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Buthionine sulfoximine diverts the melanogenesis pathway toward the production of more soluble and degradable pigments

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ABSTRACT

Buthionine sulfoximine (BSO) is a specific inhibitor of γ-glutamylcysteine synthetase, thus blocking the synthesis of glutathione (GSH). It is known that this makes that BSO affects melanin synthesis because of the role of thiols in melanogenesis. However, BSO may also react with the intermediate oxidation products of melanogenesis, a possibility that has not been investigated from the initial steps of the pathway. We created in vitro conditions simulating eumelanogenesis (oxidation of L-DOPA in the absence of GSH) and pheomelanogenesis (oxidation of L-DOPA in the presence of GSH) under presence or absence of BSO. BSO made that eumelanogenesis results in pigments more soluble and less resistant to degradation by hydrogen peroxide than pigments obtained without BSO. A similar but less marked effect was observed for pheomelanogenesis only at subsaturating concentrations of GSH. These results suggest that BSO diverts the melanogenesis pathway toward the production of more soluble and degradable pigments.

Buthionine sulfoximine (BSO) is a specific inhibitor of γ-glutamylcysteine synthetase, the enzyme that catalyzes the rate-limiting step in the synthesis of glutathione (GSH), where two of its three constitutive amino acids (glutamate and cysteine) are bonded.[1] BSO thus decreases intracellular GSH levels with no side, toxic effects.[2–4] The important antioxidant activity of GSH makes that BSO inhibits the growth of different tumour cell lines and increases their sensitivity to antineoplastic drugs.[5] The inhibitory effect of BSO is particularly high against melanoma-derived cell lines,[6] as melanoma may be dependent on the role of GSH and its linked enzymes in melanin synthesis.[7] However, the effect of BSO on the synthesis of different types of melanin has received little attention. This may have important consequences for understanding possible side effects of BSO use.

The first step in the melanogenesis pathway, catalyzed by the enzyme tyrosinase, is the oxidation of the amino acid L-tyrosine to L-dopaquinone (see Fig. 1) which undergoes an intramolecular cyclization of the amino group to give L-dopachrome via L-cyclodopa, which in turn suffers a redox exchange with L-dopaquinone that produces L-dopachrome and 3,4-dihydroxy-L-phenylalanine (DOPA).[8] In this process, the recruiting of L-DOPA to be re-oxidized to L-dopaquinone is again catalyzed by tyrosinase, so that half of the DOPA oxidized to L-dopaquinone is reduced back to DOPA. L-Dopaquinone is the common precursor of the two connected biosynthetic routes in melanogenesis that lead to the formation of either eumelanin or pheomelanin.[9] In the absence (or below certain concentration) of sulfhydryl groups from thiol compounds such as cysteine or GSH, dopachrome evolves to two dihydroxyindoles, DHICA (5,6-dihydroxyindole-2-carboxylic acid) or DHI (5,6-dihydroxyindole) by tautomerization or decarboxylation, respectively. The resulting DHI/DHICA ratios depend on the level of dopachrome tautomerase activity and/or the presence of some metal ions.[10] DHICA and DHI are further oxidized and polymerized to form eumelanin (Fig. 1). In the presence (or above certain concentration) of sulfhydryl-containing compounds, these conjugate with L-dopaquinone to generate mainly 5-*S*-cysteinyldopa (in the presence of cysteine) or 5-*S*-glutathionyldopa (in the presence of GSH). These and other thiol–DOPA conjugates are further oxidized and polymerized to form pheomelanin[8,9,11] (Fig. 1). Given this biochemical process, the reduction of GSH levels exerted by BSO should decrease pheomelanin production and increase eumelanin production.

However, the effect of BSO on the synthesis of melanins may not be only mediated by the inhibition of γ-glutamylcysteine synthetase and cysteine production, because the S=NH group of BSO

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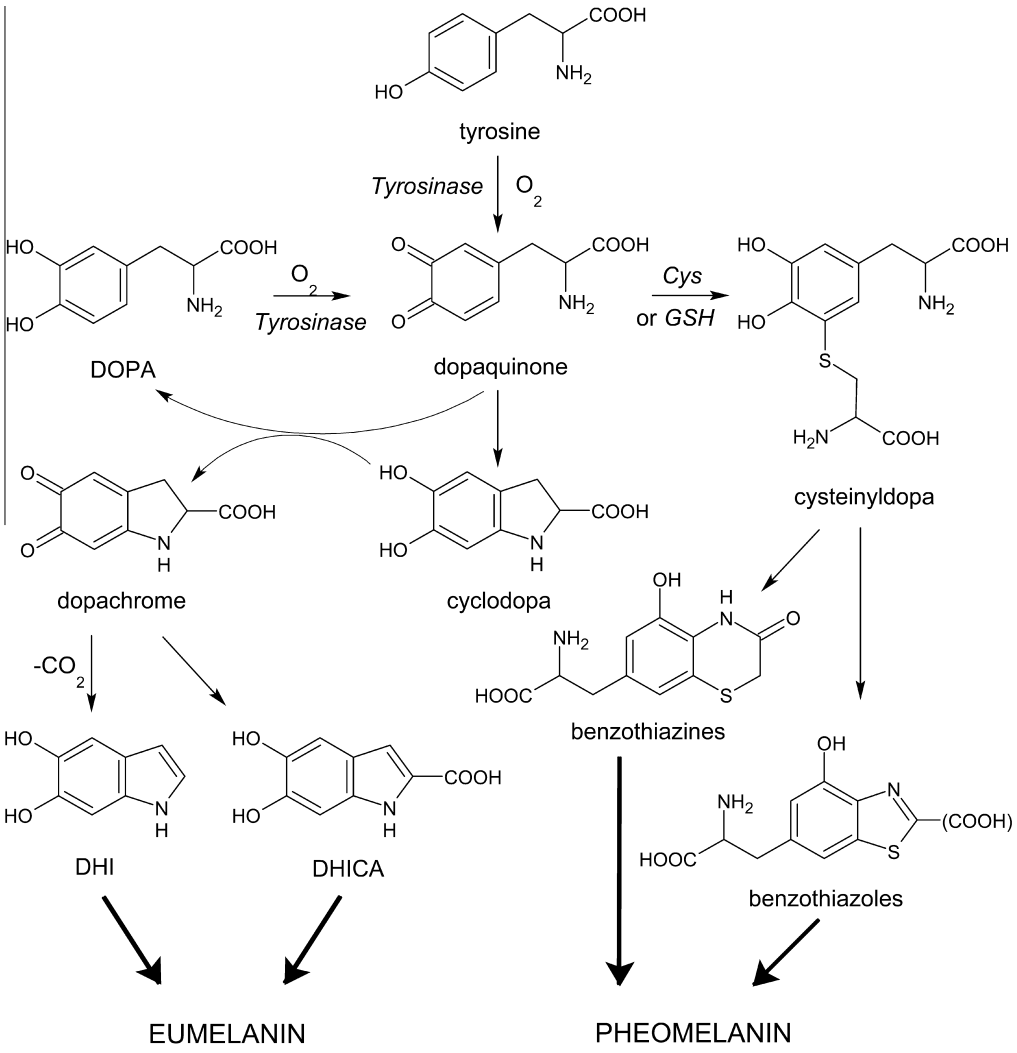

Figure 1. Schematic representation of the melanogenesis pathway. Tyrosinase catalyzes the oxidation of L-tyrosine to L-dopaquinone, which is the common precursor of the two connected biosynthetic routes in melanogenesis that lead to the formation of either eumelanin or pheomelanin depending on the absence or availability of thiol-containing agents. Adapted from Ito et al. (2011).

may also behave as the sulfhydryl groups (S–H) of cysteine and GSH, and thus BSO may react directly with some of the intermediate oxidation products of the melanogenesis pathway and that would affect the production of pigments. To investigate the latter possibility, we conducted an in vitro experiment to go deeper into the chemical reactivity properties of BSO. Benathan and co-workers[12,13] have previously reported increases in the pheomelanin precursor 5-*S*-cysteinyldopa and decreases in total pigmentation in in vivo cells exposed to BSO. However, the possibility that the S=NH group of BSO reacts with melanogenesis intermediates has never been explored. The expectation should be that BSO reacts with dopaquinone to form BSO–DOPA conjugates and thus diverts the melanogenesis route, decreasing the synthesis of both pheomelanin and eumelanin similarly to other agents containing sulfhydryl groups.[14]

To investigate the possibility that BSO reacts with intermediates of the melanogenesis pathway, we reproduced under in vitro conditions the initial steps of the melanogenesis pathway oxidizing L-DOPA with tyrosinase in the presence or absence of GSH, and in the presence or absence of BSO. The oxidation of L-DOPA without GSH should thus be similar to eumelanogenesis, while the oxidation of L-DOPA in the presence of GSH should simulate pheomelanogenesis.[11] We used GSH instead of cysteine as a sulfhydryl compound because the former is more abundant in intracellular media than free cysteine, so pheomelanogenesis in the presence of GSH may resemble more closely the in vivo situation.[9] In any case, 5-*S*-glutathionyldopa, which is the major thiol-conjugated species formed when L-dopaquinone reacts with GSH, releases 5-*S*-cysteinyldopa,[15] the main intermediate of the monomeric subunits for pheomelanin, after the action of a dipeptidase and the pathway then progresses as when only cysteine is present.[9]

To explore the influence of BSO on melanogenesis, five solutions were prepared in cryogenic vials all containing 10 mM L-DOPA[11,16] and 90 mM BSO (DL-buthionine-(*S*,*R*)-sulfoximine) in 1 ml of saline phosphate buffer (4 mM $KHCO_3$, 2 mM $CaCl_2$ $2H_2O$, 20 mM $NaHCO_3$, 138 mM NaCl and 2 mM KCl), pH 7.4. One of these solutions did not contain GSH, other two solutions contained GSH at concentrations (0.65 and 3.2 mM) that were lower than the concentration of the substrate L-DOPA, and finally other two solutions contained GSH at concentrations (16.3 and 81.3 mM) that were higher than the concentration of the substrate L-DOPA but lower than the one of BSO. 36.1 μg mushroom tyrosinase (1715 units/mg) diluted in 20 μl of the saline phosphate buffer were added to all tubes. Other five solutions were prepared as explained before, except that these did not contain BSO, thus serving as appropriate controls for standard eu- or pheomelanogenesis. All solutions were made in duplicates. All products were purchased from Sigma-Aldrich (St. Louis, MO, USA).