Studies on the Effects of Various Topical Phototoxic Drugs and UVA on Melanocytes of C57 BL Mice

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One hundred sixteen C57 BL mice were painted with either 8-MOP, 5-MOP or TMP solution in concentrations of 0.02%, 0.1%, and 0.5% each and UVA irradiated. Skin biopsies were performed at 1, 3, 5 and 7 weeks after UVA irradiation.

The results measuring the number, area, and perimeter of the melanocytes after topical PUVA were higher in the TMP-painted group than in the 8-MOP or 5-MOP painted groups. In all groups, the weekly changes showed an increasing value through five weeks. In comparing the drug concentrations used, 0.1% of chemicals produced the same or higher values than 0.5%.

There have been few studies on the effects of 5-MOP in photochemotherapy. In this study 5-MOP produced a pigment-producing effect similar to 8-MOP. Therefore, if topical PUVA with 5-MOP is used in clinical practice, we could expect a significant therapeutic effect in vitiligo. (Ann Dermatol 3 :(1) 15—22, 1991)

Key Words: Melanocytes, 8-MOP, 5-MOP, TMP, Topical PUVA

Vitiligo is an acquired, chronic and progressive skin disorder of unknown etiology characterized by destruction and loss of melanin-forming melanocytes resulting is depigmented patches on the skin.

Although topical and systemic corticosteroids have been the mainstay of repigmentation therapy, systemic complications limit long-term steroid use. Therefore, systemic or topical PUVA, that is, UV irradiation after ingestion or topical application of phototoxic drugs, has become more widely used.[1-2]

In topical PUVA, it is very difficult to control the length of ultraviolet exposure for pigment deposition while minimizing toxicity. In the treatment of vitiligo, complications of phototoxicity include erythema or vesicle formation.

Therefore, optimal therapy involves the selection of drugs with proper concentration to produce good repigmentation with minimal side effects.

8-Methoxypsoralen (8-MOP) and 4, 5, 8-trimethylpsoralen (TMP) are phototoxic drugs most frequently used in systemic phototherapy along with the recent addition of 5-methoxypsoralen (5-MOP). However, in topical photochemotherapy, TMP and 8-MOP in concentrations of 0.01-1% are currently recommended[2-7]. Although there have been reports on the pigment producing effect of topical photochemotherapy using 5-MOP, we could not find literature on the optimal concentrations of this chemical. Grimes et al. (1982)[6] and Park and Whang (1989)[8] have reported that low concentrations (0.1%) of 8-MOP and TMP are as effective in pigment formation as high concentrations.

The purpose of this study was to evaluate repigmentation response in vitiligo using various

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concentrations (0.02%, 0.1%, 0.5%) of 8-MOP, 5-MOP and TMP in topical photochemotherapy.

MATERIALS AND METHODS

Materials

Experimental animal

One hundred-sixteen mature black male mice of the C57 BL strain weighing 25 gm to 30 gm were divided into nine experimental test groups and one control group. The experimental groups of mice were painted with 8-MOP, 5-MOP or TMP solution in concentrations of 0.02%, 0.1% or 0.5% each. The control mice were painted with a base solution.

Light source

UVA was irradiated on the dorsal surface of the ears of C57 BL mice at a distance of 14cm with an intensity of 50mw/cm^2 using the Sellas (Dr. Sellmeier Co., Gevelaberg, Germany) that emits strong UVA. The wavelength of the lamp ranged from 320 to 420nm, with the highest wave length at 365nm. The intensity was measured with IL 442 radiometer (Ultralite Enterprises Inc., Lawrenceville, Georgia). To obviate the effect of UVB, a Mylar sheath UVB filter was installed.

Phototoxic drug

Pure powders of 8-MOP (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), 5-MOP (Sigma Chemical Co., St Louis, Missouri, U.S.A.) and TMP (Sigma Chemical Co., St Louis, Missouri, U.S.A.) were each dissolved in 70% ethylalcohol and diluted to a 1% solution using distilled water. This solution was then diluted to concentrations of 0.02%, 0.1% and 0.5%.

Methods

Topical application with phototoxic drugs and UV irradiation.

In the experimental group, 0.1ml of phototoxic drugs in concentrations of 0.02%, 0.1% and 0.5% was homogeneously applied to the dorsa of both ears of the mice using a micropipette. In the control group only, the base solution was applied using the same method. Twice weekly for seven weeks the mice were exposed to UVA radiation for 30 minutes after topical application. The amounts of UVA exposure during each exposure were 150mJ/cm^2 during the first week, 200mJ/cm^2 the second week, 250mJ/cm^2 the third week, and 300mJ/cm^2 during the fourth week.

Skin biopsy and split-dopa stain.

After the first, third, fifth and seventh weeks of irradiation, three mice from each experimental test group and two mice from the control group were sacrificed under anesthesia with ether. Skin biopsies were performed on both ears of the mice, and the biopsy specimens were washed in physiologic saline for 1 minute. The specimens were immediately transferred to EDTA solution and incubated for 2 hours at 37°C in a CO_2 incubator, and the epidermis was separated. The epidermis was then washed in isotonic saline solution for one minute, fixed in ten percent formalin and then transferred to a dopa solution at 37°C for one hour. A fresh dopa solution was used for an additional seven hours. When the color of the epidermis changed to brown, it was placed on a glass slide and mounted in Permount to be examined by light microscopy.

The measurement of the number, area and perimeter of the melanocytes.

The grid was inserted over the ocular lens in a 400 power light microscope and the number of melanocytes per mm^2 was counted in ten areas of each sample. The area and perimeter of five melanocytes on each slide were measured using the Optomax V program image analyzer (Analytical Measuring System Co., England). When the melanocyte was observed under a 400 power light microscope attached to a video camera, the image of the cells was seen on the monitor. After the selection of proper gray level and correction of artifacts, the area and perimeter of the melanocytes were measured.

The significance of the result was judged by the Student-Newmann procedure of multiple comparison test.

RESULTS

The number of melanocytes

After three weeks of photochemotherapy, significantly more melanocytes were seen in the TMP group than in the 5-MOP and 8-MOP groups ($P>0.05$), except for the 0.02% groups at three weeks. Comparing the 8-MOP and 5-MOP groups, the number of melanocytes was significantly increased in the 0.1% and 0.5% 5-MOP groups at the fifth week and the 0.1% 8-MOP group at the seventh week ($p>0.05$). In a comparative study between the photoxic drugs at 0.1% concentration, a significant difference in the number of melanocytes occurred in the fifth, sixth, third, and first weeks in decreasing order. The comparison of melanocyte numbers between different concentrations after five weeks of photochemotherapy showed a significant difference in decreasing order at 0.1%, 0.5%, 0.02% (Table 1, Fig. 1, Fig. 2, Fig. 3, Fig. 4, Fig. 5).

The areas of melanocytes

The area of the melanocytes was larger in the TMP group at a concentration of 0.5% after the first and third weeks of photochemotherapy, at 0.1% and 0.5% after five weeks, and at all con-

Table 1. The number of melanocytes according to weeks after application of psoralens in various concentrations

Weeks	1			3			5			7		
Concentrations (%) Kind of psoralen	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5
TMP	33.3 ±2.6	50.0 ±6.3	51.7 ±6.1	42.5 ±4.2	83.3 ±5.2	74.2 ±3.8	65.8 ±4.9	120.8 ±6.6	105.8 ±7.4	68.3 ±6.8	100.8 ±7.4	73.3 ±6.8
8-MOP	30.0 ±6.3	46.7 ±7.5	54.2 ±7.4	40.0 ±4.5	57.5 ±5.2	60.8 ±7.4	56.7 ±7.5	91.7 ±5.2	79.2 9.7	55.0 ±4.5	74.2 ±4.9	64.2 ±5.8
5-MOP	28.3 ±6.1	45.8 ±8.0	51.7 ±4.1	36.7 ±4.1	60.0 ±5.5	57.5 ±5.2	60.0 ±5.5	104.1 ±10.7	90.8 ±8.0	57.5 ±7.6	65.8 ±6.6	60.8 ±9.2
P-Value+	NS*	NS	NS	NS	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.02

Values are mean±SD (μ/mm^2)

\+ The significance of difference of the number of melanocytes among TMP, 8-MOP and 5-MOP Groups was calculated by multiple comparison test

* NS indicates not significant

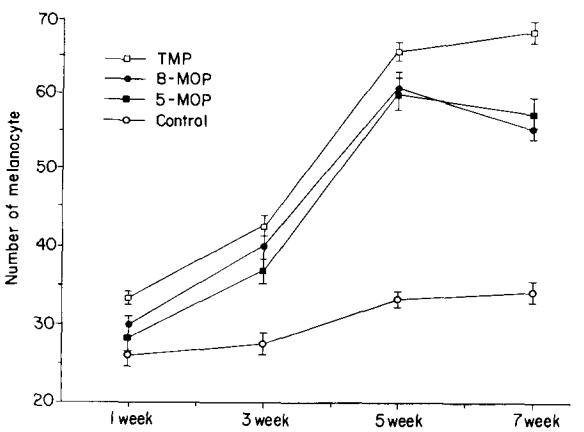

Fig. 1. The change of melanocyte numbers at various weeks after topical photochemotherapy using 3 different psoralen derivatives of 0.02%

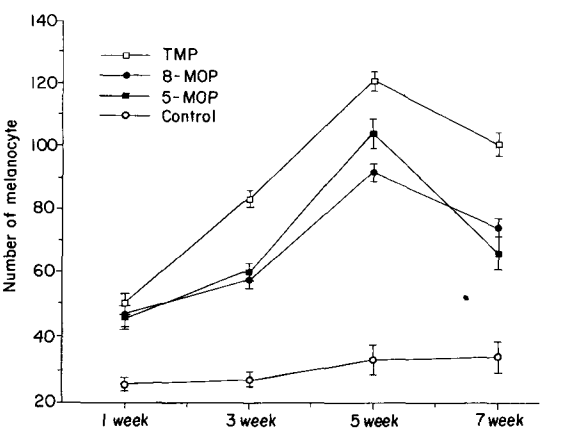

Fig. 2. The change of melanocyte numbers at various weeks after topical photochemotherapy using 3 different psoralen derivatives of 0.1%

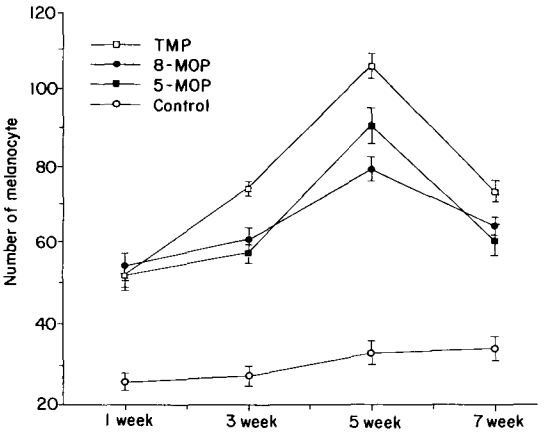

Fig. 3. The change of melanocyte numbers at various weeks after topical photochemotherapy using 3 different psoralen derivatives of 0.5%.

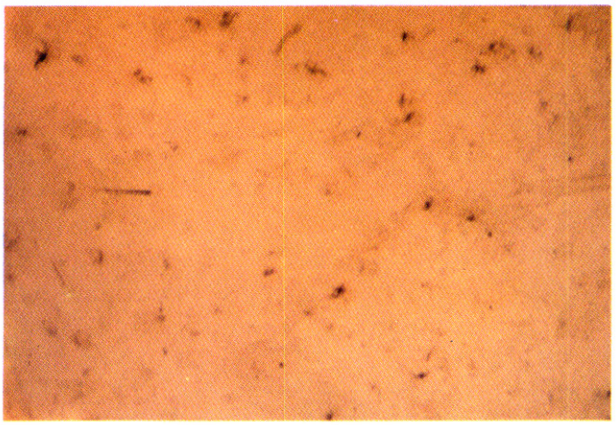

Fig. 4. Epidermal melanocytes of mouse skin before UV light irradiation. Melanocytes are few in number and dispersed (Dopa stain, ×20).

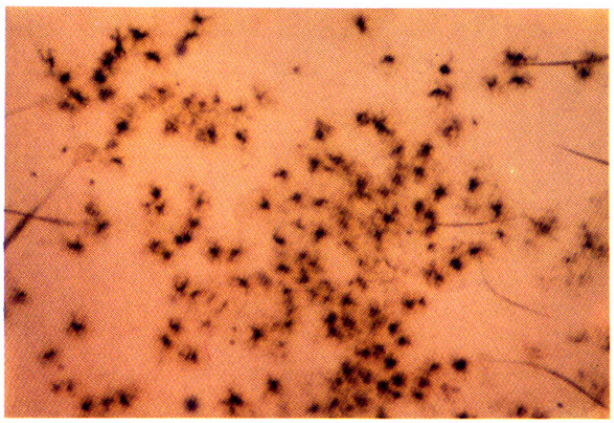

Fig. 5. Epidermal melanocytes after 5 weeks of topical PUVA in TMP painted group. Melanocytes are increased in number and enlarged (Dopa stain, ×20).

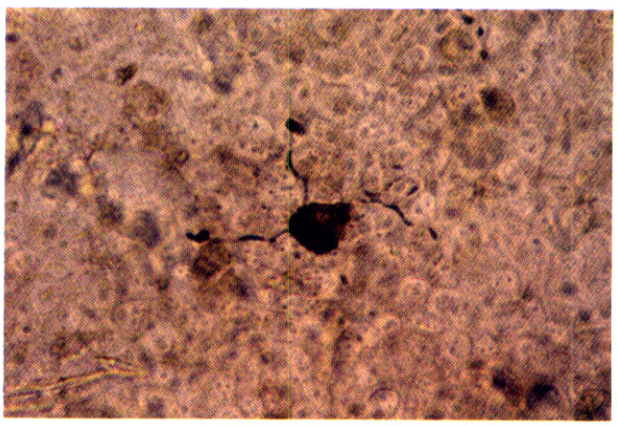

Fig. 6. Epidermal melanocytes before UV irradiation. Melanocytes are smaller and dendrites are poorly developed (Dopa stain, ×400).

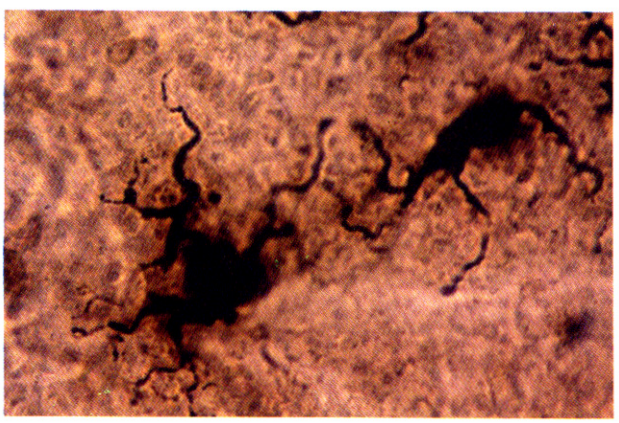

Fig. 7. Epidermal melanocytes after 5 weeks of topical PUVA in TMP painted group. Melanocytes are very large and the number, length, thickness and branches of dendrites are much increased (Dopa stain, ×400).

centrations after seven weeks ($P>0.05$) than the other experimental groups. Comparing the 8-MOP and 5-MOP groups, the melanocyte area was significantly larger in the 8-MOP group after seven weeks. Also, larger cells were observed in the 0.1% 5-MOP group compared to the 8-MOP group after five weeks ($P>0.05$). In a comparative study between the phototoxic drugs at 0.1% concentration, a significant difference in melanocytes area occurred in the fifth, third, seventh, and first weeks in decreasing order. The concentrations after five weeks of photochemotherapy showed a significant difference of melanocytes area, in decreasing order of 0.1%, 0.5%, 0.02% (Table 2, Fig. 6, Fig. 7).

The perimeter of melanocytes

The perimeter of melanocytes was the longest in the TMP group at all concentrations after three weeks of photochemotherapy and in the 0.02% and 0.5% groups after five weeks ($P>0.05$). However, an unexpectedly lower value was obtained in the 0.1% group after one week ($P>0.05$). Comparing the 8-MOP and 5-MOP groups, the perimeter of melanocytes was significantly longer in the 0.5% 8-MOP group after one week, in the 0.1% and 0.5% groups after five weeks ($P>0.05$). In a comparative study between the phototoxic drugs at 0.1% concentration, a significant difference was noted in the fifth, seventh, third and first weeks in decreasing order. The concentrations after five weeks of photochemotherapy showed a significant difference in decreasing order of 0.1%, 0.5%, 0.02% (Table 3, Fig. 6, Fig. 7).

DISCUSSION

Psoralens are chemical compounds derived from certain plants. The medical use of certain plants activated by sunlight dates back to the treatment of vitiligo by the ancient Egyptians as early as 1500 BC and by Indians around 1400 BC.[9] Psoralens

Table 2. The area of melanocytes according to weeks after application of psoralens in various concentrations

Weeks	1			3			5			7		
Concentrations (%) Kind of psoralen	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5
TMP	148.1 ±35.3	166.7 ±45.4	259.3 ±68.4	188.9 ±26.9	348.1 ±29.4	328.9 ±17.0	237.7 ±19.2	416.5 ±35.6	360.3 ±28.5	256.5 ±26.3	99.8 ±31.1	233.0 ±34.8
8-MOP	153.3 ±28.7	147.8 ±23.1	245.1 ±32.0	175.0 ±42.0	269.6 ±43.7	290.6 ±56.8	204.4 ±44.0	280.1 ±24.9	313.9 ±75.6	213.5 ±51.3	73.3 ±77.1	225.7 ±41.1
5-MOP	154.9 ±42.1	184.2 ±61.3	168.7 ±38.4	218.1 ±34.9	308.6 ±84.1	296.2 ±90.1	190.9 ±64.2	317.3 ±41.0	288.3 ±55.7	214.9 ±51.8	33.7 ±42.5	186.1 ±49.4
P-Value+	NS*	NS	P<0.01	P<0.05	NS	P<0.03	NS	P<0.01	P<0.01	P<0.05	P<0.01	P<0.04

Values are mean±SD (μ/mm^2)

+ The significance of difference of the area of melanocytes among TMP, 8-MOP and 5-MOP groups was calculated by multiple comparison test * NS indicates not significant

Table 3. The perimeter of melanocytes according to weeks after application of psoralens in various concentrations

Weeks	1			3			5			7		
Concentrations (%) Kind of psoralen	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5	0.02	0.1	0.5
TMP	88.7 ±18.2	97.6 ±26.0	168.4 ±44.4	189.7 ±8.1	265.3 ±24.6	245.8 ±28.9	191.5 ±27.6	281.1 ±31.7	277.3 ±38.8	173.9 ±35.1	251.2 ±22.8	233.0 ±34.8
8-MOP	109.0 ±30.5	185.3 ±48.8	178.6 ±46.6	132.4 ±35.0	177.0 ±43.8	196.1 ±49.0	198.4 ±35.4	242.5 ±28.9	212.3 ±36.6	192.1 ±47.0	236.3 ±50.2	212.4 ±40.2
5-MOP	98.6 ±27.7	121.1 ±33.3	112.4 ±33.6	215.7 ±53.5	234.0 ±60.9	223.3 ±59.6	147.5 ±50.9	252.5 ±39.1	189.7 ±34.8	174.9 ±56.9	223.8 ±47.4	186.1 ±49.4
P-Value+	NS*	NS	NS	NS	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.03	P<0.03

Values are mean±SD (μ/m)

+ The significance of difference of the perimeter of melanocytes among TMP, 8-MOP and 5-MOP groups was calculated by multiple comparison test

* NS indicates not significant

attracted worldwide attention as therapeutic agents when first used for the treatment of vitiligo in 1948 by EL Mofty.[10-11]

Bergapten (5-MOP) was isolated from bergamot oil by Kalbrunner in 1834.[12] 8-MOP was extracted from Ammi majus by Fahmy & EL Mofty in 1947. In the mid-1960s the synthetic furocoumarin, trimethlpsoralen or TMP, was developed.[9] In 1967 Pathak[13] measured the effect of photosensitivity of 8-MOP, 5-MOP and TMP and reported that these materials induced pigment formation on the skin.

In the treatment of vitiligo, psoralen has been used with sunlight. Photochemotherapy gained wide use after Parrish developed a powerful UVA source which irradiated the whole body and could be easily controlled.

If large areas of skin are involved, the used of topical PUVA is more difficult to manage than systemic PUVA. Topical PUVA is more likely to induce side effects such as erythema and vesicle. However, if the dose of irradiation is well controlled, topical PUVA is more useful in the treatment of small areas of vitiligo, minimizing the local or systemic side effects caused by unnecessarily large amounts of irradiation.

A number of changes can occur in the epidermal melanocytes in human or experimental animals, if they are exposed to UV light following oral or topical application of a phototoxic drug. The changes include increased number and size of melanocytes and increased number and length of dendrites which can be identified with Dopa staining.[15-21]

This study compared the pigment-producing effect of various phototoxic drugs by measuring the number, area and perimeter of melanocytes. We performed this experiment for seven weeks to judge the effect of long-term topical PUVA on melanocytes. As the study progressed, pigment production increased. However, in 0.1% and 0.5% concentration, pigment production decreased at the seventh week after topical PUVA. This result is compatible with the study by Mitchelle[22] in 1963 showing that over-exposure with UV radiation decreased the number of melanocytes. Pathak[13] compared the effect of pigment deposition occurring at different concentrations of 8-MOP and TMP. He reported that a higher concentration induced more rapid reaction, but, because a lower concentration could also induce pigment deposition during long-term treatment, there was no correlation between the concentration of phototoxic drug and the effect of pigment deposition.

Two kinds of psoralens are currently in routine topical use, TMP and 8-MOP the most potent oral photosensitizing psoralen, 8-MOP, is less effective topically than TMP.[23] This may be due to differences in water/lipid solubility or to more rapid metabolism of TMP than 8-MOP.[24] 5-MOP, an oral phototoxic drug, is said to be at least as effective as 8-MOP when given in high doses or when high doses of UVA irradiation are used. With therapeutic doeses of 5-MOP, side effects such as erythema, blister, or nausea rarely occur.[25-27] The decreased phototoxicity seen in the 5-MOP when compared to 8-MOP may be due to its reduced concentrations in the epidermis.[29] 5-MOP induces pigment formation faster than 8-MOP, and, unlike 8-MOP, without the occurrence of erythema. Although 5-MOP has been rarely used in topical PUVA, it is strongly melanogenic and may be used in the treatment of vitiligo and photosensitive disease.[30]

We compared the effect of pigment deposition in topical PUVA using 8-MOP, 5-MOP and TMP. There was no consistent difference after one week of topical PUVA, but after three weeks TMP showed more pigment producing effect than 8-MOP in topical PUVA. 5-MOP, which is rarely used in topical PUVA, produced an effect similar to 8-MOP. Therefore, we suggest it can be effectively used in topical PUVA therapy.

The recommended concentration of phototoxic drug in topical PUVA varies from 0.01% to 1%. Generally 0.15% and, occasionally, 1% concentrations are used. In this study, we chose the concentrations of 0.02%, 0.1%, and 0.5% and compared pigment production in the fifth week when the effects are most pronounced, Griems et al.[6] assessed the efficacy of topical 8-MOP in varying concentrations in 73 vitiligo patients and reported low dose 8-MOP (0.1%) was as effective as high dose 8-MOP (0.5%, 0.3%) except in recalcitrant areas. To evaluate the various concen-

trations of TMP in producing pigmentation, Park and Whang.[8] measured the number, area and perimeter of the melanocyte of mice after painting TMP and UVA irradiation. They concluded that the concentration of TMP is not proportionate to the pigment-producing effect. Low concentrations of TMP were recommended in treating vitiligo, which usually necessitates long-term therapy. We found that 0.1% concentration produced better results than 0.02% or 0.5%. When a high concentration of phototoxic drugs was used, pigment production was greatly reduced after long-term topical PUVA. Consequently, we think low concentration with its low phototoxic reaction is better than higher concentration in treating vitiligo.

In conclusion, measurement of the number, area, and perimeter of the melanocytes after topical PUVA were increased in the TMP group compared to 8-MOP or 5-MOP groups. There were no differences between in the 8-MOP and 5-MOP groups. In comparing drug concentration used, 0.1% produced the same or higher values than 0.5%. If topical PUVA with 5-MOP is applied in clinical treatment of vitiligo, we would expect almost same therapeutic effect as TMP 8-MOP.

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