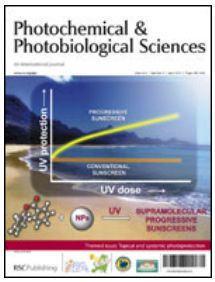

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Mice drinking goji berry juice (*Lycium barbarum*) are protected from UV radiation-induced skin damage *via* antioxidant pathways

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The goji berry, *Lycium barbarum*, has long been recognised in traditional Chinese medicine for various therapeutic properties based on its antioxidant and immune-modulating effects. This study describes the potential for orally consumed goji berry juice to alter the photodamage induced in the skin of mice by acute solar simulated UV (SSUV) irradiation. In Skh:hr-1 hairless mice, 5% goji berry juice significantly reduced the inflammatory oedema of the sunburn reaction. Dilutions of goji berry juice between 1% and 10% dose-dependently protected against SSUV-induced immunosuppression, and against suppression induced by the mediator, *cis*-urocanic acid, measured by the contact hypersensitivity reaction. The immune protection could not be ascribed to either the minor excipients in the goji juice, pear and apple juice, nor the vitamin C content, nor the preservative, and appeared to be a property of the goji berry itself. Antioxidant activity in the skin was demonstrated by the significant protection by 5% goji juice against lipid peroxidation induced by UVA radiation. Furthermore, two known inducible endogenous skin antioxidants, haem oxygenase-1 and metallothionein, were found to be involved in the photoimmune protection. The results suggest that consumption of this juice could provide additional photoprotection for susceptible humans.

Introduction

Lycium barbarum, family Solanaceae (includes potato, tomato, eggplant, chilli pepper), is a deciduous woody perennial found in many countries that produces bright red berries 1–2 cm long, known as goji berries. The goji berry, also known as Chinese wolfberry, has a long history of health applications that 'nourish the blood, enrich the yin' in Chinese medicine dating back to the 7th century Tang dynasty. Several published studies in the Chinese literature report medicinal benefits such as antioxidant properties, protection against inflammatory diseases, vision-related diseases, neurological diseases, as well as anticancer and immunomodulatory effects. However there are scant studies that have confirmed these reports in the western medical literature.

The composition of the goji berry is of interest, with analysis reported mainly in the Chinese literature.[1–4] The fruit is unusually rich in water-soluble peptide-conjugated polysaccharides that represent up to 40% of the fresh fruit pulp. This fraction, known as the *L. barbarum* polysaccharides, or LBP, to which is attributed most of the biological effects of the fruit, has been extracted and fractionated, and has been the predominantly studied form of the goji berry in the last decade. In addition to this water-soluble fraction, however, high levels of the B vitamins (riboflavin, thiamine, nicotinic acid), ascorbic acid, carotenoids and numerous polyphenolic phytochemicals and phytosterols have been described. Many of these components are well recognised as meaningful health-promoting antioxidants available in the human diet.

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Several studies have reported strong antioxidant properties of LBP in animal disease models.[5,6] Furthermore, LBP has immune-modulating properties[7] that have been linked with the antioxidant activity,[8] and has potentiated immune responsiveness in animals in association with an inhibition of the growth of a transplanted tumour.[9] Interestingly, the antioxidant properties of the purified LBP fraction in orally fed rabbits was found to be inferior to the antioxidant effect of cruder extracts that contained additional components from the fruit, indicating that ingredients other than the LBP also provide biological advantages.[10] Therefore it is noteworthy that a recent study in aged (55–72 years) humans drinking whole goji berry juice has reported improvements in serum biomarkers of antioxidant activity such as significantly elevated superoxide dismutase and glutathione peroxidase, and decreased malondialdehyde indicative of reduced lipid peroxidation.[11] These studies therefore demonstrate that the goji berry has significant and complex antioxidant properties when included in the human diet.

In view of the established contribution of oxidative stress to the inflammatory UV radiation-induced sunburn reaction, to the subsequent suppression of T cell mediated immune function, and to the development of photocarcinogenesis,[12] we hypothesise that goji berry juice will have ameliorating effects against UV radiation-induced skin damage when fed as a dietary supplement. Therefore this project aims to provide evidence from murine models for the potential for orally consumed goji berry juice to inhibit those forms of cutaneous UV-induced photodamage that are understood to comprise a prerequisite for UV-induced skin cancer development. The study examines the effect of oral goji berry juice on UV radiation-induced inflammation, measured as the oedema component of the sunburn reaction, and on immunosuppression measured by the contact hypersensitivity

(CHS) reaction. Oxidative damage will be assayed by the degree of lipid peroxidation in the skin, and the possible involvement of two cutaneous molecules that are induced by oxidative stress and that have photoimmune-protective properties, namely haem oxygenase-1[13] and metallothionein,[14] will also be measured.

Materials and methods

Mice

Inbred female Skh:hr-1 hairless albino mice, mice with a null mutation for metallothionein-I,II (MT−/−), and their wild type 129Ola/C57BL/6J control mice (MT+/+) were obtained at 8 to 12 weeks of age from the Veterinary Science breeding colonies at the University of Sydney. The mice were maintained in conventional wire-topped cages at 25 °C room temperature, with ambient lighting provided by gold fluorescent tubes (F40GO, GEC, Hobart, Tasmania, Australia) that do not emit measurable UV radiation, on a 12 h on/off regime. Cage bedding was compressed paper (Fibrecycle Pty. Ltd., Mudgeeraba, Queensland, Australia), and the mice were fed stock rodent pellets (Gordons Specialty Stockfeeds, Yanderra, New South Wales, Australia) and tap water *ad libitum*. Fur on the dorsal skin of MT−/− and MT+/+ mice was shaved with electric clippers (Oster Clippers, #42 fine blade; Oster Manufacturing, Milwaukee, WI) one day before SSUV irradiation, and on the abdomen one day before contact sensitisation (for CHS induction, as below). All procedures were approved by the University of Sydney Animal Ethics Committee and complied with the current New South Wales Animal Welfare Act.

Goji berry and other fruit juices

Goji berry juice was provided as Himalayan Goji Juice, distributed by FreeLife International, Phoenix, AZ. This product contains 89% goji juice and approximately 8% other fruit juices (grape, pear, apple and pear puree) added for flavour, and 50 mg per 100 mL ascorbic acid, as well as the preservatives benzoic acid and sorbic acid. A sample of the goji berry juice without the preservatives was also provided. A tetrapack of JustJuice apple/pear (no added sugar, preservative-free, containing 15 mg per 100 mL ascorbic acid) was purchased (Woolworths supermarket). The fruit juices were diluted with pre-boiled and cooled tap water at 1, 2, 5 or 10%, and were offered to the mice freshly prepared each day. Some groups of mice received the pear/apple juice supplemented with a high concentration of 100 mg per 100 mL ascorbic acid (Sigma). The mice found the juices palatable, and the consumption rate was the same for water or fruit juice, approximately 5 mL per mouse per day. Therefore mice drinking 1–10% goji berry juice were consuming approximately 2.0–20 mL undiluted juice per kg body weight daily. The recommended daily dose for humans[11] is approximately equivalent (120 mL daily, or around 1.7 mL per kg body weight), considering the higher metabolic rate of the mouse.

UV radiation

The solar simulated radiation (SSUV; 290–400 nm) was produced by a planar array of 7 fluorescent tubes comprising one UVB tube (Philips TL40W 12R/S, Eindhoven, The Netherlands) flanked by 2 sets of 3 UVA tubes (Hitachi 40 W F40T 10/BL, Tokyo, Japan) held in a reflective batten and filtered through a 0.125 mm sheet of cellulose acetate film (Grafix Plastics, Cleveland, OH) to eliminate wavelengths below 290 nm. The UVA (320–400 nm) waveband, recognised as the most effective for induction of cutaneous oxidative stress, was isolated from the SSUV source by replacing the UVB tube with a seventh UVA tube, and filtering the radiation through a sheet of 6 mm window glass. Irradiance was measured with an IL 1500 radiometer (International Light, Newburyport, MA) with irradiation detectors for UVB and UVA that were calibrated to the source emission spectra.

Mice in treatment groups of 5 were irradiated on the dorsum with a single SSUV exposure of 3× the minimal oedematous dose (MEdD), restrained in their boxes by the covering cellulose acetate film. The MEdD of SSUV for the Skh:hr-1 mouse has been established previously to be 1.33 kJ m^{-2} of UVB and 21.3 kJ m^{-2} of UVA, and for the shaved MT+/+ mouse to be approximately twice this dose.[15] This exposure dose resulted in a transient marked erythema and oedema without blistering or burning, that resolved by 96 h after exposure. For induction of oxidative damage to the skin, mice were irradiated with 400 kJ m^{-2} UVA alone, which did not result in any grossly visible skin changes. Temperature during UV exposure was stabilised by surrounding curtains and an electric fan.

Measurement of skin inflammation/oedema

Mice were pre-fed 5% goji berry juice for 7 days prior to SSUV irradiation, and continuing for the following 96 h. The mid-dorsal skinfold thickness was measured non-invasively immediately before and at repeated intervals after SSUV exposure, using a spring micrometer (Interapid, Zurich, Switzerland), and the average change recorded for each group of 5 Skh:hr-1 mice. Statistical significance of the differences between treatments was obtained using Student's *t*-test.

Induction of contact hypersensitivity (CHS)

Groups of 5 mice were pre-fed goji berry juice or other fruit juices diluted to concentrations between 1–10% in the drinking water (pre-boiled tap water) for 7 days prior to SSUV irradiation, or *cis*-urocanic acid treatment, and continuing until sensitisation. Control mice were fed pre-boiled tap water only. On days 8 and 9 after UV irradiation, or *cis*-urocanic acid treatment, mice were sensitised by application of 0.1 mL of 2% oxazolone (Sigma Aldrich Corp., Castle Hill, New South Wales, Australia) in ethanol on the unirradiated dorsal skin. Mice were challenged on day 15 with 5 µL 0.2% oxazolone/ethanol applied to both surfaces of each pinna, immediately after measuring the pre-challenge ear thicknesses with the spring micrometer (Interapid). Ear thickness was then measured repeatedly every two hours between 16 and 24 h post-challenge to identify the time point of maximum ear thickness in the unirradiated control mice. Effects of the treatments were presented at this time point as the group average ear swelling that was calculated by the difference in the average ear thicknesses immediately before and after challenge. The percentage immunosuppression represents the degree of reduction of the response compared with the unirradiated control mice. Statistical significance of the differences between treatments was obtained using Student's *t*-test.

Treatment of mice with *cis*-urocanic acid

In some experiments, the mice were treated with topical application of the immunosuppressive mediator, *cis*-urocanic acid. The *cis* isomer was produced by UVB-irradiation of a thin layer of a 4% solution of *trans*-urocanic acid (Sigma Aldrich Corp., Castle Hill, NSW, Australia) in dimethyl sulfoxide (DMSO), resulting in an equilibrium mixture of approximately 50% of each isomer, referred to as '*cis*-urocanic acid' here, that was diluted into a simple cosmetic oil-in-water emulsion to provide 0.2% urocanic acids and 5% DMSO, as previously described.[14] Aliquots of 0.1 mL (0.2 mg urocanic acids) were applied to the mouse dorsum 3 times within 24 h, to simulate the effect of SSUV irradiation. Control mice received the lotion vehicle.

Involvement of haem oxygenase (HO) activity

Groups of 5 mice were pre-fed 5% goji berry juice in the drinking water for 7 days prior to SSUV irradiation, and continuing until sensitisation. Haem oxygenase enzyme activity was inhibited by the subcutaneous injection of 20 μmol per kg body weight of tin protoporphyrin-IX (SnPP; Porphyrin Products, Logan, UT) diluted in phosphate buffered saline, pH 7.2, into the lower abdominal skin immediately after SSUV irradiation and again 24 h later, as previously described.[13] The control treatment was the injection of phosphate buffered saline alone.

Activation of the HO-1 gene was assessed by the presence of HO-1 mRNA in skin extracts, identified by reverse transcriptase polymerase chain reaction (RT-PCR). Groups of 3 mice were fed 5% goji berry juice for increasing periods, and skin samples taken at daily intervals for 4 days, RNA extracted, and the HO-1 mRNA expression compared with water-drinking mice. RT-PCR analysis was performed as previously described,[16] including β-actin as housekeeping gene.

Induction of lipid peroxidation

Lipid peroxidation was induced by exposure of groups of 5 mice to 400 kJ m^{-2} of UVA radiation, after pre-feeding 5% goji berry juice for 7 days, and continuing for 24 h post-irradiation, when mice were euthanised and dorsal skin excised and snap frozen in liquid nitrogen. Skin samples were then cut into 10 μm slices using a cryostat and were suspended at 40% wet weight in the reaction buffer of the commercial TBARS assay kit (Cayman Chemicals, Ann Arbor, MI) containing in addition 1 mM dithiothreitol (Sigma) and 0.2% butylated hydroxytoluene/ethanol. The production of thiobarbituric acid-related species was quantitated as malondialdehyde using a standard curve of tetraethoxypropane.

Results

Goji berry juice inhibits inflammation/oedema of sunburn

In the Skh:hr-1 mouse irradiated with 3 MEdD of SSUV there is a marked sunburn inflammatory reaction that develops during the following 48 h, and is illustrated by the peak in the increase in mid-dorsal skinfold thickness at this time point, represented by the oedema component of this reaction as a 2.0-fold increase in skinfold thickness in water-drinking mice (Fig. 1). The increased skinfold thickness subsided subsequently. Mice drinking goji berry juice (5%) were found to have a significantly reduced oedema at each measurement point until 48 h, that was highly significant ($P < 0.001$) at the peak of the oedema response (1.6-fold increase in skinfold thickness).

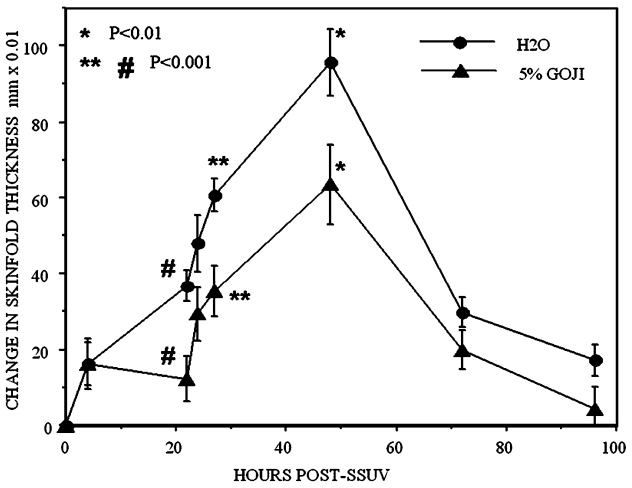

Fig. 1 Average change in mid-dorsal skinfold thickness following SSUV irradiation (3 MEdD) in groups of 5 Skh:hr-1 mice fed water or 5% goji berry juice. Matching symbols indicate statistically significant differences.

Goji berry juice inhibits suppression of CHS by SSUV and *cis*-urocanic acid

The induction of CHS to oxazolone resulted in an approximate doubling of the ear thickness in the unirradiated Skh:hr-1 mice drinking water. The consumption of goji berry juice at all doses 2–10% did not significantly alter this ear swelling response (Fig. 2). Irradiation with 3 MEdD of SSUV markedly reduced the CHS response in the mice drinking water by 54%. However there was a dose-dependent protection against this suppression of CHS in mice drinking 2% and 5% goji berry juice (suppression reduced to 38%, $P < 0.05$, and 17%, $P < 0.001$, respectively), highly significant

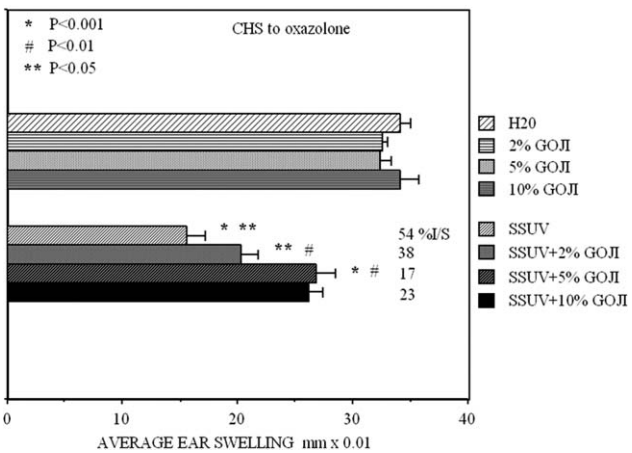

Fig. 2 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 Skh:hr-1 mice drinking water or goji berry juice at 2%, 5% or 10% dilution, and the effect of irradiation with 3 MEdD of SSUV. Matching symbols indicate statistically significant differences.

for 5% goji berry juice. The increase in the protection between 2% and 5% was also significant ($P < 0.01$). Drinking 10% goji berry juice did not afford further protection against SSUV compared with 5% juice.

To probe the mechanism of goji berry juice protection, mice were treated by topical application of the immunosuppressive UV photoproduct, *cis*-urocanic acid, to replace SSUV irradiation. The CHS response to oxazolone was strongly suppressed by 60% by this treatment (Fig. 3). In mice drinking 5% goji berry juice, however, there was complete protection against *cis*-urocanic acid immunosuppression, indicating that this immunosuppressive mediator is a major target for goji berry juice. This result also demonstrated that oral consumption of goji berry juice does not provide a sunscreen-like UV-absorbing effect at the skin target tissue.

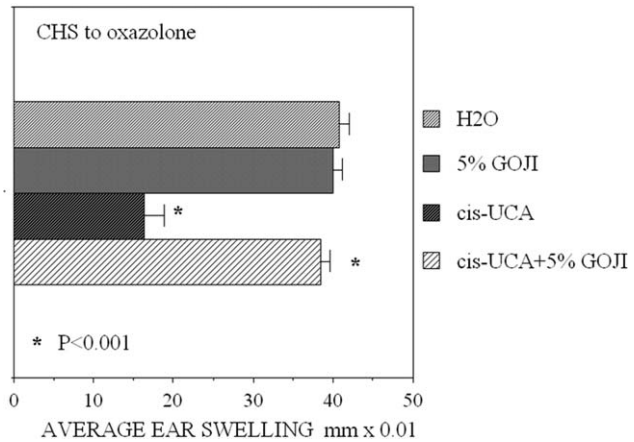

Fig. 3 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 Skh:hr-1 mice drinking water or 5% goji berry juice, and the effect of topical treatment with *cis*-urocanic acid lotion ('*cis*-UCA'). Matching symbols indicate a statistically significant difference.

Goji berry juice photoimmune protection is not attributable to vitamin C, pear/apple juice content, or preservative

To assess the possible contributory effects of the minor fruit juices and high vitamin C content of the goji berry juice, Skh:hr-1 mice were fed pear/apple juice (preservative-free) with and without a high vitamin C additive. Neither the pear/apple juice at 2% or 5%, nor the high vitamin C content had any significant effect on the CHS responses, nor did they significantly alter the suppression of CHS (43%) by the SSUV irradiation (Fig. 4). Therefore the photoimmune protection by goji berry juice appears to be a property of the goji berry itself.

A sample of the goji berry juice without preservative was also tested at concentrations between 1–10%. A dose-dependent protection against suppression of CHS by SSUV was observed between 2–10% (Fig. 5). Interestingly, the absence of the preservatives allowed stronger protection by 10% goji berry juice, so that the CHS reaction remained not statistically different from unirradiated mice. Thus this experiment suggested that the inclusion of the preservatives restricted the photoimmune protective properties of the goji berry juice.

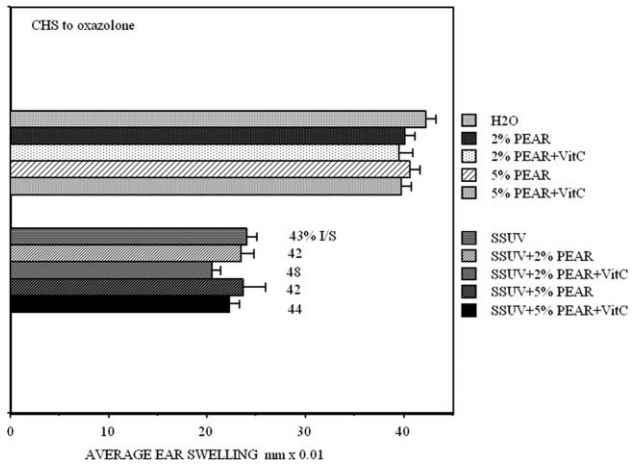

Fig. 4 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 Skh:hr-1 mice drinking water or 2% or 5% pear/apple juice alone or with additional ascorbic acid ('Vit C') at 1 mg mL^{-1}, and the effect of irradiation with 3 MEdD of SSUV. Neither pear/apple juice nor additional ascorbic acid significantly altered the suppression of the response.

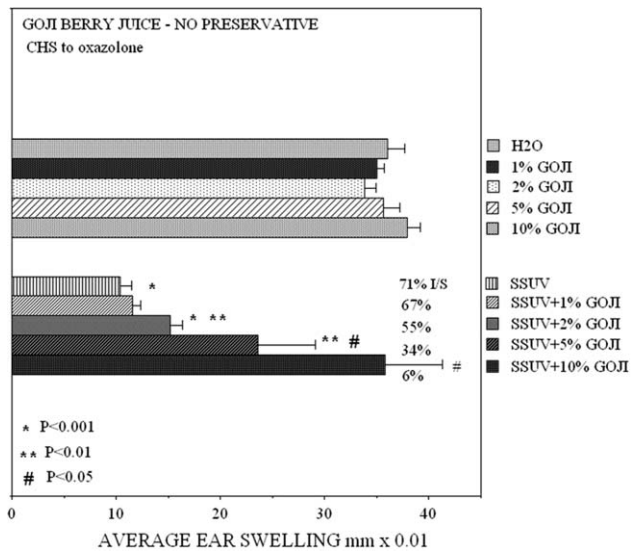

Fig. 5 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 Skh:hr-1 mice drinking water or preservative-free goji berry juice at 1%, 2%, 5% or 10% dilution, and the effect of irradiation with 3 MEdD of SSUV. Matching symbols indicate statistically significant differences.

Goji berry juice protects against UVA-induced lipid peroxidation

Lipid peroxidation was apparent in the untreated Skh:hr-1 mouse skin, possibly as a result of auto-oxidation during the processing. However lipid peroxidation was significantly induced by UVA irradiation and was measured as approximately 25% increase in malondialdehyde formation in the mouse skin (Fig. 6). Mice drinking 5% goji berry juice (preservative free) had the normal level of lipid peroxidation which remained not significantly different following UVA irradiation. Thus goji berry juice consumption was a strong inhibitor of UVA-induced lipid peroxidation in the skin.

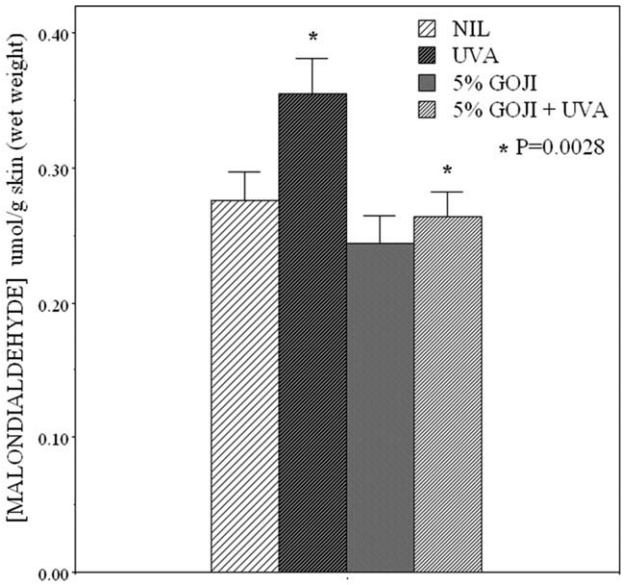

Fig. 6 Lipid peroxidation measured as the average production of malondialdehyde by dorsal skin extracts from 5 Skh:hr-1 mice drinking water or 5% goji berry juice at 24 h after irradiation with 400 kJ m^{-2} UVA. Symbols indicate a statistically significant difference.

Haem oxygenase activity is involved in photoimmune protection by goji berry juice

Skh:hr-1 mice were treated with the specific inhibitor of haem oxygenase activity, SnPP, to assess the contribution of this photoimmune protective enzyme to goji berry juice effects on CHS. The injection of SnPP was not immunomodulatory itself (Fig. 7), whether mice were drinking water or goji berry juice. Mice strongly immunosuppressed (59% suppression) by SSUV were significantly protected by 5% goji berry juice (29% suppression) as anticipated, however the SnPP treatment in these mice completely abrogated the goji berry juice immunoprotection (62% suppression). Thus haem oxygenase activity appears to be an essential contributor to goji berry juice photoimmune protection.

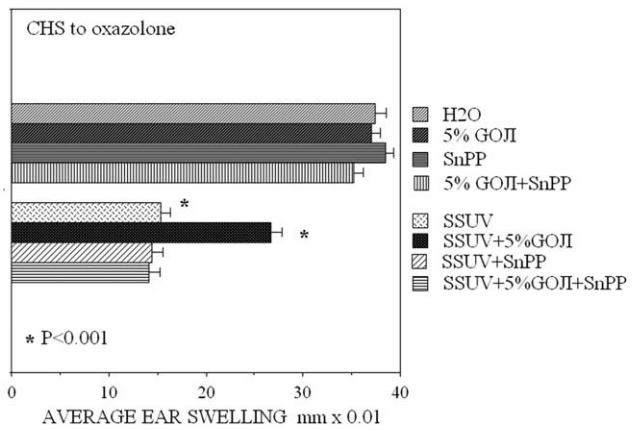

Fig. 7 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 Skh:hr-1 mice drinking water or 5% goji berry juice, and the effect of irradiation with 3 MEdD of SSUV in the presence or absence of the inhibitor of haem oxygenase activity (SnPP). Symbols indicate a statistically significant difference.

RT-PCR analysis of cutaneous HO-1 showed the absence of this mRNA in mice drinking water (Fig. 8), consistent with previous observations.[16] However HO-1 mRNA was strongly expressed in the skin of mice drinking 5% goji berry juice by 24 h, and continued to be strongly expressed as the mice continued to consume the juice for at least 4 days. The upregulation of HO-1 gene activity appears to be consistent with the requirement for HO enzyme activity for immune protection by goji berry juice.

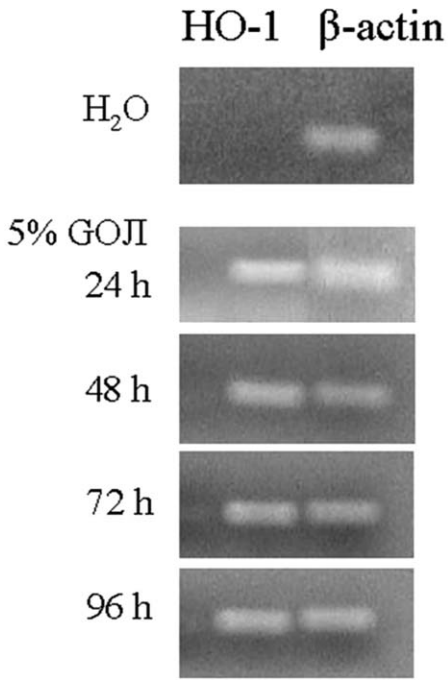

Fig. 8 RT-PCR analysis of haem oxygenase-1 mRNA, with β-actin as housekeeping gene, in extracts from dorsal skin of Skh:hr-1 mice drinking water or 5% goji berry juice for 1-4 days.

Metallothionein is involved in photoimmune protection by goji berry juice

A possible role for involvement of the cysteine-rich protein molecule, metallothionein, in the goji berry juice photoimmune protection was sought by comparing the response in MT deficient mice (MT−/−) with MT replete normal mice (MT+/+). The CHS response in the MT−/− mice was slightly stronger than in the MT+/+ mice (average ear swelling 36.6 and 32.5 mm × 0.01, respectively), not considered to be relevant, as goji berry juice consumption slightly decreased the reaction in the MT−/− mice (ear swelling 33.8 mm × 0.01) but slightly increased it in MT+/+ mice (ear swelling 35.3 mm × 0.01). The CHS response was similarly suppressed by SSUV irradiation in both strains of mice (37% and 33%, respectively). However, while 5% goji berry juice partially but significantly ($P < 0.001$) reduced this immunosuppression in the normal MT+/+ mice (17% suppression), it failed to affect the immunosuppression in the MT−/− mice, which remained as immunosuppressed as SSUV-irradiated MT−/− mice drinking water (Fig. 9). Thus metallothionein, known for its antioxidant capacity, also contributes to the photoimmune protective properties of goji berry juice.

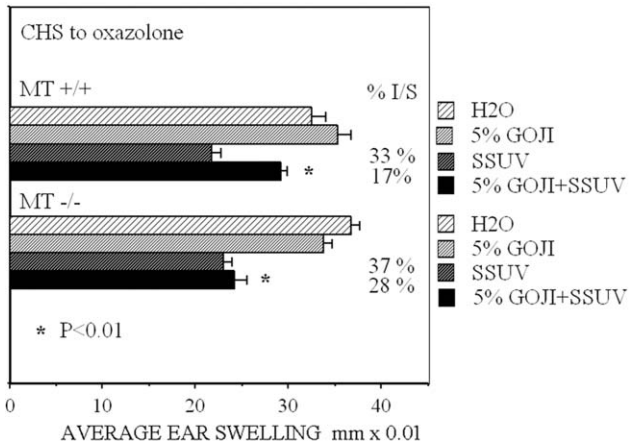

Fig. 9 Contact hypersensitivity responses to oxazolone measured as the average ear swelling in groups of 5 metallothionein wild type (MT+/+) mice or metallothionein knockout (MT−/−) mice drinking water or 5% goji berry juice, and the effect of irradiation with 3 MEdD of SSUV. Symbols indicate a statistically significant difference.

Discussion

These experiments have demonstrated significant protective effects of orally consumed goji berry juice against cutaneous photodamage in the mouse. The inflammatory sunburn reaction was strongly reduced, and the suppression of CHS was dose-dependently reduced. The photoimmune protection was shown to be independent of any possible UV-absorbing properties of the goji berry juice that might have migrated into the outer skin strata, because suppression of CHS by topical *cis*-urocanic acid was also prevented in mice drinking 5% goji berry juice. Minor components of other fruit juices (pear and apple) did not contribute to the goji berry juice protection, nor did additional vitamin C, in spite of published reports ascribing photoimmune protection to topically applied vitamin C preparations in other animal models.[17,18] The preservatives that were included in the first goji berry juice samples likewise did not contribute to the photoimmune protection. Omission of the preservatives was found actually to improve the photoimmune protective property, and UV-irradiated mice drinking 10% goji berry juice produced strong CHS responses not significantly different from the unirradiated mice. Therefore the anti-inflammatory and immune protective effects observed here in the hairless mouse appear to result from the goji berry alone. The response of the skin to oral consumption of the juice also suggests that goji berry juice has a systemic efficacy that might be active in many peripheral tissues of the body.

In agreement with reports from other models,[5,6,10,19,20] we have shown that the goji berry juice has antioxidant activity in the skin, by its potential to reduce lipid peroxidation induced by UVA irradiation. This broad indicator of oxidative stress led to the examination of possible involvement of HO-1 and metallothionein, which are inducible endogenous molecules with both antioxidant and photoimmune protective attributes in the skin.[13,14,21] Treatment of mice with SnPP inhibits the enzyme action of both the constitutive HO-2 and the inducible HO-1 isoforms, but only the HO-1 protein has been found to be associated with photoimmune protection.[13] Past studies have shown that HO-1 mRNA and protein are absent from untreated hairless mouse skin.[16] Here SnPP totally prevented the photoimmune protection of 5% goji berry juice and, in agreement, the expression of HO-1 mRNA was upregulated by 5% goji berry juice consumption. However HO-1 mRNA expression remained elevated in mice drinking the juice for at least 4 days. Such evidence for a prolonged response of the HO-1 gene in mice continuously fed goji juice is not consistent with HO-1 gene activation after UVA irradiation, when a state of refractoriness to repetitive activation has been observed in both cultured human skin cells and in the immune responses of mice.[22,23] We speculate that the HO-1 response to goji berry juice may not be due solely to oxidative stress, but that this protective stress enzyme is upregulated by another mechanism, which future studies might identify. The recent study of oral goji berry juice consumption resulting in elevated levels of circulating antioxidant enzymes in elderly humans[11] also suggests that such endogenous defence molecules might respond positively to specific components of the goji berry.

In addition, the goji berry juice photoimmune protection was observed to be inhibited in the metallothionein-deficient mouse. Deletion of metallothionein-I,-II has previously been shown to exacerbate both cutaneous oxidative stress and the suppression of CHS in mice by UVB irradiation.[14,21] Here the MT−/− mice did not show exacerbated suppression of CHS in response to SSUV irradiation, and we speculate that the UVA component of SSUV provided the immune protection in the absence of metallothionein.[15] A future study should confirm whether metallothionein is induced in the skin of mice drinking goji berry juice, but it is clear from the present experiment that photoimmune protection of mice by goji berry juice is partially dependent on metallothionein.

This study is the first to identify the amelioration of photodamage in the skin by oral goji berry juice consumption. The results are in agreement with a small number of reports in which the LBP fraction has been observed to enhance antioxidant activity and decrease lipid peroxidation in aged animals[5] or in streptozotocin-treated rats.[6] The photoimmune protection is consistent with reports of the upregulation by LBP of pro-inflammatory cytokines in cultured rodent or human immune cells,[7,8] since the photoimmune suppressed state results largely from a dysregulated predominance of anti-inflammatory Th-2 cytokines over the Th-1 cytokines in the skin.[24] The examination of the cutaneous cytokine expression in UV-irradiated goji berry juice-drinking mice is currently underway.

How the peptide-conjugated goji polysaccharides might act remains unclear. However glycated polysaccharides also occur in the cell walls of bacteria, yeasts and fungi, and mammalian cells are known to carry receptors for such glucans/glycans, particularly on primary defence cells of the immune system[25] where their activation is immunostimulatory and has been recognised for medicinal and therapeutic effects related to immune function and cancer protection.[26–28] The other identified components of the goji berry also provide potential immune regulatory and anticancer possibilities. For example, vitamin C, nicotinic acid, lutein, lycopene, polyphenolic compounds have all been reported in a variety of animal studies, to provide protection against UV radiation-induced immune suppression and skin tumour development, *via* antioxidant, anti-inflammatory or cell cycle regulatory pathways.[12,18,29–32]

In summary, this study has revealed, in the mouse, properties of oral goji berry juice that offer significant protection against UV radiation-induced inflammatory sunburn, immune suppression and oxidative stress, factors recognised as prerequisite contributors to photocarcinogenesis. Consistent data has been reported by others in a small number of *in vitro* and *in vivo* human studies. Therefore goji berry juice might prove to be a useful adjunct to topical photoprotective strategies, in preventing skin cancer development in susceptible humans.

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