Review paper

MELANOGENESIS AND HYDROQUINONE

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SUMMARY

Mammalian melanocytes synthesize melanin in specific intracellular organelles, the melanosomes, which are afterward carried along melanocytic dendrites, injected into keratinocytes and, finally, degraded and eliminated by the epidermal turnover. Melanogenesis, i.e., the synthesis of melanosomes and melanin, is a cascade of events controlled by factors which are either internal and external to the melanocyte. Melanins are divided into eumelanins, pheomelanins, mixed melanins, tricochromes, neuromelanins, and, recently oxymelanins. The role of sulphydrilic compounds (cystein and glutation) for determining the kind of melanin, in particular for pheomelanins, does not seem to be due to their absolute presence or absence, but rather to their concentration in a certain moment. Eumelanogenesis and pheomelanogenesis are conditioned by a balance between tyrosinase and natural melanogenic inhibitors, as lactic acid, ascorbic acid and glutathione.

Among agents that are capable to interfere with melanocytic metabolism there are various cytotoxic compounds, some of which are selective for the melanocyte, as hydroquinone (HQ) and his derivatives. HQ is a compound capable of inhibiting melanogenesis acting as a substrate for tyrosinase. This competitive action does not seem to inactivate tyrosinase, thus the activity is reversible. The tendency of HQ to undergo hydroxylation and dehydrogenation, with formation of highly reacting quinones, i.e., hydroxybenzoquinone, p-benzoquinone and their derivatives, may suggest a biochemical base to understand the melanocytic cytotoxic effects, interfering with melanization and causing focal degradation of melanosomes. The complex activity of HQ on pigmentation, although not yet completely known, might also explain the main side-effects of topically-applied HQ, i.e., toxic depigmentation and exogenous ochronosis, as the active concentration is next to the toxic one.

KEY WORDS:
melanogenesis, hydroquinone, selective effects

MELANOGENESIS

Skin pigmentation due to melanin is regulated by two components: 1) the pigmentation constitutively expressed, according with the genetic program, independed of exposure to ultraviolet (UV) rays (constitutional skin tanning); 2) the pigmentation due to direct exposure to UV (immediate and

delayed tanning). Mammalian melanocytes synthesize melanin in specific intracellular organelles, the melanosomes, which are afterward carried along melanocytic dendrites, injected into keratinocytes and, finally, degraded and eliminated by the epidermal turnover.

Pigmentation control is complex, as shown by the wide range of colours in all species of animals. Genetic control of pigmentation involves, in mice, more than 60 loci, divided into two main groups: 1) loci controlling melanoblasts' survival and differentiation during embryogenesis, and 2) loci controlling the phenotypic differentiation of melanocytes, e.g., the c-locus for tyrosinase, the a-locus for quantity and distribution of pheomelanins and eumelanins and the b-locus for eumelanins. Functionally, these loci can be divided into four major classes: class A defines migration, proliferation and survival of melanocytes; class B controls quantity of melanin produced; class C determines the type of melanin synthesized; class D establishes shape and ultrastructural features of melanocytes (1).

Moreover, melanocytes are an excellent model for evaluating hormonal modulatory activity on proliferation and differentiation; in fact, many studies have shown that these cells are a target not only for the melanocytic stimulating hormone (MSH), but even for insulin, glucocorticoids and prostaglandins. Also inflammatory and immunogenic cytokines interact with melanocytes controlling pigmentation, growth, differentiation, immunologic susceptibility and cytotoxicity, production of cytokines and matrix proteins and cell movement (2). For instance, interleukin-1 (IL-1) alpha and beta, IL-6, TNF-alpha and granulocyte/monocyte colony stimulating factor (GM-CSF) are capable of inhibiting the melanocytic response, without modifying the basal tyrosinase activity to MSH-alpha, prostaglandins E1 and E2, and to isobuthyl-methyl-xanthine. Melanocytes synthesize arachidonic acid metabolites and various cytokines, suggesting their active role in the skin immune system (3). Moreover, it is well known that for a normal growth of cultured melanocytic cells various growth factors are required, as insulin, alpha-MSH, basic fibroblast growth factor and the phorbol esther TPA (4).

Melanogenesis, i.e. synthesis of melanosomes and melanin, is a cascade of events controlled by factors which are either internal or external to the melanocyte (Tab. I). The membranous structure of melanosomes regulates the recognition and selection of these informations and thus the differentiation of melanosomes as well as the kind of melanin. In the melanosome two main components can be distinguished: the lipidic one, initially located at the external surface, and the proteic one, constituting the central part of the melanosome. The former is very important for functional regulation, whereas the latter, including tyrosinase and his regulatory factors, for structural differentiation. Melanogenesis is conditioned by UV, hormones and by the substrate available.

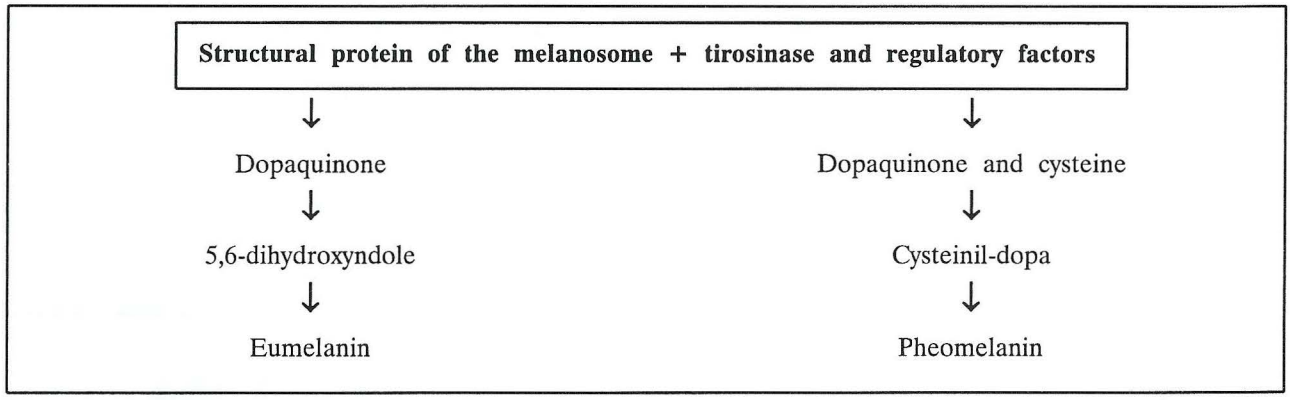
Melanins are distinguished into eumelanins, pheomelanins, mixed melanins, tricochromes, neuromelanins, and, recently oxymelanins (5). The role of sulphydrilic compounds (cystein and glutation) for determining the kind of melanin, in particular for pheomelanins, does not seem to be due to their absolute presence or absence, but rather to their concentration at a certain moment. Eumelanogenesis and pheomelanogenesis are conditioned by a balance between tyrosinase and natural melanogenic inhibitors, as lactic acid, ascorbic acid and glutathione (6). Morphogenesis of melanosomes could not follow, from the beginning, the eumelanic or the pheomelanic pathway, but both types may coexist, in so-called "mosaic melanosomes", before definitive differentiation. Variation of melanogenesis type could be controlled by structural and functional availability of the vesico-globular bodies associated with HMSA5 glicoproteins, as the number of these bodies is different in eumelanogenesis and pheomelanogenesis; they could play an important role not only in the structural organization of the melanosome matrix, but also as carriers of pre- or post-tyrosinase regulatory factors (7).

HYDROQUINONE

Among agents that are capable to interfere with melanocytic metabolism there are various cytotoxic compounds, some of which are selective for the melanocyte, as hydroquinone (HQ) and his derivatives. HQ is one of the most common compounds used for treatment of melasma, chloasma and other disorders characterized by increased cutaneous pigmentation, i.e., cutaneous melanoses.

The activity of HQ was known since 1936, when Oettel (8) showed that black hairs of cats treated with HQ per os gradually cleared, becoming gray. Martin and Ansbacher (9) confirmed the HQ activity in young mice, determining loss of hair pigment reversible in a period comprised between 4 and 20 weeks. Further contributions, during the subsequent years, demonstrated that even topical application, as well as local injection of HQ, is effective to clear skin and hair pigmentation of mouse, guinea pig

Table I: The steps of melanogenesis



and man.

Histologic and electron-microscopy investigations focused the specific action of HQ on skin and hair melanocytes. Previous studies had suggested that the main action of HQ is inhibition of melanocytic tyrosinase, with subsequent decreasing of melanin production and depigmentation (10). Further investigations revealed that HQ acts also as cytotoxic agent (11-12). Jimbow confirmed, with ultrastructural investigations, that HQ activity is selective for follicular and skin melanocytes; moreover HQ can induce inflammation with activation of Langerhans' cells (13).

Lerner and coworkers (14) demonstrated that HQ is less effective than tyrosine and DOPA as a substrate for mammalian tyrosinase, but it could participate as a substrate in the partial melanization in presence of tyrosine. In fact, HQ oxidation may give rise to free radicals (semi-quinones-like) capable to disrupt the membrane by enzymatic oxidation. It could be conceivable that HQ, topically applied or locally injected, may generate free radicals within the tyrosine-tyrosinase system in melanosome and melanocytes. These radicals could interfere with tyrosine oxidation and melanosome melanization causing focal degradation of the matrix and changes of melanosome membrane. Riley (15) demonstrated that phenolic derivatives may generate, by means of enzymatic oxidation by tyrosinase, free radicals which can cause lipidic oxidation and cellular damage. Jimbow (13) showed that HQ attacks nuclear and cytoplasmic membranes of melanocytes, with disruption and degradation of membranous organelles up to necrosis. Hu (12), analyzing in vitro melanosome movements in melanocytic dendrites in order to evaluate the effectiveness of HQ, found reduced movements at concentrations of 1.25 - 2.5 mcg/ml. These concentrations inhibited also DNA and RNA synthesis. Penney et al. (16) revealed a selective inhibition of HQ on melanocyte metabolism compared to other cell-types, demonstrating a primary effect on RNA synthesis, suggesting that the depigmenting activity of HQ is prevalently due to a direct action on melanocyte metabolism more than a specific effect on melanin synthesis. Morphological effects of HQ are documented by reduction of melanized melanosomes and by the presence of abnormally pigmented melanosomes, these latters show an altered core and are rapidly degraded by keratinocytes. The direct cellular damages of melanocytes are disruption and lysis of membranous organelles, up to cellular necrosis.

Table II. Possible mechanisms of hidroquinone activity

a) competitive substrate for tyrosinase;
b) chemical link with the intermediate compounds of melanin synthesis, blocking melanin formation;
c) focal degradation of melanosomes, by means of formation of free radicals in melanin and/or intermediate compounds.

The abnormal melanization induced by HQ could be due to the following actions:

a) HQ, similar to tyrosine, could act as a competitive substrate for tyrosinase;
b) HQ could be chemically linked with the intermediate compounds of melanin synthesis, blocking melanin formation;
c) HQ could cause focal degradation of melanosomes, by means of formation of free radicals in melanin and/or intermediate compounds.

The enzyme tyrosinase seems to mediate almost all the effects of HQ on skin depigmentation, but the biochemical events that give rise to melanotoxic effects are not completely understood.

CONCLUSIONS

Finally, also on the basis of the most recent "in vitro" contributions (17-19), we can draw the following considerations: a) HQ is a compound capable of inhibiting melanogenesis acting as a substrate for tyrosinase (17-18); b) this competitive action does not seem to inactivate tyrosinase, thus the activity is reversible; c) the tendency of HQ to undergo hydroxylation and dehydrogenation, with formation of highly reacting quinones, i.e., hydroxybenzoquinone, p-benzoquinone and their derivatives, may suggest a biochemical base to understand the melanocytic cytotoxic effects, interfering with melanization and causing focal degradation of melanosomes.

Moreover, the complex activity of HQ on pigmentation (Tab. II), although not yet completely known, might also explain the main side-effects of topically-applied HQ, i.e., toxic depigmentation and exogenous ochronosis, as the active concentration of HQ is next to the toxic one.

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