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Report

An open-label, single-arm trial of the safety and efficacy of a novel preparation of glutathione as a skin-lightening agent in Filipino women

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Abstract

Background Glutathione (GSH) is a naturally occurring thiol that has been reported to cause skin lightening in a manner for which several mechanisms have been proposed. Highest plasma concentrations are achieved with IV administration but are accompanied by greater levels of risk. Oral administration has been less successful in elevating plasma GSH levels.

Objectives The use of a lozenge containing GSH was investigated in order to evaluate the buccal mucosa as a route for GSH administration. Substances that are absorbed through the buccal route go directly into the systemic circulation, effectively bypassing the gastrointestinal tract.

Methods Thirty Filipino females with Fitzpatrick skin types IV or V received a glutathione-containing lozenge daily for eight weeks.

Results Findings showed a significant decrease in melanin indices from baseline to endpoint that became evident in as little as two weeks. There were no serious adverse events, and laboratory examination findings remained normal.

Conclusions The authors conclude that the lozenge containing glutathione was safe and effective in lightening the skin of Filipino women.

Introduction

Glutathione (GSH) is the most abundant low-molecular weight intracellular thiol in mammals.[1–3] It is a tripeptide composed of the amino acids L-cysteine (L-Cys), glutamate, and glycine. Glutathione is one of the five network antioxidants and has been recognized as a master antioxidant. It is involved in many important biological processes such as DNA and protein synthesis and the protection of cells through the metabolism of xenobiotics and carcinogens.[1,3] It also functions to detoxify electrophilic compounds and acts as a reductant in the detoxification of peroxides, protecting cells from intracellular free radicals and reactive oxygen species (ROS).[1,2] Glutathione also serves as a reservoir for the amino acid cysteine.[3]

Local markets in the Philippines have seen an upsurge in various products containing GSH that are available as topical, oral, or parenteral preparations. It is widely advertised in mass media that GSH supplementation, through either oral or parenteral routes, can result in generalized skin lightening as well as other beneficial changes in the skin.

Glutathione as a skin-lightening agent

At high concentrations, glutathione can influence melanogenesis by several proposed mechanisms. These include: (i) a shift in the production of pheomelanin over eumelanin; (ii) the effects of glutathione on tyrosinase; and (iii) the quenching of ROS and free radicals that influence tyrosinase activation.[4–6]

In a cell-free system, thiols like GSH can shift the production of eumelanin to pheomelanin by rapidly and spontaneously conjugating with L-dopaquinone to produce glutathionyldopa, or by acting as a reservoir of L-Cys that conjugates with L-dopaquinone to produce cysteinyldopa. These two thioldopa substrates serve as the precursor of the lighter pigment, pheomelanin. When the concentration of these sulfhydryl compounds is low, L-dopaquinone is preferentially converted to dopachrome, initiating the eumelanogenic pathway. These events suggest that the availability of thiols like GSH and L-Cys regulates the switch in the production of either pheomelanin or eumelanin.[4–10]

As an antioxidant, GSH can influence melanogenesis by chelating copper ions on the active site of tyrosinase,

leading to the inactivation of the enzyme.[6,10,11] The sulfhydryl group of GSH (and L-Cys) is responsible for the inactivation, which has also been demonstrated in melanoma cells, in which the addition of GSH and cysteine resulted in inactivation of tyrosinase.[4,7,11] Furthermore, the depletion of GSH by buthionine-S-sulfoxime (an inhibitor of γ-glutamylcysteine synthetase which regenerates GSH) in melanoma cells resulted in a dose-dependent, parallel increase in tyrosinase activity and increase in eumelanin production.[12] Glutathione has been shown to block the formation of a molecular form of tyrosinase (T3), resulting in the inhibition of transfer of another molecular form of tyrosinase (T1) into premelanosomes.[4] It has the ability to inhibit tyrosinase glycosylation, resulting in disruption of the maturation and transfer of tyrosinase from Golgi-endoplasmic reticulum lysosome-coated vesicles into the premelanosomes.[6,13]

Glutathione is able to quench free radicals and ROS.[5,6] Hong *et al.*[14] demonstrated that intracellular ROS produced by paraquat was effectively suppressed by extracellular GSH at concentrations of 1–10 mM in a dose-dependent manner. This concentration, however, is higher than the normal concentration of GSH normally found in the general circulation, which is measured in μM.[14] As tyrosinase activity is known to be stimulated by ROS and free radicals, it is logical to presume that tyrosinase activity will decrease with increases in GSH levels.

Glutathione absorption

Parenteral administration, specifically through the IV route, has been said to provide faster and better results because it avoids the first-pass metabolism seen with the oral route. However, safety continues to be a concern as there have been reports of adverse reactions. These range from mild, transient headaches and skin eruptions to severe drug reactions that have required hospitalization, as per the Philippine Dermatological Society's Advisory entitled Intravenous Glutathione as a Skin Whitening Agent, circulated on June 15, 2011.

Intravenously administered GSH is not directly utilized by cells but is cleaved into its component amino acids and is resynthesized to GSH intracellularly after transmembrane transport.[1] Hong *et al.*[14] demonstrated that after IV administration, most GSH was immediately oxidized and disappeared from the circulation with a half-life of 10 minutes, and the rest was broken down into its three component amino acids.

By contrast, oral ingestion has been reported to produce variable results in terms of skin lightening, although most information is based on anecdotal evidence and testimonials. It was previously believed that the glutathione molecule could not pass through the intestinal lining of the gastrointestinal tract and had to be broken down into its component amino acids before absorption. Animal studies have shown that the intact glutathione molecule can be absorbed through the small intestine and thus can raise plasma glutathione levels, which was not observed when equal amounts of the component amino acids were given.[2,3,5,15] Plasma GSH levels increased three-fold 90 minutes after administration and remained elevated for 2–3 hours thereafter.[3] Sodium-dependent and sodium-independent carrier systems requiring no energy were found to be responsible for this.[15,16] Other animal studies, however, have demonstrated that enteral intake of GSH precursors, specifically glutamine and cysteine (in the form of *N*-acetyl-L-cys), increase intracellular GSH levels.[1]

Caveats of oral ingestion of GSH capsules include the possibility that the capsule will not be digested, and thus glutathione will not be made available for absorption in the small intestine. At the other end of the spectrum is the potential for the capsule to be digested too early in the stomach, preventing the glutathione from reaching the small intestine.

In order to maximize absorption of the glutathione molecule, the buccal mucosa may be a useful route of administration. Substances that are absorbed through the buccal route go directly into the systemic circulation. An *in vivo* study indirectly demonstrated that GSH could be absorbed through the buccal mucosa, although the sample size was small and no similar studies have been conducted to support or refute the results.[15,16] Complications of sublingual and buccal medications are rare but can include inflammation of the mucous membranes.

A novel preparation of glutathione, with absorption using the oral mucosal route, has thus been formulated to facilitate optimum absorption into the bloodstream without the side effects of IV administration. The product investigated in this study is a lozenge containing a proprietary mixture of reduced L-glutathione, selenium, vitamin C, vitamin D3, vitamin E, and grape seed extract.

The main objective of this study was to determine the safety and efficacy of using a lozenge containing glutathione in the lightening of skin color in Filipino females.

Materials and methods

This open-label, single-arm pilot study enrolled 34 subjects, 30 of whom completed the study. Subjects were healthy women (aged 22–42 years) who worked in a tertiary hospital as medical secretaries or hospital personnel. They had Fitzpatrick skin types IV or V, with melanin indices of ≥ 20 out of a maximum value of 99. Informed consent was obtained prior to a subject's inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki.

Reasons for exclusion were: known hypersensitivity to GSH or any of the components of the product under investigation;

use of oral or IV GSH in the previous four weeks; current intake of any medications including vitamin supplements and herbal medications; use of a skin-lightening product on the test sites in the previous four weeks; presence of any abnormal pigmentation or skin lesion over the areas to be tested that might impair test results; current pregnancy, nursing, lactation, or active attempts to conceive during the time of the trial; treatment with another investigational product within one month or plans to participate in another clinical trial; current use of oral contraceptive pills or current receipt of hormone replacement therapy; smoking; frequent consumption of alcoholic beverages; presence of mouth sores or pathologies in the oral cavity that might prevent the proper use of the product under investigation; regular participation in vigorous activities (e.g. sports) that include strenuous exercise; occupational or leisure activities that involve daily sun exposure between 10.00 hours and 15.00 hours; a history of autoimmune disease or immunodeficiency; major surgery within the previous four weeks; active or recent systemic infection in the previous four weeks; and any disorder that might prevent compliance.

Subjects were given identical bottles containing 30 lozenges of the product under investigation, each of which contained 500 mg of GSH. They were instructed to put one lozenge in the mouth against the inner cheek (buccal mucosa) every morning and to keep it there until it completely dissolved.

Clinical evaluation was performed at baseline and every two weeks over a period of eight weeks. A portable mexameter (Skin Pigment Analyzer 99; Courage & Khazaka Electronic GmbH, Cologne, Germany) was used to determine the melanin index of each subject. Values ranged from 0 (no detectable pigment) to 99 (darkest pigmentation possible). Melanin indices were taken on a sun-exposed area (extensor surface of the right wrist) and a sun-protected area (mid-sternum). Three readings were taken on each spot, and the average of the three readings was recorded. Laboratory examinations were performed at baseline and at the end of the study. These included a complete blood count (CBC) and liver function tests (serum glutamate-pyruvate transaminase [SGPT] and serum glutamic oxaloacetic transaminase [SGOT]).

Study participants were asked to describe any change in skin color at the endpoint using the categories listed in Table 1 for reference. Adverse effects occurring at any time in the course of the study were recorded.

Table 1 Global assessment scores in 30 subjects

Degree of skin lightening	Score	*n* (%)
None	0	0
Mild change	1	3 (10%)
Moderate	2	27 (90%)
Obvious	3	0
Very marked	4	0

Primary outcome measures were the change in melanin indices from baseline to endpoint, the presence of abnormalities in the CBC and liver enzymes from baseline to endpoint and adverse effects as reported by the subjects.

Secondary outcome measures included the change in melanin indices at 2-week intervals for the duration of the study and the subject's subjective assessment using the 5-point scale for skin lightening (Table 1).

Statistical analysis

A Student's *t*-test was used to determine if there was a statistically significant change in the skin color of the subjects from baseline to endpoint and at 2-week intervals. A *P*-value of ≤ 0.05 was considered to indicate statistical significance. Statistical analysis was conducted using Analyse-it Version 3.90.5 (Analyse-it Software Ltd, Leeds, UK).

Results

Sun-exposed skin

At the end of this pilot study (week 8), all subjects (100%) showed a significant decrease in melanin index from baseline ($P < 0.0001$). Successive readings showed a statistically significant, steady decrease in comparison with the preceding readings at intervals of two weeks. Skin-lightening effects, as measured by decreases in the melanin index, were evident after only two weeks of regular use (Fig. 1).

Sun-protected skin

At the end of this pilot study (week 8), all subjects (100%) showed a significant decrease in melanin index from baseline ($P < 0.0001$). Successive readings showed a statistically significant, steady decrease in 26 (87%) subjects in comparison with preceding readings obtained at intervals of two weeks, except at week 6. Thus, the skin-lightening effect was evident even on sun-protected skin. Four (13%) of the 30 subjects showed slightly elevated melanin indices at the end of the study (week 8) in comparison with baseline values. However, such increases in melanin indices were not statistically significant (Fig. 2).

Safety

None of the subjects experienced any serious adverse effects for the entire duration of the study. One subject, who dropped out of the study complained of soreness in the gums caused by use of the product. Another subject who dropped out complained that the lozenge was sour and was chalky in texture. Complete blood count, SGPT, and SGOT values remained normal at the end of the study.

Subjects' global assessment

All subjects reported that they noticed skin lightening at the end of the study. Twenty-seven of the subjects (90%)

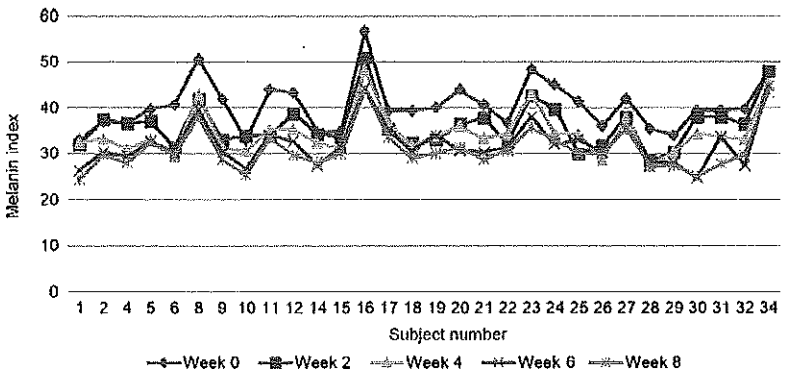

Figure 1 Melanin indices in sun-exposed skin (n = 30; Student's t-test, d.f. 29, $P < 0.0001$)

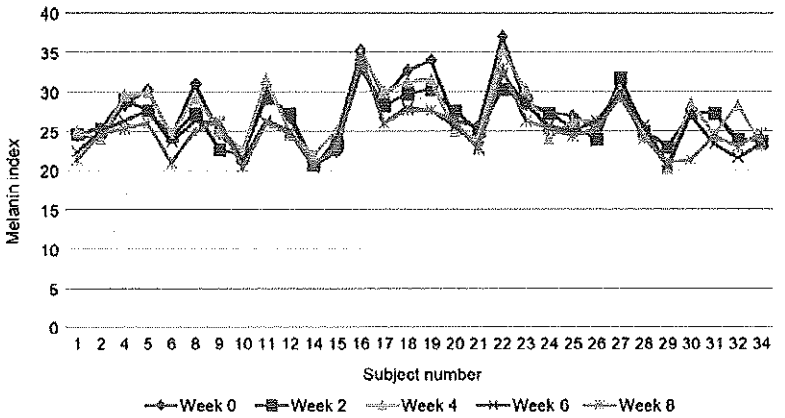

Figure 2 Melanin indices in sun-protected skin (n = 30; Student's t-test, d.f. 29, $P < 0.0001$)

noted moderate skin lightening or gave a score of 2 out of 4, and three noted mild skin lightening (10%) or gave a score of 1 out of 4. The average score was thus computed at 1.9/4.0 (Table 1).

Discussion

To our knowledge, this is the first study to evaluate the skin-lightening effect of glutathione using the oral mucosa as a route of administration. The skin-lightening effect of GSH, as measured by a decrease in the melanin index, was evident after as little as two weeks of daily use, and the melanin index continued to show a steady decrease over time when measured at 2-week intervals. This change was evident in both sun-exposed and sun-protected areas, a finding also demonstrated in a study carried out in Thailand.[5] This supports the role of GSH as an anti-melanogenic agent through several mechanisms, such as the shift in the production of eumelanins to pheomelanins, that affect the whole body, regardless of sun exposure.

There are a few advantages of using the oral mucosa as the site of absorption of glutathione. It eliminates the disadvantages imposed by oral intake, such as the decreased ability of GSH to pass through the gastrointestinal tract as an intact molecule. It also eliminates the need to use the IV route in order for the glutathione molecule to reach the systemic circulation at greater concentrations, thereby avoiding the risks associated with IV access. However, local side effects can be expected, as in the case of one subject who reported soreness of the gums caused by sucking the lozenge. Transferring the lozenge from one side of the mouth to the other to prevent the onset of soreness can minimize these incidents. Another disadvantage may refer to the flavor or texture of the lozenge, which may be disagreeable for some individuals and thus deter continued usage.

Although plasma GSH levels were not measured in the current study, it is presumed that the main ingredient in the lozenge responsible for the skin-lightening effect was GSH. The other ingredients, mostly antioxidants, may have contributed to the skin-lightening effect through the quenching of free radicals and ROS, leading to decreased tyrosinase activity. It is therefore recommended that a placebo-controlled study be conducted to ascertain that the lightening effect can be attributed to GSH alone.

The study recruited only women because it is generally Filipino women, rather than men, who wish to lighten

their skin color. Another reason for including only women was to exclude the effects of hormonal differences between the sexes that might affect melanogenesis. The study results, therefore, may only be applicable to females.

Clinically, the study subjects reported only mild to moderate skin lightening. None reported an obvious skin lightening easily apparent to other observers. The study ran for only eight weeks, and it is possible that the constant and continued use of the GSH lozenge may result in a greater and sustained skin-lightening effect. Although this was outwith the scope of this pilot study, it would be useful to know when the skin color of subjects reverted to pre-trial levels after discontinuation of the product under investigation.

Conclusions

Glutathione when taken through the mucosal route is safe and effective in lightening the skin color in Filipino women in both sun-exposed and sun-protected sites. Although the decreases in melanin indices were statistically significant, the subjective assessment of the subjects revealed only mild to moderate skin-lightening effects.

It is recommended that a placebo-controlled, randomized clinical trial with a larger sample size and longer duration be undertaken in order to establish the safety and efficacy of GSH as a skin-lightening agent. It is also recommended that subjects are followed up after the termination of the study to determine how long the skin-lightening effects last after discontinuation of the GSH lozenge.

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