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Glutathione as a depigmenting agent: an overview

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Synopsis

Glutathione is an ubiquitous compound found in our bodies. Aside from its many ascribed biologic functions, it has also been implicated in skin lightening. We review *in vitro* and *in vivo* studies that show evidence of its involvement in the melanogenic pathway and shed light on the its anti-melanogenic effect. Proposed mechanisms of action include: (a) direct inactivation of the enzyme tyrosinase by binding with the copper-containing active site of the enzyme; (b) mediating the switch mechanism from eumelanin to phaeomelanin production; (c) quenching of free radicals and peroxides that contribute to tyrosinase activation and melanin formation; and d) modulation of depigmenting abilities of melanocytotoxic agents. These concepts supported by the various experimental evidence presented form basis for future research in the use of glutathione in the treatment of pigmentary disorders.

Résumé

Le glutathion est un composé ubiquitaire présent dans nos organismes. En plus de ses fonctions biologiques déjà décrites, il a été impliqué dans le blanchissement de la peau. Nous passons en revue les études *in vitro* et *in vivo* qui apportent des preuves de son implication dans la voie de la mélanogénèse et des possibles explications de son effet anti mélanogénétique. Les mécanismes proposés de cette action incluent: (a) une inactivation directe de l'enzyme tyrosinase par liaison au Cuivre du site actif de l'enzyme; (b) influencer le mécanisme de bascule entre la production d'eumélanine vers la phaeomélanine; (c) extinction des radicaux libres et des peroxydes qui contribuent à activer la tyrosinase; et (d) modulation des capacités dépigmentantes des agents mélanocytotoxiques. Les concepts appuyés par les diverses démonstrations expérimentales présentées ici forment les bases des recherches futures sur l'utilisation du glutathion dans le traitement des désordres pigmentaires.

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Introduction

Melanogenesis is a highly complex process influenced by various internal and external factors. Sulfhydryl-containing compounds are determinants of the quality of pigmentation produced by mammalian melanocytes. Glutathione, the most abundant thiol in the body, is believed instrumental in producing skin lightening [1]. Although other thiols like cysteine play equally significant roles in melanogeneis, this article deals mainly with concepts, theories and experimental evidence that elucidate the role of glutathione in the inhibition of melanization.

Glutathione synthesis and metabolism

Glutathione is formed by three amino acids namely glutamate, cysteine, and glycine. Its synthesis and utilization are linked by the gamma-glutamyl cycle. It is an ubiquitous compound containing a biologically active sulfhydryl (SH) group which allows for interactions with a variety

of biochemical systems. The functions ascribed to glutathione include: (a) maintenance of the SH groups of proteins and other molecules; (b) destruction of hydrogen peroxide and other free radicals; (c) catalyst for disulfide exchange reactions; (d) coenzyme for some enzymes; (e) detoxification of foreign compounds; and (f) translocation of amino acids across cell membranes [2].

Glutathione exists in cells mostly in the reduced form (GSH). GSH is constantly being oxidized in cells forming oxidized glutathione (GSSG) and its supply is replenished by the action of glutathione reductase (GR) [3]. The relative concentrations of these intermediates may be modulated by both internal (hormones, inflammation) [4, 5] and external (UV, heat, chemicals) [6] factors. In recent years, the role of GSH and other thiols in melanocyte metabolism and human pigmentation has been investigated.

Early concepts about effects of thiols on pigmentation

Early studies on pigmentation have documented important relationships between thiols and skin color. Sulfhydryl-containing compounds in water extracts of human epidermis were observed to prevent the formation of melanin from tyrosine by tyrosinase [7, 8]. Rothman *et al.* [7] postulated that oxidation of this sulfhydryl compound by X-ray, UV, heat, inflammation inactivated it and removed its inhibitory effect on tyrosinase leading to hyperpigmentation. Halprin *et al.* [3] presented convincing physical, chemical, and biologic evidence that the sulfhydryl compound referred to by Rothman *et al.* was the tripeptide glutathione. He also demonstrated significantly lower levels of GSH in human black skin compared to white skin. Lower levels of GSH were documented in black skin areas compared to red and yellow skin in tortoiseshell guinea pigs [9]. These observations linked low levels of glutathione with production of darker pigment.

The mechanisms by which the thiols influence melanogenesis are complex. We discuss their direct effects on tyrosinase that inhibit melanin production and their indirect effects by mediating the switch from eumelanogenesis to phaeomelanogenesis.

Thiols and their effects on the enzyme tyrosinase

Because the inhibition of mammalian melanogenesis by sulfhydryl compounds is believed to involve critical steps in the tyrosinase metabolic pathway, we review this briefly. Tyrosinase is a multifunctional copper-containing enzyme which catalyzes the first step of the melanin pathway (see Fig. 1). It converts L-tyrosine to L-dopa then subsequently to dopaquinone. At this point, melanocytes may either enter into the classical pathway leading to eumelanin (darker pigment) formation or, through certain switch mechanisms involving the formation of thiol-dopa conjugates, enter the alternative pathway forming phaeomelanins and mixed-melanins called trichromes (lighter pigments) [10].

Because tyrosinase is the rate-limiting step in melanin formation, inhibiting it is a major step to reduce pigment production. Evidence shows that glutathione may affect tyrosinase directly, interfere with its cellular transport processes, or indirectly inactivate it through modulation of cysteine levels.

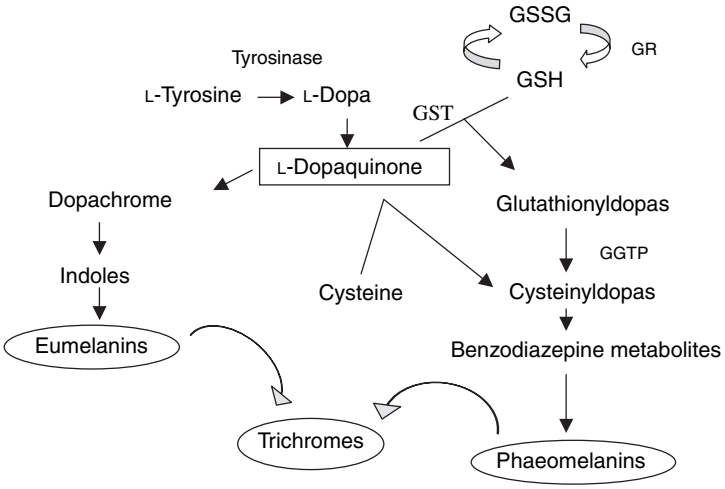

Figure 1 Interpretation of the interaction of thiols in the tyrosinase pathway. Reduced glutathione (GSH) and cysteine bind with dopaquinone to produce thiol-dopas to favor phaeomelanogenesis. Glutathione-*S*-transferase (GST) catalyzed binding of GSH and dopaquinone. Glutathione reductase (GR) replenishes supply of GSH in the cells. Gamma-glutamyl transpeptidase (GGTP) converts glutathionyldopas to cystathionyldopas for entry into the pathway.

Thiols chelate copper in tyrosinase, thereby, effectively inactivating it

Sulfhydryl compounds effectively inhibit mammalian tyrosinase. The thiol groups were demonstrated to chelate copper ions on the active site of the enzyme, leading to its inactivation [11]. This inactivation was found to be reversible by the addition of copper sulfate, underlining the protective effect of copper on tyrosinase activity [12]. Several experiments show that addition of glutathione and another thiol cysteine caused inactivation of melanoma tyrosinase in a dose-dependent manner [13, 14].

Some studies, however, point out that this may not be the main mechanism by which thiols inhibit melanin formation. Seiji *et al.* [15] showed that the pK_i value of the glutathione levels in the blood of humans (6×10^{-4} M) produced no inhibition of tyrosinase activity. Also, the regular occurrence of cysteinyldopas, a thiol compound, in melanin-forming cells indicates that tyrosinase is capable of activity in an environment containing cysteine or cysteine-containing peptides such as glutathione [16].

Glutathione interferes will cellular transport of tyrosinase

Tyrosinase exists in three molecular forms, T1, T2, and T3 depending on their maturation process inside intracellular melanogenic compartments [17]. Transport of tyrosinase into premelanosomes is necessary for initiation of melanogenesis *in vivo* [18]. Glutathione blocks formation of T3 tyrosinase by inhibiting the active transfer of T1 tyrosinase from GERL-coated vesicles to premelanosomes. This finding along with electromicroscopic differences in premelanosome size and structure in glutathione-treated cells led authors to concluded that glutathione inhibits recovery of melanogenesis after glycosylation inhibition [18, 19].

Alpha tocopherol, and hydrocoumarins, proven biologic upregulators of GSH, are believed to exert their antimelanogenic effects, not by direct inhibition of tyrosinase activity, but by blocking tyrosinase transfer to premelanosomes [20].

Glutathione depletion enhances tyrosinase activity and increases melanin content

Prota [1] documented that decreased glutathione concentration was associated with the following findings: enhanced tyrosine hydroxylation favoring L-dopaquinone formation; slower rate of reaction between L-dopaquinone and thiols; and lower levels of thiol-dopa conjugates. The end result is the preferential conversion of L-dopaquinone to L-dopachrome, increasing eumelanogenesis.

Using L-buthionine-*S*,*R*-sulfoximine (BSO) to deplete GSH in human melanoma cells, del Marmol *et al.* demonstrated that tyrosinase activity was increased by 158% that 14C-melanin formation (reflective of global eumelanogenesis) increased to 400% that of control. As BSO has no direct effect on the enzyme, they postulate the activation paralleled the depletion of GSH [21] . These findings support earlier observations relating lower GSH levels to black skin in human and animal studies [3, 9].

GSH is an antioxidant that quenches free radicals and peroxides which have been shown to induce tyrosinase activity. It is postulated that the quenching effects of GSH contribute to its antimelanogenic activity [22]. It is logical to presume that depletion of GSH allows for increased tyrosinase activity and melanogenesis through the uninhibited action of free radicals.

Effect of glutathione on the phaeomelanin pathway

In reality, most mammalian melanin pigments are mixed and consist of eumelanins and pheaomelanins, either as copolymers or mixtures[23]. At a critical point in the tyrosinase pathway, thiols (cysteine and glutathione) react with dopaquinone enabling the switch from eumelanogenesis to pheaomelanogenesis resulting in lighter skin pigmentation [10,14]. In the absence of thiols on the other hand, dopaquinone is rearranged to indole derivatives proceeding to eumelanin formation (see Fig. 1) [16].

Cysteine quickly binds with dopaquinone through a non-enzymatic reaction to give rise to cysteinyldopas. These undergo oxidative cyclization of their cysteinyl residues and are then converted to benzothiazine metabolites and ultimately to phaeomelanins or mixed type melanins called trichromes [23, 25]. Glutathione likewise combines with dopaquinone to form glutathionyldopas [26]. Whether this reaction proceeds through a non-enzymatic conjugation or through the enzyme glutathione-*S*-transferase is a matter of dispute [27]. These glutathionyldopas in turn undergo enzymatic hydrolysis, by the enzymes Υ-glutamyl transpeptidase (and possibly dipeptidases) to form

cysteinyldopas that subsequently enter the pheaomelanogenic pathway [28].

The switch from eumelanogenesis to phaeomelanogenesis is influenced by the modification of the ratio between cysteine and glutathione levels. Phaeomelanogenesis preferentially proceeds under conditions of high cysteine concentrations and low tyrosinase activity [29]. Being the major physiologic reservoir of cysteine, glutathione concentrations influence cysteine levels and may influence this switching process as well [30].

The effect of GSH levels on depigmenting agents

Many depigmenting chemicals are melanocytotoxic. They are oxidized in the cell to produce highly toxic intermediates such as quinones. These quinones destroy melanocytes ultimately resulting in loss of pigment [31]. Glutathione is believed to protect the melanocyte from such reactions through its antioxidant properties [32]. The enzyme glutathione-*S*-transferase(GST) mediates this cytoprotective action by conjugating intracellular GSH with toxic species and free radicals [22]. Factors that depress cellular levels of GSH or decrease the activity of GST likewise lead to impairment of this cytoprotective effect [33]. Several studies show that by depleting intracellular GSH levels the ability of melanocytotoxic agents to produce depigmentation becomes enhanced [34–37] (see Table I).

Bolognia *et al.* [35] showed that BSO and cystamine, both chemical depletors of GSH [38,39], enhanced the depigmenting effect of hydroquinone (HQ), a known melanocytotoxic agent on black and yellow hairless mice.

N-acetyl-4-*S*-CAP is a lipophilic drug that effectively inhibits melanogenesis by being a competitive

Table I Effects of glutathione levels on depigmenting abilities of some chemicals

Author (year) [reference]	Depigmenting agents	Results	Theories/concepts
Bolognia *et al.* (1995) [35]	Hydroquinone cystamine and BSO; *In vitro*: on murine and human melanoma cell lines; *In vivo*: black and yellow hairless guinea pigs	Decreased hair pigmentation and epidermal melanin content	Decrease in GSH led to loss of cellular antioxidant protection thus enhancement of melanocytotoxic effect of HQ
Yonemoto *et al.* (1983) [34]	TBC; *In vitro*: human melanoma cell line	Decrease in eumelanin, sulfur-content, and cell color and increased enzymatic activity of GR and GGTP	Increased activity of GR and GGTP promote phaeomelanogenesis
Yamamamura *et al.* (2002) [20]	Hydrocoumarins and α-tocopherol; *In vitro*: cultured normal human melanocytes	Increased intracellular GSH in treated cells	Antimelanogenic activity may be a combination of antioxidant property as well as promoter of GSH synthesis
Alena *et al.* (1995) [36]	NASCAP; *In vivo*: multiple intraperitoneal injections in black and yellow mice; modulation of GSH by BSO and NAC	Dose-dependent depigmenting potency in parallel to tissue eumelanin content and tissue glutathione content was enhanced by BSO and abolished by NAC	Tissue glutathione content protects cells from melanocytotoxicity of NASCAP and may be involved in melanogenesis switch mechanisms
Kasraee *et al.* (2003)* [37]	ATRA	Enhancement of depigmenting effect of hyroquinone and hydroxyanisole	Impairment of GSH-dependent cytoprotection by ATRA leads to enhancement of melanocytotoxic effects

ATRA, all-*trans*-retinoic acid; BSO, buthionine-l-sulfoximine; GGTP, gamma-glutamyl transpeptidase; GSH, reduced glutathione; GR, glutathione reductase; NAC, *N*-acetylcysteine; NASCAP, *N*-acetyl-4-*S*-cysteaminylphenol; TBC, 4-tertiary butyl catechol.
*Review article.

inhibitor of tyrosinase and by increasing the cytotoxic potential of orthoquinone intermediates [40]. It has been shown to be cytotoxic to melanocytes in both *in vitro* and *in vivo* studies, leading to loss of pigmentation. It also conjugates with glutathione and produces a relative depletion of glutathione in the cytosol [41]. Alena *et al.* [36] demonstrated that BSO also potentiated its skin lightening effects. Moreover, they proved that by upregulating GSH by adding *N*-acetylcysteine (NAC), there was complete reversal of the process, leading to repigmentation.

Among the retinoids, all-*trans*-retinoic acid (ATRA) is the most potent inhibitor of GST activity. It also reduces intracellular GSH in certain cells in a time and dose-dependent manner [42]. The fact that ATRA synergistically enhances the depigmenting effects of some melanocytotoxic agents is well established [43]. Aside from the well-known fact that ATRA increases epidermal turnover, it is postulated that the enhancement of the depigmenting effect of hydroquinone and hydroxyanisole by ATRA may also result from the impairment of glutathione-dependent cytoprotection [37].

On the other hand, some depigmenting agents increase levels of GSH. 4-Tertiary butyl catechol (TBC), a chemical that causes vitiligo in the skin of man and animals [44], enhances the activity of the enzymes glutathione reductase (GR) and gamma glutamyl transpeptidase(GGTP) in *in vitro* studies. These enzymes increase production of GSH and cystathionyldopa (5-SCD), both of which favor pheaomelanogenesis. Electromicroscopic studies confirm formation of phaeomelanosomes in areas depigmented by TBC and assays for melanin production likewise confirm decrease in eumelanogenesis [34].

Conclusion

These concepts supported by the various experimental evidence presented form basis for future research in the use of glutathione in the treatment of pigmentary disorders. Direction would be toward: (1) increasing cellular levels of reduced glutathione to promote phaeomelanogenesis ; (2) promoting GSH-mediated inhibition of tyrosinase activity through any of the proposed mechanisms; (3) increasing the antioxidant effects of GSH to quench tyrosinase-inducing free radicals; and (4) using glutathione-depleting agents like BSO in conjunction with known depigmenting agents to potentiate cytotoxic effect on melanocytes to lighten skin tone. We await randomized, double blind, clinical trials in humans to give definitive evidence for glutathione as an anti-melanogenic agent.

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