Latest Insights into Skin Hyperpigmentation

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Hyperpigmentary problems, including postinflammatory hyperpigmentation, solar lentigos, and melasma, occur widely in the human population and are thus of broad interest for control. On the basis of genomic and proteomic understanding of the melanocyte and melanogenesis, there are potentially hundreds of proteins and other effectors involved in pigmentation. This knowledge, although complex, should prove most useful in identifying specific abnormalities that lead to the hyperpigmentary problems. Also available are new laboratory screening methods and skin color measurement tools that are increasing the pace at which materials can be screened and evaluated clinically for their effectiveness. Combined with a clear consumer need for effective pigmentation control agents, advanced pigmentary system understanding and new research capabilities are setting the stage for future technological advancements.

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INTRODUCTION

There is great diversity in the color of human skin across the globe, from the very pale color of Celtic skin to the very dark skin types present in regions such as sub-Saharan Africa. Across this array of skin colors, there are also many disorders of the pigmentary system, resulting in problems ranging from hypopigmentation to hyperpigmentation. Regardless of the nature of the problem, the general desire is for uniformity of skin color. This short review will focus on some specific hyperpigmentary problems (postinflammatory hyperpigmentation (PIH), solar lentigos, and melasma), investigative methods to measure and understand the problems, and treatment approaches.

DISCUSSION

Hyperpigmentary disorders and their causes

Postinflammatory hyperpigmentation. Skin insults that result in inflammation can induce PIH, particularly in people with dark skin (Pandya and Guevara, 2000; Cayce *et al.*, 2004; Stratigos and Katsambas, 2004; Halder and Nordlund, 2006). Among such insults are acne lesions, ingrown hairs, scratches, insect bites, and surfactant damage. As an example of the latter, exposure of human forearm skin to the surfactant sodium lauryl sulfate under patch for a few hours will produce erythema within a day, resulting in hyperpigmentation over the course of 1–2 weeks even in Caucasian skin, and the anti-inflammatory phytosterol will prevent this PIH (Table 1).

Even the most common cause of hyperpigmentation (sunlight exposure of skin) is more likely a postinflammatory response to UV damage to skin (Gilchrest *et al.*, 1998; Abdel-Malek and Kadekaro, 2006). That response may be the result of an obvious inflammatory acute event such as sunburn or of repeated suberythemal exposures to UV. Although in the latter, there may not be visible erythema, histologically, such exposed skin has elevated inflammatory cell content, yielding a ''subclinical'' inflammatory process.

Inflammation may result in hyperpigmentation through several mechanisms. Among them is direct stimulation of melanocytes by inflammatory mediators such as IL-1-α, endothelin-1, and/or stem cell factor (Sriwiriyanont *et al.*, 2006; Unver *et al.*, 2006). Reactive oxygen species, such as superoxide and nitric oxide, generated in damaged skin (for example, from UV exposure) or released as by-products from inflammatory cells are also known stimulators of melanocytes. Additionally, damage induced to epidermal cells can lead to release of endocrine inducers of pigmentation, such as α-melanocyte-stimulating hormone. The resulting hyperpigmentation induced by all these effects provides some measure of protection against subsequent insult, as melanin has both UV absorption and reactive oxygen species scavenging capacity.

The melanin produced during an inflammatory event can enter the dermis where it is engulfed by macrophages, producing ''melanophages'' (Halder and Nordlund, 2006). These cells are often retained in the upper dermis for prolonged periods, as removal of dermal melanin apparently is a very slow process. Thus, PIH can be a very long-lived problem for the skin.

Solar (actinic) lentigos. These hyperpigmented spots are also known as lentigines, age spots, and liver spots. They occur on

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Table 1. PIH on the forearm

Test agent	Erythema grade (day 2)	PIH grade (day 11)
Vehicle	2.09	0.93
5% phytosterol	1.71*	0.55*

PIH, postinflammatory hyperpigmentation; SLS, sodium lauryl sulfate.
A 20% solution of SLS was applied to the forearm skin of Caucasian subjects (n=19) under occlusive patch (0.2 ml solution in a 19-mm-diameter chamber patch). The patch was removed after 1–4 h, depending on the individual subject responsiveness. After washing the site to remove surface SLS, the skin was treated topically twice daily for 5 days with test agent. The skin was graded (0–4 grading scales) daily for erythema and pigmentation (PIH) for 11 days (Unpublished work, D.L. Bissett).
*Statistically significantly different ($P<0.05$) versus vehicle.

sun-exposed parts of the body (in particular, the hands, arms, and face), and thus most likely occur due to chronic exposure of skin to UV and the resultant chronic inflammation. Their dark appearance may also result, at least in part, in dermal melanophages that have been observed histologically to lie beneath the lentigines. Presumably, these observations indicate that there has been a change in the genetic expression of both the keratinocytes and the melanocyte(s) within the spot, as compared with the melanocytes in the surrounding nonspot skin. There are often drastic modifications of the epidermal architecture, which may, in part, be the result of the chronic UV exposure damage associated with spot development. The mRNA levels of melanogenesis-associated genes (for example, POMC (proopiomelanocortin), tyrosinase, TYRP1 (tyrosinase-related protein 1), DCT (dopachrome tautomerase), Pmel-17, P, MITF (microphthalmia-associated transcription factor)) are all increased in actinic lentigos. There is also an accentuation of the epidermal endothelin cascade (endothelin-1, endothelin-converting enzyme 1-α, endothelin-B receptor), and a role for stem cell factor in hyperpigmentation (solar lentigos in particular) has been identified (Kadono *et al.*, 2001; Imokawa, 2006; Unver *et al.*, 2006). Additionally, alterations in the epidermal–melanin axis and factor XIII1 melanophages have been observed. Many of these changes appear to be permanent, as these spots persist even when further UV exposure is avoided (Motakawa *et al.*, 2005). The details of these apparent genomic expression changes have not been defined.

Melasma. This hyperpigmentary disorder is not well understood (Lee *et al.*, 2006). It occurs typically as symmetrical lesions on the face, primarily in darker skin type females at puberty or later in life. Sunlight exposure is probably a factor in the development of melasma, as it occurs on the face (a sun-exposed body site) and as the condition worsens in the summer. Most melasma sufferers have a hypersensitivity to UV radiation, that is, they display a lower minimum erythemal dose, and even brief exposures to sunlight can stimulate hyperpigmentation. There is also a hormonal component, likely progesterone, as episodes of melasma are often associated with pregnancy and the use of hormonal birth control. In melasma lesions, there is excess melanin present in both the epidermis and upper dermis (associated with extravascular macrophages). As there is only a slight increase in number of melanocytes, the abnormality appears to be in function of the skin cells, in particular increased expression of α-melanocyte-stimulating hormone in keratinocytes and overexpression of stem cell factor in fibroblasts and its receptor C-kit in melanocytes of the involved skin (Imokawa, 2004, 2006; Kang *et al.*, 2006). In contrast to PIH, there is no apparent inflammatory phase involved in its development. Additionally, there is more likely a genetic component that predisposes individuals to melasma, although the specific genetic basis for it is not defined.

Genomics and proteomics of pigmentation

There are approximately 1500 gene products (proteins) expressed in melanosomes of all developmental stages, with 600 of them being expressed at any given time and 100 of them apparently unique to the melanosome (Chi *et al.*, 2006). Added to this are many other proteins (membrane-associated, cytoskeletal, transport, and so on) involved in pigmentation in both the melanocyte and the keratinocyte, indicating the complexity of the pigmentary process and many opportunities to further understand it. Although the basic process (for example, stimulation of melanocytes and conversion of tyrosine to melanin) is well studied, there are many regulatory elements that have emerged from recent research involved in signaling, in the transport of melanosomes within the melanocyte, and the transfer of melanosomes to the keratinocyte (Figure 1).

Less well studied are the events that occur in the keratinocyte once melanosomes have been transferred there. In addition to the melanosome engulfment process itself, presumably there are intracellular signals, regulatory elements, and transport mechanisms to distribute the melanosomes within keratinocyte. There is the process of melanin degradation to produce ''melanin dust,'' an apparently enzymatic process which is more active in lighter skin versus darker skin individuals (Chen *et al.*, 2006). This is an area ripe for further study.

Pigmentation control agents

As noted above, as there are many processes and proteins involved in the pigmentary process, there is a wide array of targets against which to screen for pigmentation control agents (Boissy, 2003; Nakayama *et al.*, 2005). Among the many targets are inhibitors of melanocyte stimulation (for example, antioxidants, anti-inflammatory agents), cell receptor antagonists (for example, α-melanocyte-stimulating hormone antagonists), inhibitors of melanin synthesis enzymes (for example, tyrosinase, TRP (tyrosinase-related protein)-1, TRP-2), inhibition of melanosome transport within the melanocyte and transfer to the keratinocyte (for example, PAR-2 antagonists), and activators of melanin degradation within the keratinocyte.

A classic target is inhibition of tyrosinase, the first enzyme in the conversion of tyrosine to melanin (Nakayama *et al.*, 2005; Land *et al.*, 2006). Agents such as hydroquinone, kojic

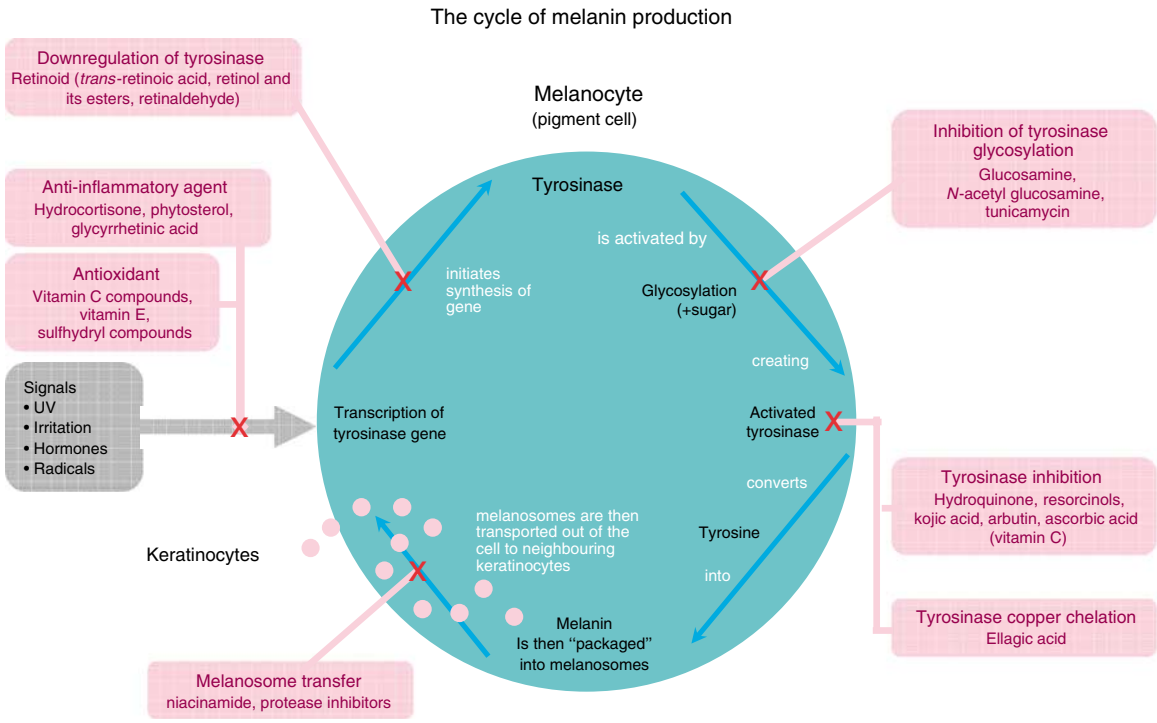

Figure 1. The cycle provides the mechanism of action of various pigmentation control targets and effective agents as described in the published literature.

acid, arbutin, ascorbic acid, ellagic acid, sulfhydryl compounds, and resorcinols are effective in interfering with this process. However, as several of these materials also have other effects, it is difficult to directly connect a specific mechanism to the observed effect on pigmentation. For example, sulfhydryl compounds are also effective antioxidants. Table 2 overviews a short list of the many possible targets and a few agents effective against them.

In recent work, niacinamide and NAG (*N*-acetyl glucosamine) have been determined to be effective in reducing melanin production in culture (Hakozaki *et al.*, 2002; Bissett *et al.*, 2006). *In vitro,* NAG reduces production of melanin by inhibiting activation of tyrosinase, whereas niacinamide inhibits melanosome transfer from melanocytes to keratinocytes. When formulated with other cosmetic ingredients as a skin moisturizer product (Bissett *et al.*, 2004, 2007; Kimball *et al.*, 2006), a NAG/niacinamide-containing product diminishes the appearance of hyperpigmentation (Figure 2). Another new effective topical agent is deoxyarbutin, a tyrosinase inhibitor (Hamed *et al.*, 2006).

Table 2. Pigmentation control targets and effective agents

Pigmentation control target	Effective agent
Tyrosinase inhibition	Hydroquinone, resorcinols, kojic acid, arbutin, ascorbic acid (vitamin C), deoxyarbutin
Tyrosinase copper chelation	Ellagic acid
Inhibition of tyrosinase glycosylation	Glucosamine, *N*-acetyl glucosamine, tunicamycin
Melanosome transfer	Niacinamide, protease inhibitors
Downregulation of tyrosinase	Retinoid (*trans*-retinoic acid, retinol and its esters, retinaldehyde)
Antioxidant	Vitamin C compounds, vitamin E, sulfhydryl compounds
Anti-inflammatory agent	Hydrocortisone, phytosterol, glycyrrhetinic acid
Increase epidermal turnover	Retinoids, salicylic acid

Laboratory tools

Many of the targets noted above can be investigated in simple mechanism-specific solution assays or melanocyte cell culture systems in the laboratory. These models permit screening of potentially large numbers of compounds for their inhibitory and stimulatory effects on the specific processes being evaluated (Land *et al.*, 2006). For example, one screening assay involves a simple mixture of tyrosinase and tyrosine in which a brown product (melanin) quickly appears and can be quantified colorimetrically. Tyrosinase inhibitors, of course, reduce the melanin produced. A simple assay of this type can be readily performed in a 96-well plate, allowing rapid robotic high-throughput screening of thousands of compounds. Other assays, such as those involving melanocytes, are more complex, but even those can be constructed in a multiwell plate format for moderate throughput screening of potentially hundreds of compounds. Establishing an array

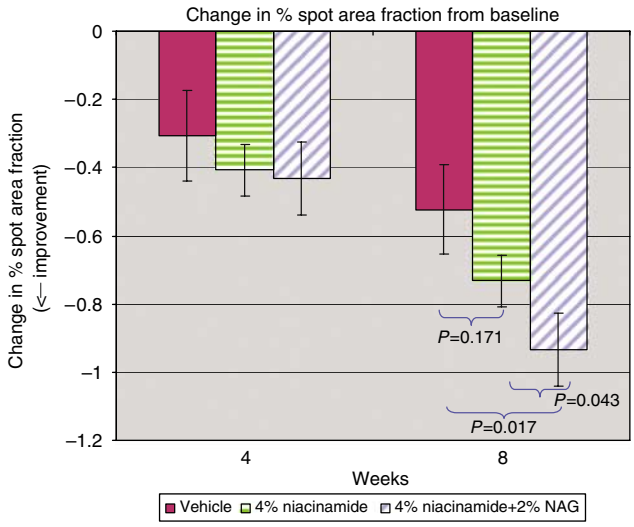

Figure 2. **Computer image analysis of Caucasian facial digital images ($n = 35$) for change in spot area fraction.** More negative numbers indicate reduction in hyperpigmentation (improvement). The *P*-value is for 4% N + 2% NAG versus 4% N. N, niacinamide; NAG, *N*-acetyl glucosamine.

of such simple assays permits the screening of a substantial library of compounds through all these assays to identify promising candidates quickly.

Another useful laboratory model that has emerged over the past few years is the skin equivalent culture (Costello, 2000; Klausner *et al.*, 2000). These three-dimensional cultures can contain both dermal and epidermal compartments, with fibroblasts, keratinocytes, melanocytes, and potentially other cell types. When they are raised to the air–liquid interface, the keratinocytes will differentiate to form a stratum corneum structure, permitting topical dosing with simple solutions or even complex emulsion formulations or commercial skin care products. Another particular advantage of these cultures is that they are not mechanism specific—as all the pigmentation machinery is present in the cultures, they potentially can be responsive to materials that affect any of the pigmentation targets. These cultures are available from commercial suppliers. Although these are useful tools for material evaluation, they are relatively low throughput, particularly due to the high cost of the cultures. The final proof of value, although, requires progressing materials from the laboratory to clinical testing to demonstrate on-skin activity.

Additionally, a new imaging method has emerged that specifically measures the melanin chromophore (Moncrieff *et al.*, 2002; Preece *et al.*, 2003; Matts *et al.*, 2007). This noninvasive method is a useful addition to the existing clinical tools to detect changes in skin melanin content and specifically distribution, regardless of the presence of other skin chromophores (for example, hemoglobin).

CONCLUSION

An increased understanding of the pigmentation process provides a basis for establishing targets against which to screen new compounds to identify those that may be effective pigmentation control agents. With the advances in laboratory and clinical methodology, the screening process can occur much more rapidly than in the past. In addition, there continues to be a clear consumer need for new and effective pigmentation control agents, particularly as the long-used over-the-counter technology hydroquinone, which was already banned in Europe and Japan, is likely soon to be banned in the United States over concerns such as cytotoxicity. This fuels the need for additional options effective for problems of skin hyperpigmentation.

CONFLICT OF INTEREST

J.-P. Ortonne received an honorarium for consultant's services from Procter & Gamble towards the preparation of this article. D.L. Bissett is an employee of Procter & Gamble.

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