STUDIES ON EXPERIMENTAL CONDITIONS FOR DETECTING PHOTOTOXIC POTENTIALS OF DRUGS IN Balb/c MICE

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Abstract.....This study was designed to establish a procedure for detecting the phototoxicity of drugs in an animal model. Experimental conditions in relation to intensity distribution of ultraviolet-A (UVA), duration of irradiation, and suitable region for irradiation were investigated. One black light gave a wide constant-energy region when the distance from the light source to the irradiation area was 15 cm. The intensity distribution of a bank of 10 black lights formed a pattern like the contour map of a truncated cone in the irradiation area. In phototoxic studies, Balb/c strain mice were orally administered chlorpromazine and nalidixic acid, clinically known as photosensitizers, and were immediately exposed to UVA irradiation. The optimal irradiation time was 4 hours at an energy of 20 Joules/cm², which with a high frequency caused erythema on the surface of the ears in the central area, which received about 1.5 mW/sec·cm², but no reaction occurred in the surrounding area (0.5–0.8 mW/sec·cm²). These results indicate that it is important to select a suitable irradiation area and sufficient intensity of irradiation in order to determine whether a drug has phototoxic potential.

Key words : Phototoxicity, mouse, intensity, distribution, UVA.

INTRODUCTION

Ultraviolet light is the light generally used for causing phototoxic reactions in animal models. There are a number of reports on the prediction of drug photosensitivity which state that black lights offer the advantages of its ease of use, inexpensiveness and suitability for photopatch testing (Harber et al., 1974). Black lights, a type of low pressure mercury arc emitting in the 300 to 400 nm range, are

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usually placed in parallel as a source of long-wave ultraviolet-A (UVA).

Most reports mention the types of light sources and of detectors, and the total irradiation energy, but they rarely refer to the intensity distribution of the photoenergy in the irradiation area. Generally, phototoxic reactions occur when chemicals with chromophores in reactive tissue absorb a considerable amount of radiation (Epstein and Wintroub, 1985). The conditions for ultraviolet irradiation must be set up carefully so that the animals will be exposed equally to the energy. Although Epstein (1985) mentions the need to take into account several disadvantages of *in vivo* testing, factors such as whether enough ultraviolet light is reaching the appropriate target tissue, the appropriateness of the wavelengths and the timing of the irradiation. But these could be improved by methodological means.

This paper indicates the position of the irradiated area with suitable UVA intensity, and a simple method for inducing the phototoxic potential of the photosensitizers chlorpromazine and nalidixic acid (Baes, 1968 ; Birkett et al., 1969 ; Ramsay and Obreshfova, 1974 ; Epstein, 1968) in Balb/c strain mice.

MATERIALS AND METHODS

Light source characterization :

An apparatus was devised to act as a UVA radiation source with reference to the method of Jordan (1982). The light source was a bank of 10 Toshiba type FL20SBLB "Black Light" tubes (diameter, 32.5 mm ; length, 58 cm, Tokyo, Japan), emitting radiation in the 300 to 400 nm range, fixed onto a wooden board (70×70 cm). The tubes were placed in a parallel arrangement at 6.5 cm intervals. In order to characterize the UVA intensity distribution of the black light, Y-and X-axes were assigned for the irradiation area so that the Y-axis was parallel with the tubes and the X-axis was at 90° to them. The UVA intensity at 365 nm was quantified in the irradiation area at distances of 10 and 15 cm from the light.

The output of the source was quantified with a model UVX Digital Radiometer fitted with a UVX-36 sensor responding at 365 nm (UVP Inc, San Gabriel, CA USA). A 3 mm-thick pane of glass (Floatglass, Asahi Glass, Tokyo, Japan) was used as a filter to eliminate wavelengths below 320 nm.

Chemicals :

Chlorpromazine (CPZ) and nalidixic acid (NA) were used. They were extracted from commercial tablets or powder in our institute. Their purity was 99% or more.

Experimental animals :

Albino mice, Balb/c strain, female, 5 to 6 weeks old and weighing 17.6 to 21.2 g (Charles River Japan Inc., Kanagawa, Japan) were used in the study. The animals were housed 5-6 per cage in plastic cages with free access to food (MB-1, Funabashi Farm, Chiba, Japan) and tap water in an air conditioned room (temperature : $23 \pm 2^\circ\text{C}$, relative humidity $55 \pm 15\%$).

Irradiation :

Mice were placed individually in a plastic box ($24 \times 16 \times 4$ cm³, with a stainless steel wire screen at the bottom and wide slits at the top for ventilation) with 12 partitioned chambers ($4 \times 8 \times 4$ cm³) (Kerdell, 1987). The box was covered with 3 mm-thick window pane. The distance from the light to the backs of the mice was 15 cm. An electric fan was placed away from the light source so that its breeze, whose speed was set at about 2 m/sec, would prevent the temperature in the box from rising.

Phototoxic study :

A phototoxic study was performed according to a modification of the methods of Kuzuna (1983), Ljunggren (1978) and Lim (1986). Mice, fasted overnight, were orally administered CPZ dissolved in distilled water, or NA suspended in 0.5% sodium carboxymethylcellulose, and were immediately exposed to UVA for 4 hours (about 20 Joules/cm²). The ears of the exposed mice were observed 0, 24 and 48 hours after irradiation. Two groups of mice were used as controls. The one received the test compound without UVA irradiation, and the other received the UVA irradiation without the test compound. The phototoxic reaction was judged to be positive when the ears exhibited significant erythema or obvious reddening.

RESULTS

Intensity distribution of UVA in the irradiated area :

Fig. 1 shows the intensity distributions on the Y-axis at distances of 10 and 15 cm, both symmetrical curves with plateaus of high intensity in the center region. The 15 cm distance yielded a wider region of high intensity (Y-axis between 18 and 38 cm), while at a distance of 10 cm, the intensity was much higher but was constant over a very small area.

Fig. 2 shows the horizontal intensity distribution in the UVA irradiated area. The lights were in a parallel arrangement as indicated by the black arrows. The open circles indicate positions of measurement, and sites of equivalent intensity are linked with solid lines. The result is a contour map of a truncated cone with a constant intensity about 1.5 mW/sec·cm². Fig. 3 shows the vertical intensity distribution along the dashed lines indicated by open arrows L-1 and L-2 in Fig. 2. The center field (L-1) shows a similar intensity between about 15 and 45 cm, but the peripheral field (L-2) shows a very low intensity. The area of the center field was about one-tenth of the area covered by the bank of 10 black lights.

Phototoxic study :

1. Optimal UVA photoenergy for dose for phototoxic study

To optimize the phototoxicity study conditions, mice (n=6) were given CPZ (10 mg/kg) or NA (200 mg/kg) orally and were immediately exposed to UVA for 0, 1, 2, 4 or 8 hours at the center field of the irradiation area. Table 1 shows that 4-hr irradiation of UVA was optimal for inducing very frequent phototoxic reaction to CPZ or NA with erythema on the surface of the ears. The total photoenergy dose

Fig. 1. Vertical distribution of the UVA intensity of one black light in the irradiation area. UVA intensity was measured below the light at distance 15 (○) and 10 (△) cm along the Y-axis. A glass filter was fitted to the sensor.

Fig. 2. Horizontal distribution of UVA intensity of a bank of 10 black lights in the irradiation area. Black lights were arranged in parallel, as indicated by the black arrows. Photoenergy was quantified 15 cm below the lights.

Fig. 3. Vertical distribution of the UVA intensity on sections L-1 and L-2, indicated by the dashed lines in Fig. 2. Photoenergy was quantified 15 cm below the lights.

was about 20 Joules/cm² over a 4-hr irradiation. The prolonged, 8-hr irradiation, however, was considered to be too long for detection of the phototoxic potency of both drugs, since it resulted in edema in all mice at the 48-hr observation point, and a lower energy level did not induce sufficient phototoxic reaction. The two control groups (n=4) showed no response to either 4 or 8-hr irradiation.

2. Comparison of reactions in mice placed in center and peripheral fields

To confirm the effects of intensity or position, Balb/c mice (n=4) treated with NA or CPZ were exposed to UVA in the center field (intensity : 1.5 mW/sec·cm², X-axis : 22 to 38 cm, Y-axis : 24 to 32 cm in Fig. 2) and the peripheral field (0.5–0.8 mW/sec·cm², 22 to 38 cm, –4 to 4 cm) for 4 hr. The animals in the center all showed positive reactions, but those in the peripheral area were all negative (Table 2).

DISCUSSION

Several methods have become available for phototoxic study, *in vivo* as well as *in vitro*. For the *in vivo* techniques, mice (albino or hairless), and guinea pigs are the

Table 1. Optimum UVA irradiation time (dose) in center field ($1.5\text{mW/sec}\cdot\text{cm}^2$) for phototoxic reaction on ears of Balb/c strain mice.

Drug	Dose (mg/kg)	UVA (hr)	No. of mice used	No. of mice with erythema		
				0	24	48hr
CPZ	10	1	6	0	0	0
		2	6	0	0	2
		4	6	0	3	6
		8	6	6	6	6 ^{a)}
NA	200	1	6	3	0	0
		2	6	6	0	3
		4	6	6	6	4
		8	6	6	6	6 ^{a)}
Control	0	4	4	0	0	0
		8	4	0	0	0
CPZ	10	0	4	0	0	0
NA	200	0	4	0	0	0

^{a)} with edema in all mice**Table 2.** Comparison of reactions of mice placed in center of field and in surrounding areas.

Drug	Dose (mg/kg)	Position	Intensity ($\text{mW/sec}\cdot\text{cm}^2$)	UVA (hr)	No. of mice used	No. of mice with erythema		
						0	24	48hr
CPZ	10	Center	1.5	4	4	4	4	4
		Surrounding	0.5-0.8	4	4	0	0	0
NA	200	Center	1.5	4	4	4	4	4
		Surrounding	0.5-0.8	4	4	0	0	0

most common experimental animals, and phototoxic effects have been measured by registering erythema and/or edema and necrosis of ears and tail (Ljunggren and Moller, 1975). Although some attempts to quantify the response have been made (Ljunggren and Moller, 1978, 1976 : Scott et al., 1970 : Ljunggren, 1984), few authors have reported the precise UV intensity distribution in the irradiated area.

The sun has been replaced as a light source in radiation studies with artificial light sources, e.g., mercury lamps, several kinds of fluorescent tubes, since the sun's photoenergy varies with the weather. On the other hand, artificial light sources must be placed near to the animals, because of their low photoenergy levels. For this reason, it is important to confirm the location of the constant-intensity region in the irradiation experiment, and the irradiated animals must be carefully positioned.

As black light is widely used in phototoxic and/or photoallergic studies, we devised an irradiation apparatus and measured the distribution of UVA intensity using a bank of 10 black lights, with the intention of optimizing the accuracy of phototoxic studies. When only one black light was used, the pattern of UVA intensity on the Y-axis was flat over a wide region in the center when the distance was 15 cm. At 10 cm, the UVA power was strong, but the constant energy region was very small, so that a distance 10 cm would not be useful in practice for irradiating many animals with a bank of black lights. In the case of a bank of 10 tubes, as shown in Figs. 2 and 3, the intensity distribution at a distance 15 cm resembled the shape of a contour map of a truncated cone. The region of constant photoenergy was concentrated in the central area ($24 \times 16 \text{ cm}^2$), where up to 12 mice were used for phototoxic and/or photoallergic study in one irradiation.

To confirm the intensity of UVA in the center area required to induce a phototoxic reaction to CPZ and NA, well known clinically as photosensitizers (Baes, 1968 ; Birkett, 1969 ; Ramsay, 1974 ; Epstein, 1968), the optimum dose was found using Balb/c mice. When a 4-hour irradiation was carried out at $1.5 \text{ mW/sec}\cdot\text{cm}^2$, the erythema reactions to CPZ resembled those obtained by the quantitative mouse tail technique (Ljunggren, 1976). These irradiation conditions were nearly equivalent to sunbathing for 4 to 5 hours in the summer in Tokyo (lat. $35^\circ 40' \text{N}$), and were thought to involve sufficient energy for UVA to penetrate the epidermal tissue to reach the blood stream and/or the reactive tissue.

With regard to the effects of position or UVA intensity, a comparison was made of reactivity between the center field and the periphery. A phototoxic reaction was obtained in the former, but not in the surrounding area, which indicates that it is important to place the animals judiciously so that they receive enough irradiation energy to allow detection of the phototoxic potential of a test drug. This study is expected to be useful in the detection of drug phototoxicity.

REFERENCES

- Baes, H. (1968) : Photosensitivity caused by nalidixic acid. *Dermatologica*, **136**, 61-64.
- Birkett, D. A., Garretts, M. and Stevenson, C. J. (1969) : Phototoxic bullous eruptions due to nalidixic acid. *Br. J. Derm.*, **81**, 342-343.
- Epstein, J. H. and Wintroub, B. U. (1985) : Photosensitivity due to drugs. *Drugs*, **30**, 42-57.
- Epstein, S. (1968) : Chlorpromazine photosensitivity : phototoxic and photoallergic reactions. *Arch. Dermatol.*, **98**, 354-363.
- Harber, L. C., Bickers, D. R., Epstein, J. H., et al. (1974) : Report on ultraviolet light sources. *Arch. Dermatol.*, **109**, 833-839.
- Jordan, W. P., Jr. (1982) : The guinea pig as a model for predicting photoallergic contact dermatitis. *Contact Dermatitis*, **8**, 109-116.
- Kerdel, F. A., Soter, N. A. and Lim, H. W. (1987) : *In vivo* mediator release and degranulation of mast cells in hematoporphyrin derivative-induced phototoxicity in mice. *J. Invest. Dermatol.*, **88**, 277-280.
- Kuzuna, S., Tamura, S. and Kawai, K. (1983) : Phototoxicity of anti-inflammatory agents, evaluated by ear skin erythema in mice. *Ensho (JPN)*, **3**, 515-516.
- Lim, H. W., Hagan, M. and Gigli, I. (1986) : Phototoxicity induced by hematoporphyrin derivative in C5-deficient, mast cell-deficient and leukopenic mice. *Photochem. Photobiol.*, **44**, 178-180.
- Ljunggren, B. (1984) : The mouse tail phototoxicity test. *Photodermatology*, **1**, 96-100.
- Ljunggren, B. and Moller, H. (1975) : Studies on the phototoxic potency of chlorpromazine and other phenothiazines. Photochemotherapy, basis technique and side effects. Proceedings of the German-Swedish symposium on photomedicine in Oberursel/W. Germany on April 23rd-25th, 43-47.
- Ljunggren, B. and Moller, H. (1976) : Phototoxic reaction to chlorpromazine as studied with the quantitative mouse tail technique. *Acta Dermatovener (Stockholm)*, **56**, 373-376.
- Ljunggren, B. and Moller, H. (1978) : Drug phototoxicity in mice. *Acta Dermatovener (Stockholm)*, **58**, 125-130.
- Ramsay, C. A. and Obreshfova, B. (1974) : Photosensitivity from nalidixic acid. *Br. J. Derma.*, **91**, 523-528.
- Scott, C. W., Stasse, J. and Bonomo, R. (1970) : Evaluation of the phototoxic potential of topically applied agents using long-wave ultraviolet light. *J. Invest. Dermatol.*, **55**, 335-338.