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ORIGINAL ARTICLE

Glutathione as an oral whitening agent: A randomized, double-blind, placebo-controlled study

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Abstract

Objective: To determine whether orally administered glutathione, 500 mg per day for 4 weeks, affects the skin melanin index, when compared with placebo. *Methods*: This randomized, double-blind, two-arm, placebo-controlled study was set in the King Chulalongkorn Memorial Hospital, Bangkok, Thailand, a teaching hospital affiliated with a medical school. Sixty otherwise healthy medical students were randomized to receive either glutathione capsules, 500 mg/day in two divided doses, or placebo for 4 weeks. The main outcome was mean reduction of melanin indices measured at six different sites. Several secondary outcomes, including UV spots, were recorded by VISIA™. Efficacies of glutathione and placebo were compared by ANCOVA with baseline values as co-variates. *Results*: Sixty participants enrolled and completed the study. At 4 weeks, the melanin indices decreased consistently at all six sites in subjects who received glutathione. The reductions were statistically significantly greater than those receiving placebo at two sites, namely the right side of the face and the sun-exposed left forearm (p-values = 0.021 and 0.036, respectively). This was similarly reflected in the changes in the number of UV spots, as measured by VISIA. Both glutathione and placebo were very well tolerated. *Conclusion*: Oral glutathione administration results in a lightening of skin color in a small number of subjects. However, long-term safety has not been established and warrants more extensive clinical trials.

Key words: *glutathione, melanin, melanin index, melanogenesis, whitening*

Introduction

The grass is always greener on the other side of the fence. Many fair-skinned individuals do all they can just to be tanned, while people with skin of color are continually in search of a miracle whitening or lightening agent.

Topical, oral and even intravenous ‘whitening’ agents of various natures and mechanisms of action are widely available. Numerous topical agents acting on various steps before, during or after melanin biosynthesis (1) and sunscreening agents are widely used for facial lightening purposes.

Because total-body skin lightning is often desired, oral agents have also been widely popular. One such agent used in many parts of Asia, especially Japan, is tranexamic acid. However, the safety of long-term use of this plasmin inhibitor has never been adequately demonstrated. Another interesting agent is glutathione, a cysteine-glycine-glutamate tri-peptide, which exerts several effects on melanogenesis through different mechanisms involving the functions and cellular transport of tyrosinase, the rate-limiting step enzyme in melanin formation (2). Importantly, it is well-known that when glutathione or cysteine is added to melanocytes or melanoma cell lines, the melanogenetic pathway is shifted from eumelanin towards pheomelanin formation.

After oral dosing, glutathione is not well absorbed from the gastrointestinal tract and intravenous administration has thus been used in many counties, especially in southeast Asia. Recently, there has been a

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surge in the use of intravenous glutathione administration in Thailand, prompting the authorities to ban the use of such a modality for fear of severe adverse reactions, including anaphylaxis. We thus performed this randomized, double-blind, placebo-controlled study to investigate the role of oral glutathione as a lightening agent.

Materials and methods

This was a double-blind, randomized, placebo-controlled study, performed in accordance with the Helsinki declaration and approved by the IRB of Faculty of Medicine, Chulalongkorn University. Informed consent was obtained from each participant prior to the start of treatment.

Subjects

Otherwise healthy medical students were eligible for this study. The inclusion criteria were as follows: aged 18 years or older and with an understanding of all the information given by a written consent form. Personal or family history of skin cancer, especially melanoma, consumption of any preparations containing glutathione within 1 month of enrollment, pigmentary disorders or any dermatoses, which may affect the measurement within the study areas, were our exclusion criteria.

Treatment

Subjects were block-randomized to receive either glutathione (250 mg) or placebo capsules (JSP Pharmaceuticals, Bangkok, Thailand), which were identical in appearance and packaged in identical-looking containers. The capsules were taken twice daily on an empty stomach for 4 weeks. Subjects were withdrawn from the study if they started any medications or supplements, which may affect melanogenesis, became pregnant, developed serious adverse effects or drug allergy, or reported significant changes in outdoor activities.

Compliance was assessed by counting the remaining capsules at each follow-up visit.

Assessment

The primary outcome measured was the melanin index, determined by Mexameter (Courage-Khazaka Electronic, Koln, Germany). All measurements were done in triplicate at six sites to represent the skin of the sun-exposed and sun-protected areas as follows:

Sun-exposed areas:

- Face – left and right sides, 2.5 cm caudally from the lateral canthi.
- Extensor surfaces of the forearms, left and right, 7 cm above the ulnar styloid processes.

Sun protected areas:

- Upper, inner arms, left and right, 10 cm from the axillary vault.

Standardized digital photographs were taken by the VISIA™ CR system (Canfield Scientific, Fairfield, NJ, USA), the software for which was also used to quantitatively evaluate UV spots, pores, and evenness on the left and right sides of the face.

For global evaluation, subjects were asked to grade the overall response using a 4-point rating scale: 4 = very satisfactory; 3 = moderately satisfactory; 2 = minimally satisfactory; and 1 = not satisfactory. They were also questioned regarding adverse events and whether or not they thought the events were related to therapy.

All measurements and questions were performed and asked at baseline and repeated at the end of the study (i.e. 4 weeks).

Statistical analysis

Results are presented as mean ± SD with their corresponding 95% confidence intervals (CIs). The paired *t*-test was used to compare baseline values with those of the final visit. Efficacies of glutathione and placebo were compared by ANCOVA, with the baseline values as covariates. The statistical significance level was defined as a p-value < 0.05 (two-tailed). Analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Sixty medical students, 18 males and 42 females, aged between 19 and 22, enrolled and completed the study. Other than baseline skin evenness on the right side of the face on VISIA analysis, which showed a statistically significant difference ($p = 0.039$, Levene's test for equality of variances), the glutathione and placebo groups were well matched. The characteristics of subjects are detailed in Table I.

Melanin indices: Baseline vs end of study

The baseline melanin indices at all corresponding test sites were not statistically significantly different

Table I. Demographic data.

Characteristics	Glutathione	Control
Sex, male/female	8/22	10/20
Age, years, mean ± SD (range)	20.6 ± 6.03 (19–22)	19.7 ± 0.75 (19–22)
Skin phototype		
II	13	10
III	12	14
IV	4	6
V	1	0

between the glutathione and placebo groups (data not shown). There was a consistent and statistically significant reduction of melanin indices at all six sites in subjects who received glutathione (Figure 1). Figure 2 shows the representative clinical photographs taken at baseline and 4 weeks. On the contrary, there were actually increases in melanin indices in the facial areas in those receiving placebo (Figure 1). At the other four sites, there were reductions in melanin indices; however, these did not reach statistically significant levels (Figure 1).

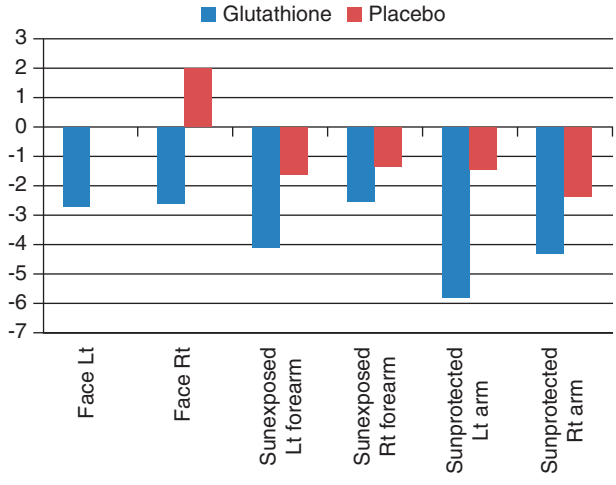

Figure 1. Changes in melanin indices (negative numbers denote decrease in melanin indices).

VISIA analysis: Baseline vs end of study

There were no statistically significant differences of baseline features on the face, as measured by VISIA analysis between the glutathione and placebo groups (data not shown). At 4 weeks, there was a minimal increase in the number of UV spots in subjects receiving oral glutathione. On the other hand, there were statistically significant increases in the number of UV spots on both sides of the face in the placebo group (p-values = 0.012 and 0.006 for the left and right sides, respectively) (Figure 3), which mirrored the changes in melanin indices.

Comparison between glutathione and placebo

Melanin indices. Using analysis of co-variance (ANCOVA), it was clearly seen that there were greater reductions in melanin indices in the glutathione group as compared to controls on all sun-exposed areas. This is especially true for the right side of the face and the left forearm, where statistically significant differences could be demonstrated (p-values = 0.021 and 0.036, respectively) (Figure 4).

VISIA analysis. Similarly, when baseline values were adjusted, a significantly smaller number of UV spots developed in subjects on glutathione treatment (Figure 5). Interestingly, this treatment also resulted in an increase in skin evenness and a reduction in pore sizes. However, these latter two changes were not statistically significant when compared to placebo (Figure 5).

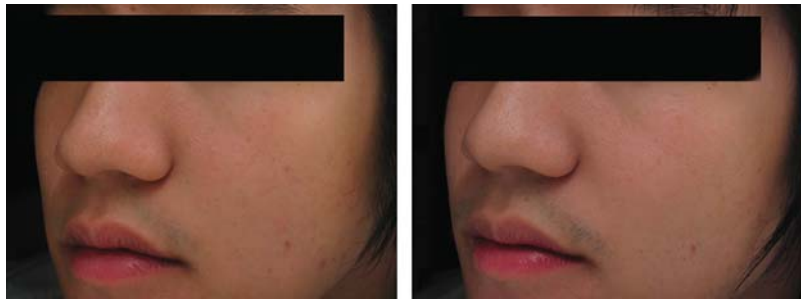

Figure 2. Digital photography comparing baseline (left) with 4 weeks of glutathione treatment (right).

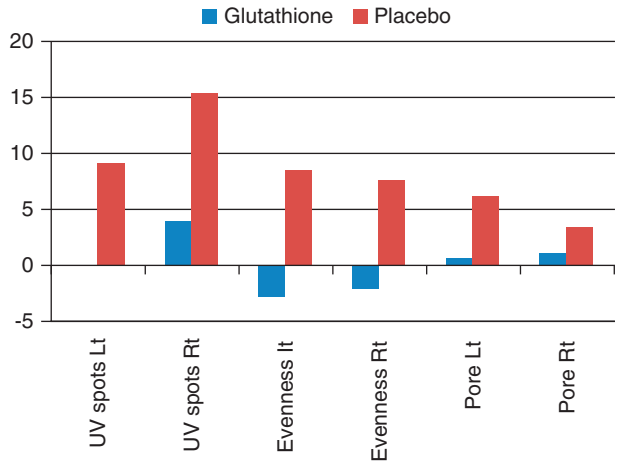

Figure 3. Changes in VISIA parameters (negative numbers denote decrease in parameters).

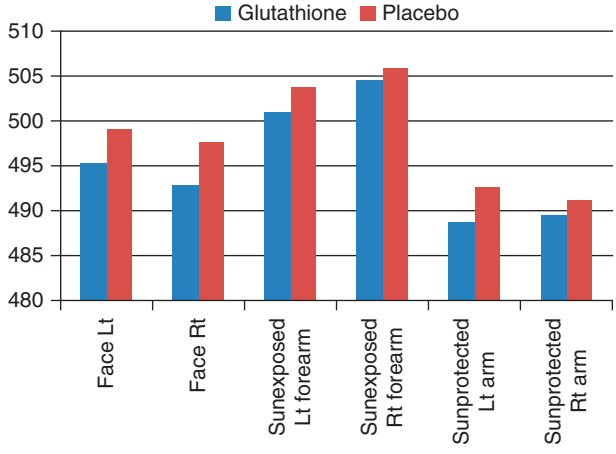

Figure 4. Comparison of melanin indices at the end of the study with baseline values adjusted using ANCOVA.

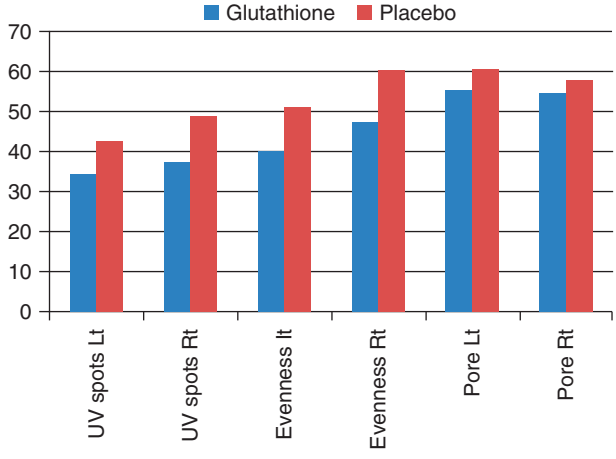

Figure 5. Comparison of VISIA parameters at the end of the study with baseline values adjusted using ANCOVA.

Global evaluation

With the maximum possible score of 4, subjects in the glutathione group reported an average satisfaction score of 3.06, in contrast to only 2.13 in the placebo group. Seven subjects in the glutathione group reported that there were remarks that their skin became fairer, while only two in the control group stated the same.

Adverse events

Flatulence was reported during the first few days in one subject in the glutathione group and constipation in one subject receiving placebo. The treatments were otherwise well tolerated.

Discussion

Our study demonstrated that glutathione, when taken orally, has a skin lightening effect, which could be demonstrated in several body regions, both sun-exposed (i.e. the face and the extensor surfaces of the forearms) and sun-protected skin of the upper arms. The fact that glutathione affects mainly the melanin indices and UV spots in the sun-exposed areas is very interesting. This fits well with the hypothesis that it affects only new melanogenesis and really not the existing pigment.

Melanogenesis is under tight control of several enzymes involving several steps (3–5). It is also under the influence of several physical as well as chemical stimuli, such as temperature, pH, or the presence of precursors and intermediates, and co-factors. Amongst these, glutathione is one well-known peptide that can modulate the pathway. It has clearly been shown that the presence and the amounts of L-cysteine and glutathione govern the types of melanin produced (6) such that when cysteine or glutathione is added to melanocytes and melanoma cell lines, melanogenesis is shifted towards pheomelanin formation (7–9). As pointed out previously, glutathione also affects melanogenesis through other mechanisms involving tyrosinase function and cellular transport. This was recently comprehensively reviewed by Villarama and Maibach (2). Moreover, existing mostly in a reduced form, glutathione is a potent antioxidant which plays a significant role in counteracting the effects of reactive oxygen species produced in the cells. This may also play an important part in skin lightening.

Several oral agents have been used to improve facial hyperpigmentation as well as to induce general skin lightening. These agents modulate pigmentation

through different mechanisms. Alpha-tocopherol acts as an anti-oxidant (10), while tranexamic acid depletes keratinocytes' pool of arachidonic acid, a molecule involved in UV-induced melanogenesis (11,12). However, being a plasmin inhibitor, long-term use of this anti-hemorrhagic drug as an oral lightening agent raises a valid safety concern. Glutathione, on the other hand, is synthesized in the cytosol of all mammalian cells (13). This sulfhydryl-containing compound is available in a number of fresh as well as freshly frozen fruits, and meats. When taken orally, it can be absorbed as an intact tri-peptide from the gastrointestinal tract, at least in animal models (14,15). However, even when large oral doses are administered, most of the absorbed glutathione remains within the gut luminal cells and only small and transient increases of glutathione (GSH) can be detected in the general circulation (16). We were thus rather surprised that a skin lightening effect was observed in this study.

There are some limitations in our study. First, plasma glutathione levels were not measured. Second, we limited our study period to only 4 weeks. Albeit short, our results showed that at least seven out of 30 subjects who received oral glutathione could see that their skin became fairer. Whether continuing treatment longer than 4 weeks will further improve the lightening efficacy remains elucidative. Also, we did not follow up on the participants to determine when the skin melanin indices return to their baseline values. Finally, medical students were chosen to represent a young, otherwise healthy population. The study was conducted during their college time to ensure that sun exposures were minimal and also to increase the compliance, which proved to be a successful strategy, resulting in no drop-outs. Our results may thus be applicable to only young, otherwise healthy Asian individuals.

Eumelanin has a protective effect against ultraviolet radiation (9,17,18), as has clearly been shown in culture systems that pigmented melanocytes are protected from UV-induced DNA damage (19). On the contrary, pheomelanin photosensitizes UV-induced DNA damage in cultured human melanocytes (20), which may account, at least in part, for the higher incidences of melanoma in fair-skinned Caucasians. Of course, the ethnic differences in melanin contents together with different geographic locations and sun-exposure habits result in different incidences of skin cancers, including melanoma. It is not known at this point whether switching the normal machinery from eumelanin to pheomelanin formation through the use of an extraneous agent will also result in similar radicals as seen in the fairer skin types, especially if this process is continued for an extended period of time. We believe that the grass should be greener on our side and it might as well be better if we remain our true color.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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