Antinuclear Antibodies in Mice Induced by Long Wave Ultraviolet Radiation (UVA)

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PUVA therapy has been reported to induce antinuclear antibodies (ANA). The generation of ANA following ultraviolet irradiation was studied experimentally in albino mice. When treated with long wave ultraviolet radiation (UVA) from blacklight fluorescent tubes a significant number of animals developed positive ANA titres, whereas no change was noted in groups, treated with PUVA, 8-methoxypsoralen only or medium wave ultraviolet irradiation (UVB) respectively. The tendency for UVA-irradiated mice to develop ANA was stronger when higher ANA titres were compared. UVA induces ANA in mice, and PUVA-induced ANA may be due to the UVA component of this therapy. *Key words: Auto-antibodies; PUVA.* (Received April 18, 1984.)

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Photochemotherapy with 8-methoxypsoralen and long wave ultraviolet radiation (PUVA) was introduced in 1974 for the treatment of psoriasis, and has since then become an established and effective form of therapy (1). The indications have been extended and include today also other skin diseases. Short term side effects are well known and usually harmless while the long term risks still have to be evaluated. The development of antinuclear antibodies (ANA) during PUVA therapy has been reported (2–6). The pathogenesis of the PUVA-induced ANA is not clear. This study was performed in order to elucidate the mechanism.

MATERIAL AND METHODS

This study is based on four experiments.

Experiment 1

Female albino mice (NMRI, Anticimex, Sollentuna, Sweden) weighing about 30 g were kept in a dark room in order to avoid light exposure. Five or six animals were kept together in each cage and the mice were individually marked with carbol fuchsin on their backs allowing them to be identified. The animals were divided into 5 groups; 4 treatment groups each consisting of 26 animals and one control group of 50 animals. The treatment groups were given PUVA treatment, long wave ultraviolet irradiation (UVA) only, psoralen only and medium wave ultraviolet irradiation (UVB) respectively. Treatments were given twice a week for 6 weeks.

A. *PUVA treatment.* 9 mg 8-methoxypsoralen was dissolved in 100 ml of a solution consisting of ethanol 50 g, glycerol 25 g, pektin 5 g, water 420 g and 0.5 ml (1.5 mg/kg bodyweight) of this solution was injected intraperitoneally in the lower left quadrant. The tails of the animals were then exposed for one hour to UVA radiation from two blackligt tubes (Philips TL 40 W/08) at a distance of 12 cm as described elsewhere (7). The measured average intensity of radiation was 2.75 mW/cm^2. A pilot study had shown the chosen combination of psoralen and UVA doses to be the highest applicable for repeated treatments without giving macroscopic inflammatory changes of the tails.

B. *Psoralen treatment.* The animals in this group were given 8-methoxypsoralen as described above without UVA exposure.

C. *UVA irradiation.* The same light source as described in A was used and the tails were exposed for one hour.

D. *UVB irradiation.* The tails of the animals were exposed to UVB radiation for 7.5min from two fluorescent tubes (Westinghouse Sun-lamp 40 W). A pilot study had shown this dose of radiation to be

the highest possible for repeated treatments in order to avoid visible inflammatory changes of the tails. One animal in this group died during the study.

E. *The control group* did not receive any treatment at all. One animal in this group died during the study.

Experiment 2

Male albino mice (Balb/C, Anticimex, Sollentuna Sweden) weighing about 30 g were used in this study. ANA tests were performed and analysed a few weeks before the experiment and only animals without ANA prior to the study participated. Animals with ANA (about 10%) were excluded and the remaining mice were divided into 3 groups. Two groups consisted of 100 animals each and these were given PUVA treatment and UVA irradiation respectively. The control group consisted of 115 animals. Animals were kept and treated in the same way as in experiment 1 except that the mice in the UVA-irrediated group were exposed for 3 hours. Treatments were given twice a week for 7 weeks. During the study 9 animals died (2 in the PUVA group; 4 in the UVA group; 3 in the control group).

Experiment 3

Thirty-six animals of the same type and weight as in experiment 2 were divided into 2 groups. Only animals without ANA prior to the treatment participated. The experimental conditions for the 17 control animals and 19 UVA-irradiated animals were the same as in experiment 1. UVA irradiation was given for 5 hours three times a week (totally 22 treatments).

Experiment 4

Two hundred female albino mice as in experiment 1 were divided into 2 equal groups. Only animals without ANA before treatment were selected from a pool (about 10% with ANA). The control group and the UVA irradiated group were handled as in the before-mentioned experiments. UVA irradiation was given for 5 hours three times a week (totally 19 treatments).

Table I shows data concerning the UVA-irradiated mice in the four experiments.

Blood samples

Blood samples were drawn immediately before and after the treatment period. One mm of the tip of the tail of the mouse was excised and the blood collected by a Pasteur pipette using the capillary force. About 15 µl of blood was required for the assay. The blood samples were centrifuged and the sera were frozen and kept at −20°C until all samples from one experiment were analysed at the same time.

ANA test

ANA were detected by the indirect immunofluorescence test using cryostat sections of snap-frozen rat kidney (6 µm) (8). Whenever a positive reaction was detected in sera diluted 1/5, duplicate serial dilutions were made. The fluorescein isothiocyanate conjugate, conforming to standard requirements, had an antibody content of 2.6 mg/ml and a F/P quotient of 4.8×10^{-3}. It was used in a dilution determined by performance test (usually 1/20). The preparations were read in a Leitz Dialux 20 EB

Table I. *Data concerning the long wave ultraviolet (UVA) irradiated mice in the four experiments*

Only animals without antinuclear antibodies (ANA) before treatment are included

Exp.	Strain of mice	Sex	Number of mice		Irradiation time (h)	Number of treatments	Total dose (Joule/cm^2)
			UVA	Control			
1	NMRI	F	19	27	1	12	118.8
2	Balb/C	M	96	112	3	14	415.8
3	Balb/C	M	19	17	5	22	1 089.0
4	NMRI	F	100	100	5	19	940.5
Total			234	256			

* indicates ANA titre.

immunofluorescence microscope for incident illumination equipped with a filter system and with a HBO 50 W mercury lamp. The magnification used was ×312. Detectable ANA means that there are ANA in a titre of 8 or more. A negative test means that there are no ANA in the serum when it is diluted to 1/8; thus the ANA titre is less than 8.

Statistical methods

The X^2 test and Kruskal-Wallis one-way analysis of variance (9) were used for the statistical calculations.

RESULTS

Table II shows data regarding the presence of ANA before and after treatment in experiment 1. About one third of the animals had ANA before the start and the numbers of animals developing ANA during the study in the different groups were to small to permit any statistically confirmed conclusions. The results of the ANA tests in experiment 1, however, revealed information on factors possibly influencing the development of ANA. There was a tendency towards higher titres in the PUVA- and UVA-treated animals. This tendency was more marked when attention was paid also to ANA titre canges.

Table III shows the titre changes in animals with ANA before or after treatment. When the figures for the various treatment groups in Table III are compared to the figures of the control group using a nonparametric statistical method (9) a significant difference is noted only for the UVA-irradiated group ($H=6.1$; $0.02>p>0.01$) in relation to the control group. There is also a significant difference when the UVA and UVB-irradiated groups are compared ($H=4.7$; $0.05>p>0.02$).

The results of experiment 1 indicated that UVA irradiation may be a promoting factor for the production of ANA. Therefore, the interest in the following experiments was focused on the influence of this part of the UV spectrum.

Table I shows the combined results of all four experiments concerning the development of ANA. Only animals with negative ANA test before treatment are included. In all experiments, except number 2, there is a slight predominance for the mice in the UVA-irradiated groups to develop ANA in comparison with the animals in the control groups. This tendency is stronger with higher ANA titres, but in no single experiment the differences are statistically significant. When the results of all experiments are added, the predominance of the UVA-irradiated animals in developing ANA is statistically significant when animals with ANA titres of at least 16 are compared ($X^2=4.8$; $0.05>p>0.02$). The significance is higher when mice with ANA titres of at least 32 are compared ($X^2=10.0$; $0.01>p>0.001$).

Number of mice developing ANA

UVA				Control			
8*	16	32	≥64	8	16	32	≥64
1	0	1	2	2	0	0	1
1	0	0	0	1	0	0	0
0	1	2	1	2	1	0	0
2	2	6	1	1	4	0	0
4	3	9	4	6	5	0	1

The ANA titres were generally low. Among animals with negative ANA test before treatment the highest titre, 512, was noticed in the UVA-irradiated group. In the control groups 12 mice developed ANA and of these 9 (75%) showed a homogenous staining pattern while the remaining 3 animals (25%) showed a nucleolar pattern. The corresponding figures for the UVA-irradiated groups were 11 (55%) with homogenous pattern and 9 (45%) with nucleolar pattern.

DISCUSSION

Since the PUVA therapy was introduced, a number of adverse effects have been reported such as the development of ANA (2–6). Considering the immunological effects of PUVA therapy (10) it seemed possible that the combination of psoralen and UVA irradiation was responsible also for the ANA production.

Most UVA sources produce some emission in the shorter wavelength ranges also. Because of the much greater sensitivity of most biological systems to UVB, even minor contamination of the UVA emission spectrum by shorter wavelengths may complicate the interpretation of the results (11). In clinical practice the light source in the PUVA equipment is generally used without a glass pane, thus permitting the UVB radiation component to reach the skin of the patients. For this reason our experiments were designed without glass filtering, despite the fact that the fluorescent tubes emit 1 to 2% of their energy in the UVB range. A group of mice irradiated with UVB was included in experiment 1 to determine whether the UVB part of the spectrum played any role in the development of ANA. The amount of UVB energy was chosen as high as possible without causing any macroscopic inflammatory effects. From the lack of ANA development in this

Table II. *Experiment 1. Results of antinuclear antibody (ANA) tests*

Treatment group	Total number of mice	Number of mice with ANA		Number of mice developing ANA during treatment
		Before treatment	After treatment	
PUVA	26	4	9	5 19%
Psoralen	26	6	8	2 8%
UVA	26	7	11	4 15%
UVB	25	9	9	0 0%
Control	49	22	25	3 6%

Table III. *Experiment 1. Numbers of mice in different treatment groups with regard to ANA titre changes during treatment*

Only animals with ANA before or after treatment are included

	Changes of ANA (titre steps)							
	−1	0	+1	+2	+3	+4	>+4	*n*
PUVA group	1	2	1	2	2		1	9
Psoralen group		3	2	2	1			8
UVA group		3	2	1	2	2	1	11
UVB group	1	4	4					9
Control group	2	13	6	3		1		25

group it is likely that the amount of UVB energy received in the UVA group is not causing the ANA. The differences in reactivity in ANA between the UVA- and UVB-irradiated mice as shown in Tables II and III are thus likely to be due to the UVA irradiation. As both this difference and the difference in ANA titre steps between the UVA and the control groups were significant (Table III), further experiments were focused on the effect of UVA irradiation.

When the results of the four experiments were added, UVA irradiation was shown to induce ANA in mice negative before treatment (Table I). The difference vs. controls is more significant for higher ANA titres.

The total UVA doses given were high but on the other hand the area treated (tail) was rather small. However, the generation of ANA has been reported in PUVA-treated patients, when only the palms have been irradiated (3). The mice of the NMRI strain seem to be more sensitive to UVA irradiation (experiments 1 and 4 in Table I) and here the large amount of UVA energy delivered in experiment 4 did not increase the number of ANA-positive mice. On the other hand the number of animals developing ANA increased in the Balb/C strain, when the UVA dose was increased (experiments 2 and 3 in Table I).

Ultraviolet radiation of UVB type can induce cutaneous lupus erythematosus (LE) and exacerbate SLE (12). Some strains of mice have a pronounced ability to develop ANA and these have therefore been used in studies concerning SLE (13–15). Mice immunized with ultraviolet (UV) irradiated deoxyribonucleic acid (DNA), and then given whole body UV irradiation developed cutaneous lesions resembling SLE with deposition of IgG and complement (15). The action spectrum of UV irradiation induced damage to nuclear DNA has been investigated in vivo (16). In this study UV irradiation with wavelengths 254 nm to 305 nm was shown to induce DNA damage while irradiation with 310 nm, 320 nm and 330 nm did not. UVB radiation has been shown to alter the immunogenic properties of DNA (17).

PUVA has also been shown to yield psoralen-DNA photadducts that are immunogenic (18).

Much less is known about the biological effects of UVA irradiation alone. UVA has been shown to induce DNA damage in *E. coli* (19) and to produce mutants in cultures of this microorganism (20). Lately UVA-induced DNA breaks in cultured human fibroblasts have been reported (21–22). The effects of UVA irradiation and PUVA treatment on the DNA of cultured human fibroblasts differ as PUVA treatment apart from DNA breaks also induces mono- and bifunctional adducts. The immunogenic properties of the UVA- and PUVA-damaged DNA do not necessarily need to be the same. Antinuclear antibodies are a heterogenous group of immunoglobulins with antigenic specificity directed towards a variety of macromolecular constituents of mammalian cell nuclei (23). UVA- and PUVA-induced ANA may thus also differ in properties. The UVA radiation only may, however, be responsible for the generation of ANA in PUVA-treated patients. Whether the UVA-induced ANA reported here is of any pathogenetic importance or not has to be evaluated.

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REFERENCES

1. Melski JW, Tanenbaum L, Parrish JA, Fitzpatrick TB, Bleich HL, and 28 participating investigators. Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. J Invest Dermatol 1977; 68: 328–335.

2. Bjellerup M, Bruze M, Forsgren A, Krook G, Ljunggren B. Antinukleära antikroppar under PUVA-behandling. Hygiea (Proceedings of the Annual Meeting of the Swedish Medical Society) 1978; 87: 126.
3. Bjellerup M, Bruze M, Forsgren A, Krook G, Ljunggren B. Antinuclear antibodies during PUVA therapy. Acta Derm Venereol (Stockh) 1979; 59: 73–75.
4. Bruze M, Ljunggren B. Antinuclear antibodies appearing during PUVA therapy. Acta Derm Venereol (Stockh) 1985; 65: ●●–●●.
5. Kubba R, Steck WD. Antinuclear antibodies associated with PUVA therapy, abstracted. Clin Res 1978; 26: 572.
6. Kubba R, Steck WD, Clogh JD. Antinuclear antibodies and PUVA photochemotherapy. Arch Dermatol 1981; 117: 474–477.
7. Ljunggren B, Möller H. Phototoxic reaction to chlorpromazine as studied with the quantitative mouse tail technique. Acta Derm Venereol (Stockh) 1976; 56: 373–376.
8. Bergqvist R. Immunofluorescence. Evaluation of technique and reagents with special reference to methodological standardization. Thesis. Karolinska Institutet, Stockholm 1974.
9. Siegel S. Non parametric statistics for the behavioral sciences. Tokyo: Kōgakusha Company Ltd, 1956; 184–193.
10. Morison WL. Phototherapy and photochemotherapy. In: Parrish JA, ed. Photoimmunology. New York: Plenum Medical Book Company, 1983; 273–289.
11. Greaves MW, Vella Briffa D. UV-A and the skin. Br J Dermatol 1981; 105: 477–482.
12. Tuffanelli DL. Lupus erythematosus. Am Acad Dermatol 1981; 4: 127–142.
13. Howie JB, Helyer BJ. The immunology and pathology of N.Z.B. mice. Adv Immunol 1968; 9: 215–266.
14. Ten Veen JH, Feltkamp TEW. Studies on drug induced lupus erythematosus in mice. I. Drug induced antinuclear antibodies (ANA). Clin Exp Immunol 1972; 11: 265–276.
15. Natali PG, Tan EM. Experimental skin lesions in mice resembling systemic lupus erythematosus. Arthritis Rheum 1973; 16: 579–589.
16. Tan EM, Freeman RG, Stoughton RD. Action spectrum of ultraviolet light-induced damage to nuclear DNA in vivo. J Invest Dermatol 1970; 55: 439–443.
17. Davis P. Antibodies to UV DNA and photosensitivity. Br J Dermatol 1977; 97: 197–200.
18. Zarebska Z, Jarzabeh-Chorzelska M, Rzesa G, Chorzelski T. Antigenicity of DNA induced by photoaddition of 8-methoxypsoralen. Photochem Photobiol 1978; 27: 37–42.
19. Tyrell RM. Induction of pyrimidine dimers in bacterial DNA by 265 nm radiation. Photochem Photobiol 1973; 17: 69–73.
20. Kubitschek HE. Mutagenesis by near-visible light. Science 1967; 155: 545–546.
21. Bredberg A. DNA damage in human skin fibroblasts exposed to UVA light used in clinical PUVA treatment. J Invest Dermatol 1981; 76: 449–451.
22. Bredberg A, Lambert B, Söderhäll S. Induction and repair of psoralen crosslinks in DNA of normal human and xeroderma pigmentosum fibroblasts. Mutat Res 1982; 93: 221–234.
23. Sontheimer RV, Deng J-S, Gilliam JN. Antinuclear and anticytoplasmic antibodies. Concepts and misconceptions. J Am Acad Dermatol 1983; 9: 335–343.