Korean J. Ginseng Sci.
Vol. 13, No. 2, 248–253(1989)

Effect of 13-*cis*-Retinoic Acid and Ginseng Saponin on Hyperkeratinization of Guinea Pig Skin*

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(Received October 17, 1989)

Abstract□The effects of 13-*cis*-retinoic acid and ginseng saponin from Korean red ginseng on hyperkeratinization of guinea pig skin were investigated by means of enzymatic analysis and light microscopic observation. To induce hyperkeratinization, hexadecance was topically applied to the dorsal skin of female guinea pigs every other day for eight days and 13-*cis* retinoic acid or ginseng saponin solution was administered orally or topically applied daily during the experimental period. As a result, both topical application of ginseng saponin and oral administration of 13-*cis*-retinoic acid showed preventive effects on hyperkeratinization while topical application of 13-*cis*-retinoic acid inhibited normal epidermal cell proliferation and reduced epidermal enzyme activities such as LDH, ICD and G6PDH below the levels in a normal epidermis. It is suggested that topical application of ginseng saponin and oral administration of 13-*cis*-retinoic acid may have beneficial effects against hyperkeratinization possibly by controlling epidermal proliferation and enzyme activities related to epidermal energy metabolism.

Keywords□13-*cis*-retinoic acid, ginseng saponin, hyperkeratinization, guinea pig skin.

Introduction

In modern cosmetic development, there is a trend toward formulations containing substances with some therapeutic efficacy. With this point of view, ginseng is particularly interesting and valuable plant for application because of its mysterious effects in biological systems including skin,[1-3] non-toxic, non-irritating[4,5] and water-soluble properties which make it easy incorporation into cosmetic and pharmaceutical products.

Skin is the largest organ of the body as determined by its wet weight or by its surface area and an active site of the biotransformation of a variety of endogeneous substances such as steroid hormones, cholesterol and foreign xenobiotics[6]. Skin has a most complex structure which is nourished by a dense network of blood capillaries in the dermis and affected by various factors[7]. Aging of skin, especially, being inevitable and irrevocable, is a subject of vital importance to all. Ginseng extract showed activating action on sagging and wrinkled skin as well as on dry, greasy and acneic skin[8]. Anguelakava *et al.*[9] suggested that active components of ginseng for cutaneous bioactivation may be estrone, estradiol and estriol in lipid fraction of ginseng. Recently liposomes made of phospholipid and ginseng extract or ginseng saponin were developed for enhancing general metabolic events in skin aging such as loss of elasticity, hydration and circulatory derangements[10-12].

Even though ginseng has been widely used for skin disorders in Oriental medicine and is applied for modern cosmetic products, mechanism of action of ginseng has not been clarified. Therefore, this study was designed to determine the effect of ginseng saponin, main effective component of ginseng, on experimentally induced hyperkeratinization in guinea pig skin. In addition, the effect of ginseng saponin was compared with that of 13-cis-retinoic acid which is used for treatment of cutaneous disor-

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ders and keratinization[13].

Materials and Methods

Materials

n-Hexadecane, 13-cis-retinoic acid, triethanolamine, glucose-6-phosphate, NADP, lactate dehydrogenase test kit, isocitrate dehydrogenase test kit, hematoxylin and eosin were obtained from Sigma Chemical Co. (St. Louis, MO). Paraplast tissue embedding medium was purchased from Monoject Co. (St. Louis, MO). All other reagents used were of guaranteed reagent grade commercially available. Ginseng saponin was prepared from water-saturated buthanol fraction of Korean red ginseng, followed by repeated filtration after adding activated charcoal and methanol to obtain a pure yellow powder[14-15].

Animal treatment

Female Hartley guinea pigs, 450-500g, from Sam Yuk Animal Breeding Lab. (Osan, Korea), were kept under conventional laboratory conditions with commercial laboratory chow (Jeil Animal Food, Ind.), tap water and fresh vegetables ad libitum and used after 10 day of acclimation. Dorsal hairs, over an area of approximately 8 cm^2, were shaved with electric clipper 1 day prior to the experiment. 0.5 m*l* of 13-cis-retinoic acid (0.2% alcoholic solution) or ginseng saponin (2% alcoholic solution) were topically applied to dorsal skin of guinea pig 1 hr before the application of n-hexadecane (2 m*l*/kg B.W.) every day for 8 days. Oral administration of 0.5 m*l* 13-cis-retinoic acid (0.2% dissolved in corn oil) was also given to guinea pig daily 1 hr before topical application of n-hexadecane. n-Hexadecane was administered every other day during experimental period. 0.5 m*l* of ethanol was topically applied daily as a vehicle (Fig. 1). To minimize chemical and photodegradation, all procedures were performed under reduced lighting and 13-cis-retinoic acid solutions were prepared freshly. Animals were housed under subdued light and were killed by cervical dislocation at the 8th day of experiment.

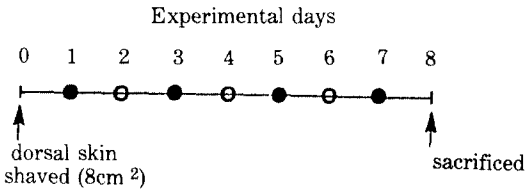

Fig. 1. The experimental model is shown. Twenty five female guinea pigs were divided into five groups; vehicle group, hexadecane group, hexadecane group with topical application of 13-cis-retinoic acid or ginseng saponin and hexadecane group with oral administration of 13-cis-retinoic acid. Closed circles (●) denote the days of n-hexadecane and 13-cis-retinoic acid or ginseng saponin while open circles (⊖) are the days of 13-cis-retinoic acid or ginseng saponin application.

Epidermal separation for enzyme assay

The treated areas of skin were excised, and the skin was immedeately immersed in ice-cold water, placed in water bath (55 °C, 30 sec), cooled in ice water and then blotted dry with filter paper. After stratum corneum of epidermis was removed with forceps, epidermis was scraped off from dermis with scalpel blade on cold glass plate[16], homogenized in 0.1M triethanolamine buffer (pH 7.6) and centrifuged (2,000rpm, 5 min, 0-4 °C) to obtain a soluble epidermal extract. Glucose-6-phosphate dehydrogenase activity in epidermal extract was determined by the method described in Biochemica information (Boehringer Mannheim Biochemica, W. Germany). Epidermal lactate dehydrogenase and isocitrate dehydrogenase activities were measured by commercial test kits (Sigma Chemical Col, St. Louis, MO). Protein concentration was determined by Lowry *et al.*[17] using bovine serum albumin as a standard.

Skin histology

Biopsies (4 mm diameter) were taken from treated areas of dorsal skin. They were fixed in 10% neutral buffered formalin and dehydrated with a graded series of alcohols before embedding in paraffin. Sections, 5μ in thickness, were stained with hematoxylin and eosin and viewed under the light microscope. Microphotographs were taken a Nikon Labphot Microscope (Tokyo, Japan).

Results and Discussion

Enzyme activities in guinea pig epidermis

Topical application of various noncarcinogenic hydrocarbons and mineral oils to mammalian skin led to hyperplasia and hyperkeratinization[18,19]. Of the saturated, straight-chained hydrocarbons, hexadecane is reported to induce the most marked changes in the skin of guinea pigs; reddening, cellular hypertrophy, maked increases in epidermal mitotic activity, hyperplasia of vital layers, and marked keratinization of the epidermal surface and hair follicles with an abnormal type of keratin[20].

As shown in Table 1, hexadecane treatment significantly increased epidermal enzyme activities such as LDH, ICD, and G6PDH. Lactate dehydrogenase (LDH) is quite active in mammalian epidermis[21] and reflects well epidermal metabolic activity. Isocitrate dehydrogenase (ICD), which belongs to aerobic krebs cycle and is one of the sources of NADPH in the cell[22]. Glucose-6-phosphate dehydrogenase is a key enzyme of the hexose monophosphate shunt pathway, in which ribose and NADPH are produced and used for cell metabolism[23]. The activity of this enzyme is correlated to epidermal cell proliferation. With this regards, hexadecane produced epidermal cell proliferation possibly by activating enzyme activities. Both oral administration of 13-cis-retinoic acid and topical application of ginseng saponin inhibited abnormal increment in enzyme activities induced by hexadecane. However, topical application of 13-cis-retinoic acid even reduced epidermal enzyme activities below the levels shown in control epidermis treated with ethanol solution alone.

Retinoids are widely used in dermatological therapy despite their mechanisms of action in skin are unknown. Among their effects on the skin the well known ability of retinoids is to induce epidermal hyperplasia[24]. And they also has a tendency to attribute to toxicity or as secondary to inhibition of epidermal differentiation which has been observed in some studies *in vitro*[25]. In this study, it is thought that topical application of 0.2% 13-cis-retinoic acid solution inhibits normal epidermal cellular proliferation, which is supported by the fact that oral use of 13-cis-retinoic acid shows great effectiveness against severe recalcitrant nodulocystic acne but the drug is not effective topically[26].

Histological findings

The skin is composed of two principal layers; epidermis(E) and dermis(D). The epidermis is thin and consists of five layers or zones; stratum basal which rests on a basement membrane, stratum spinosum, stratum granulosum, stratum lucidium and outer stratum corneum (SC) of dead, flattened cells which are constantly desquamating off the surface[27] (Fig. 2). Keratinization is the process whereby the living cells undergoing mitosis in the stratum basale (SB) and one of the daughter cells become converted to the dead cells of the stratum corneum (SC)[28]. As shown in Fig. 3 and 4, hexadecane induced hyperkeratinization by increasing epidermal

Table 1. Enzyme activities in female quinea pig epidermis

Treatment	LDH	ICD	G6PDH
Control	395.2 ± 118.5	34.1 ± 8.1	0.5175 ± 0.3189
Hexadecane (HD)	1008.3 ± 226.5	54.1 ± 21.4	1.9459 ± 0.8800
HD + 13-cis-RA[1]	175.6 ± 40.9**	5.8 ± 3.5**	0.0588 ± 0.0194**
HD + 13-cis-RA[2]	505.8 ± 119.2**	23.5 ± 15.2*	0.9067 ± 0.1350*
HD + Saponin	648.4 ± 258.7*	28.0 ± 10.4*	1.0151 ± 0.4913*

Values represent mean ± SD. An asterisk indicates values significantly different from hexadecane group. *, $p<0.05$, **, $p<0.005$. Enzyme activities are expressed as nmole/min/mg for G6PDH (glucose-6-phosphate dehydrogenase), B-B units/mg for LDH (lactate dehydrogenase) and Sigma units/mg for ICD (isocitrate dehydrogenase). 1. Topical application, 2. Oral administration.

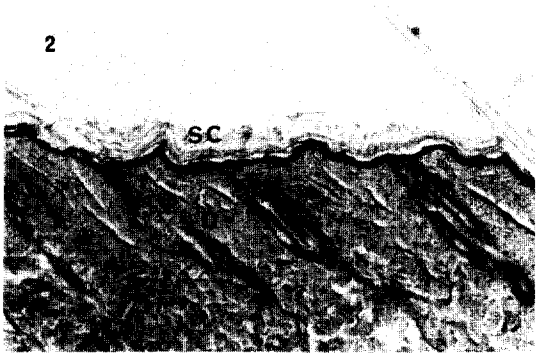

Fig. 2. Light micrograph of whole skin of control guinea pig. The skin is composed of epidermis (E) and dermis (D). The outmost stratum corneum (SC) of epidermis appear anucleated and filled with a fibrous material (×40).

Fig. 3. Hexadecane treatment resulted in mitosis in stratum basale (SB) and daughter cells become converted to the dead cells of the stratum corneum (SC) (×40).

mitotic activity and forming abnormal type of keratin. Oral administration of 13-cis-retinoic acid reduced the thickness of hexadecane-induced enlarged epidermis even though epidermis had large numbers of cornified cells (stratum corneum) (Fig. 5).

Fig. 4. Increased abnormal type of keratin was shown in stratum corneum (SC) of hexadecane treated skin (×40).

Fig. 5. Oral administration of 13-cis-retinoic acid reduced the thickness of hexadecane-induced enlarged epidermis (×40).

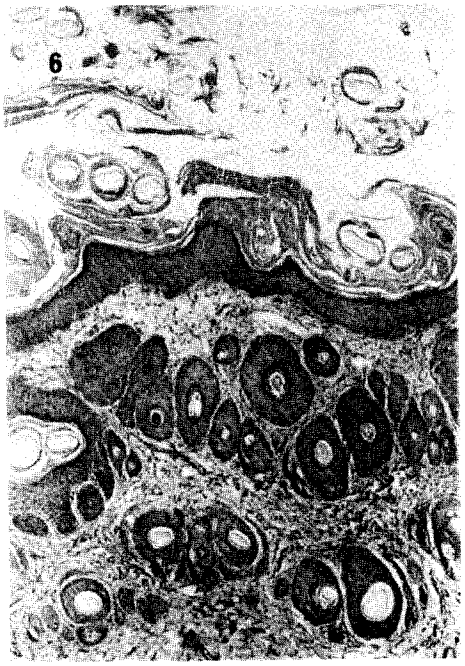

Fig. 6. Topical application of ginseng saponin showed preventive effect against hyperkeratinization by reducing epidermal thickness (×40).

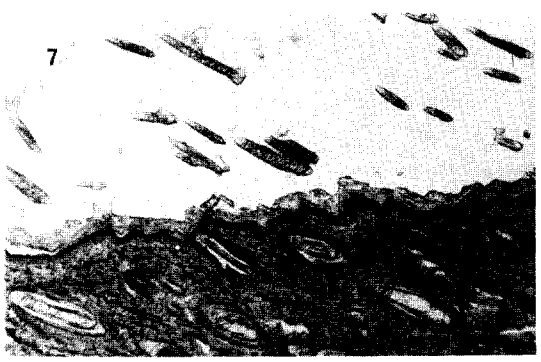

Fig. 7. 13-cis-retinoic acid applied skin had abnormally thinner epidermis than that shown in control epidermis (×40).

Retinoids are used for certain diseases of the skin such as acne, psoriasis, Darier's disease and ichthyosis by increasing cell turnover, resulting in the production of a less cohesive horny cell layer, which is more easily peeled off[26]. When ginseng saponin solution was topically applied to dorsal skin of hexadecane treated guinea pig, similar effect or more beneficial effect was shown against hyperkeratinization (Fig. 6). However, topical application of 13-cis-retinoic acid resulted in thinner epidermis than that shown in control epidermis (Fig. 7).

In conclusion, topical application of ginseng saponin and oral administration of 13-cis-retinoic acid may have protective effects against experimentally-induced hyperkeratinization by controlling epidermal cell proliferation and energy-related enzyme activities in epidermis.

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