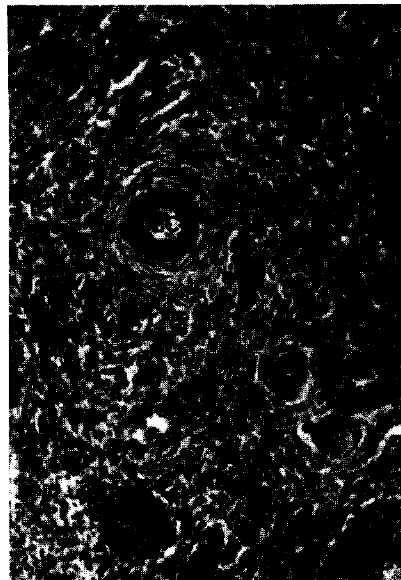

Fig. 7. Encapsulating cords (EC) of epithelium overlying the inflamed area were appeared in bacteria-injected ear tissue (×100).

Fig. 6. Bacteria-injected ear tissue at day 21. Hyperplastic epidermis (E), infiltration of neutrophils, macrophages and lymphocytes, adipocytes (A) and hyperplastic pilosebaceous follicles (arrows) were shown. Commedone (CM) containing keratin mass and necrotic inflammatory material was protruded at the surface of the skin (×100).

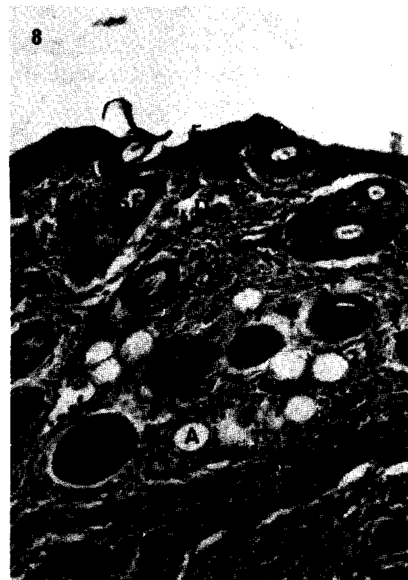

Fig. 8. Ginseng saponin-treated rat ear tissue with bacteria injection at day 21. Hyperplastic sebaceous folicles (SF), adipocyte (A), hyperplastic epidermis and encapsulating cords (EC) of epithelium were exherted (×100).

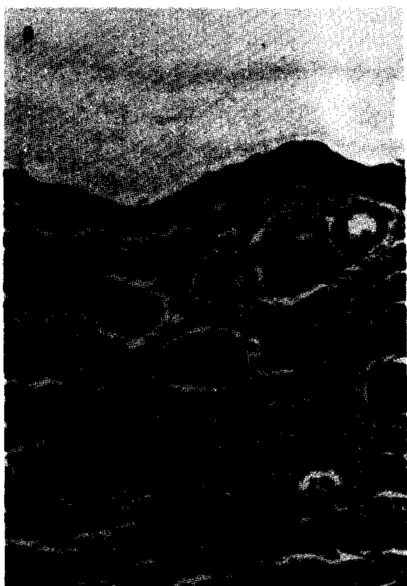

Fig. 9. Ginseng extract-treated ear tissue with bacteria injection at day 21. No histological changes other than bacteria-induced inflammation were shown (×100).

Fig. 10. Benzoyl peroxide-applied ear tissue with bacteria injection at day 21. Benzoyl peroxide did not change bacteria-induced inflammatory alterations (×100)

inflammed area were shown. Treatment of ginseng saponin (Fig. 8), ginseng extract (Fig. 9) and benzoyl peroxide (Fig. 10) did not change these bacteria-induced alterations in ear tissues. Hyperplastic sebaceous follicles(SF), adipocyte(A), encapsulating cords(EC) of epithelium, hyperplastic epidermis and infiltration of foreign cells such as macrophages, neutrophils and lymphocytes were also shown. These kinds of histological changes by P. acnes-injection were also reported by De Young *et al.*[16].

Conclusively, ginseng saponin and extract from Korean red ginseng reduced ear thickness and modified lipid constituents of P. acnes-injected rat ear tissues and these effects were similar to that of benzoyl peroxide. Even though marked histological changes on inflammatory lesions could not be detected in ginseng-treated groups, Korean red ginseng showed a possibility of reduce in the risk of acne development in humans.

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