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Effects of a turmeric extract (*Curcuma longa*) on chronic ultraviolet B irradiation-induced skin damage in melanin-possessing hairless mice

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ABSTRACT

Turmeric (the rhizomes of *Curcuma longa* L., Zingiberacease) is widely used as a dietary pigment and spice, and has been traditionally used for the treatment of inflammation, skin wounds and hepatic disorders in Ayurvedic, Unani and Chinese medicine. Although the topical application or oral administration of turmeric is used to improve skin trouble, there is no evidence to support this effect. The aim of this study was to clarify whether turmeric prevents chronic ultraviolet B (UVB)-irradiated skin damage. We examined the effects of a turmeric extract on skin damage including changes in skin thickness and elasticity, pigmentation and wrinkling caused by long-term, low-dose ultraviolet B irradiation in melanin-possessing hairless mice. The extract (at 300 or 1000 mg/kg, twice daily) prevented an increase in skin thickness and a reduction in skin elasticity induced by chronic UVB exposure. It also prevented the formation of wrinkles and melanin (at 1000 mg/kg, twice daily) as well as increases in the diameter and length of skin blood vessels and in the expression of matrix metalloproteinase-2 (MMP-2). Prevention of UVB-induced skin aging by turmeric may be due to the inhibition of increases in MMP-2 expression caused by chronic irradiation.

Introduction

Recently, there are many reports that substances isolated from many dietary herbals such as grape seed proantocyanidins, green tea catechin, tomato paste (lycopene), pine pycnogenol, and vitamin C etc. are used as oral administration for the protection against ultraviolet (UV)-induced skin damage. Mittal et al. (2003) reported that dietary feeding of proanthocyanidins (0.2 and 0.5%) from grape seeds prevents UVB-induced photocarcinogenesis in SKH-1 hairless mice. It has been reported that orally administered green tea polyphenols prevent UVB-induced oxidative damage and photocarcinogenesis in SKH-1 hairless mice (Vayalil et al. 2004; Mantena et al. 2005). The oral administration of mixture of vitamin C, vitamin E, pycnogenol and evening primrose oil also prevented UVB-induced skin wrinkle formation (Cho et al. 2007). Furthermore, in clinical study, the oral administration of dietary tomato paste (including lycopene), and French martitime pine bark extract pycnogenol protected against UV-induced erythema in humans (Stahl et al. 2001; Saliou et al. 2001). A powder of the rhizomes of *Curcuma longa* L. (Zingiberacease), turmeric is commonly used as a dietary pigment and spice. It has also been used traditionally in Asian medicine for the treatment of inflammation, skin wounds, hepatic and biliary disorders, coughing, and certain tumors. Curcumin supplemented with cosmetics and skin care products/ lotion are avialble in several parts of the world (Baliga and Katiyar 2006). Although the topical application or oral administration of turmeric has long been used to treat skin-aging caused by exposure to the sun, no relevant experimental data exists. An increase in skin thickness and reduction in skin elasticity are part of a process known as photoaging, which is characterized by histological changes such as damage to collagen fibers and the excessive deposition of abnormal elastic fibers (Sams and Smith 1961; Smith et al. 1962; Uitto et al. 1989). In this study, we examined the effects of a turmeric extract on chronic UVB irradiation-induced skin damage including changes in skin thickness and elasticity, pigmentation, and wrinkling in melanin-possessing hairless mice.

Materials and methods

Materials

Tissue protein extraction reagent (T-PER) was purchased from Pierce Co. (Rockford, IL, USA). Coomassie Brilliant Blue 250 was obtained from Sigma Co. (Tokyo, Japan). All other chemicals were of reagent grade and purchased from Wako Pure Chemical Co. (Osaka, Japan).

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Plant materials

The turmeric extract (the rizomes of *Curcuma longa* L., Zingiberacease, Lot No. 080911AG) was supplied by Nihon Funmatsu Pharmacy Co. Ltd. (Osaka, Japan). The high performance liquid chromatography (HPLC) analysis of turmeric extract was performed by the methods of Jayaprakasha et al. (2002) using a Hitachi HPLC system (Pump: L-7100, UV-Detector: L-7400, Column oven: L-7300, Integrator: D-7500, Hitachi Co., Tokyo, Japan) under the following conditions: The elution was carried out with gradient solvent systems with a flow rate of 1.0 ml/min, monitoring wavelength at 425 nm, and COSMOCIL 5C18-AR-II column (250 × 4.6 mm I.D.) (Nacalai Tesque Co., Kyoto, Japan). The mobile phase consisted of methanol (A), 2% acetic acid (B), and acetonitrile(C). HPLC profile of turmeric extract was determined using the above solvents programmed linearly from 45 to 65% acetonitrile in B for 0–15 min. The gradient then went from 65 to 45% acetonitrile in B for 15–20 min, with a constant of 5% A. (Fig. 1). Curcumin was obtained from Sigma-Aldrich Japan (Tokyo, Japan). Desmethoxycurcumin and bisdesmethoxycurcumin were purchased from US Pharmacopeia Co. Inc. (Rockville, USA). The % contents of curcumin, desmethoxycurcumin and bisdesmeetjoxycurcumin of the turmeric extract used in this study were 2.3, 0.59 and 0.36%, respectively, by the calculation from standard curve for authentic samples. A voucher sample (Lot. No. 080911 AG) has been deposited at the Division of Biochemical Pharmacology, Department of Basic Medical Research, Ehime University Graduate School of Medicine.

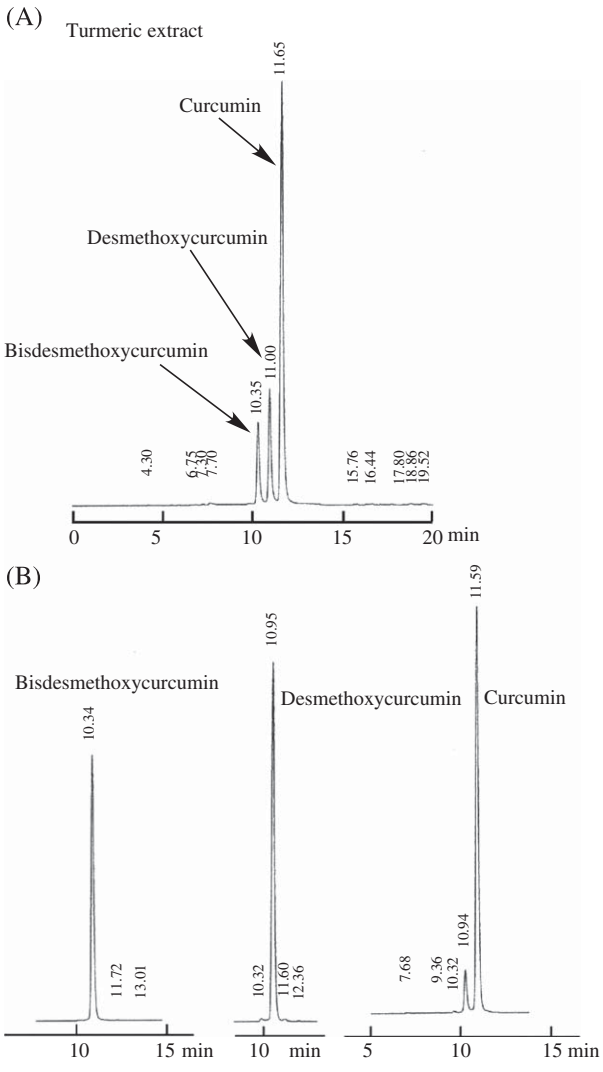

Fig. 1. HPLC chromatogram of the turmeric extract (an extract of the roots of *Curcuma longa* L.). (A): HPLC profile of the turmeric extract; (B): HPLC profile of desmethoxycurcumin, bisdesmethoxycurcumin and curcumin.

Animals

Male hairless Hos: HRM mice (melanin-possessing hairless mice) (5 weeks old) were purchased from Hoshino Laboratory Animals Co. Ltd. (Saitama, Japan), housed for 1 week in a temperature-controlled room at 25 ± 1 °C and 60% relative humidity, and given free access to a standard laboratory diet and water before the experiments. Mice were treated according to the Ethical Guidelines of the Animal Center, Graduate School of Medicine, Ehime University, and the experimental protocol was approved by the Animal Studies Committee of Ehime University.

Measurement of skin thickness, elasticity, and wrinkles in ultraviolet B (UVB) irradiated mice

To examine the effects of the turmeric extract on skin thickness, elasticity, and wrinkles, a UVB lamp (15 W type, UV maximum wavelength 312 nm; UV intensity 100 μW per cm^2, Ieda Boeki Co., Tokyo, Japan) was used. The period of irradiation was varied to control the UVB energy applied to the dorsal region. The minimal erythema dose (MED) per mouse was about 36 mJ per cm^2. The turmeric extract (300 or 1000 mg/kg, twice daily) was administered orally every day for 19 weeks. The initial dose of UVB was set at 36 mJ per cm^2, but was subsequently increased to 54 mJ per cm^2 at weeks 1–4, 72 mJ per cm^2 at weeks 4–7, 108 mJ per cm^2 at weeks 7–10, 144 mJ per cm^2 at weeks 10–13, 168 mJ per cm^2 at weeks 13–16, and finally 180 mJ per cm^2 at weeks 16–19. The frequency of UVB irradiation was set at three times per week before the oral administration of distilled water (control) or the indicated amounts of the turmeric extract. Wrinkles began to be observed macroscopically in the dorsal region from about 7 weeks after the initiation of UV irradiation. Skin thickness and elasticity were measured every week using a Quick Mini Caliper (Mitutoyo Co., Kanagawa, Japan) and Digimatic Caliper (Mitutoyo Co., Kanagawa, Japan), respectively. To evaluate the formation of wrinkles, each mouse was anesthetized with an intraperitoneal injection of pentobarbital (50 mg per kg body weight) at 8 and 11 weeks, and then the UVB-irradiated dorsal area (site of wrinkles) was photographed. The degree of wrinkling was assessed from the photograph according to the grading scale described in Table 1, whereas the name of the animal group was kept blind. This is a modification of the evaluation of wrinkling in hairless mice described by Bissett et al. (1987). Since inflammation of the skin

Table 1
Effects of the turmeric extract on skin wrinkles induced by chronic UVB irradiation in melanin-possessing hairless mice.

Treatment	Wrinkle score	
	8 weeks	11 weeks
Normal mice	0 ± 0*	0 ± 0*
Vehicle-treated UVB-irradiated mice (control)	2.6 ± 0.8	5.2 ± 0.6
+Turmeric extract (300 mg/kg, twice daily)	4.0 ± 1.0	3.8 ± 1.0
+Turmeric extract (1000 mg/kg, twice daily)	2.2 ± 0.7	2.2 ± 0.6*

The Grading of wrinkles was as follows; Grade 0, no coarse wrinkles; Grade 2, a few shallow, coarse wrinkle across the back (Bisset's Grade 1); Grade 4, Shallow, coarse wrinkles across the entire back (Bisset's Grade 2); Grade 6, Some deep, long wrinkles across the back (Bisset's Grade 3).
Values are means ± S.E. for five mice.

* Significantly different from UVB-irradiated mice (control), $p < 0.05$.