Chyawanprash, a formulation of traditional Ayurvedic medicine, shows a protective effect on skin photoaging in hairless mice

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ABSTRACT

OBJECTIVE: Chronic exposure to ultraviolet (UV) radiation induces skin photoaging (premature skin aging). UV irradiation generates reactive oxygen species (ROS), which are shown to play a pivotal role in skin photoaging. Ayurveda is a holistic traditional medical system, and Chyawanprash is one of the most popular formulations in Ayurveda. Since maintenance of the function and appearance of skin is important, we examined whether Chyawanprash has a protective effect on skin photoaging.

METHODS: To examine the effect of Chyawanprash on skin photoaging, hairless mice were administered with Chyawanprash in drinking water for 3 weeks, and then repeatedly exposed to ultraviolet light B (UVB) irradiation (225 or 450 mJ/cm²) to induce skin photoaging. To further examine the function of Chyawanprash, its effects were examined in cells cultured in vitro. Chyawanprash was added in culture medium, and examined for the effect on the growth of human keratinocytes, and for the ability to eliminate ROS which generated by paraquat (50 μmol/L) in HeLa cells.

RESULTS: UVB irradiation caused symptoms such as rough skin, erythema, and edema on the skin in hairless mice, but administration of Chyawanprash relieved these symptoms. Further, Chyawanprash significantly suppressed epidermal thickening, a typical marker of skin photoaging, in mice. We then analyzed the effect of Chyawanprash in human cells in culture, and found that Chyawanprash enhanced the growth of human keratinocytes, and efficiently eliminated ROS, which are causally involved in skin photoaging, in HeLa cells.

CONCLUSION: These findings suggested that Chyawanprash may have beneficial effects on slowing skin photoaging.

Keywords: medicine, Ayurvedic; Chyawanprash; photoaging of skin; reactive oxygen species

1 Introduction

Human beings have successfully used natural plants as a source for treatment of diseases and injuries from ancient times. The accumulation of clinical and research data has led to the establishment of various regional medical systems. Among them, Ayurveda, which means the science of longevity in Sanskrit, was established approximately 5000 years ago in the Indian subcontinent. Ayurveda is a holistic medical system and also is an effective healthcare system; thus, it is recommended as a reliable and economical medical system by the World Health Organization (WHO)[1]. A great variety of herbs are used for various purposes in Ayurveda systems. The effective ingredients of these herbs are now being identified by use of modern analytical methods and assay systems.

Rasayana, which constitutes one of the major categories of Ayurveda, aims to maintain youth, vigor and vitality in the body. Among the Rasayana recipes, Chyawanprash (also spelled Chyavanaprash, Chyavanaprasha, Chyavanaprasam, etc) is a comprehensive herbal tonic with multiple health benefits, and is widely consumed as a dietary health supplement in India[2,3]. Chyawanprash strengthens the functions of the liver, heart, immune system and the respiratory system[4-6]; improves memory and learning ability[7,8]; reduces postprandial glycemia and blood cholesterol[9] and shows antioxidant, antimutagenic and anticarcinogenic activities[10–12].

Since Chyawanprash has been shown to have various beneficial effects on human health, we examined whether it also shows a protective effect on skin photoaging (premature skin aging), which is induced by ultraviolet (UV) irradiation[13]. Skin photoaging is characterized by wrinkles, laxity, uneven pigmentation, altered connective tissue, increased skin thickness and other symptoms[14]. UV radiation from sunlight is divided into three categories: ultraviolet light A (UVA: 320–400 nm), ultraviolet light B (UVB: 290–320 nm), and ultraviolet light C (UVC: 100–290 nm). UVC light is mostly absorbed by the ozone layer and weakly affects skin; UVB light affects the superficial layer of the skin and causes sunburn; UVA light is less energetic but penetrates deeper in the skin than UVB light[15]. Exposure to UVB radiation strongly induces skin photoaging in hairless mice[16-20]. Here we report the protective effect of oral administration of Chyawanprash against skin photoaging in hairless mice and also its beneficial effects on the growth of human cells in culture.

2 Materials and methods

2.1 Chyawanprash

The recipe for Chyawanprash employed in this study was derived from the Chyabanprash recipe described in Bangladesh National Ayurvedic Formulary of Ayurvedic Medicine[24] with minor modifications. The mixture of 32 dried herbs (300 g) was extracted with 1 L of hot water for 5 h, filtered, concentrated to half the volume and again filtered, yielding the liquid form of Chyawanprash. The herbs included in the recipe were Adhatoda vasica (bark), Aegle marmelos (root bark), Asparagus racemosus (root), Boerhaavia diffusa (whole herb), Cinnamonomum tamala (leaf), Cinnamonomum zeylanicum (bark), Desmotrichum fimbriatum (root), Dsmodium gangeticum (whole herb), Elettaria cardamomum (seed), Emblica officinalis (fruit), Gmelina arborea (root bark), Hedychiump spicaturn (rhizome), Ipomoea paniculata (rhizome), Mesua ferrea (flower), Nymphaea cyanea (flower), Phaseolus trilobus (whole herb), Phyllanthus niruri (whole herb), Piper longum (seed), Premna integrifolia (root bark), Proxylim indicum (root bark), Pierocarpus santalinus (tree), Rhus succedanea (fruit), Solanum xanthocarpum (whole herb), Solanum dulcamara (root), Solanum indicum (whole herb), Stereospermum suaveolens (root bark), Teramnus labialis (whole herb), Terminalia chebula (fruit), Tinospora crispa (stem), Uricia lagopoides (whole herb), Vitis vinifera (fruit) and Withania somnifera (root). This sample was used in experiments after filter sterilization and appropriate dilution.

2.2 Animals

All animal experiments were approved by the Ethics Committee of Yokohama City University and were conducted according to the institutional guidelines. Six-week-old female hairless mice (HR-1) were purchased from SLC Co. Ltd. (Japan) and were housed in plastic cages at a temperature of 22 (± 1) °C, under a 12:12 light-dark cycle using artificial illumination (light on 8:00 a.m.–8:00 p.m.). The mice were provided with pelleted diet (Oriental Yeast, Japan) and drinking water ad libitum. Six-week-old female hairless mice (HR-1) were purchased from SLC Co. Ltd. (Japan) and were housed in plastic cages at a temperature of 22 (± 1) °C, under a 12:12 light-dark cycle using artificial illumination (light on 8:00 a.m.–8:00 p.m.). The mice were provided with pelleted diet (Oriental Yeast, Japan) and drinking water ad libitum.

Chyawanprash was added to the water at a dilution of 1:20 (v/v). Chyawanprash was orally administered to the mice for 3 weeks prior to their random division into 6 groups of 3 mice. The dorsal skin of the mouse was exposed to UV lamps (Royal Philips Electronics) at 0, 225 and 450 mJ/cm². Chyawanprash was administered throughout the experiment.

2.3 Histological analysis

The mice were sacrificed at the end of experiment (day 31) and skin samples were obtained from the dorsal part, fixed with 4% formaldehyde, and embedded in paraffin. Fivemicrometer thick slices were sectioned, deparaffinized and stained with haematoxylin and eosin (H&E). The epidermal thickness was randomly measured in at least 50 independent areas in the H&E-stained skin sections.

2.4 Cell culture

Human cervical keratinocytes were cultured in...
plastic dishes containing medium comprised of a 4:1 mixture of MCDB 153 medium (Sigma-Aldrich) and human keratinocyte serum-free medium (DS Pharma Biomedical), under 5% CO₂, and 95% humidity at 37 °C. To determine the growth of keratinocytes, the cells (1 × 10⁴ cells) were plated onto 60-mm dishes and cultured with serial dilutions of Chyawanprash for 2 weeks. Formed colonies were stained with Coomassie brilliant blue (CBB). Similarly, HeLa cells were cultured in Eagle’s medium (MEM) supplemented with 5% fetal calf serum (Hyclone). To determine the resistance to oxidative stress in HeLa cells, the cells (3 × 10⁴ cells) were plated onto 35-mm dishes and cultured with serial dilutions of Chyawanprash in the presence of paraquat (50 μmol/L) for 2 weeks. Formed colonies were stained with CBB.

2.5 Reactive oxygen species assay

To generate reactive oxygen species (ROS) in HeLa cells, the cells were treated with 50 μmol/L of paraquat for 3 days. After washing twice with phosphate-buffered saline (PBS), cells were incubated in serum-free medium supplemented with 5 μmol/L of a ROS-sensitive fluorescence probe, 2',7'-dichloro-fluorescein diacetate (H₂DCFDA; Molecular probes), for 30 min, at 37 °C, in the dark. Cells were then washed twice with PBS and the fluorescence signals were photographed with a fluorescence microscope (IX-70, Olympus, Japan) equipped with a standard filter set for fluorescein.

2.6 Methylene blue reduction activity assay

Five microliters of Chyawanprash or vitamin C (1.6%, w/v) were incubated with 1 mL of a methylene blue solution (62.5 μmol/L) for 5 min. Reduction of methylene blue was monitored using a spectrophotometer at the wavelength of 665 nm (Ultrospec 2000, Pharmacia, USA).

2.7 Statistical analysis

Statistical significance was determined by one-way analysis of variance followed by the Tukey-Kramer test. P<0.05 was considered statistically significant.

3 Results

3.1 Protective effect of Chyawanprash on skin photoaging in mice

We administered Chyawanprash orally to hairless mice for 3 weeks and exposed them to UVB irradiation as shown in Figure 1A. The mice continued to receive Chyawanprash throughout the experiment. Administration of Chyawanprash did not largely affect food and water intake in mice, and did not cause macroscopic changes in the skin surface of mice when they were not exposed to UVB irradiation (Figure 1B). Upon UVB irradiation, the control mice that were not treated with Chyawanprash showed symptoms such as rough skin, erythema and edema; however, the mice that were treated with Chyawanprash showed less severe symptoms: e.g., edema on the skin of the mice exposed to UVB irradiation (450 mJ/cm²) was much smaller in the mice treated with Chyawanprash than without Chyawanprash (Figure 1B).

Since epidermal hyperplasia is a reliable marker of skin photoaging, we next analyzed the biopsied skin tissue stained with H&E. UVB irradiation caused an increase in epidermal thickness (Figure 1C and D). Chyawanprash significantly suppressed the epidermal thickening (Figure 1C and D). These observations indicated that oral administration of Chyawanprash showed a protective effect on skin photoaging in hairless mice.

3.2 Antioxidant activity of Chyawanprash

ROS, which is generated by UVB irradiation, has been demonstrated to play pivotal roles in skin photoaging through activating many signaling pathways, as well as damaging cellular components. We then examined whether Chyawanprash had an ability to eliminate ROS in living cells. For this, we cultured HeLa cells with paraquat, which generates ROS in cells, and examined the levels of ROS with a ROS-sensitive fluorescent probe, H₂DCFDA. Paraoquat increased the ROS levels in cells, but simultaneous addition of Chyawanprash clearly decreased them, indicating that Chyawanprash efficiently eliminated ROS (Figure 2A). We next examined whether Chyawanprash showed a protective effect against paraquat-derived oxidative stress in cells. Addition of paraquat inhibited the growth of HeLa cells, however the simultaneous addition of Chyawanprash greatly restored the growth of HeLa cells treated with paraquat (Figure 2B). These findings suggested that Chyawanprash showed a protective effect against oxidative stress, probably through eliminating ROS. We further measured the reducing activity of Chyawanprash with methylene blue, and found that the Chyawanprash formula used in this study showed a reducing activity roughly equivalent to a 3-percent solution of vitamin C (Figure 2C). Thus, Chyawanprash showed strong antioxidant activity, which would likely play an important role in suppression of skin photoaging.

3.3 Effect of Chyawanprash on the growth of keratinocytes in culture

We finally examined the effect of Chyawanprash on the growth of keratinocytes because epidermal hyperplasia is caused by the augmented growth of keratinocytes in the epidermis. For this, we plated human keratinocytes onto plastic dishes at a low-cell density and cultured them with and without Chyawanprash. Interestingly, addition of Chyawanprash markedly enhanced the growth of keratinocytes (Figure 3). This observation suggested that the suppression of epidermal thickening by Chyawanprash was not due to its inhibitory effect on the growth of...
Figure 1  The effect of Chyawanprash (CHY) on skin photoaging in hairless mice
A: Time-course of the experiment is shown. Hairless mice were treated with or without Chyawanprash for 3 weeks and exposed to UVB irradiation five times at the days indicated. B: Photographs of mice at the day 25 are shown. Arrows indicate edema on the dorsal skin of mice. C: H&E staining of the dorsal skin tissues of the mice exposed to UVB irradiation. Arrows indicate the epidermis of the skin. D: The epidermal thickness was measured. Error bars indicate standard deviation. *: statistical significance at $P<0.05$; H&E: haematoxylin and eosin; UVB: ultraviolet B.

Figure 2  Antioxidant activity of Chyawanprash (CHY)
A: HeLa cells were cultured with 50 μmol/L of paraquat (PQ) in the presence or absence of Chyawanprash (diluted at $3 \times 10^{-5}$) for 3 days, and ROS was detected with a fluorescent probe, H$_2$DCFDA. B: HeLa cells were cultured with 50 μmol/L of paraquat (PQ) in the presence or absence of Chyawanprash (diluted at $3 \times 10^{-5}$) for 2 weeks. Formed colonies were stained with CBB. C: Reducing activities of Chyawanprash and vitamin C (VC) were determined. The activity is expressed as a value relative to that of vitamin C (1.6%); H$_2$DCFDA: 2′,7′-dichloro-fluorescein diacetate; CBB: Coomassie brilliant blue.
keratinocytes because Chyawanprash showed positive effects on the growth of keratinocytes in culture.

4 Discussion

In this study, we have shown that oral administration of Chyawanprash showed a protective effect on skin photoaging induced by UVB irradiation in hairless mice. It is well established that ROS generated by UVB irradiation plays pivotal roles in skin photoaging. Besides damaging cellular macromolecules, ROS activates an epidermal growth factor receptor (EGFR) and MAP kinases in epidermal keratinocytes and fibroblasts. Activation of these signaling pathways leads to the activation of the AP-1 and NF-κB transcription factors, and eventually results in decreased collagen synthesis, increased matrix metalloproteinases that degrade extracellular matrix and increased production of inflammatory cytokines. These events, caused by ROS, collectively induce the phenotype of skin photoaging. Thus, we examined the antioxidant activity of Chyawanprash, and found that it efficiently eliminated ROS in cells. We also have shown that Chyawanprash protected cells from the toxic effects of oxidative stress. These findings are consistent with the result obtained from the in vitro experiment by Jose et al. Chyawanprash is constituted by many kinds of herbs, and one major constituent of Chyawanprash is Emblica officinalis (Amla), which contains a high amount of antioxidants. Thereby, the antioxidant activity of E. officinalis, together with that of other herbs, would likely play an important role in suppression of skin photoaging in mice.

A number of experiments show that the extracts derived from natural plants, when orally administered, show a protective effect against skin photoaging in mice. These include herbs such as the rhizomes of Curcuma longa L. (Turmeric), Dalbergia odorifera T. Chen, Eucommia ulmoides, Panax ginseng, Kaempferia parviflora Wall. ex. Baker (black ginger), Vigna angularis (azuki bean), Coriandrum sativum L. (coriander), Mangifera indica L. (mango), Cyclopia intermedia (Honeybush) and green tea. Importantly, many of these are herbs that are used in traditional medicine and possess an antioxidant activity. Thus, the antioxidant activity in these plants would play an important role in protection from skin photoaging.

Additionally, in this study, we have shown that Chyawanprash stimulated the growth of keratinocytes in culture. To the best of our knowledge, this is the first report that describes the positive effect of Chyawanprash on keratinocyte growth in vitro. However, the growth-stimulating effect of Chyawanprash on keratinocytes in vitro appears to be inconsistent with its growth-suppressive effect on keratinocytes in UVB-irradiated mice. But this does not necessarily deny the growth-stimulating effect of Chyawanprash on keratinocytes under normal conditions in vitro, given that hyperproliferation of keratinocytes induced by UVB irradiation is a rapid and strong response, and thus may mask the growth of keratinocytes stimulated by Chyawanprash under normal conditions. Since appropriate stimulation of the growth of keratinocytes can be beneficial to skin health, verification of the in vivo effect of Chyawanprash on the growth of keratinocytes under normal conditions is of great importance. If Chyawanprash stimulates the growth of keratinocytes under normal conditions in vitro, it would be possible to speculate that Chyawanprash may regulate the growth of keratinocytes through two distinct ways: stimulation of the growth of keratinocytes under normal conditions and suppression of hyperproliferation of keratinocytes under UV irradiation-induced stress conditions.

Although Chyawanprash is a well-known Ayurvedic tonic and is consumed daily as a dietary supplement, the chemical components responsible for its beneficial effects on human health are largely unidentified. This may be largely due to a lack of simple assay systems to detect its effects. In this study, we have shown that Chyawanprash has an antioxidant activity and a growth-stimulating activity in keratinocytes in vitro. These assay systems would facilitate the identification of the chemicals beneficial to human health, and identification of such chemicals would provide molecular basis for the functions of the herbs used in traditional medicine.

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6 Competing interests

Chyawanprash was provided by Ichiban Life Corporation.
REFERENCES


Submission Guide

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