

AUTORADIOGRAPHIC LOCALIZATION OF 8-METHOXYPsorALEN IN PSORIASIS SKIN *IN VITRO*

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Abstract. Slices of psoriatic (Pso) skin were incubated with tritiated 8-methoxypsoralen (8-MOP) and exposed to UVA irradiation. The photobinding of 8-MOP was studied by autoradiography at the cellular level and in the different layers of the epidermis and in the dermis. Silver grains were found in the nucleus and the cytoplasm of the various cell types. Keratin, collagen and lipoproteins were also labelled. The upper malpighian cells and the parakeratotic cells showed a greater degree of labelling than did the basal layers. In skin incubated with [³H]8-MOP and unirradiated, no measurable labelling was detected. These results suggest that the targets responsible for the therapeutic activity of 8-MOP might be not only nucleic acids, but also proteins.

Key words: 8-methoxypsoralen; UVA; Psoriasis; Autoradiography

The use of 8-MOP associated with UVA irradiation is well established for the treatment of psoriasis (Pso) (1, 7, 10, 11). It is assumed that the therapeutic effect is due to the photoreaction of 8-MOP with DNA, as shown by many studies on prokaryotic and eukaryotic cells (see general review (9)).

Cellular localization of 8-MOP and trimethylpsoralen (TMP) by using autoradiographic techniques has been reported in guinea pig skin (2) and in human fibroblasts (1). Both studies showed a preferential photobinding of the drugs in the nucleus. By contrast, when using fluorescence microscopy, the fluorescence of 8-MOP was found in the stratum corneum, in cellular membranes, and in the cytoplasm of the cells of the malpighian layers (5). Recently Cech et al. (3), studying the photobinding of 8-MOP and TMP in the epidermal DNA of guinea pigs treated with therapeutic doses, suggested the possibility of targets other than DNA.

In the present work, using autoradiography, we have investigated under *in vitro* conditions the localization of 8-MOP in Pso skin irradiated with

UVA light. We report that without UVA irradiation, there was no apparent binding of 8-MOP, whereas after irradiation there was a nuclear and a cytoplasmic binding in 90 to 100 % of the cells of the different layers of the epidermis, with a higher incorporation in the upper layers, and in the dermal cells. Proteins such as keratin, collagen and lipoproteins were also labelled.

MATERIAL AND METHODS

Patients. Biopsies were performed on 2 non-treated patients suffering from acute Pso. The lesions were typical of the disease, according to clinical and pathological criteria. Biopsies from the skin of one normal subject were studied under the same experimental conditions. The punch biopsies (4 mm) were obtained after local anaesthesia with 2% Xylocaine without adrenalin.

[³H]8-MOP labelling. Skin sections (perpendicular to stratum corneum) 0.7 mm in thickness, were incubated in the dark for 1 hour at room temperature in 1 ml phosphate-buffered saline containing 38 μ Ci/ml of [³H]8-MOP (spec. act. 790 mCi/mM; C.E.A., Saclay, France). Specimens were irradiated with 5 J cm⁻² of UVA light (Sylvania lamp used for PUVA therapy in Waldmann 180 apparatus). Control experiments were performed on [³H]8-MOP-treated skin without UVA irradiation.

Autoradiography. The specimens were fixed with glutaraldehyde-osmium tetroxide, dehydrated, embedded and sectioned according to a technique described in detail elsewhere (4). Autoradiographs of 1 μ m sections, using Ilford K2 emulsion, were prepared and developed after 3 weeks of exposure, as reported previously (6). For each experiment, the number of silver grains was counted in 100 cells of whole epidermis and in 100 cells in each layer of the epidermis, i.e., basal, malpighian, upper malpighian, and parakeratotic layers for Pso epidermis, and basal malpighian, and granular layers for normal epidermis. The binding of 8-MOP was also evaluated in cells of the dermal inflammatory infiltrate (100 cells counted). In order to avoid quantitative differences due to the differences between cell surface (parakeratotic cells versus basal cells), the number of silver grains was also evaluated for known areas (2500 μ m²).



Fig. 1. Autoradiograph of a section of psoriatic skin biopsy material incubated with [^3H]8-MOP ($38 \mu\text{Ci/ml}$ [^3H]8-MOP) for 1 h and irradiated with UVA light (5 J cm^{-2}). Many silver grains are present in epidermal cells of all the layers. They are distributed equivalently between nucleus and cytoplasm. Parakeratotic cells show a greater degree of photobinding than the cells of the basal layer. $\times 515$.

RESULTS

Non-irradiated biopsies of normal and Pso skin incubated with [^3H]8-MOP did not show any labelling. After irradiation with 5 J cm^{-2} 90 to 100% of the epidermal cells were labelled in both normal and Pso skin. The silver grains were found equally distributed between the nucleus and the cytoplasm. The [^3H]8-MOP photobinding in one Pso biopsy is illustrated in Fig. 1. In the dermis, fibroblasts, histiocytes, endothelial cells and, in Pso, cells of the inflammatory infiltrate were also labelled, as well as collagen, keratin of hair follicles and lipoproteins of the fatty lobules (Fig. 2 A, B, C).

Tables I and II summarize and give the quantitative values of the experimental results. The photobinding of 8-MOP in all the layers of the epidermis

is dependent upon UVA irradiation (Table I). Table II gives the average number of grains in each layer of the epidermis.

The degree of binding of 8-MOP per cell in the

Table I. Binding of [^3H]8-MOP in normal and Pso epidermis

	Average number of grains/cell		
	Normal	Psoriatic ^a	
		Case 1	Case 2
Unirradiated	0.4 $\pm$ 0.09	0.4 $\pm$ 0.08	0.5 $\pm$ 0.06
Irradiated (5 J/cm ²)	2.8 $\pm$ 0.1 ^b	5.0 $\pm$ 0.3	6.1 $\pm$ 0.3

^a Parakeratotic cells were not counted.

^b 200 cells counted.

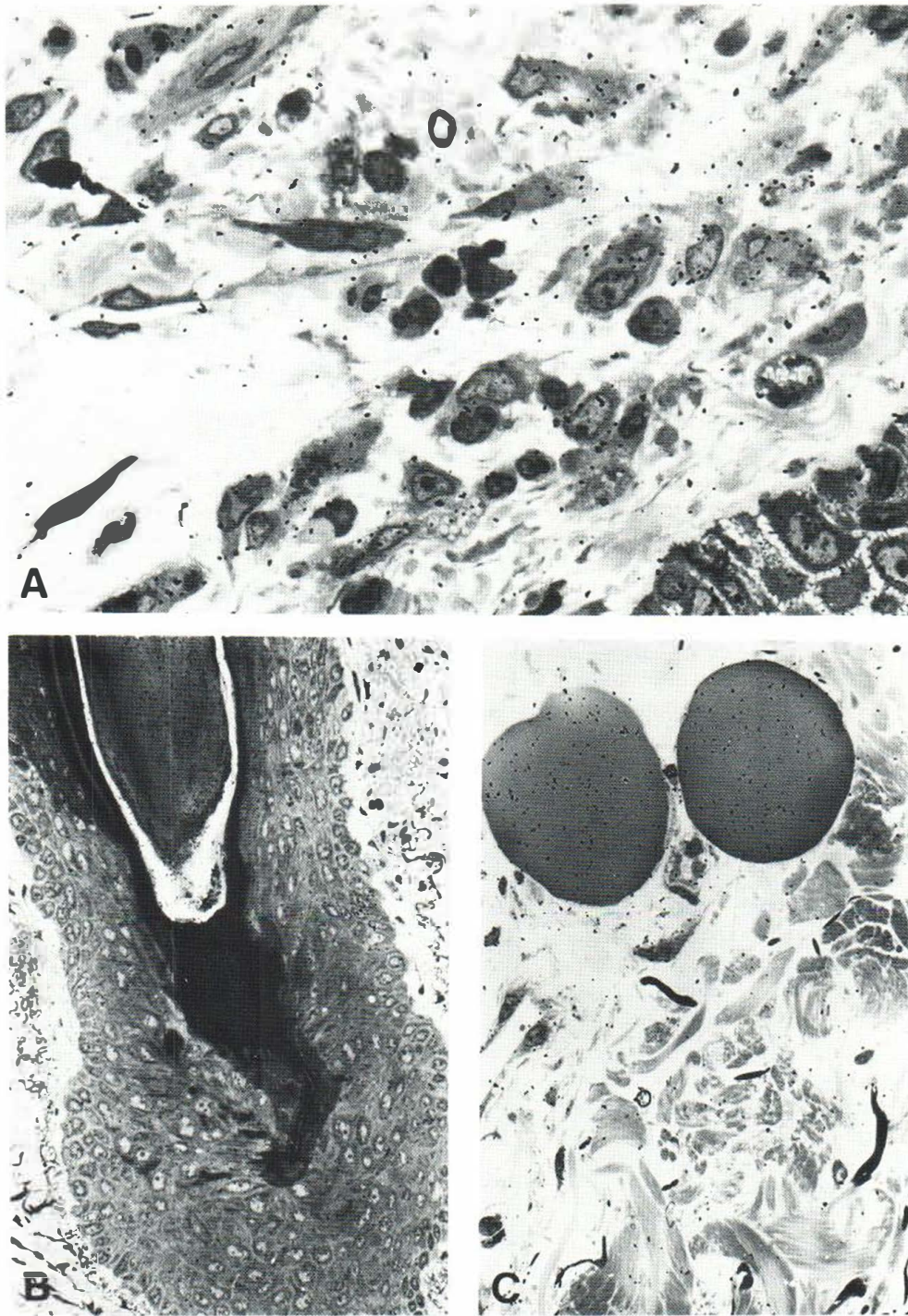


Fig. 2. Autoradiographs of psoriatic skin incubated with [^3H]8-MOP and UVA light, as in Fig. 1. (A) All the cells in the inflammatory infiltrate are labelled in both nucleus and cytoplasm. Average number of grains per cell: case 1, 4.5 ± 0.2 ; case 2, 3.9 ± 0.2 . $\times 1140$. (B) Keratin of hair folli-

cle, intensely labelled. Mean number of grains per $2500 \mu\text{m}^2$ of keratin: 230. $\times 890$. (C) Fatty lobules are labelled. Mean number of grains per $2500 \mu\text{m}^2$ of lobules: 130. $\times 890$. Under the same experimental conditions, the mean number of grains per $2500 \mu\text{m}^2$ of epidermis was 140.

Table II. Localization of [^3H]8-MOP in the different layers of normal and Pso epidermis after UVA irradiation (5 J/cm 2)

	Average number of grains/cell				
	Basal cells	Malpighian cells	Upper malpighian cells	Granular cells	Parakeratotic cells ^a
Normal	2.0 $\pm$ 0.1	2.5 $\pm$ 0.2		3.4 $\pm$ 0.2	
Psoriasis					
Case 1	3.6 $\pm$ 0.2	5.1 $\pm$ 0.4	6.7 $\pm$ 0.3		13.4 $\pm$ 0.6
Case 2	5.2 $\pm$ 0.2	7.7 $\pm$ 0.4	9.4 $\pm$ 0.4		

^a In psoriasis only.

upper malpighian and parakeratotic cells was significantly ($P < 0.01$) greater than in the basal layer, the average number of grains per a known area (2 500 μm^2) of each layer being twice as high in upper malpighian and parakeratotic layers as in the basal layer.

DISCUSSION

By means of an autoradiographic technique, the present study showed the cytological distribution of the [^3H]8-MOP photobinding in the different cell types of both normal or Pso skin.

Many reports deal with the localization of various psoralens in animal (2, 3, 8, 12) and human skin (5) and in cultured cells (1, 13).

To the best of our knowledge, this is the first study undertaken on skin from human subjects suffering from Pso.

Under our experimental conditions, the exposure of the skin to the 8-MOP was comparable for all the different cell types of the skin, while with PUVA therapy the distributions may not be identical, depending on the way of administering of the drug.

It must also be pointed out that the fixation, washing, dehydration and embedding, necessary for the preparation of the specimens for autoradiography, allow one to detect only those molecules of 8-MOP which have been firmly bound to cell structures. The weak types of binding not detected in our experimental conditions may also play a role in the PUVA therapy.

In samples exposed to 8-MOP, but not irradiated, no 'binding' of the drug was detected. In irradiated samples, 90 to 100% of the cells were labelled in epidermis as well as in the dermis, and silver grains were present in equal amounts in both nucleus and cytoplasm. This result is in complete agreement

with the work of Bredberg et al. (1) who reported an equal distribution of 8-MOP in nuclei and cytoplasm of fibroblasts irradiated with UV doses lower than 6 J cm $^{-2}$. This finding may also indicate the preferential binding of psoralens to nucleic acids (DNA and RNA) as reported previously in autoradiographic studies (1, 2) and after cell fractionation (8). However, proteins such as keratin, collagen and lipoproteins of the fatty lobules were also labelled with [^3H]8-MOP.

In psoriasis, we have noticed a higher incorporation of 8-MOP in the upper layers (parakeratotic cells) compared with the basal layer. This increased photobinding might reflect the process of keratinization taking place in the upper layers of the epidermis. However, cytochemical studies involving enzymatic extractions of RNA and proteins are necessary to confirm this hypothesis.

All these data lend support to the fact that targets other than nucleic acids may be involved in PUVA therapy, as recently suggested by Cech et al. (3). Our study may represent a good model by which to study the localization and the differential affinity of 8-MOP for the various components of the skin.

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