

SHORT REPORTS

Thymidine Labelling of Epidermal Melanocytes in UV-irradiated Skin

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Abstract. By using autoradiography and a technique of repeated ³H-thymidine injections, it was found that there is a normal turnover of epidermal melanocytes in C57BL mice, and that the numerical increase in the population of active melanocytes following repeated UV irradiation is essentially the result of cell division.

Key words: Autoradiography; Melanocytes; Mitosis; UV irradiation

Repeated ultraviolet (UV) irradiation of the skin induces a considerable increase in the number of active, melanin-producing melanocytes in the epidermis (6, 8). The mechanism for this increase is still not known. Mitosis of normal melanocytes has rarely been demonstrated in vivo (4, 8, 9). Therefore, other processes have been suggested to account for this increase, such as activation of pre-existing, melano-genetically inactive melanocytes, or migration of dermal melanocytes into the epidermis (5, 9). In this study the role of mitosis in the increase of the melanocyte population following UV irradiation has been investigated using a technique of repeated labelling with ³H-thymidine.

MATERIALS AND METHODS

Male mice of a pigmented strain (C57BL/6J, Jackson Laboratories), were used. In this strain melanocytes are normally found in less hairy areas, such as the soles, the

tail and the outer portion of the ears. Three mice had their right ears irradiated daily at 10:30 a.m. for 8, 10, and 11 days respectively. The light source was a fluorescent sun lamp (Westinghouse FS, wavelength spectrum 290-350 nm), and the daily dose was 1×10^6 ergs/cm². The left ears were covered and used as controls. Starting on the second day of irradiation, the animals were injected intraperitoneally four times a day (9:00 a.m., 1:00, 5:00 and 10:00 p.m.), with 50 μ Ci methyl-³H-thymidine (spec. act. 40-60 Ci/mole, New England Nuclear). The animals were sacrificed 4 hours after the last injection.

Skin samples were taken from symmetrically opposite regions of the dorsal side of the control and irradiated ears. Skin samples from the lower halves of the ears were incubated in 2 N NaBr and the dermis was removed. The epidermal sheets were then incubated in buffered DOPA to demonstrate melanocytes and were mounted on slides with basal layer facing up (10). Skin samples from the upper halves were fixed in 3% glutaraldehyde, DOPA incubated, and embedded in Epon. 1 μ m thick serial sections were prepared for autoradiography using Ilford K5 emulsion. After 21 days' exposure the slides were developed and counterstained with toluidine blue.

RESULTS AND DISCUSSION

Macroscopically, the irradiated skin showed a clear increase in pigmentation. This difference was associated with a 4-6 fold increase in the number of melanocytes in the stimulated ears (Table I). These figures are based on cell counts from ten 1 mm² fields in the lower halves of the ears. Although the number of melanocytes varies in different parts of the ear, the population increases by approximately the same factor in all parts following UV irradiation (unpublished data).

Due to the DOPA reaction the melanocytes were easily distinguishable from the keratinocytes in the autoradiographs. In the irradiated ears 70-85% of the melanocytes showed a marked accumulation of silver grains over the nucleus (Table I). The grains were distinguishable from the melanosomes by their differing location, colour and focal plane. Labelled cells had 15-40 grains over the nucleus (Fig. 1A), while unlabelled cells had no more than background activity (3 grains or less, Fig. 1B). These findings were consistent in all sections of the same nucleus.

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Table 1. *Melanocyte response to repeated UV irradiation*

Days of irradiation	Melanocytes/mm ² ±S.E.		Labelled melanocytes/Total
	Control ear	Irrad. ear	
8	22±3	78±13	41/57
10	15±3	95±9	56/65
11	40±3	150±10	35/50

Some melanocytes in the control ears were also labelled. However, since the melanocytes population density was very low in the unirradiated control ears, too few melanocytes have been studied to allow a reliable estimate of the percentage of labelled cells. Preliminary data from the tail skin is in qualitative agreement with the results obtained from the ears.

While ³H-thymidine labelling has proved to be a useful method for determining mitotic activity, incorporation of ³H-thymidine during repair of UV-damaged DNA molecules is a complication to be considered in the interpretation of the present results (1, 7). It seems unlikely, however, that such a process can explain our findings. DNA repair seems

to be a fast process *in vivo*, and the injection schedule was chosen to minimize the amount of labelled compound available for this process (2). The incorporation of ³H-thymidine during DNA repair seems to be uniform, and even after an optimally timed intradermal injection this labelling is very sparse in comparison with the dense accumulation of silver grains over cells in DNA replication phase (1, 2, 3, 7). In contrast to this, the labelling of melanocytes in the present study was essentially of an all-or-nothing character. The number of grains in labelled melanocytes was the same as in labelled differentiated keratinocytes, and melanocytes and keratinocytes labelled to the same degree were also found in the unirradiated control ears. Thus, it seems reasonable to conclude that the labelling of melanocytes in the present study was due to incorporation of ³H-thymidine during DNA replication.

Since 70–85% of the epidermal melanocytes in the stimulated ears were labelled, it can be concluded that the numerical increase in melanocytes following repeated UV irradiation was essentially a result of cell division. Labelled melanocytes were also found in the epidermis of the unirradiated control ears, indicating a turnover of melanocytes in non-stimulated skin as well. Whether the dividing cells are differentiated melanin-producing melanocytes or precursor cells cannot be determined from the present study. Experiments to answer this question are in preparation.

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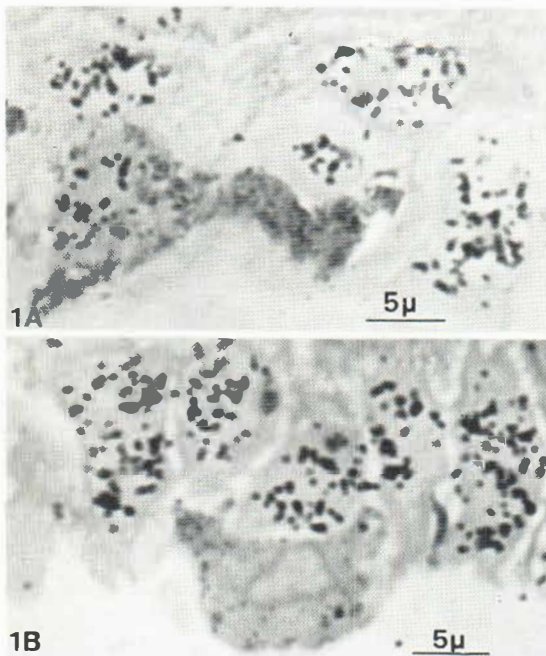


Fig. 1. Autoradiographs of a labelled (A) and an unlabelled (B) melanocyte from UV-irradiated ear.

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HL-A Histocompatibility Antigens and Cutaneous Reactions to Practolol

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The occurrence of psoriasiform cutaneous eruptions in patients treated with practolol, as first observed by Felix et al. (1), has become a well-known side effect and for general use the drug has now been withdrawn from the market. The mechanisms underlying the outbreak of the psoriasiform eruptions remain unknown, but it seems conceivable that beta-blockade per se in these patients might lead to a reduced level of epidermal cyclic AMP as seen in psoriatic epidermal tissue (4).

Psoriasis vulgaris is known to occur about five

Table I.

Initials	Sex	Age	HL-A type
E. W.-M.	♀	72	1, 2, 13, 8
E. H. P.	♀	66	1, 2, 8, 17
T. H. O.	♀	60	1, 9, 12, 5, 315
H. K.	♀	72	2, W26, 12, 22, AJ
B. K.	♀	44	1, 2, W15, 8, UPS
G. J.	♀	50	3, 9, 7
E. H.	♀	70	9, 11, W5, 8, 315
O. G.	♀	61	9, W32, 12, W15, AJ, 315

times more often in individuals possessing HL-A 13 and/or 17 than in those lacking these antigens. However, it is entirely unknown how these antigens confer the increased risk of psoriasis. In order to investigate the possibility that HL-A 13 and 17 might operate through a mechanism(s) involving cyclic AMP, we have HL-A typed 8 patients with practolol-induced cutaneous eruptions. None of these suffered from psoriasis or had any family history of psoriasis. Five of the 8 patients presented with a distinctive psoriasiform cutaneous eruption. Further clinical details have been described previously (2). The HL-A phenotypes of the patients are given in Table I. The HL-A 17 antigen was found in one patient and HL-A 13 in another. Both of these 2 patients presented with a psoriasiform cutaneous eruption. HL-A 13 and/or 17 were not present in the remaining 6 patients.

In psoriasis vulgaris about 50% of the patients carry either HL-A 13 or HL-A 17 as compared with only 11.7% of 1967 control persons. The frequency (25%) of these antigens in the present series of patients is far from significantly different from that in controls. This is true even when considering only the 5 patients with definite psoriasiform eruptions. Nevertheless, 2 (40%) of these were carrying the psoriasis-associated HL-A antigens, and thus a study of a larger group of patients would seem warranted in order to confirm or disprove the possible association. If confirmed, it may support the concept that HL-A can interfere with the receptors for ligands on the cell surface (3).

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