The Study on the Effects of Psoralen Derivatives on Epidermal Melanocytes in C57 BL Mice after Topical Photochemotherapy

Seung Min Lee, M.D., Seung Kyung Hann, M.D., Yoon Kee Park, M.D.

Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea

Background: **Monofunctional psoralens plus UVA radiation are not severely phototoxic and have less mutagenic activity than bifunctional psoralens plus UVA radiation.**

Objective: **The purpose of this study was to evaluate pigment producing effect using various concentrations(0.02%, 0.1%, 0.5%) of monofunctional psoralens such as angelicin, khellin and comparing it's effect with TMP in topical photochemotherapy.**

Method: **Ninty three C57BL mice were painted with either angelicin, khellin or TMP solution in concentrations of 0.02%, 0.1% and 0.5% each and were UVA irradiated.**

Skin biopsies were performed at 1,3,5 weeks after UVA irradiation. The pigment producing effects were measured by the number, area and perimeter of the melanocytes after topical PUVA.

Results: **The comparison of melanocyte numbers between different psoralens after five weeks of photochemotherapy showed a significant difference in decreasing order of TMP, khellin and angelicin. The area and perimeter of melanocytes were larger in the TMP group after five weeks photochemotherapy than the other group. However in the khellin and angelicin group, the area and perimeter of melanocytes were not increased by increasing the frequency of the UVA irradiation.**

Conclusion: **The number, area and perimeter of melanocytes after topical PUVA increased in the TMP group compared to angelicin or khellin group. We expect the clinical application of angelicin and khellin in vitiligo is possible considering the result of the study of pigment producing effect with a higher concentration and higher dose of UVA.**

(Ann Dermatol 6:(1) 1~8, 1994)

Key Words: Angelicin, Khellin, Topical Photochemotherapy, Trimethylpsoralen(TMP)

Photochemotherapy was introduced into modern dermatology by El-Mofty in 1948[1] and it took another 30 years before photochemotherapy with oral psoralen and UVA(PUVA) was used in the treatment of vitiligo and psoriasis. Psoralens are divided into bifunctional and monofunctional psoralens. Monofunctional psoralen plus UVA radiation are not severely phototoxic and have less mutagenic activity than bifunctional psoralens plus UVA radiation[2]. For these reasons, they have received considerable attention in recent years as potential alternative therapeutic agents for vitiligo and psoriasis.

Angelicin which is an angular monofunctional furocoumarins has recently been proposed as an alternative photochemotherapeutic agent[2] and, in general it is less phototoxic. Khellin has been widely used in the 1940s and 1950s for long-term treatment of angina pectoris and has some structural similarities to 8-methoxypsoralen. In 1982

Receive April 14, 1993
Accepted for Publication October 4, 1993
Reprint request to: Seung Min Lee, M.D., Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea.

Annals of Dermatology
Vol. 6, No. 1, January 1994

Abdel-Fattach et al[3] reported encouraging results using khellin as a new photochemotherapeutic regimen. After oral administration of khellin followed by exposure to sunlight, they observed repigmentation of vitiliginous skin in an Egyptian patient. 4,5,8-trimethylpsoralen(TMP) is a phototoxic drug most frequently used in systemic and topical photochemotherapy along with 8-methoxypsoralen (8-MOP). It's pigment producing effect is well documented compared to 8-methoxypsoralen and 5-methoxypsoralen.

The purpoe of this study was to evaluate the pigment producing effect using various concentrations(0.02%, 0.1%, 0.5%) of monofunctional psoralens such as angelicin, khellin and comparing its effect with TMP in topical photochemotherapy (Fig. 1).

MATERIALS AND METHODS

Experimental animal

Ninty three mature black male mice of the C57 BL strain were divided into nine photochemotherapy groups and a control group. The photochemotherapy groups of mice were painted with angelicin, khellin or TMP solution in the order of the 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 the C57 BL mice at a distance of 14cm using Sellas UVA machine(Dr. Sellmeier Co.,Gevelaberg, Germany).

The wave length of the lamp ranged from 320 to 420 nm. The peak wave length being at 365 nm. The intensity was measured with IL442 radiometer(Ultralite Enterprises Inc., Lawrenceville, Georgia). The obviate the effect of UVB, a mylar sheath UVB filter was installed.

Phototoxic drugs

Pure powder of angelicin(Department of Dermatology, Harvard Medical School, Boston, U.S. A. Offer), khellin(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% ethyl alcohol and were diluted to a 1% solution using distilled water. This solution was then diluted to concentrations of 0.02%, 0.1% and 0. 5%.

Topical application with phototoxic drugs and UV irradiation

In the photochemotherapy 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 micropippet. Three times weekly for five weeks the mice were exposed to UVA radiation for 30minutes after topical application. The amounts of UVA exposure during each exposure were 300mJ/cm^2 during the first week, 350mJ/cm^2 in the second week and 400mJ/cm^2 in the third, fourth and fifth week. In the control group only the base solution was applied and UVA radiation was performed in the same manner.

Skin biopsy and split-dopa stain

After the first, third and fifth weeks of irradiation, three mice from each photochemotherapy group and control group were sacrificed under anesthesia with chloral hydrate. 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 37C° in a CO_2 incubator, and the epidermis was separated. The epidermis was then washed in isotonic saline solution for 1 minute, fixed in 10% formalin and then transferred to a dopa solution at 37C° for 1 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 melanocytes were 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 statistical analysis of the results between the photochemotherapy groups were judged by Duncan's multiple range test and the significances of the results between the photochemotherapy groups and the control group were judged by Paired t-test.

RESULTS

The number of melanocytes

In the photochemotherapy groups there was a marked increase in the number of melanocytes compared with the control group. There was a positive correlation between number of melanocytes and frequency of UVA irradiation. Significantly more melanocytes were seen in the TMP group after five weeks of photochemotherapy but there was no difference between concentrations. However, there were significant differences between concentrations in the angelicin and khellin group after three weeks of treatment.

The comparison of melanocyte numbers between different psoralens after five weeks of photochemotherapy showed a significant difference in decreasing order at TMP, khellin, angelicin(Table 1, Fig. 2, 3).

The area of melanocytes

The area of melanocytes in the control group increased after the first and third weeks of irradiation and decreased after fifth week of irradiation. Comparing the results between the photochemotherapy groups and control group, it was shown that the area of melanocyte was significantly increased in the angelicin group after one and three weeks of photochemotherapy and the TMP group after three and five weeks of photochemotherapy. The area of melanocytes was larger in the TMP group after five weeks of photochemotherapy than any other group. The area of melanocytes in khellin and angelicin groups was not increased by increasing the frequency of the UVA irradiation and increasing the concentration of psoralens(Table 2).

The perimeter of melanocytes

The perimeter of melanocytes in the control group was increased in the third week of irradiation and decreased in the fifth week of irradiation. After one week of photochemotherapy significantly larger melanocytes were seen in the khellin and angelicin group, but after the third and fifth week of photochemotherapy the perimeter of melanocytes had decreased or didn't change.

The perimeter of melanocytes was the largest in the TMP group in all concentrations after three and five weeks of photochemotherapy. The perimeters of melanocytes in khellin and angelicin groups were not increased by increasing the frequency of UVA irradiation but they were increased by increasing the concentration of psoralens(Table 3).

DISCUSSION

The parent compound psoralen and many of its derivatives are naturally occurring compounds found in a large number of plants. 8-methoxypsoralen(8-MOP) is obtained from the seeds of a plant that grows in the Nile Valley, called Ammi majus, EI Mofty at the University of Cairo first used a purified psoralen for the treatment of vitiligo in 1947[1]. 4,5,8-trimethylpsoralen(TMP) is a synthetic compound introduced in 1964 for therapy of vitiligo. 5-methoxypsoralen(5-MOP) is now undergoing trials in the United States[4]. The study to determine the efficacy of 5-MOP in the treatment of vitiligo in Korean people showed that 5-MOP PUVA therapy has been quite promising and the side effects of this treatment was minimal compared to 8-MOP or TMP PUVA therapy[5].

There are two groups in furocoumarins, linear furocoumarins and angular furocoumarins. Photoactive psoralens intercalate between the bases of DNA in the absence of radiation. Absorption of photons by psoralen(furocoumarin 4[1],5[1] or 3,4 double bond) act on a pyrimidine molecule(5,6 double bond) to give a monofunctional adducts. With some compounds, including 8-MOP and TMP, a second photon can be absorbed, resulting in a crosslink to a pyrimidine molecule on the sister strand of DNA. Such crosslinks are also called bifunctional adducts. Cells can repair psoralen photoadducts, but crosslinks are more difficult to repair than are monofunctional adducts[4,6-9]. The

Table 1. Effects of various psoralens and their concentrations in topical photochemotherapy on the melanocyte numbers in C57 BL Mice. The comparison of melanocyte numbers between different psoralens after five weeks of photochemotherapy showed a significant difference in decreaseing order at TMP, khellin, angelicin.

Psoralens	concentrations (%)	1 week	3 week	5 week	p value
Angelicin	0.02*	41.0±13.4$^+$	53.8±12.8$^+$	64.0± 12.2$^+$	<0.001
	0.1*	51.2±12.8$^+$	60.8±11.5$^+$	76.2± 14.7$^+$	<0.001
	0.5*	55.0±12.2$^+$	70.4±12.2$^+$	80.6± 12.8$^+$	<0.001
Khellin	0.02*	56.3±13.4$^+$	61.4± 8.3$^+$	96.0± 12.8$^+$	<0.001
	0.1*	65.3±11.5$^+$	64.0±12.2$^+$	99.8± 12.8$^+$	<0.001
	0.5*	65.9±12.2$^+$	76.2±11.0$^+$	90.9± -9.6$^+$	<0.001
TMP	0.02*	77.9±22.4$^+$	80.6± 8.3$^+$	145.9±-39.7$^+$	<0.001
	0.1*	88.3±23.0$^+$	110.1±15.4$^+$	147.8±-23.7$^+$	<0.001
	0.5*	81.3±23.0$^+$	87.0±11.5$^+$	149.1± 32.0$^+$	<0.001
UVA alone		31.4±12.2$^+$	40.3±10.2$^+$	51.8± 10.9$^+$	<0.001

values are mean ± SD(/mm^2).
+ The significance of difference of the number of melanocytes among angelicin, khellin and TMP groups was calculated by Duncan's multiple range test. P<0.05
* The significance of difference of the number of melanocytes by weeks was calculated by Duncan's multiple range test. P<0.05

Table 2. Effects of various psoralens and their concentrations in topical photochemotherapy on the melanocyte areas in C57 BL Mice. The area of melanocytes was larger in the TMP group after five weeks of photochemotherapy. The area of melanocytes in khellin and angelicin groups was not increased by increasing the frequency of the UVA irradiation and increasing the concentration of psoralens.

Psoralens	concentrations (%)	1 week	3 week	5 week	p value
Angelicin	0.02*	330.1±123.9$^+$	175.3±71.4$^+$	171.3± 66.8$^+$	<0.001
	0.1*	288.5±105.7$^+$	173.3±52.1$^+$	201.0± 72.0$^+$	<0.001
	0.5*	333.7±162.7$^+$	179.2±76.2$^+$	213.9± 71.4$^+$	<0.001
Khellin	0.02*	410.1±132.1$^+$	163.2±52.8$^+$	197.6± 55.1$^+$	<0.001
	0.1*	401.7±128.4$^+$	176.8±69.1$^+$	197.0± 59.9$^+$	<0.001
	0.5*	316.3± 66.9$^+$	213.9±84.4$^+$	195.7± 64.8$^+$	<0.001
TMP	0.02*	216.5± 98.6$^+$	254.9±77.8$^+$	314.4± 93.3$^+$	<0.001
	0.1	278.6± 92.5$^+$	251.9±92.2$^+$	304.1± 85.1$^+$	NS
	0.5*	428.1±210.2$^+$	278.2±95.2$^+$	308.8±117.9$^+$	<0.001
UVA alone		176.2± 62.3$^+$	217.4±65.9$^+$	163.0± 61.1$^+$	

Valuse are mean ±SD(μmm^2).
+ The significance of difference of the area of melanocytes among angelicin, Khellin and TMP groups was calculated by Duncan's multiple range test. P<0.05
* The significance of difference of the area of melanocytes by weeks was calculated by Duncan's mulitiple range test. P<0.05
NS means no significance.

PUVA reaction produces suppression of DNA and RNA synthesis in cells through this mechanism and this effect was originally considered to be its mechanism of action in the treatment of psoriasis.

Angelicin, angular monofunctional furocoumarin, due to their angular structure, only one of their two photoreactive sites can be aligned with a pyrimidine base of DNA. Thus they have less phototoxicity and less genotoxicity[10-13] and 4,4[1], 6-trimethylangelicin show strong antiproliferative activity without erythema[14].

Khellin is a naturally occuring furochromne isolated from *Ammi visnaga*. In 1982, Abdel-Fattah

Table 3. Effects of various psoralens and their concentrations in topical photochemotherapy on the melanocyte perimeter in C57 BL Mice. The perimeter of melanocytes were largest in the TMP group in all concentrations after three and five weeks of photochemotherapy. The perimeter of melanocytes in the khellin and angelicin groups were not increased by increasing the frequency of the UV radiation but they were increased by increasing the concentrations of psoralens.

Psoralens	concentrations (%)	1 week	3 week	5 week	p value
Angelicin	0.02*	124.9±29.1^{+}	89.0± 24.2^{+}	89.6± 17.2^{+}	<0.001
	0.1*	127.0±35.6^{+}	98.7± 27.7^{+}	102.4± 26.5	<0.001
	0.5*	144.4±52.9	106.5± 38.5	109.5± 25.5^{+}	<0.001
Khellin	0.02*	145.0±28.9^{+}	96.6± 34.9^{+}	103.1± 26.1^{+}	<0.001
	0.1*	163.4±48.2^{+}	107.9± 27.8	105.9± 24.8	<0.001
	0.5*	129.0±28.7	109.5± 28.0^{+}	109.3± 22.4^{+}	<0.001
TMP	0.02	100.2±37.9^{+}	113.6± 28.1^{+}	116.4± 32.0^{+}	NS
	0.1	109.2±30.6^{+}	133.3± 38.3^{+}	146.1±154.2	NS
	0.5	130.1±44.3	155.8±184.4	129.1± 39.7^{+}	NS
UVA alone		77.4±24.1	129.4± 28.5	94.0± 28.0	

Values are mean±SD(mm)
+ The significance of difference of the area of melanocytes among angelicin, khellin and TMP groups was calculated by Duncan's multiple range test. P<0.05
* The significance of difference of the area of melanocytes by weeks was calculated by Duncan's multiple range test. P<0.05
NS means no significance.

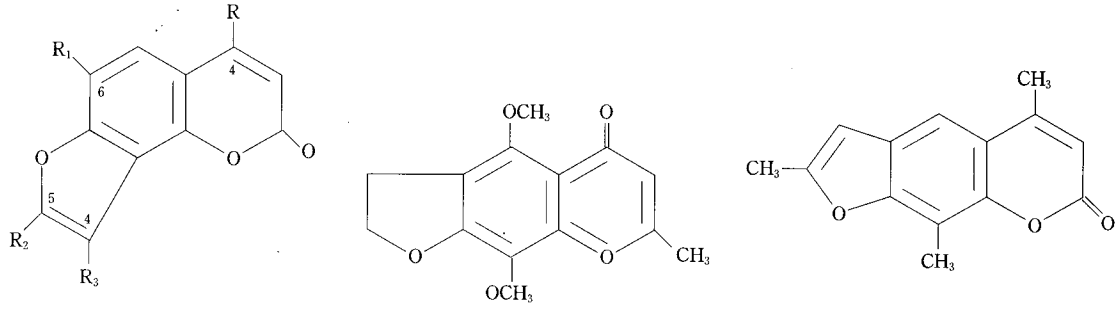

Fig. 1. Chemical structure of angelicin, khellin and trimethyl psoralen(TMP).

et al, presented a preliminary report on the sucessful use of oral khellin followed by exposure to natural light in a patient with vitiligo[3]. Hönigsmann and Ortel[15] also reported that khellin plus artificial UVA was at least as effective as PUVA. The major advantage of khellin photochemotherapy is that it does not provoke phototoxic skin reactions. The study of the pigment producing effect of khellin compared with TMP in Korea[16] showed that khellin is slightly less effective than TMP at the same effective dose, but is quite effective for pigment producing effect and that the degree of the production is dose related.

This study compared the pigment producing effect of monofunctional psoralens like angelicin and khellin and bifunctional ones like TMP by measuring the number, area and perimeter of melanocytes. We performed this experiment for five weeks to judge the effect of topical PUVA on melanocytes.

In the angelicin and khellin goup, there was an increase in the number of melanocytes compared with the control group. However there was no change or a decrease in the area and peimeter of melanocytes compared with the control group. There was a positive correlation between the number of melanocytes and the frequency of UVA radiation in these groups. As the study progressed, pigment production of TMP was increased. The comparison of melanocyte numbers

Annals of Dermatology
Vol. 6, No. 1, January 1994

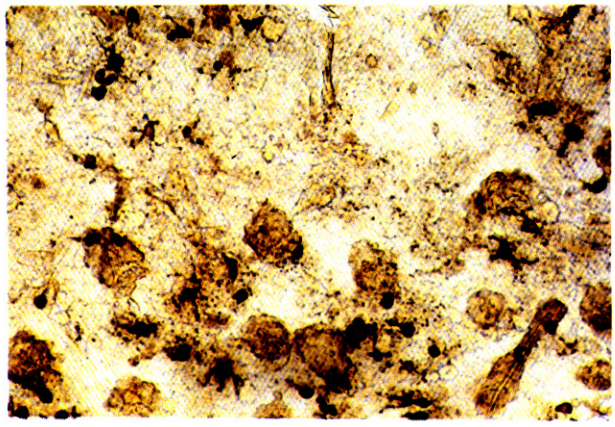

Fig. 2 a. Epidermal melanocytes after 1 week of topical PUVA using khellin.(Dopa stain, ×100).

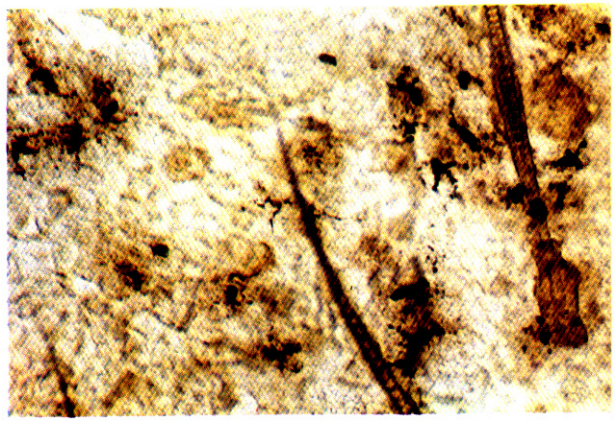

Fig. 2 b. Epidermal melanocytes after 1 week of topical PUVA in angelicin painted group (Dopa stain, ×100).

between different psoralens after five weeks of photochemotherapy showed a significant difference in decreasing order of TMP, khellin and angelicin. The comparison of melanocyte area and perimeter after five weeks of photochemotherapy showed that TMP was the most potent topical photosensitising psoralen. However in 0.5% concentrations of TMP pigment production did not change statistically at the fifth week of topical PUVA compared to 0.1% concentration. This result is compatible with the study by Mitchelle in 1963[17], Park et al in 1985[18] and in 1991[19] showed that over exposure with UV radiation decreased the number of melanocytes and a higher concentration of TMP did not always induced higher pigment producing effects. In 0.5% concentrations of angelicin and khellin numbers and perimeters of melanocytes were increased at the fifth weeks of

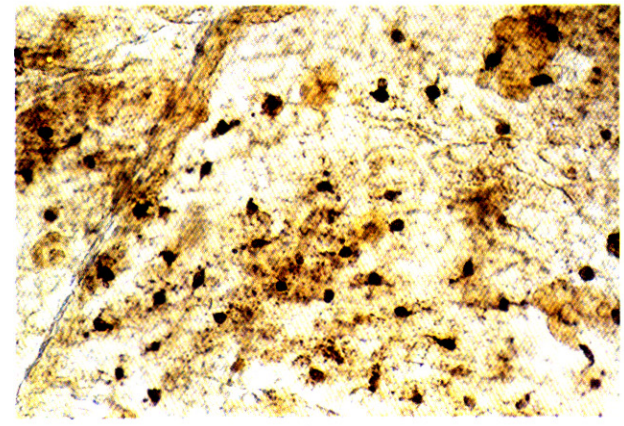

Fig. 3 a. Epidemal melanocytes after 5 weeks of topical PUVA using khellin Melanocyes are increased in number and dendrites are well developed (Dopa stain, ×100).

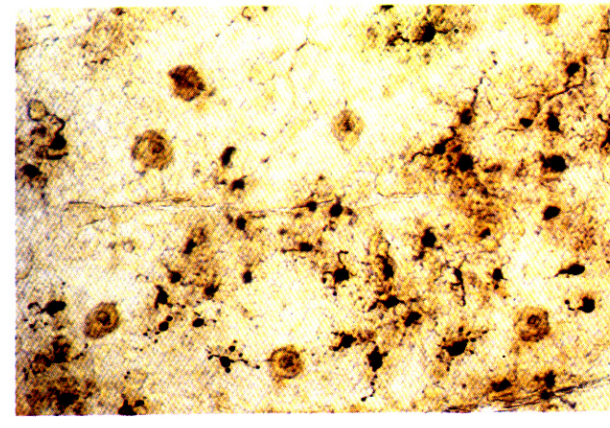

Fig. 3 b. Epidermal melanocytes after 5 weeks of topical PUVA using angelicin. Melanocytes are increased in number and dendrites are well developed (Dopa stain, ×100).

photochemotherapy compared to 0.1% concentrations.

In the study of the effect of khellin on epidermal melanocytes in systemic photochemotherapy with dosages of 5mg/kg and 20mg/kg injected intraperitoneally and irradiated with UVA dose of 4J/cm^2 three times a week for 4weeks, there was a marked increase in the number, area and perimeter of melanocytes compared with the control group[16]. Our results show that in the khellin and angelicin group, there was a positive correlation between the number of melanocytes and frequency of UVA radiation, but no relation was observed in area and perimeter of melanocytes.

In the view of present findings, it may be as-

sumed that the skin concentration of psoralen was enough to produce proliferation of melanocytes but was not enough to produce any growth of melanocytes in a short time. It may also be assumed that oral administration is more effective in rapidly raising the pharmacological skin concentration than topical application.

This study shows that TMP is the most potent pigment producting psoralen, and angelicin and khellin are less effective than TMP topically. It is possible that the doses of khellin and angelicin used in our experiment were not enough to produce changes and this is likely as it has been shown that the khellin therapeutic dose is 100mg. As monofunctional psoralens like angelicin and khellin are less phototoxic and less genotoxic, we think photochemotherapy with higher concentrations and higher dose of UVA using these monofunctional psoralens could increase the pigment producing effect.

In conclusion, the number, area and perimeter of melanocytes after topical PUVA increased more in the TMP group compared to angelicin or khellin group. We expect the clinical application of angelicin and khellin in vitiligo is possible in pigment producing effect with a higher concentration and higher dose of UVA.

REFERENCES

1. El Mofty AM: A preliminary clinical report on the treatment of leukoderma with Ammi majus Linn. JR Egypt Med Assoc 31: 651-665,1948.
2. Dall' Acqua F: Furocoumarin photosensitising and its main biological implications. In Hönigsmann H, Stingl G. Therapeutic photomedicine. Karger Swizerland, 1986,99 137-163.
3. Abdel Fattach A, Aboul-Enein MN, Wassel G, El-Menshawi B:An approach to the treatment of vitiligo by khellin. Dermatologica 65:136-140, 1982.
4. Morison WL, Fitzpatrick TB: Phototherapy and Photochemotherapy of skin disease.: Psoralens and UVA radiation. 2nd eds Raven press, New York, 1991, pp43-52.
5. Hann KS, Cho MY, Im SB, Park YK: Treatment of vitiligo with oral 5-methoxypsoralen. J Dermatol 18:324-329, 1991.
6. Rodighiero G, Musajo L, Dall'Acqua, Marciani S, Caporal G, Ciavatta L:Mechanism of skin photosensitization by furocoumarins. Photoreactivity of various furocoumarins with native DNA and with ribosomal RNA. Biochim Biophys Acta 217:40-49, 1970.
7. Baccichetti F, Antonello C, Carlassare F, Gia O, Palumbo M, Bordin F:Photobiological properties of some water-soluble 5-MOP and 8-MOP derivatives. Photodermatology 2:221-228, 1985.
8. Damaret JP, Brunie S, Ballini JP, Vigny P:Geometry of intercalation of psoralens in DNA approached by molecular mechanics. Photochem Photobiol 50:7-21, 1989.
9. Hönigsmann H,Wolff K,Fitzpatrick MA,Parrish JA:Oral chemotherapy with psoralens and UVA (PUVA):Principle and practice. In Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM,Austien KF,eds. Dermatology in general medicine. McGraw-Hill-book company, New York, 1987, pp 1533-1558.
10. Guiotto A, Rodighiero P,Manzini P,Pastorini P, Bordin F, Baccichetti F, Carlassare F, Vedaldi D, Dall' Acqua F, Tamare M, Recehia G, Christofolini M:6-Methylangelicins:a new series of potential photochemotherapeutic agents for the treatment of psoriasis. J Medicin Chem 27:959-967, 1984.
11. Averbeck D:Relationship between lesions photoinduced by mono and bifunctional furocoumarins in DNA and genotoxic effects in diploid yeast. Mutat Res 151:217-233, 1985.
12. Averbeck D and Moustacchi E: Decreased photoinduced mutagenecity of monofunctional as opposed to bifunctional furocoumarins in yeast. Photochem Photobiol 31:475-478, 1980.
13. Baccichetti F, Carlassare F, Bordin F, Guiotto A, Rodighiero P, Vedaldi D, Tamaro M, Dall' Acqua F: 4, 4', 6-Trimethylangelicin, a new very photoreactive and non skin phototoxic monofunctional furocoumarin. Photochem Photobiol 39:525-529, 1984.
14. Cristofolini M, Recchia G, Boi S, Piscioli F, Bording F, Baccichetti F, Carlassare F, Tamaro M, Pani B, Babudri N, Guiotto A, Rodighiero P, Vedaldi D, Dall'Acqua F:6-methylangelicin:new monofunctional photochemotherapeutic agents for psoriasis. Br J Dermatol 122:513-524, 1990.
15. Hönigsmann H, Ortel B:Khellin Photochemo therapy of vitiligo. Photodermatology 2:193-194, 1985.
16. Yoon MS, Hong KT, Park YK:A study on the effect of various dosage of khellin on epidermal melanocytes and a comparison with trimethylpsoralen in systemic photochemotherapy.Kor J Dermatol 26:828-838,1988.

17. Mitchell RE:The effect of prolonged solar radiation on melanocytes of the human epidermis. J Invest Dermatol 41:199-212, 1963.
18. Park YK, Whang KC:The study on the pigment producing effect of various concentrations of trimethylpsoralen in topical photochemotherapy. Kor J Dermatol 25:234-239, 1987.
19. Park YK, Kim YC, Hann SK, Cho MY, Koo SW:Studies on the effects of various topical phototoxic drugs and UVA on melanocytes of C57 BL Mice. Ann Dermatol :15-22, 1991.20. Abeysekera BF, Abramowski Z, Towers GHN:Genotoxicity of the natural furochromones, Khellin and Visnagin and the identification of a Khellin-Thymine photoadduct. Photochem Photobiol 38:311-315, 1983.
21. Ortel B, Tanew A, Honigsman H, Wolff K: Khellin-UVA phototherapy of vitiligo. Photochem Photobiol 39:52s, 1984.
22. Erickson KL, Montagna W: The induction of melanogenesis by ultraviolet light in the pigment system of Rhesus monkey. J Invest Dermatol 65: 279-284. 1975.
23. Pathak MA, Wodren LR, Kaufman KD: The effect of structural alterations on the photosensitizing potency of furocoumarins(psoralens) and related compounds. J Invest Dermatol 48-103-118, 1967.
24. Rosdahl IK, Swanbeck G:Effects of PUVA on the epidermal melanocyte population in psoriatic patients. Acta Dermatol Venerol 60:21-26, 1980.
25. Rosdahl IK, Szabo G:Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. J Invest Dermatol 70:143-148, 1978.
26. Sato T, Kawada A:Mitotic activity of hairless mouse epidermal melanocytes:Its role in increase of melanocyte during UV radiation. J Invest Dermatol 58:392-395, 1972a.
27. Snell RS: The effect of ultraviolet irradiation on melanogenesis. J Invest Dermatol 40:127-132, 1963a.
28. Snell RS: The effect of X-ray irradiation on melanocytes in the skin. J Invest Dermatol 40:233-241, 1963b.