

Augmented Glutathione Absorption from Oral Mucosa and its Effect on Skin Pigmentation: A Clinical Review

Dave Krishan Sharma¹, Peeyush Sharma²

¹Department of Surgery, Northwick Park Hospital, London, UK; ²Department of Surgery, North Middlesex Hospital, London, UK

Correspondence: Peeyush Sharma, North Middlesex Hospital, British Institute of Aesthetic Plastic Surgery, Maxwell Road, Peterborough, Cambridgeshire, PE2 7JE, United Kingdom, Tel +44 01733 396171, Email drsharmafrs@gmail.com

Abstract: Treatment of dark skin with glutathione has become popular due to its depigmenting properties and low toxicity. Glutathione has been used topically, orally and parenterally in the management of dark skin. There are no clear published guidelines for management of skin pigmentation despite some clinical trials of shorter duration and small sample sizes. We examined published scientific and patient data to generate guidance for the clinician for managing hyperpigmentation using glutathione by orobuccal route. Various aspects of glutathione bioavailability were examined when administered by oral routes. Absorption of glutathione from the gastrointestinal tract is poor. Some trials have favored administering high oral doses to achieve therapeutic effect. General consensus remains against treatment of hyperpigmentation with glutathione by the oral route. Clinical and experimental evidence supporting significant glutathione absorption from orobuccal mucosa was examined. The latter is superior to the oral route since glutathione passes directly into systemic circulation resulting in a much higher rate of absorption compared to that achieved by oral intake. High blood levels thus achieved have therapeutic value. Treatment of hyperpigmentation with glutathione by the orobuccal route using hydroxypropyl cellulose (HPC) film was reviewed to formulate clinical guidance from published data. A future randomized, double-blind, placebo-controlled trial should study treatment of hyperpigmentation with glutathione using oral dispersible HPC film, with longer-term follow-up and larger sample size. This paper will hopefully offer broad guidance for the clinician on use of glutathione for hyperpigmentation management, until outcomes of larger, longer duration trials become available.

Keywords: glutathione, skin, pigmentation, mucosa, absorption, orobuccal, oral dispersible film, hyperpigmentation

Introduction

Glutathione is a well known food supplement due to its perceived health benefits, in particular, for skin whitening, and several over the counter products are available on the market. Its use is being popularized by the lay press and in some cases there has been excessive usage of glutathione by the public leading to some national drug control authorities in South East Asia restricting its sale and usage. Clinical trial data in the literature examining the biological effects of glutathione intake is fragmented with no clear guidelines for the clinician especially in the management of skin pigmentation issues. As a result the clinicians use arbitrary empirical dosage schedules which lead to inconsistent results. Administration, dosage, bioavailability and other aspects of glutathione therapy, as applied to the treatment of hyperpigmentation of skin, have not been examined systematically. Our group has an interest in glutathione and it is our hope to examine its various effects using scientific methods which will provide much needed guidance to physicians. For the purposes of this review paper, we have chosen to examine the effect of glutathione on skin pigmentation and the best routes to administer it, in order to achieve high enough blood levels to be of therapeutic benefit.

Although lighter skin color is desirable in many cultures especially in Asia, Africa, and other places, tanned skin is suggestive of affluence and good health in Europe and other parts of the world. But attitudes to skin color are highly dependent upon cultural values. Women of Asian and African descent and some from other races with darker skin, eg

those with Fitzpatrick skin type IV to VI, commonly seek skin lightening treatments. Prevalence of pigmentary disorders is also higher in people with darker skin, which sometimes makes it difficult to manage these cases. Owing to the desire for lighter, fairer skin the demand for depigmenting agents is growing with several products available to buy over the counter especially in Asian countries. There are many agents with a well known depigmenting effect like pycnogenol, orchid extract, marine algae extract, cinnamic acid, soy, aloesin, *Boswellia*, hydroquinone, glycolic acid, arbutin, kojic acid, ascorbate, tocopherol, niacinamide, etc. Due to the high demand for skin lightening remedies, the growth of available products has mushroomed. Most of the products available to buy over the counter are untested and have unpredictable effects. These products range from topical agents to those which can be taken orally.

Glutathione has a role in many cellular processes and is used for the treatment of several diseases. It has many health benefits and also a well-established role as a skin lightening agent. Most cells are capable of glutathione synthesis especially hepatocytes since glutathione reductase enzyme is present in these cells. Glutathione reductase enzyme converts the oxidized form of glutathione to the reduced and biologically active reduced form (GSH). Therefore it is not an essential dietary supplement. However, the ability of cells to synthesize glutathione declines in certain disease states as well as part of ageing, probably due to lower levels of glutathione reductase enzyme. Therefore, exogenous supplements may be needed.^{1,2} Glutathione helps remove reactive oxygen species like superoxide, hydrogen peroxide, etc, which may damage DNA, RNA, and proteins and may even cause cell death. Mitochondrial function is thus impaired, which is characteristic of various diseases.²⁻⁴

There have been some trials on the effect of glutathione on skin pigmentation and these provide some clinical data. Watanabe et al used topical oxidized glutathione (GSSG) on 30 subjects in a split face randomized double blind placebo-controlled trial.⁵ They showed a significant reduction in melanin index over a 10-week study period. Arjinpathana and Asawanonda conducted a randomized double-blind placebo-controlled study on 60 subjects who were given oral glutathione and studied over a four-week period.⁶ They showed some reduction in melanin index also, but the study did not measure blood levels of glutathione. Handog et al, in a single-arm study administered glutathione as lozenges for oral absorption in 30 women over an eight-week period and showed a significant reduction in melanin index.⁷ Bruggeman et al conducted a placebo controlled trial using an orobuccal route for glutathione absorption thereby seeking to increase blood levels.⁸ Patients were given a solution with 200 mg glutathione or placebo. The solution was held in the mouth for 90 seconds and then swallowed. Blood samples were collected and glutathione levels determined. They found that glutathione absorption from oral mucosa massively and rapidly increased serum concentration. Campolo et al conducted a randomized placebo-controlled trial on 16 male subjects with cardiovascular risk factors. They treated the subjects with 200 mg per day of a sublingual formulation of glutathione. They studied peripheral vascular function, hepatic function, lipid profile and oxidative stress on the subjects after four weeks of treatment.⁹ They found that sublingual administration of glutathione significantly increased the blood levels and after four weeks of treatment, beneficial therapeutic effects were discernible. They did not study the effects on skin pigmentation, but this study did reaffirm the rapid absorption of reduced glutathione from oral mucosa to reach high serum concentrations which can have therapeutic value in many areas.

Review papers which looked at the few available clinical trials have not offered any clear guidelines for the clinician seeking advice on best practice for the management of patients with hyperpigmentation.¹¹⁻¹³ We therefore decided to review the literature and try to correlate absorption routes, bioavailability and clinical effectiveness of glutathione to formulate some guidance for the practitioner trying to treat hyperpigmentation, in particular using the orobuccal route using newer methods like the oral dispersible film. The objective of this work was to bring together all scientific and patient-related data regarding oral absorption, dosage, and clinical effects in a fusion which has clinical relevance to serve as guidance for the clinician before more robust and well-designed trials can be conducted to provide the many answers which still elude us about this very promising treatment. This paper is not intended to be a comprehensive review of glutathione, but rather its clinically relevant aspects which are of interest to the clinician treating hyperpigmentation.

Molecular Structure

Glutathione (γ -glutamyl-cysteinylglycine) is a naturally occurring low molecular weight, water soluble thiol-tripeptide formed of three amino acids (glutamate, cysteine, and glycine). Its molecular formula is $C_{10}H_{17}N_3O_6S$. Its mono-isotopic

mass is 307.083801 Da. Its IUPAC name is (2S)-2-amino-4-[[[(1R)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl]butanoic acid. Its boiling point is 754.5°C and melting point is 195°C. Glutathione has two mutually interconvertible forms, reduced glutathione (GSH) and oxidized glutathione (GSSG). The sulfhydryl group from cysteine is the biologically active part of the molecule.¹⁴ The sulfhydryl group interacts with different biochemical systems, therefore, the active form of glutathione is called GSH. GSH is oxidized during peroxide disposal by glutathione peroxidase to GSSG (two molecules of GSH bound by disulfide-bond). GSSG is reduced back to GSH by glutathione reductase enzyme. Intracellular ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) ranges from 10 to 100. GSH is involved in nutrient metabolism, antioxidant defence, and regulation of cellular metabolic functions ranging from gene expression, DNA, and protein synthesis to signal transduction, cell proliferation, and apoptosis. Bachhawat and Yadav have reviewed glutathione metabolism in the light of newly available evidence and presented a comprehensive understanding of this very important component of the intracellular redox system.¹⁰

Biological Activity

Glutathione is an effective antioxidant and the reduced form GSH is mainly intracellular where it acts as a strong antioxidant. Intracellular GSH is a defence mechanism against toxins and xenobiotics. GSH is oxidized to GSSG by glutathione peroxidase. GSSG is reduced by glutathione reductase back to GSH. Intracellular redox balance is thus maintained.¹⁵ Being a powerful antioxidant, glutathione plays a critical role in several metabolic pathways: DNA and protein synthesis, amino acid transport, enzyme activation, immune system function, scavenging free radicals eg hydrogen peroxide, cofactor in Phase I cytochrome P450 enzyme-mediated detoxification, amino acid translocation across cell membranes, detoxification of xenobiotics, maintenance of sulfhydryl groups of proteins, mitochondrial function and maintenance of mitochondrial DNA, catalysis of exchange reactions and many more.^{10,16,17}

Health Benefits of Glutathione

It has been found that low levels of glutathione exist in several diseases. There is already a body of published data on several of these diseases like cystic fibrosis, HIV, emphysema, asthma, cancer, chemotherapy, some allergic disorders, drug toxicity, autism, metabolic disorders, Alzheimers disease, etc, where low levels of glutathione have been found to coexist.¹⁸⁻²⁰ In addition, health benefits of glutathione are being increasingly recognized.^{21,22} Therapeutic uses of glutathione in various diseases are being explored and it is possible that we will see establishment of a clear role for glutathione in the management of various conditions.^{15,23} GSH depletion in the brain is a common finding in patients with neurodegenerative conditions eg Alzheimer's disease and Parkinson's disease, which can cause neurodegeneration prior to disease onset. This fact is causing some excitement in the hope that GSH may have a role in management and cessation of neurodegenerative processes involved in these conditions.²⁴ Supply of glutathione declines with advancing age, arguably because the body cannot synthesize enough. Lower glutathione levels appear to accompany poor state of health. Glutathione is the most abundant nonprotein thiol compound in the body with numerous intracellular functions, therefore, it plays several vital roles in cellular physiology.

Skin Pigmentation and Glutathione

Melanogenesis begins with the conversion of L-tyrosine to DOPA-quinone under the effect of tyrosinase enzyme (Figure 1). Intermediates are formed by addition of cysteine or glutathione to DOPA-quinone. This is followed by transformations and polymerization to the final product, pheomelanin.²⁵ There are two types of melanin in skin, black-brown eumelanin and yellow-red pheomelanin. Ratio of eumelanin and pheomelanin determines skin color.²⁶ Higher levels of pheomelanin are associated with lighter skin color and higher levels of eumelanin cause darker skin color. Ultraviolet radiation increases generation of reactive oxygen and nitrogen species, which in turn enhance tyrosinase activity and thereby synthesis of melanin species within cells. Oral antioxidants like glutathione reduce melanogenesis by suppressing these free radicals. Glutathione suppresses tyrosinase in three ways: direct inhibition through chelation of copper, interference with the transfer of tyrosinase to premelanosomes, tyrosinase inhibition indirectly by antioxidant effect.²⁷ Glutathione thus shifts the equation of melanogenesis from eumelanin to pheomelanin through reactions between thiol groups and dopaquinone through formation of sulfhydryl-dopa conjugates.²⁸ Chung et al

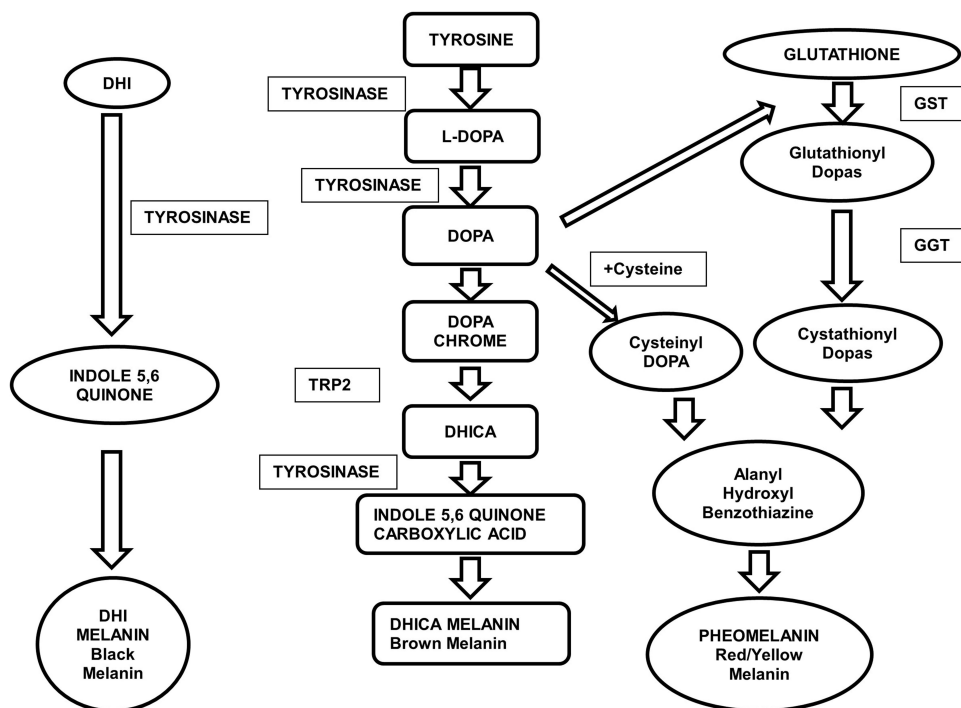


Figure 1 Melanin synthesis (Raper–Mason pathway). Role of glutathione in shifting the equation of melanin synthesis from eumelanin to pheomelanin.

Abbreviations: DHI, 5,6-dihydroxyindole; L-DOPA, Levo DOPA; DOPA, 3,4-dihydroxyphenyl alanine; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; GST, glutathione-S-transferase; GGT, gamma glutamyl transpeptidase; TRP2, tyrosinase-related protein 2.

investigated antimelanogenic effects of GSH and its derivatives *in vitro*. They concluded that the esterified GSH derivative was effective in reducing melanin and tyrosinase activity and raised the pheomelanin/eumelanin ratio without affecting cellular viability.²⁹ High blood levels of glutathione are consistent with inhibition of pigmentary disorders. There is some evidence in the literature regarding this. Wiraguna et al studied serum glutathione levels and tried to correlate them with melasma area severity index score (MASI).³⁰ They showed a significant negative linear relationship between MASI scores against plasma glutathione. Other authors have also studied the relationship between glutathione levels and skin pigmentation.³¹ Glutathione administration by parenteral route is an effective way to raise serum levels of this substance to adequate therapeutic levels however it is impractical and not easy to administer safely and uniformly. Oral route is a safe and effective way to administer glutathione, but since the latter is not adequately absorbed from the gastrointestinal tract, the oral route is ineffective as a therapeutic alternative. The orobuccal route, however, is very effective and glutathione is rapidly absorbed directly into systemic circulation with rapid rise in serum concentration, as we shall see in the following sections. With the arrival of oral dispersible film impregnated with glutathione, this can become the method of choice for treatment of hyperpigmentation.

Glutathione Absorption

Orally administered glutathione GSH is primarily absorbed in the upper jejunum. Circulating glutathione is cleared by kidneys. Hydrolysis of glutathione GSH by γ -glutamyltransferase results in poor absorption from the gastrointestinal tract. This leads to low serum levels of glutathione.³² Even oral intake of high doses of glutathione does not lead to high enough serum levels to be of therapeutic value. As such, dietary glutathione GSH is not a major determinant for rise in its blood levels.³³ In a study of GSH systemic availability by Witschi et al showed that it is impossible to boost serum GSH level to a clinically beneficial level even after oral administration of a high single dose of 3 g.³⁴ This fact limits the therapeutic use of glutathione GSH by the oral route. Therapeutic levels of glutathione in blood are difficult to arrive at since glutathione, especially GSH, exists in the serum as well as erythrocytes and leucocytes. Michelet et al have studied whole blood and plasma glutathione in subjects of various ages and life styles.³⁵ The exact significance of both the

former and the latter in therapeutic terms in the management of hyperpigmentation is unknown except what can be gathered from observational studies.³⁰ The preferred route for administration of glutathione GSH without having to resort to injection, is absorption from the orobuccal mucosa. It is well known that many active substances are absorbed from the oral mucosa. Oral mucosa has a rich vascular supply and lymphatic drainage, therefore, absorption from it can bypass intestinal and hepatic degradation leading to a high serum level which can be of therapeutic value. We shall look at this in greater detail.

Oral Mucosa

The buccal mucosa is unique in that it has a rich vascular supply as well as excellent lymphatic drainage. Those substances that can and are absorbed from the orobuccal mucosa, enter the systemic circulation without the need to pass through the intestinal absorptive mechanisms, portal circulation or the liver.³⁶ The orobuccal absorptive surface area is approximately 200 cm², supplied by a rich vascular network.³⁷ Average saliva secretion is about 1500 mL per day, about 10 mL/h, variably. Due to the large absorptive area and a rich vascular supply, the orobuccal mucosa allows a rapid absorption of glutathione and other substances by passive transport through or around the cells into the abundant capillary circulation. The absorption of glutathione and other substances from the gastrointestinal tract³⁸ and oral mucosa³⁹ has been the subject of some studies and it is now well known that orally administered glutathione cannot be relied upon for therapeutic purposes. Buonocore et al conducted a study on in vitro and in vivo absorption of glutathione GSH. The in vitro study aimed to investigate the penetration of GSH through reconstituted human oral epithelium. The objective of the in vivo study was to evaluate glutathione GSH absorption in 15 healthy volunteers.³³ The in vitro study showed penetration and fast absorption of glutathione through reconstituted oral epithelium, from 55% after 10 min to about 70% after 30 min. Kinetics of passage of glutathione through epithelium in vitro thus depicted a progressive absorption gradient saturated over time.

In vivo study by the same group³³ showed fast absorption of glutathione from orobuccal mucosa. Glutathione GSH concentration in blood increased after absorption from the orobuccal mucosa, the change being statistically significant compared to basal levels. This means that glutathione GSH blood levels increase very quickly and significantly, when administered and absorbed from orobuccal mucosa. This fact can be used for therapeutic use of this route for substances like glutathione that are poorly absorbed from the gastrointestinal tract.

This inevitably leads us to the design of orobuccal formulations for administration of various drugs especially glutathione GSH. One such new orobuccal formulation is oral dispersible film (ODF) with glutathione GSH. On the basis of the published data reviewed in this paper it is reasonable to extrapolate the findings of experimental and clinical studies to administration of glutathione GSH through the ODF, as a suitable, easy to administer, and reliable formulation.

Therapy and Dosage

Oral administration is the easiest and most common method for administration of drugs especially for longer-term usage. As demonstrated in several earlier studies, oral administration of glutathione does not lead to high enough serum concentration to be of any therapeutic value at all due to extremely poor absorption from the gastrointestinal tract.^{33,34,38} It is now well known that the orobuccal mucosa offers another route through which glutathione can be administered and it can reach high enough levels to be of value therapeutically. Evidence from experimental studies forms the basis of administration of glutathione for absorption from orobuccal mucosa as mentioned in the previous section.

Dosage of glutathione GSH depends on the indication. Glutathione may be used for treatment of diverse conditions including autism, inborn errors of metabolism, male infertility, cystic fibrosis to improve airway clearance, atherosclerosis, diabetes, immune-stimulation, liver diseases, memory loss, Parkinson's disease, Alzheimer's disease, melasma, hyperpigmentation, etc. Three commonly used routes for administration of glutathione are parenteral, topical, and oral. Bioavailability of each route is different. Here we will consider only hyperpigmentation treatment by the orobuccal route. In determining the dosage, we can first look at studies where oral administration has generated some experience on safety levels at varying doses. Such studies are not available. In a study on the depigmenting effects of glutathione, Weschawalit et al administered 250 mg glutathione daily, both reduced GSH and oxidized GSSG forms, to 60 volunteers for 12 weeks showing a good depigmenting effect without any adverse effects.⁴⁰ Glutathione is quite nontoxic, its lethal dose being

quite high, and it is generally well tolerated.^{40,41} However, high doses of glutathione for prolonged periods may cause chronic toxicity and carry some risks like zinc depletion, hypersensitivity, drug interactions, teratogenicity, etc. and this is more likely to happen when administered parenterally in an uncontrolled manner.^{42–45} Since absorption of glutathione from the gastrointestinal tract is poor, the dose can be lower when administered in an orobuccal formulation. Absorption by this route is significantly higher as recent evidence suggests.^{7–9,33} Therefore, it is safe to say that glutathione can be administered using the orobuccal route at a dose of 100–400 mg daily, variably depending upon severity of hyperpigmentation, for about 10 to 12 weeks. Safety of glutathione GSH when taken by the orobuccal route for an indefinite period of time, needs to be determined in a clinical trial especially using the oral dispersible film formulation.

Oral Dispersible Film

The increasing use of orobuccal route for administration of certain substances like glutathione, for reasons explained in previous sections, has led to efforts at refinement of methods to achieve it. One of the methods is by the use of an oral dispersible film made of hydroxypropyl cellulose (HPC), which is a nontoxic substance widely used in the pharmaceutical industry for diverse applications.^{46–48} These films are fast dissolving, nontoxic and can be used for drug delivery. HPC film is ideally suited for administration of glutathione by the orobuccal route. We have come across a product that offers glutathione impregnated HPC film for application to buccal mucosa from where glutathione is directly absorbed into the systemic circulation (Lyuh Esther Glutathione Direct, Bio 360, Korea). The film has a surface area of about 9–10 cm² and may assist in more rapid absorption from the orobuccal mucosa, after the film absorbs moisture and releases glutathione. It can be postulated on the basis of available evidence from earlier studies³³ that presenting a film impregnated with for example glutathione, over a large surface area of oral mucosa will hasten absorption. We are planning a clinical trial to evaluate treatment of hyperpigmentation with glutathione using this method.

Discussion

Glutathione is a major and important antioxidant. Its role in several intracellular processes is well known.^{15–17} Glutathione is synthesized in the body as part of a major redox system however the ability to synthesize it apparently declines with age.^{15,51} This is evidenced by the lower levels of glutathione in several diseases as discussed earlier. Glutathione is also involved in controlling melanin synthesis. Owing to the preference for a fairer complexion in many parts of the world due to personal and social reasons, there has been a huge interest in glutathione as a depigmenting agent. Therefore, many therapeutic modalities have emerged ranging from topical applications to parenteral infusions. For safety reasons some governments have banned some of these therapeutic agents as the potential for misuse was perceived to be high especially the parenteral use of glutathione for skin whitening practised in some Asian countries.⁴⁹ There are now several trials in the literature documenting a positive role of orally administered glutathione for the treatment of hyperpigmentation, despite its poor absorption.^{6,7,12,13,33,40} Several review papers have tried to pull together evidence supporting the role of glutathione in reducing skin pigmentation.^{11–13} Nearly all trials are conducted over shorter durations of time and there is no long-term data available on the effect of orally administered glutathione on skin pigmentation. Long-term data is important in view of the fact that those individuals, predominantly women, who seek treatment need to be managed over longer periods of time to maintain effect and as part of their personal preference and self-esteem.

In the present paper we have reviewed all relevant scientific aspects of orally administered glutathione as applied to the treatment of hyperpigmentation. The review of published data is not necessarily replete in absolute terms because only data of clinical relevance in the management of hyperpigmentation has been included. The role of glutathione, especially the reduced GSH form, in reducing pigmentary changes of skin is now well established as seen from the many studies, but no consensus has emerged on dosage and routes of administration.^{5–7,33} The effect of glutathione on skin pigment in trials is measured by melanin index which yields numeric data. One also needs to consider the subjectively perceived change from a darker to a lighter skin color. There is no mention of patient satisfaction with their appearance posttreatment in any of the trials. It is also important to ascertain how long the depigmenting effect thus achieved would last and what the long-term effects would be on skin color by repetitive treatments lasting several weeks. None of the published trials offer that information. There is thus a clear need for a trial over a longer period looking at these areas.

Design of such a trial should be given some consideration because the goals here are multiple: first to determine how long the effect would last, second to determine effectiveness of a repeat treatment, and third the general health of the patient and how well they tolerate the repeat treatments.

Glutathione is a safe and effective agent. Its role in the management of several important conditions like autism, cystic fibrosis, drug induced hepatic injury, HIV, and many others^{18–21,50} is just becoming apparent, but the main stumbling block for its therapeutic potential is the route of administration. Orally administered glutathione is only sparingly absorbed due to its rapid hydrolysis by γ -glutamyltransferase present in intestinal mucosa, hepatocytes and cholangiocytes. Therefore, it is not possible to boost blood levels of glutathione GSH to a clinically beneficial level even after oral administration of high doses.^{32–34} There is some published evidence on oral treatment with glutathione especially its absorption from the gastrointestinal tract.^{33,34} After oral administration, absorption of glutathione is very poor mainly due to hydrolysis by intestinal and hepatic γ -glutamyltransferase as mentioned above, and for that reason, most therapeutic approaches using the oral route struggle to achieve results and the patients seek less safe treatments like intravenous treatment often under uncontrolled situations which may not be medically supervised, in some countries. For patients seeking a lighter skin there is always the option of parenteral glutathione, but the most popular route of administration is oral. Intravenous glutathione for skin lightening especially at high doses has the potential of serious toxicity and although there are no serious adverse events reported in the literature, advisories against this treatment have been issued in the past. The food and drug administration of Philippines clearly states the following in an advisory: “Side effects on the use of injectable glutathione for skin lightening include toxic effects on the liver, kidneys, and nervous system. Also of concern is the possibility of Stevens Johnson Syndrome”.⁴⁹ It is advisable to avoid parenteral glutathione until more is known about the pharmacodynamics of parenterally administered glutathione. This becomes especially relevant when there are superior options like administration by the orobuccal route, whereby high serum levels are rapidly achieved.^{7–9} This route has not been seriously considered as a widely prescribed option for skin lightening treatments. Equally we now know that glutathione GSH has a therapeutic role in the management of other diseases also, therefore, the oral dispersible film method for orobuccal administration assumes huge significance.^{18–21}

As has been discussed, glutathione is rapidly absorbed by the orobuccal route and reaches high enough levels in the blood to be therapeutically efficacious. Studies comparing gastrointestinal versus orobuccal absorption have demonstrated that the latter is far superior in that glutathione enters the circulation directly and rapidly reaches high levels.^{7–9,33,39} Therapeutic value of this route, although known for some time, needs further evaluation. Some recent studies have made a beginning in this direction.^{7–9,33} However, the rate of absorption per square centimeter and the evaluation of oral films versus lozenges in terms of achieving the highest rate of absorption, etc need further study. At the present level of our knowledge, it is safe to conclude that the orobuccal route can be used for treatment of hyperpigmentation with glutathione GSH at a dose of 100–400±50 mg per day for periods of between 10 and 12 weeks, depending upon indication, and it is hoped the treatment can be repeated to maintain effect. This recommendation is based on an evaluation of the published clinical data on dosage and toxicology related considerations.^{40,42–45} Having established this we have to consider that there is no uniform formulation for the administration of glutathione GSH by the orobuccal route in that people have used gels, lozenges, liquids, etc. The availability of oral dispersible HPC film aims to make orobuccal administration of glutathione GSH easy and uniform for all patients.

Orobuccal administration of glutathione is thus superior, but we do not know much about this route and its long-term reliability. It is, however, well worth investigating the orobuccal absorption of glutathione in view of what we prefer to call intestinal inflation in that over 90% of it is destroyed and eliminated in the gastrointestinal tract. We do know that the orobuccal mucosa offers a large surface area of about 200 cm². The orobuccal mucosa is richly supplied by vasculature and its lymphatic drainage is equally impressive. The most valuable property of the orobuccal mucosa is that drugs that can be absorbed, enter the systemic circulation directly by passive transport.^{36,38,39} Compared to oral administration, whereby under 10% actually enters the blood stream,⁵² orobuccal administration leads to over 80% absorption directly into the systemic circulation.³³ It is thereby safe to say on the basis of published data, that absorption from the orobuccal route, for example by using oral dispersible film, is over 80% greater as compared to absorption from the gastrointestinal tract. It is also noteworthy that blood and tissue levels of glutathione rise slowly and cumulatively only when large doses of it are administered orally.⁵² The study by Richie et al gives us important insights into rise in concentrations of

glutathione in erythrocytes, plasma, lymphocytes and buccal cells over time. It was seen that the most significant rises in tissue glutathione occurred in the high-dose group after about six months.⁵² While the rate of increase in tissue levels of glutathione depends upon dose and time elapsed, the real value of administration by the orobuccal route lies in that, firstly smaller doses can be used and secondly, its immediate availability to deal with oxidative stress and toxins if any. Glutathione therapy is safe and occurrence of adverse events like pruritus, macular erythema, red spots on the skin, and tiredness, etc is rare.⁴⁰

Glutathione absorption by the orobuccal route is thus a highly prized tool in the armamentarium of the practitioner, but one that is poorly understood and its reliability for long-term drug delivery, eg that of glutathione, needs investigation. It would be highly desirable to conduct a trial studying absorption of glutathione from the human orobuccal mucosa comparing administration in the form of a lozenge versus an HPC film which theoretically offers more rapid absorption over a larger surface area. Such a trial to study treatment of hyperpigmentation with glutathione must also study long-term effects of single treatment, maintenance of effect over time and value of at least one repeat treatment. This must be a randomized, double-blind, placebo-controlled trial with a longer-term follow-up and large sample size, to evaluate efficacy, reliability and safety of skin-lightening effect of glutathione administered by the orobuccal route. Until the outcome of such a trial becomes available, we must use our clinical judgement and best practice on the basis of published clinical data, as offered in this work. The present paper will hopefully offer that crucial link in the decision-making process that most clinicians seek.

Conclusion

In this paper we have examined clinically relevant published data on the best treatment methods to achieve high serum and tissue levels of glutathione to treat hyperpigmentation. It is concluded that earlier trials are of short duration, on small sample sizes and did not document patient satisfaction, duration of depigmenting effect or need for repeat treatments. None of the earlier trials have documented how well the patient population tolerated the various dosage regimen either. Therefore we conclude that firstly, a future trial should address all these areas. Glutathione is an important agent for the treatment of hyperpigmentation and also for the treatment of a number of other diseases where it has an important role to play. Secondly, in view of the poor absorption of glutathione from the gastrointestinal tract and the relative unavailability of other effective and easy methods of administration, the orobuccal route should be the preferred choice. The effectiveness of the orobuccal route for glutathione administration in rapidly achieving high serum concentrations is well documented. The orobuccal administration of glutathione can be standardized by using the HPC oral dispersible film. Thirdly, on the basis of published and empirical data, we would like to offer some guidance to clinicians regarding dosage of glutathione GSH. A dose of 100 to 400±50 mg per day, in single or divided doses, for periods of between 10 and 12 weeks can be administered by the orobuccal route, preferably using the oral dispersible film, depending upon severity of hyperpigmentation. This treatment advice is based on consideration of all aspects of glutathione GSH absorption and its safety, until results of a more comprehensive trial become available.

Acknowledgments

Evaluation of published data was carried out jointly by authors. A standardized approach was used. Authors are grateful to Miss Uma Sharma, for help with literature search, spell checking and composing the layout of the paper.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lu SC. Regulation of glutathione synthesis. *Mol Aspects Med.* 2009;30(1–2):42–59. doi:10.1016/j.mam.2008.05.005
2. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr.* 2004;134(3):489–492. doi:10.1093/jn/134.3.489
3. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation.* 2001;104(22):2673–2678. doi:10.1161/hc4601.099485

4. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med.* 1997;23(1):134–147. doi:10.1016/S0891-5849(96)00629-6
5. Watanabe F, Hashizume E, Chan GP, Kamimura A. Skin lightening and skin-condition-improving effects of topical oxidized glutathione: a double-blind and placebo-controlled clinical trial in healthy women. *Clin Cosmet Investig Dermatol.* 2014;7:267–274. doi:10.2147/CCID.S68424
6. Arjinpathana N, Asawanonda P. Glutathione as an oral lightening agent: a randomized, double-blind, placebo controlled study. *J Dermatolog Treat.* 2012;23:97–102. doi:10.3109/09546631003801619
7. Handog EB, Datuin MS, Singzon IA. 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. *Int J Dermatol.* 2016;55:153–157. doi:10.1111/ijd.12999
8. Bruggeman BK, Storo KE, Fair HM, Wommack AJ, Carriker CR, Smoliga JM. The absorptive effects of orobuccal non-liposomal nano-sized glutathione on blood glutathione parameters in healthy individuals: a pilot study. *PLoS One.* 2019;14(4):e0215815. doi:10.1371/journal.pone.0215815
9. Campolo J, Bernardi S, Cozzi L, et al. Medium-term effect of sublingual l-glutathione supplementation on flow-mediated dilation in subjects with cardiovascular risk factors. *Nutrition.* 2017;38:41–47. doi:10.1016/j.nut.2016.12.018
10. Bachhawat AK, Yadav S. The glutathione cycle: glutathione metabolism beyond the γ -glutamyl cycle. *IUBMB Life.* 2018;70(7):585–592. doi:10.1002/iub.1756
11. Sonthalia S, Daultabad D, Sarkar R. Glutathione as a skin whitening agent: facts, myths, evidence and controversies. *Indian J Dermatol Venereol Leprol.* 2016;82:262–272. doi:10.4103/0378-6323.179088
12. Sitohang IBS, Ninditya S. Systemic glutathione as a skin-whitening agent in adult. *Dermatol Res Pract.* 2020;2020:6. doi:10.1155/2020/8547960
13. Dilokthornsakul W, Dhippayom T, Dilokthornsakul P. The clinical effect of glutathione on skin color and other related skin conditions: a systematic review. *J Cosmet Dermatol.* 2019;18(3):728–737.
14. Murray RK. Metabolism of xenobiotics. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA, editors. *Harper's Illustrated Biochemistry.* 28th ed. Michigan: McGraw-Hill; 2009:612–613.
15. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med.* 2009;30(1–2):1–12.
16. Meister A, Tate SS. Glutathione and related gamma-glutamyl compounds: biosynthesis and utilization. *Annu Rev Biochem.* 1976;45:559–604. doi:10.1146/annurev.bi.45.070176.003015
17. Hodges R, Minich D. Modulation of metabolic detoxification pathways using foods and food-derived components: a scientific review with clinical application. *J Nutr Metab.* 2015;2015:760689. doi:10.1155/2015/760689
18. Herzenberg LA, De Rosa SC, Dubs JG, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci USA.* 1997;94:1967–1972. doi:10.1073/pnas.94.5.1967
19. Grey V, Mohammed SR, Smountas AA, Bahloul R, Lands LC. Improved glutathione status in young adult patients with cystic fibrosis supplemented with whey protein. *J Cyst Fibros.* 2003;2:195–198. doi:10.1016/S1569-1993(03)00097-3
20. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother.* 2003;57:145–155. doi:10.1016/S0753-3322(03)00043-X
21. Kern JK, Geier DA, Adams JB, Garver CR, Audhya T, Geier MR. A clinical trial of glutathione supplementation in autism spectrum disorders. *Med Sci Monit.* 2011;17:CR677–82. doi:10.12659/MSM.882125
22. Pizzorno J. Glutathione. *Integr Med.* 2014;13(1):8–12.
23. Wu JH, Batist G. Glutathione and glutathione analogues; therapeutic potentials. *Biochim Biophys Acta.* 2013;1830(5):3350–3353. doi:10.1016/j.bbagen.2012.11.016
24. Aoyama K. Glutathione in the brain. *Int J Mol Sci.* 2021;22(9):5010. doi:10.3390/ijms22095010
25. Rzepka Z, Buszman E, Beberok A, Wrzeński D. From tyrosine to melanin: signaling pathways and factors regulating melanogenesis. *Postepy Hig Med Dosw.* 2016;70:695–708. doi:10.5604/17322693.1208033
26. Nordlund JJ, Boissy RE. The biology of melanocytes. In: Freinkel RK, Woodley DT, editors. *The Biology of the Skin.* New York: CRC Press; 2001:113–130.
27. Yamamura T, Onishi J, Nishiyama T. Antimelanogenic activity of hydrocoumarins in cultured normal human melanocytes by stimulating intracellular glutathione synthesis. *Arch Dermatol Res.* 2002;294:349–354. doi:10.1007/s00403-002-0345-8
28. Karg E, Odh G, Wittbjør A, Rosengren E, Rorsman H. Hydrogen peroxide as an inducer of elevated tyrosinase level in melanoma cells. *J Invest Dermatol.* 1993;100 2 Suppl:209S–13S. doi:10.1038/ijd.1993.78
29. Chung BY, Choi SR, Moon IJ, Park CW, Kim YH, Chang SE. The glutathione derivative, GSH monoethyl ester, may effectively whiten skin but GSH does not. *Int J Mol Sci.* 2016;17(5):629. doi:10.3390/ijms17050629
30. Wiraguna AAGP, Hari ED, Praharsini IGAA. Correlation between glutathione plasma with degree severity of melasma in Balinese women. *Clin Cosmet Investig Dermatol.* 2020;13:455–459. doi:10.2147/CCID.S258834
31. Villarama CD, Maibach HI. Glutathione as a depigmenting agent: an overview. *Int J Cosmet Sci.* 2005;27(3):147–153. doi:10.1111/j.1467-2494.2005.00235.x
32. Hagen TM, Wierzbicka GT, Bowman BB, Aw TY, Jones DP. Fate of dietary glutathione: disposition in the gastrointestinal tract. *Am J Physiol.* 1990;259(4 Pt 1):G530–G535. doi:10.1152/ajpgi.1990.259.4.G530
33. Buonocore D, Grosini M, Giardina S, et al. Bioavailability study of an innovative orobuccal formulation of glutathione. *Oxid Med Cell Longev.* 2016;2016:3286365. doi:10.1155/2016/3286365
34. Witschi A, Reddy S, Stofer B. The systemic availability of oral glutathione. *Eur J Clin Pharmacol.* 1992;43:667–669. doi:10.1007/BF02284971
35. Michelet F, Gueguen R, Leroy P, Wellman M, Nicolas A, Siest G. Blood and plasma glutathione measured in healthy subjects by HPLC: relation to sex, aging, biological variables, and life habits. *Clin Chem.* 1995;41(10):1509–1517. doi:10.1093/clinchem/41.10.1509
36. Moharamzadeh K, Brook IM, VanNoort R, Scutt AM, Thornhill MH. Tissue-engineered oral mucosa: a review of the scientific literature. *J Dent Res.* 2007;86(2):115–124. doi:10.1177/154405910708600203
37. Chotaliya MKB. Overview of sublingual tablets. *Pharma Sci Monit.* 2013;4:151–170.
38. Flagg EW, Coates RJ, Eley W, et al. Dietary glutathione intake in humans and the relationship between intake and plasma total glutathione level. *Nutr Cancer.* 1994;21:33–45. doi:10.1080/01635589409514302

39. Hunjan MK, Evered DF. Absorption of glutathione from the gastro-intestinal tract. *Biochim Biophys Acta*. 1985;815:184–188. doi:10.1016/0005-2736(85)90287-1
40. Weschawalit S, Thongthip S, Phutrakool P, Asawanonda P. Glutathione and its antiaging and antimelanogenic effects. *Clin Cosmet Investig Dermatol*. 2017;10:147–153. doi:10.2147/CCID.S128339
41. Kovacs-Nolan J, Rupa P, Matsui T, et al. In vitro and ex vivo uptake of glutathione (GSH) across the intestinal epithelium and fate of oral GSH after in vivo supplementation. *J Agric Food Chem*. 2014;62(39):9499–9506. doi:10.1021/jf503257w
42. Awasthi S, Bajpai KK, Piper JT, et al. Interactions of melphalan with glutathione and the role of glutathione S-transferase. *Drug Metab Dispos*. 1996;24(3):371–374.
43. Kaplowitz N. Interaction of azathioprine and glutathione in the liver of the rat. *J Pharmacol Exp Ther*. 1977;200(3):479–486.
44. Kumar A, Singh BK, Ahmad I, et al. Involvement of NADPH oxidase and glutathione in zinc-induced dopaminergic neurodegeneration in rats: similarity with paraquat neurotoxicity. *Brain Res*. 2012;15(1438):48–64. doi:10.1016/j.brainres.2011.12.028
45. Suzuki H, Miki S, Oshima M, Sado T. Chronic toxicity and teratogenicity studies of glutathione sodium salt. *Clin Rep*. 1972;6:2393.
46. Bhyan B, Jangra S, Kaur M, Singh H. Orally fast dissolving films: innovations in formulation and technology. *Int J Pharm Sci Rev Res*. 2011;9:9–15.
47. Karki S, Kim H, Na SJ, Shin D, Jo K, Lee J. Thin films as an emerging platform for drug delivery. *Asi J Pharm Sci*. 2016;11:559–574.
48. Dey P, Ghosh A. Wafers: an innovative advancement of orodispersible films. *Int J Apl Pharm*. 2016;8:1–7.
49. FDA Advisory No. 2019-182. Unsafe use of glutathione as skin lightening agent. Available from: <https://www.fda.gov/ph/fda-advisory-no-2019-182-unsafe-use-of-glutathione-as-skin-lightening-agent/>. Accessed August 24, 2022.
50. Bjørklund G, Tinkov AA, Hosnedlová B, et al. The role of glutathione redox imbalance in autism spectrum disorder: a review. *Free Radic Biol Med*. 2020;160:149–162. doi:10.1016/j.freeradbiomed.2020.07.017
51. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions. *J Amino Acids*. 2012;2012:736837. doi:10.1155/2012/736837
52. Richie JP Jr, Nichenametla S, Neidig W, et al. Randomized controlled trial of oral glutathione supplementation on body stores of glutathione. *Eur J Nutr*. 2015;54(2):251–263. doi:10.1007/s00394-014-0706-z

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