

The Haptogenic Pathogenesis of Vitiligo and the Source of the Pattern of Depigmentation

Patrick A Riley*

Totteridge Institute for Advanced Studies, London, UK

***Corresponding author:** Patrick A Riley, Totteridge Institute for Advanced Studies, The Grange, Grange Avenue, London N20 8AB, UK, Tel: (+44) 20 8445 5687; E-mail: p.riley@ucl.ac.uk**Received date:** May 03, 2014; **Accepted date:** May 23, 2014; **Published date:** May 25, 2014**Copyright:** © 2014 Riley PA, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The pathogenesis of vitiligo based on the model of depigmentation produced by topical application of phenolic compounds that are substrates for tyrosinase is outlined. The main steps in the pathway consist of the haptening of melanosomal proteins by the quinone products of the analogue phenols with the generation of neo-antigens which are able to elicit a cell-mediated immune response that results in loss of melanocytes and consequent epidermal depigmentation. The source and nature of the haptogenic compounds is unknown but may be of dietary origin. It is suggested that the pattern of depigmentation in vitiligo is determined by regional variations in tyrosinase activity. The possibility of utilizing quinone-mediated haptening as a means of targeting anti-melanoma immunotherapy is briefly discussed.

Keywords: Vitiligo; Tyrosinase; Hapten; Quinones; Auto-immunity; Melanocyte; Melanoma

telogen phase follicles escape damage by virtue of their inactive tyrosinase.

Introduction

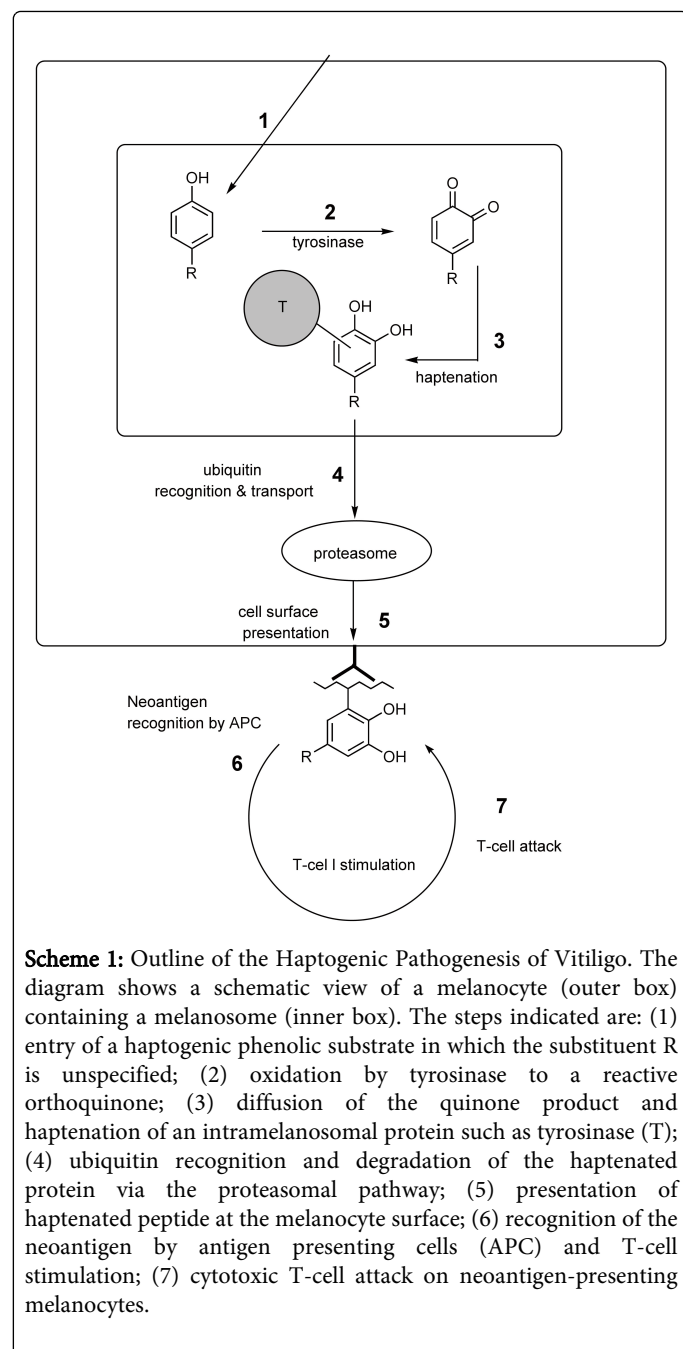
Epidermal depigmentation in vitiligo is due to the localized absence of melanogenesis [1] consequent on the absence of melanocytes in the affected areas of the skin [2]. Generally the depigmentation affects the epidermis, often leaving residual pigmentation of the hair in the affected region and this may reflect the relatively low population density of epidermal melanocytes which renders them susceptible to elimination by trauma, as shown for ischaemic depigmentation [3]. It has been known for some time that localized depigmentation follows topical application of phenolic compounds capable of acting as substrates for tyrosinase, the principal enzyme of melanogenesis [4]. Although several direct actions have been ascribed to the oxidation products of alternative tyrosinase substrates the most likely action leading to in vivo depigmentation is that they are capable of acting as haptens. It is known that the quinone products of oxidation undergo facile nucleophilic addition reactions and are able to bind to proteins [5]. In particular, it has been demonstrated that labelled 4-hydroxyanisole, a phenolic analogue of tyrosine, is covalently bound to tyrosinase [6]. Thus, the pathogenesis proposed here (summarized in Scheme 1) is based on tyrosinase-catalysed oxidation of an analogue substrate yielding a reactive quinone which binds covalently to the enzyme, or some adjacent intramelanosomal protein, causing the haptened protein to be recognized as a neoantigen and eliciting an anti-melanocytic immune response which eliminates active melanocytes in the affected region.

In general, the clinical experience regarding repigmentation has emphasized the lack of response of vitiligo to treatment (usually attempts to stimulate melanocyte activity) and where responses are observed the inference is that there remain some undamaged pigment cells capable of activation. This possibly accounts for pigment spread from hair follicles, where populations of melanocytes present in

Cell-directed immunity and the haptening model

The clinical evidence suggests that the depigmentation of vitiligo results from regional melanocyte destruction by a cell-mediated immune process characteristic of a delayed-type hypersensitivity reaction. The histopathological features of the process of melanocyte loss includes a local increase in the epidermal population of Langerhans cells, CD4+ and CD8+ lymphocytes, and other inflammatory features typical of a hypersensitivity response. It has been proposed that this response is initiated by haptening of one or more melanocyte proteins. Haptening refers to a process whereby small molecules modify the immunogenicity of other molecules [7]. The involvement of haptens in contact allergy has been extensively reviewed [8]. Haptening offers a plausible explanation for the elicitation of auto-immune responses to altered self-antigens by small reactive molecules binding to native proteins which are subsequently processed to generate neo-antigenic peptides not recognized by the immune system as 'self' components. Modified proteins, on degradation, yield peptide fragments that are presented at the cell surface and may induce cytotoxic T-cell responses [9].

To explain the melanocyte specificity of the immune response in vitiligo it is suggested that haptening takes place by covalent binding of a quinone metabolite formed by tyrosinase-catalysed oxidation of a haptogenic precursor, able to act as a substrate for the enzyme. In the skin, tyrosinase is only expressed in cells of the melanocyte lineage and the immune response is observed only in a narrow margin of actively depigmenting skin. Moreover, loss of melanocytes is dependent on the expression of melanosomal proteins, since undifferentiated melanocytes, such as those of the outer root sheath of the hair follicles which do not express tyrosinase activity [10], are spared from T cell-mediated destruction [11] (Scheme 1).



The model for the proposed hapten-initiated pathogenic mechanism is outlined in Scheme 1 and is based on the vitiligo-like depigmentation induced by known phenolic agents such as monobenzone and 4-hydroxyanisole. The pathological features of depigmentation by these agents are indistinguishable from those observed in vitiligo (including the perilesional inflammatory infiltrate) and there is clinical evidence from treating vitiligo patients with monobenzone that an identical process is involved [12]. Nordlund et al. [13] observed that in vitiligo patients treated with monobenzone only the pigmented skin exhibited signs of inflammation with itching, redness and oedema (with the characteristic histological features of a delayed-type hypersensitivity reaction), whereas the depigmented (vitiliginous) skin, devoid of melanocytes, showed no sign of

inflammation. Furthermore, patch tests applied to pigmented and depigmented skin produced an inflammatory response only within the pigmented areas. Thus there is clear evidence that the immune response is related to the presence of active melanogenesis.

Tyrosinase-catalysed conversion of phenols

Depigmenting phenolic compounds, like 4-hydroxyanisole and monobenzone are known to be oxidized by tyrosinase to give rise to the corresponding ortho-quinones [4]. The potential for quinones generated by tyrosinase activity to undergo addition reactions with nucleophilic functions such as amino and thiol groups is well documented [5]. The oxidation of monobenzone by tyrosinase from *Agaricus bisporus* has been investigated in detail. In the presence of L-cysteine, N-acetyl-L-cysteine, and sulfhydryl-containing peptides, such as reduced glutathione, or proteins, such as bovine serum albumin (BSA), addition products derived from nucleophilic addition of the thiol group to the ortho-quinone have been detected and characterized [14].

Of relevance to the mechanism outlined above are the results of a study on mouse skin applications of the catechol, 3,4-dihydroxybenzoic acid [15]. They found that the inflammatory effect of local application was much more pronounced in mouse skin with a high tyrosinase activity than in albino animals. They proposed that the tyrosinase-derived reactive quinone intermediate of hydroxybenzoic acid is haptogenic and binds to nucleophilic residues of proteins generating conjugates which are recognized as antigens. They concluded that their data strongly indicated the occurrence of a T cell-mediated cellular immune response which was due to a tyrosinase bio-activation pathway-dependent protein haptination.

An important factor in the argument regarding haptination is the reactivity of the quinones generated by tyrosinase oxidation. In general ortho-quinones are highly reactive and their range of diffusion is therefore limited. The likelihood of reaching a significant target will be reduced in the presence of high concentrations of alternative reactants, in particular reactants with high affinity to quinones such as thiols. It would be anticipated that in pheomelanotic subjects who have high intramelanosomal concentrations of cysteine that haptination would be impeded and that therefore vitiligo would be rare by comparison with eumelanotic patients.

Since ortho-quinones have limited stability, their diffusion range is short and the most proximal haptination targets are intramelanosomal proteins. In this context it is noteworthy that the melanosome contains the majority of the melanocyte antigenic proteins [16]. As the protein nearest to the source of quinones, tyrosinase may be considered especially vulnerable to haptination and, as mentioned above, in the case of 4-hydroxyanisole it has been demonstrated by radiolabelling techniques that the oxidation product binds to tyrosinase [6]. Other potential intramelanosomal haptination targets include TRP1, MART-1, gp100 and TRP2. In this respect, TRP-1 deserves attention as it has been implicated in mediating 4-tertiary butylphenol induced depigmentation [17].

Presentation of Neo-Antigens

For the HLA-A2 isotype, a good deal is known about the MHC class I peptides generated from melanosomal proteins [18]. In fact, haptination has been suggested as a means of enhancing antigenicity by testing reactivity of MART-1 reactive T cell clones after modification with quinonemethide. Further studies are needed to

identify T cell reactivity elicited specifically by haptenated peptides presented in the context of HLA-A2 and other HLA isotypes. As the HLA-type expressed by individuals will define which peptides are presented, the efficiency by which haptenized peptides are presented to the immune system, the HLA isotype expressed will likely contribute to the potential susceptibility to vitiligo by influencing the efficiency of neo-antigen presentation. The presentation mechanism proposes that the quinone-haptenated protein(s) are processed through the proteasomal pathway and are presented at the melanocyte surface invoking an immune response. Antigen presentation occurs in the form of peptides bound to HLA molecules at the cell surface, and meta-analysis of several HLA association studies strongly suggests an association of vitiligo with HLA-A2 [19].

Targeted anti-melanoma chemotherapy

If, as proposed, the pathogenesis of vitiligo involves an auto-immune reaction initiated by neo-antigens produced by tyrosinase-catalysed oxidation of a haptogenic substrate, the possibility of employing this mechanism as a targeting strategy for anti-melanoma therapy is of considerable interest. In fact, Morgan et al. [20] reported a clinical pilot study in which 4-hydroxyanisole (4HA) was administered intra-arterially to a group of melanoma patients with widespread disease unresponsive to other therapies. Despite the high doses administered, the acute responses were disappointing, but longer term follow-up showed that 45% of cases showed some degree of regression by tumors in the infused zone, although there was no evidence of a generalized tumor response or of any obvious cutaneous or ocular depigmentation. In one case there was complete regression of secondary tumors. This patient, with multiple recurrences at the site of a skin graft on the left leg, received two courses of 4HA (100 and 84 G by intra-femoral infusion) with a 4-week interval and, when seen 4 weeks after the second infusion, was tumor-free [21]. This delayed reaction would be explained by the initiation of an immune response specific to tyrosinase-expressing cells exposed to 4HA [22,23] and is entirely consistent with the model advanced here. The same process may be reflected in Lippizaner and Camargue horses as well as the Sinclair swine, where depigmentation of melanotumours is associated with 'spontaneously' regression due to an induced immune response.

The pattern of depigmentation

Given that the depigmentation of vitiligo results from an immune mechanism of the type described above there remains the question of the source of the patterning of the loss of pigment. The pattern of depigmentation has a strong tendency to occur symmetrically [24], almost in distributions that resemble dermatomes [25] and this initially led to the proposal that the source of the potential haptenic metabolite might be neurogenic, such as, for example, the autonomic neurotransmitter nor-adrenalin [26]. However, several experimental investigations, including examination of the effect of sympathectomy on the progress of depigmentation [27] failed to adduce any clear supportive evidence. The regionalization of the depigmentation is thus hard to understand. It has been suggested that the T-cell immune response is regionally limited although what mechanism might be invoked to explain such a restriction is by no means clear. Alternatively, the degree of haptening would be expected to be proportional to the tyrosinase activity and hence depigmentation would be more pronounced in areas in which melanogenesis was stimulated. Such stimulation is known to be associated with

inflammation or other causes of local hyperaemia. This would be consistent with depigmentation occurring in areas of skin prone to mild trauma and these are often symmetrically disposed. Another interesting possibility concerns factors that control the activity of tyrosinase by alteration of the oxidation state of the active center [28]. It has been shown that tyrosinase is activated by conversion of the met-tyrosinase form to the oxy-tyrosinase form by reaction with catecholic substrates and that oxidation of phenols is dependent on this activation [29]. Hence the segmental distribution of the depigmentation caused by haptogenic phenols might be influenced by the regional availability of catechols acting not as the haptogenic agents but as local tyrosinase activators.

Even if regional variation in tyrosinase activity turns out to be a satisfactory explanation of the patterning of the loss of pigment in vitiligo there remains the problem of the source of the haptenic substrate. No specific haptenogenic agent has thus far been identified there is a wide range of potential phenolic haptogens that may act as substrates for tyrosinase. Although a number of endogenous factors could be considered as possible sources it is more probable that exogenous compounds are implicated and this may ultimately involve dietary factors. These span a range of phenolic compounds, including dietary antioxidants, and include ingredients such as those present in spices [30]. Other possible extrinsic sources might include metabolic products derived from microorganisms, e.g. gut flora, and external contaminants in the atmosphere or water supply. It has to be emphasized, whilst the development of vitiligo is plausibly explained by the considerations outlined above, that in the majority of cases no specific haptenic agent has been identified. It remains possible that other pathologies may account for the characteristic pattern of depigmentation and some alternative mechanisms have been proposed, including the recent suggestion that the cause of vitiligo is a localized deficiency of zinc- α -glycoprotein [31].

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