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Effects of UV Radiation on the Ultrastructure of Human Common Pigmented Naevi and Lentigines

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In order to investigate how sunlight may affect naevi and lentigines, their melanocytes and the basement membrane, three irradiation protocols were applied directly to ten naevi and five lentigines on 2 subjects. Neither volunteer had sufficient naevi and lentigines to be able to use the three irradiation protocols on each of the subjects. Skin biopsies were fixed in glutaraldehyde followed by osmium tetroxide, thin-sectioned and examined in a Hitachi H-7000 transmission electron microscope. Following 14 consecutive single exposures of 3 MED of UVB or single exposures followed by 25 J/cm² of UVA, 350 J/cm² UVA with either 2040 or 2280 mJ/cm² UVB, the basement membrane maintained its continuity. Melanocytes were not observed on the dermal side of the epidermal-dermal junction. UVA irradiation stimulated reinforcement of the basement membrane zone by collagen fibers. Centrioles found in melanocytes following irradiation suggest that these melanocytes maybe undergoing mitosis. Dermal fibroblasts were found to contain comparatively large quantities of melanin pigment. The pigment contained in these fibroblasts may in fact constitute an additional barrier against UV irradiation.

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A large proportion of melanomas originate in pigmented naevi, whereas simple lentigines apparently lack melanocytic hyperplasia (1). Epidermal melanocytes tend to proliferate following exposure of normal skin to ultraviolet radiation (2) which causes division in normal human melanocytes in vitro (3).

Clinical, histological and ultrastructural changes in naevi attributed to sun exposure or PUVA treatment have been reported (4). Naevi excised in summer have often shown an inflammatory response (5).

Lea & Pawlowski (6–8) have shown that human, acquired pigmented naevi appear to be located only in the epidermis. Protrusions of naevi below the level of the epidermal-dermal junction (dermal naevi) in fact remain contiguous with the epidermis, being contained by the basement membrane of the epidermal-dermal junction.

MATERIAL AND METHODS

Two Caucasian volunteers, a female aged 44 and a male aged 55, were the subjects of this study. Both had skin Type 2, capable of natural tanning after sun exposure (9). Ethical considerations prohibited irradiating naevi from donors other than the authors. The regions of origin for the biopsies are listed in Table I. The diameters of irradiated naevi and lentigines did not exceed 5 mm.

Areas measuring 10 mm² including the skin lesions were delineated with white tape. These areas were exposed directly to UV, with doses ranging from 40 to 300 mJ/cm² of UVB with a constant amount of UVA (25 J/cm²).

Irradiation protocols

1) UVB only: 3 MED – 120 mJ/cm², one exposure only, with the biopsy taken 72 h following irradiation.
2) UVB: 3 MED (either 120 mJ/cm² for subject 1, or 180 mJ/cm² for subject 2), followed after 72 h by UVA (25 J/cm²), with the biopsy taken 3 h following UVA exposure.
3) UVA: 25 J/cm², followed immediately by the UVB irradiation. Fourteen consecutive exposures at 72 h intervals were carried out. UVA irradiation dose was constant, while the UVB exposures increased gradually.

The total dose of UVA reached 350 J/cm² for both subjects, whereas for UVB reached 2040 mJ/cm² for volunteer 1 and 2280 mJ/cm² for volunteer 2.

The UVA irradiation was delivered from a Houva-Lite (208/120V, 60Hz, 60A, 3-wire, single-phase), fitted with 48F72 T12 BL/HO UVA lamps, with a spectrum of 310 nm to 390 nm (National Biological Corporation). The UVA light intensity (18 mV/cm²) was measured with a model LMA 302 meter (NBC).

The UVB irradiation was generated by UBL-FS72T12/UVB/HO lamps (NBC) with a spectrum between 280 and 350 nm. The light intensity (0.6 mV/cm²) was measured with a UVB LMH06 C light meter (NBC).

Biopsy specimens of entire naevi or lentigines surrounded by a margin of normal but irradiated skin were immediately fixed (7), cut into approximately 0.5 mm cubes and processed for TEM. Ultrathin sections were cut (MT6000XL microtome, RMC Inc.) and mounted on formvar-coated single-slotted grids, stained with lead citrate and uranyl acetate and examined in an H-7000 transmission electron microscope (Hitachi Instruments, Toronto, Cana-

Table I. *Skin lesions, location, radiation*

Volunteer	Naevus =N Lentigo =L	Local	UVB only ×1	UVA UVA ×2	UVA UVB ×14
1/F	L	Leg		*	
2/F	N	Breast		*	
3/F	N	Buttock		*	
4/M	L	Chest	*		
5/M	L	Chest	*		
6/M	N	Chest	*		
7/M	N	Chest	*		
8/M	N	Thigh			*
9/M	N	Arm			*
10/M	N	Forearm			*
11/M	L	Thigh			*
12/M	L	Thigh			*
13/F	N	Arm			*
14/F	N	Arm			*
15/F	N	Arm			*

da). Control groups included non-irradiated naevus and lentigo, as well as normal, irradiated skin with no visible naevi or lentigines from the periphery of the biopsies.

RESULTS

A single exposure of naevi and lentigines to UVB only, or UVB followed by UVA, induced papular erythema. Lentigo 1 (Table I) also showed swelling and a petecchial response. There was no clinically marked hyperpigmentation. Multiple radiations initially induced erythema, followed by hyperpigmentation.

The basement membrane separating the epidermis from dermis was clearly visible and allowed no direct contact between epidermis and dermis (Fig. 1). No cell or any part of a cell was observed in the process of crossing the basement membrane. However, in naevus 15, individual, inflammatory cells had invaded the epidermis without leaving any observable openings in the epidermal-dermal junction. Thickening of the basement membrane zone was observed following prolonged radiation with UVA and UVB. Both lamina lucida and lamina densa as well as the fibrils just beneath the lamina densa appeared to be affected. In those areas where keratinocytes attached to the basement membrane, interlocking, thickened anchoring fibrils appeared to extend from the lamina densa to become incorporated into the dermal collagen. Anchoring fibrils were interlaced with larger collagen fibers, resulting in an irregular thickening of the basement membrane zone (Fig. 2). Anchoring fibrils were absent in those regions where melanocytes, their branches or naevus nests were located next to the basement membrane. The basement membrane zone often showed irregular thickening. In the post-irradiated skin, dermal papillae were well marked except in lentigines 4, 5 and 12, and naevus 10, where the epidermal-dermal junction was comparatively flat.

Widening of the intercellular spaces in the epider-

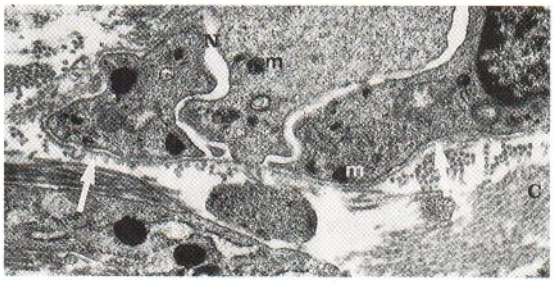

Fig. 1. Naevus 8. The basement membrane beneath the naevus nest (*N*), is continuous (*arrows*). There are no anchoring fibrils, but collagen fibers are attached to lamina densa. Well developed collagen (*C*) in the papillary layer. Melanosomes (*m*) of various shapes and sizes in the melanocytes. ×20000.

Fig. 2. Naevus 14. Continuous basement membrane separates keratinocytes from the dermis. Arches of anchoring fibrils interlaced with microfibrils and larger collagen fibers (*A*). ×24000.

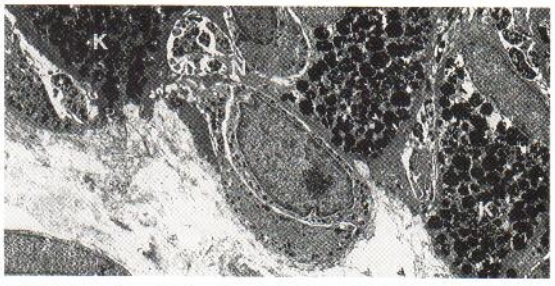

Fig. 3. Lentigo 11. Small nest (*N*) formed along the epidermal-dermal junction. Keratinocytes (*K*) filled with melanin aggregates. ×5000.

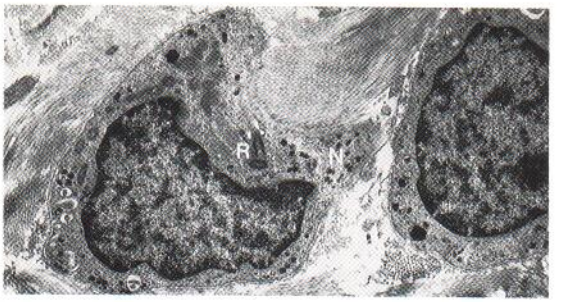

Fig. 4. Naevus 8. Fully melanized nest (*N*), protruding into the dermis. Centriole (*R*) present in the melanocyte. ×11 000.

mis as an indicator of edema was found only in naevi 2 and 3, following a single UVB, UVA exposure.

There was no obvious increase in the number of melanocytes or in the degree of melanization in naevi and lentigines when compared with non-irradiated controls. However, in lentigines which had received 14 irradiations, individual melanocytes and small nests were observed in the epidermis along the epidermal-dermal junction (Fig. 3). Rough endoplasmic reticulum (RER), Golgi complex and mitochondria with tubular cristae were prominent in melanocytes of naevi which had received extensive exposure, suggesting increased cellular metabolism. Mitotic figures were not observed, but centrioles were frequently present in UVA,UVB- and UVB,UVA-irradiated naevi (Fig. 4), indicating mitotic activity (10).

Basal and suprabasal keratinocytes had increased melanin content, especially the keratinocytes of lentigines (Fig. 3).

Superficially located naevus nests contained more melanin than those epidermal nests protruding deeply into the dermis. This melanin concentration gradient was prominent in both control and irradiated naevi. Only in naevus 9 was the deeply invaginated nest richly melanized. Neither single melanocytes nor nests of melanocytes showed signs of cellular damage.

A dermal, inflammatory infiltrate was most pronounced in lentigines after multiple irradiations but was also observed in naevi and lentigines following a single dose of either UVB (protocol 1) only or UVB and UVA (protocol 2). The infiltrate consisted mainly of neutrophils, lymphocytes and mast cells. The most characteristic changes which were observed in the blood vessels of the papillary and reticular dermis were induced by a single UVB,UVA radiation. Densely packed red blood cells, extravasations, platelet aggregates and discontinuities of some endothelial junctions were observed in small vessels.

Macrophages were rarely observed. They became more numerous following multiple irradiations and contained melanin granules and cellular debris. Most of the dermal cells were identified as fibroblasts (Fig. 5). These fibroblasts were located in the dermis just below the basement membrane. Multiple irradiations increased the electron-dense, granular content of fibroblasts. In contrast, controls as well as naevi and lentigines exposed to only a single irradiation, did not appear to increase their melanin content. All the naevi remained intra-epidermal and showed well developed nests. In contrast, lentigines showed no nest formation.

DISCUSSION

A constant low dose 25 J/cm^2 UVA radiation resulted in a borderline erythema (11). Increased doses of UVB produced a more pronounced effect on the epidermis (12, 13). The UVA dose used in this experiment may have given protection against UVB, but at the same time represented an exposure level similar to natural sunbathing for approximately 2 h. The volunteers were irradiated with UVA,UVB to simulate natural sunbathing of several hours per day for a period of 3-4 weeks.

In common acquired melanocytic naevi, the basement membrane of the epidermal-dermal junction separates the naevus from the dermis (6–8). No hemi-desmosomes were found on the epidermal side of the membrane and no anchoring fibrils on the

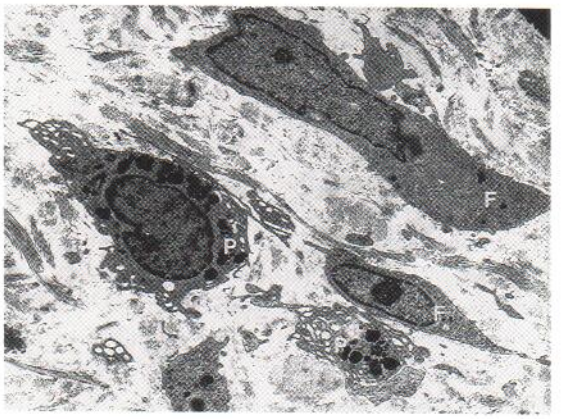

Fig. 5. Naevus 10. Macrophages (*P*) and fibroblasts (*F*) with melanin granules in the cytoplasm. ×4000.

dermal side. A lack of these structures may have created some instability of the epidermal-dermal junction, which may facilitate protrusion of a naevus into the dermis while remaining to be separated from the dermal compartment by the basement membrane of the epidermal-dermal junction. However, in contrast to normal melanocytes, melanoma cells penetrate the basement membrane (14). This ultrastructural difference may prove useful for differential diagnosis.

We did not observe free melanosomes in the skin following UVA,UVB exposure. Lysosomal characteristics of melanosomes (15) and their autophagocytosis in melanocytes suggested that melanin may be found in keratinocytes, dermal macrophages and fibroblasts.

We found that dermal fibroblasts contain comparatively large quantities of melanin granules. If skin fibroblasts are indeed viable for the life time of the dermis, as suggested by Lea & Pawlowski (16), we propose that these pigment-containing fibroblasts may in fact provide an additional, dermal barrier for protection of the underlying tissue against UV irradiation.

Various mediators participate in the skin's reaction to UV radiation and different immunological effects may occur following exposure (17). An increasing concentration of free radicals (18) which are known to damage lipid membranes and cause lysosomal instability may also cause damage to melanosomes.

The number of melanocytes in naevi did not seem to increase after irradiation. However, the presence of many centrioles, which are only rarely observed in normal melanocytes, suggested that active cell division may follow irradiation. The inflammatory reaction itself did not seem to stimulate or inhibit the proliferation of melanocytes, which supports previous observations (19).

This study affirmed that the basement membrane of the epidermal-dermal junction reacts to UV irradiation by becoming thicker. This would indicate that the average dose of "vacation sun tanning" is not enough to destroy the basement membrane of the epidermal-dermal junction. Melanocytes which form naevi do not change their epidermal location. Although lentigines usually do not form naevus-like nests, we did observe nests following UV irradiation.

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