0022-202X/78/7005-0272$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 70:272-274, 1978

Vol. 70, No. 5
Printed in U.S.A.

IDENTIFICATION OF SYSTEMIC PHOTOTOXIC DRUGS BY HUMAN INTRADERMAL ASSAY

KAYS H. KAIDBEY, M.D., AND ALBERT M. KLIGMAN, M.D., PH.D.

Duhring Laboratories, Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, U. S. A.

An improved method is presented for the detection of systemically administered photosensitizing drugs in humans. Test agents were injected intradermally in physiologic saline and then exposed to broad spectrum radiation from a Xenon solar simulator. Several clinically recognized photosensitizers were identified by this technique. The activating spectrum depended on the test drug. Sulfonamides and vinblastin were activated by sunburning erythemic radiation (UV-B), while tetracyclines, nalidixic acid and phenothiazines by near UV (UV-A), and chlorothiazide by both. It is suggested that intradermal phototesting offers a means of verifying the phototoxic potential of agents suspected clinically of provoking a photosensitivity eruption.

The importance of phototoxic drug reactions came to the fore following the widespread use of phenothiazine tranquilizers and demethylchlortetracycline. As the catalogue of new drugs has expanded, the number of agents capable of causing photosensitivity reactions has grown. Generally, the phototoxic potentiality of new drugs is recognized after and not before their introduction into commerce. This bespeaks the want of suitable laboratory assays for identifying photosensitizing compounds.

Ison and Blank utilized the skin of albino hairless mice for screening photosensitizing drugs [1]. After intraperitoneal injection of the test agents, they irradiated the animals with fluorescent blacklights. The emission from these lamps is primarily in the long ultraviolet region (UV-A, 320–400 nm). Tetracyclines, phenothiazines and 8-methoxypsoralen (8-MOP) gave positive reactions in Ison and Blank's tests, whereas chlorothiazide was negative except when the animals were exposed to sunlight filtered through window glass. Baer and Harber [2] and Sams [3], utilizing a variety of artificial UV-sources, could not evoke positive responses with agents which were already recognized as being phototoxic, for example, sulfanilamide and chlorothiazide. Sams and Epstein, however, succeeded in producing phototoxic reactions to demethylchlortetracycline, chlorpromazine and chlorothiazide in guinea pigs with natural sunlight [4].

Phototoxic responses have been experimentally produced in humans. Schorr and Monash demonstrated phototoxicity to tetracyclines following intradermal injection and exposure to sunlight [5]. Maibach, Sams and Epstein also obtained positive results with Mylar filtered sunlight but not with artificial UV-sources [6]. It became evident that a light source with high UV-A irradiance was necessary for phototesting. Subsequently, Kligman and Breit, using a Xenon solar simulator, were able to produce phototoxic reactions with most of the clinically recognized photosensitizers following intradermal injection as well as topical application to stripped skin [7]. They found that the photoactivating wavelengths for all these drugs was in the UV-A range.

Manuscript received May 2, 1977; accepted for publication November 9, 1977.

Reprint requests to: Dr. Kays H. Kaidbey, Department of Dermatology, Duhring Laboratories, Suite 203, University of Pennsylvania, 3500 Market Street, Philadelphia, Pennsylvania 19104.

Abbreviation:
8-MOP: 8-methoxypsoralen

We have reevaluated the intradermal phototesting technique and have become aware of inconsistencies which have made it necessary to amend the procedure. We report now an improved method for identifying systemic photosensitizing drugs using intradermal injections into human skin.

MATERIALS AND METHODS

Subjects

These were white, healthy adult volunteers between the ages of 21 and 32 yr. The untanned lower back served as the test site. Informed consent was obtained.

Light Source

A 150-w Xenon solar simulator with the Schott WG-320 filter was used (UV-B intensity = 12.2 mw/cm^2). This source simulated sunlight only in the sunburning portion of the spectrum [8]. The output falls sharply in the UV-A region between 360 nm and 400 nm because of filtration through a colored Corning 9863 filter, which absorbs long ultraviolet and also visible light. In order to intensify the UV-A and visible light components, the Corning filter was removed. To eliminate erythemic radiation, the Schott WG-345 filter was inserted, providing a continuous spectrum of UV-A and visible light (total flux = 130 mw/cm^2; UV-A = 30 mw/cm^2; visible light = 38 mw/cm^2; infra-red = 62 mw/cm^2). Intensity measurements were made by a calibrated thermopile (The Eppley Laboratories, Newport, R.I.).

Test Drugs

A volume of 0.1 ml was injected intradermally through a tuberculin syringe with a 27-gauge needle.

Soluble agents were dissolved in physiologic saline. These included sulfanilamide, colchicine, chlorpromazine hydrochloride and prochlorperazine sulfonate. Drugs in ampoules were diluted with saline. These were sodium tolbutamide (i.v., Orinase, Upjohn), vinblastin sulfate (Velban, Lilly), chlorothiazide (i.v., Diuril, M.S.D.), quinidine gluconate (Lilly), chlordiazepoxide hydrochloride (Librium, Roche), furosemide (Lasix, Hoechst).

Nalidixic acid was solubilized in 0.1 N NaOH and diluted to a 0.15% concentration with saline; the pH was adjusted to 7.0–7.1 with 0.1 N HCl and the solution sterilized by millipore filtration.

Insoluble agents were subjected to ultrasonification in physiologic saline (Brown sonifier) for 2 hr to provide a uniform suspension of fine particles. Drugs injected as suspensions included: griseofulvin, demethylchlortetracycline, tetracycline base and sulfapyridine. Optimal nonirritating concentrations, time intervals and light doses were estimated from previous work and from pilot studies. Controls consisted of an unirradiated drug injected site and an irradiated site injected with saline. The sites were irradiated 10 to 15 min after injection. Responses were graded 24 hr later, always in relation to the control site.

Dose-Response Studies

Two drugs, furosemide and quinidine, were selected for investigating the influence of dose on the phototoxic response. Each subject received 3 different concentrations of 1 drug intradermally, along with appropriate controls prior to irradiation as described above. The UV-A exposure for furosemide was 15.0 Joules/cm^2 and for quinidine 8.0 Joules/cm^2.

RESULTS

Phototoxic reactions were obtained with all of the drugs except sodium tolbutamide, colchicine and chlordiazepoxide (Table I). Larger concentrations of these drugs were not tested because of irritancy. The findings permit a classification of phototoxic agents into 2 categories based on action spectra.

Reactions to sulfanilamide, sulfapyridine and vinblastin sulfate were provoked only by radiation containing UV-B, but not with UV-A and visible light. An exposure of 1 Minimal Erythema Dose (about 1.6 Joules/cm^2 of UV-B) produced positive responses in the majority of subjects (Table I). The remaining drugs gave phototoxic reactions only after exposure to UV-A and visible light. The dose required varied with the test agent. Thus with quinidine an exposure of 6 min (about 10.5 Joules of UV-A/cm^2) produced positive responses while the phenothiazines and chlorothiazide required 10 min (about 18 Joules of UV-A/cm^2). Larger doses (18 min, about 32 Joules/cm^2) were needed for the tetracyclines, nalidixic acid and furosemide, and a still larger dose (22 min, about 40 Joules of UV-A/cm^2) was necessary with griseofulvin. Chlorothiazide was unusual in that reactions were obtained with both UV-A and with UV-B containing radiation (Table I). However, much larger doses of UV-A were required.

The clinical reaction pattern was the same with all these drugs. Intense erythema and edema became apparent 4–6 hr after light exposure and reached maximal intensity by 24 hr, by which time the sites were palpably infiltrated and raised. Gradual resolution occurred over the next 3–5 days. There were no immediate urticarial type responses or vesicular reactions. Irradiated control sites showed only pigmentation, except with larger exposures (22 min) where minimal erythema also developed in the majority of subjects. However, edema and infiltration were never noted.

Dose-Response Studies

Table II shows that for both furosemide and quinidine the number of subjects showing positive responses depended on the dose of the injected drug when the UV-exposure was held constant.

DISCUSSION

Intradermal phototesting in humans is a reliable technique for the identification of drugs with a photosensitizing potential. The method is simple and reproducible. A high intensity UV light source is essential. The Xenon solar simulator with the Corning 9863 filter removed is greatly suited for this purpose. A distinctive advantage is high output of long ultraviolet rays; most of the known phototoxic drugs are activated by this spectral range. We experienced many failures when we attempted to reproduce our present findings with other sources, such as blacklight fluorescent tubes, even after prolonged exposures (unpublished observations).

It should be pointed out that test agents should only be assayed at nonirritating concentrations, since a strong irritant response may obscure a phototoxic reaction. Furthermore this assay serves only as a screening procedure. A positive response indicates phototoxic potentiality but allows no firm predictions regarding comparative phototoxic activity or frequency of clinical reactions in conventional usage. Such data can only be procured by administering the test drug orally or parenterally.

TABLE I. *Phototoxicity of intradermally injected drugs*

Drug	Dose injected (μgm)	UV-A (WG-345 Filter)	Solar simulating radiation (WG-320 Filter)
Sulfanilamide	500	0/6[a]	8/8[a]
Sulfapyridine	500	0/7	8/11
Vinblastin	0.2	0/7	6/7
Nalidixic acid	150	8/10	0/8
Griseofulvin	200	7/16	0/8
Tolbutamide	500	0/8	0/8
Chlorothiazide	250	7/9	10/15
Demethylchlortetracycline	200	6/8	0/7
Tetracycline base	200	5/6	0/7
Furosemide	500	5/8	0/8
Quinidine	250	8/11	0/7
Chlorpromazine	100	5/5	0/8
Prochlorperazine	250	6/7	0/6
Colchicine	0.2	0/6	0/6
Chlordiazepoxide	100	0/7	0/8

[a] Fraction of positive responders.

TABLE II. *Influence of dose on the phototoxic responses to furosemide and quinidine*

Dose (μgm)	Furosemide[a]		Quinidine gluconate[b]	
	Number of positive responders	Dose (μgm)	Number of positive responders
1000	12/12	250	10/13
250	7/12	62.5	4/13
62.5	1/12	15.6	0/13

[a] UV-A dose for furosemide was 15.0 Joules/cm^2.
[b] UV-A dose for quinidine gluconate was 8.0 Joules/cm^2.

The solar simulator does not allow for a precise determination of action spectra. Nevertheless, by using various cut-off filters, we narrowed down the range of photoactivating wavelengths. Although most phototoxic agents, as expected, were activated by energy containing UV-A, there were important exceptions. Sulfonamides and vinblastin were activated by radiation containing UV-B, while chlorothiazide photoxicity was elicited by UV-B as well as UV-A.

There are conflicting reports concerning the action spectrum of sulfanilamide phototoxicity (Table III). Epstein [9] and Blum [10] years ago showed that the response is provoked by UV-B and can be abolished by window glass filtration. More recent reports incriminate wavelengths in the UV-A range [7,11].

The dogma that the action spectrum for photosensitizing drugs is in the UV-A region requires revision. For a few agents at least, such as sulfanilamide and vinblastin [12], the photoactivating rays lie in the UV-B range. Evidence is accumulating that this may also be true of certain agents that cause contact photoallergy. It is standard teaching that the latter are activated by UV-A. Yet Jung found that the photoallergic reaction to the laxative triacetyldiphenolisatin was caused by UV-B and not by UV-A [13]. Emmett reported a case of diphenhydramine photoallergy that was provoked by UV-B [14].

Our negative findings with tolbutamide and chlordiazepoxide are also at variance with those of certain other investigators (see Table III). It is possible that larger test concentrations of these drugs are required for eliciting phototoxic reactions. Nonetheless, it is our belief that these agents do not provoke phototoxicity. The photosensitivity eruptions imputed to tolbutamide may be photoallergic in nature.

Animal studies are useful for preliminary screening. As yet no model has been described for which proof of high sensitivity has been provided. A screening technique should enable identification of substances which have been indisputably proved to cause phototoxicity in humans. Significant species differences have been reported with regard to phototoxicity [16]. Ramsey and Obreshkova failed to obtain responses with intradermal injections of nalidixic acid in mice [17]. Blum was unable to demonstrate sulfanilamide phototoxicity in rodents, even after repeated challenge [10]. This is also congruent with our own experience. Responses are difficult to evaluate in animals, even with the use of "bluing" agents that help to visualize edema. Furthermore, drugs may be metabolized differently in animals.

In contrast to Kligman and Breit's observations [7], phototoxic reactions to sulfanilamide, tetracycline and chlorothiazide could not be regularly secured by topical application (unpublished observations). We have, therefore, abandoned topical phototesting as a screening technique for agents intended for systemic use.

Our current operating procedure is as follows: 0.1 ml of the test material is intradermally injected at a nonirritating concentration in physiologic saline. An appropriate concentration can be selected after injecting a range of concentrations into the skin of rabbits or guinea pigs. Ultrasonification is an excellent way to prepare fine, uniform suspensions of insoluble drugs. Irradiation is given 10 min after injection. Exposure to UV-A

TABLE III. *Results of phototoxicity testing for certain drugs (as reported by various authors)*

Drug, author(s) & reference number	Route of administration	UV source	Phototoxicity	Action spectrum
Sulfanilamide				
Epstein [9]	ID[a] (humans)	Mercury Arc, (hot quartz)	Yes	NI[b]
Blum [10]	ID (humans)	Mercury Arc	Yes	UV-B
Kligman & Breit [7]	ID (humans)	Xenon Arc, (solar simulator)	Yes	UV-A
Stratigos & Magnus [11]	ID (hairless mice)	Monochromator	Yes	375–400 nm 280–300 nm (questionably)
Present study	ID (humans)	Xenon Arc	Yes	UV-B
Tolbutamide				
Ison & Blank [1]	IP[c] (hairless mice)	F-40 BL lamps (300–400 nm)	No	
		Sunlight	No	
Kligman & Breit [7]	ID (humans)	Xenon Arc	Yes	UV-A
Present study	ID (humans)	Xenon Arc	No	
Chlordiazepoxide				
Ison & Davis [15]	IP (albino mice)	F-40 BL lamps (300–400 nm)	Yes	UV-A
Present study	ID (humans)	Xenon Arc	No	

[a] ID = intradermal
[b] NI = not investigated
[c] IP = intraperitoneal

and solar simulated UV should be carried out separately. Exposures to UV-A should begin with about 30 Joules/cm^2, increasing up to 40 Joules/cm^2, if necessary. For solar simulating radiation suberythemal doses are given initially (3/4 MED), increasing to 1.5 MED's. Larger doses would result in excessive erythema which may obscure a phototoxic reaction. Grading should be done 24 hr after irradiation.

REFERENCES

1. Ison AE, Blank H: Testing drug phototoxicity in hairless mice. J Invest Dermatol 49:508–511, 1967
2. Baer RL, Harber LC: Photosensitivity to drugs. Arch Dermatol 83:61–66, 1961
3. Sams WM: The experimental production of drug phototoxicity in guinea pigs: II. Using artificial light sources. Arch Dermatol 94:773–777, 1966
4. Sams WM, Epstein JH: The experimental production of drug phototoxicity in guinea pigs: I. Using sunlight. J Invest Dermatol 48:89–94, 1967
5. Schorr WF, Monash S: Photo-irradiation studies of two tetracyclines. Arch Dermatol 88:440–444, 1963
6. Maibach HI, Sams WM, Epstein, JH: Screening for drug toxicity by wavelengths greater than 3100A. Arch Dermatol 95:12–15, 1967
7. Kligman AM, Breit R: The identification of phototoxic drugs by human assay. J Invest Dermatol 51:90–99, 1968
8. Berger DS: Specification and design of solar ultraviolet simulators. J Invest Dermatol 53:192–199, 1969
9. Epstein S: Photoallergy and primary photosensitivity to sulfanilamide. J Invest Dermatol 2:43–51, 1939
10. Blum HF: Studies of photosensitivity due to sulfanilamide. J Invest Dermatol 4:159–173, 1941
11. Stratigos JD, Magnus IA: Photosensitivity by demethylchlortetracycline and sulphanilamide. Br J Dermatol 80:391–405, 1968
12. Breza TS, Halprin KM, Taylor R: Photosensitivity reaction to vinblastin. Arch Dermatol 111:1168–1170, 1975
13. Jung EG: Photoallergie durch Triacetyldipehnolisatin (TDI). Arch Klin Exper Dermatol 229:170–173, 1967
14. Emmett E: Diphenhydramine photoallergy. Arch Dermatol 110: 249–252, 1974
15. Ison AE, Davis CM: Phototoxicity of quinoline methanols and other drugs in mice and yeast. J Invest Dermatol 52:193–198, 1969
16. Morikawa F, Nakayama Y, Fukuda M, Hamano M, Yokoyama Y, Nagura T, Ishihara M, Toda K: Techniques for evaluation of phototoxicity and photoallergy in laboratory animals and man, Sunlight and Man. Edited by MA Pathak, LC Harber, M Seiji, A Kukita, and TB Fitzpatrick. University of Tokyo Press, 1974, p 529–557
17. Ramsay CA, Obreshkova E: Photosensitivity from nalidixic acid. Br J Dermatol 91:523–528, 1974