

THE FATE OF TETRACHLOROSALICYLANILIDE IN PHOTSENSITIZED GUINEA PIGS

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Abstract. The distribution and fate of 3,3',4',5-tetrachlorosalicylanilide (TCSA) in photosensitized guinea-pig skin following topical application at varying times was investigated by fluorescence microscopy in unfixed, frozen sections. When the site to which TCSA had been applied was irradiated with long-wave UV light, TCSA was detected in the entire epidermis only 3 days after application and in the horny layer up to 10 days. Unless TCSA-treated sites were exposed to light, TCSA was still recognized in the entire epidermis 10 days after application and in the horny layer even at 3 weeks. The irradiated photosensitized guinea pigs in the present study eliminated TCSA more rapidly than normal control animals in our previous work. The mechanism of persistent light reaction was discussed and we concluded that TCSA can remain in the skin for a long time, because it is a pro-hapten, not a hapten.

Key words: Tetrachlorosalicylanilide; Distribution of allergen; Photosensitization; Persistent light reactor

Investigation of the percutaneous absorption and localization of allergens is one of the most important problems in the elucidation of the mechanisms of allergic contact dermatitis. In an attempt to study this problem, investigators have used mostly radioactive chemicals or immunofluorescent methods.

In our previous paper (6), we have demonstrated the distribution and fate of halogenated salicylanilides, photocontact sensitizers, in normal guinea-pig skin, utilizing the fluorescent properties of these chemicals. In the present study, we examined the fate of TCSA in photosensitized guinea pigs to show whether there is any difference in the fate of TCSA between normal and photosensitized subjects.

The principle of the method is that fluorescent halogenated salicylanilides could be identified under fluorescence microscopy in unfixed, frozen sec-

tions taken from sites to which the chemicals had been applied topically.

An additional concern of the study was to discuss the mechanism of persistent light reaction due to halogenated salicylanilides on the basis of results obtained in the present study.

MATERIALS AND METHODS

Animals. Both male and female albino guinea pigs of the Hartley strain, weighing 300-400 g, were used for the present study.

Light sources. Toshiba FL20BLB "black lights" emitting 300-420 nm (mainly long-wave UV, peaking at 360 nm) were used, and four lamps were housed in a reflector unit. A bank of four Toshiba FL20E "sun lamps", which emit mainly erythemic radiation in a continuous spectrum peaking at 300 nm, was also used. The energy outputs of the black lights and sun lamps were approximately 4.8 and 4.0 mW/cm² respectively, at a target distance of 15 cm.

Induction of photosensitization. The nuchal area of guinea pigs was depilated and immediately afterwards a thin layer of 10% crystalline TCSA in white petrolatum was applied to a 3 cm square. One hour after application, this site was exposed to the sun-ray lamps at a distance of 30 cm for 5 min followed immediately by black light irradiation at a distance of 15 cm for 60 min. This procedure was repeated for a total of 5 exposures to the same site at 48-hour intervals. Two weeks after the last application, a 1% solution of TCSA in ethanol was applied to a depilated dorsal area followed immediately by the black-light irradiation for 15 min at a distance of 10 cm. Those guinea pigs that developed edematous erythema at 24 hours were selected as TCSA photosensitized subjects.

Preparation of unfixed, frozen sections. All biopsy specimens were frozen immediately in an acetone, dry-ice chamber (-70°C), stored at -40°C, and cut at 5-7.5 μ m on a cryostat at -25°C. Each of the sections was placed on a glass slide and air dried. Four slides were prepared from each biopsy specimen. Two slides were immersed in ethanol for 24 hours to remove unbound free TCSA before observation under a fluorescence microscope. The

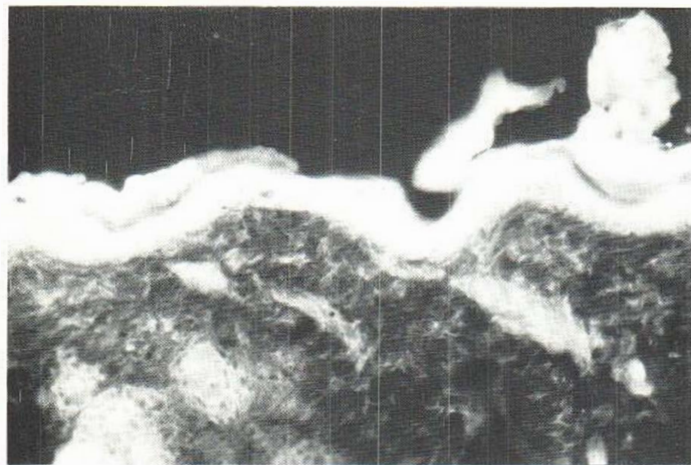


Fig. 1. Section obtained from a non-irradiated guinea pig one hour after topical application of 1% TCSA in ethanol. This and all subsequent sections are fixed and frozen ($\times 94$).

other slides were studied without ethanol extraction. The fluorescence microscope was equipped with an Osram high-pressure mercury vapor bulb, HBO200.

EXPERIMENTAL PROCEDURES AND RESULTS

All experiments were carried out in a room with fluorescent light and curtains drawn.

Five-tenths of a ml of 1% TCSA ethanol solution was applied to a 5×8 cm area on the backs of 8 photosensitized guinea pigs which had been previously depilated. Immediately afterwards, the application site of 5 subjects was irradiated with black light for 30 min at a distance of 10 cm, while the remaining 3 subjects were not irradiated.

A full-thickness biopsy was obtained from the application site of all the guinea pigs with a 5-mm punch without anesthesia, at various intervals following application: 30 min; 1, 2, 3, and 6 hours; 1, 2, 3, 5, 7, 10, 14, and 21 days.

Macroscopically, the irradiated animals were seen to have developed diffuse and mild papuloerythema with edema at the TCSA-treated sites at 24 hours and desqua-

tion was apparent 2 days afterwards. On the other hand, the non-irradiated subjects failed to develop any reaction.

In the fluorescence microscopic examination of unfixed, frozen sections, brilliant, yellow fluorescence of TCSA was clearly recognized in the entire epidermis and dermis in the 30-min to 1-day lesions. Fluorescence was also detected in the outer sheath and horny cells of hair follicles and sebaceous glands. The distribution of TCSA in the dermis was diffuse and the fluorescence was much less intense than in the epidermis. There was no difference in the distribution of TCSA between the sections from irradiated and non-irradiated animals (Figs. 1, 2).

The 2- and 3-day sections from non-irradiated guinea pigs revealed exactly the same findings as those taken earlier. On the other hand, in the sections removed from irradiated subjects in this period, the fluorescence became very faint and in some sections it was detectable only in the upper part of the epidermis.

In 5- to 10-day lesions of non-irradiated animals, the entire epidermis and the upper part of the dermis were still fluorescent, although fluorescence was less intense than that of the earlier lesions (Fig. 3). The irradiated guinea

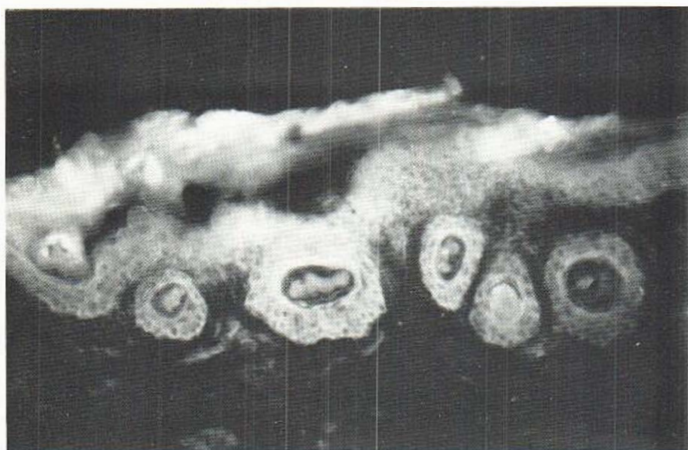


Fig. 2. Section obtained from an irradiated guinea pig one hour after application.

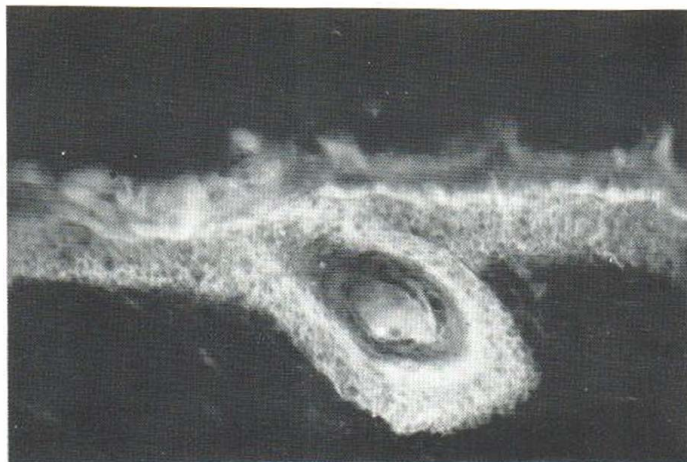


Fig. 3. Section obtained from a non-irradiated guinea pig 5 days after application.

pigs revealed fluorescence only in the horny layer of the surface epidermis and hair follicles in this period (Fig. 4).

Two and 3 weeks after application, TCSA disappeared completely from the irradiated animals, while the horny layer of the non-irradiated subjects was still fluorescent.

These results are summarized schematically in Fig. 5. The results described above were obtained in the sections not immersed in ethanol. After ethanol immersion, all the fluorescence was washed out of the sections taken from non-irradiated animals. In contrast, irradiated guinea pigs showed fluorescence in the horny layer even after extraction.

DISCUSSION

In the previous paper (6), we reported the distribution and fate of TCSA in normal guinea pigs. In normal subjects, TCSA was detectable in the entire epidermis and dermis between 5 min and 7 days or longer after topical application. It was recognized in the horny layer even after 21 days. These results were identical with the findings from non-irradiated TCSA in photosensitized guinea pigs. The fate of TCSA in normal subjects was not influenced by the irradiation with two long-wave UV. In contrast, TCSA, when irradiated *in vivo*, disappeared earlier from the skin of photosensitized guinea pigs.

Several investigators have attempted to demonstrate differences in the time or rate of disappearance of the responsible allergens from the skin of sensitized and non-sensitized control subjects. Most of them (1, 2, 5, 7, 10) were unable to find any difference. However, Geczy & Baugarten (4) observed that a greater retention of tritiated 1-fluoro-2,4-dinitrobenzene occurred in the skin of sensitized than unsensitized guinea pigs after a topical application or intradermal injection. On the other hand, Witten & March (11) found lesser

amounts of radioactivity in the skin of sensitized guinea pigs compared with control animals, following challenge with 2,4-dinitrochlorobenzene. This is in agreement with our result that irradiated photosensitized guinea pigs eliminated TCSA more rapid-

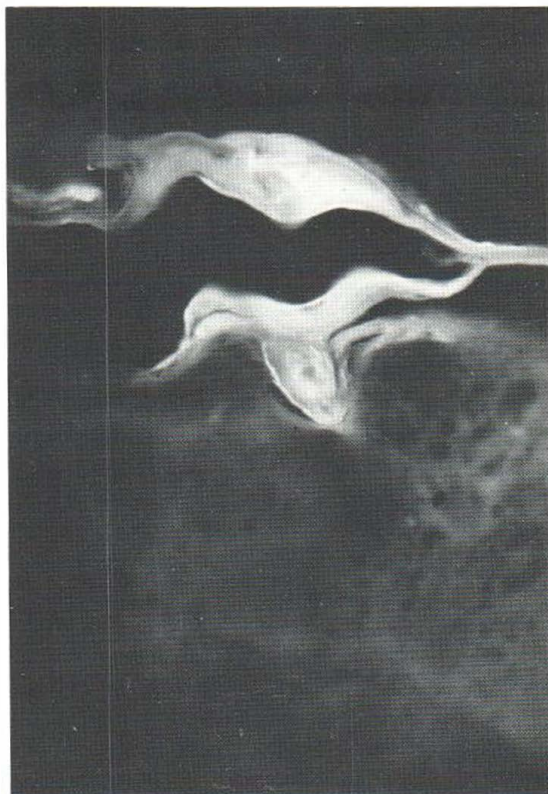


Fig. 4. Section obtained from an irradiated guinea pig 5 days after application. Fluorescence is detected only in the horny layer of the surface epidermis and hair follicle.

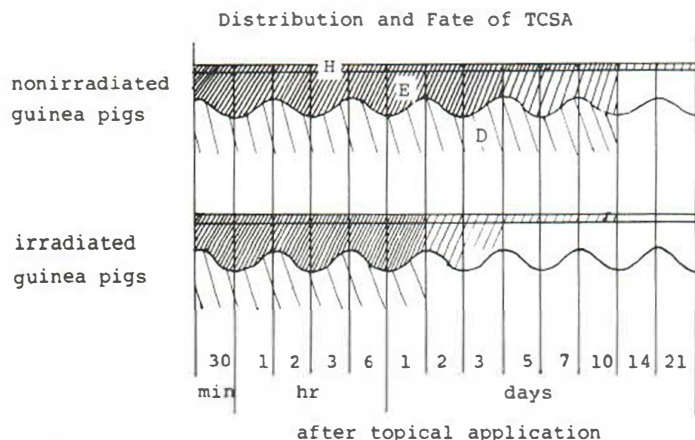


Fig. 5. Schematic summary of distribution of TCSA at various intervals after topical application of 1% TCSA in ethanol. Oblique lines represent TCSA localized by means of fluorescence microscope. H, horny layer; E, epidermis; D, dermis.

ly than normal control animals. This seems quite reasonable, because the sensitized subjects developed an inflammatory reaction at the irradiated site to which TCSA had been applied, resulting in increased desquamation in the epidermis and increased fluid flow in dermis. Consequently, the responsible allergen might be carried rapidly away from the site. However, TCSA could remain longer, unless photosensitized guinea pigs to which TCSA had been applied were irradiated.

Willis & Kligman (8) proposed that photocontact dermatitis due to TCSA is simply contact dermatitis due to photodecomposition products of TCSA. Therefore, in the photosensitized subjects, TCSA is only a prohaptens and, when irradiated, it is transformed to a true hapten which combines with a skin protein to form a complete antigen. These authors' findings are compatible with our present and previous observations that the fluorescence of TCSA was washed out of the application site in non-irradiated animals after ethanol extraction, while irradiated animals showed fluorescence in the epidermis even after the extraction. In other words, TCSA could combine with a skin component when irradiated *in vivo* and it was present in free form in the non-irradiated subjects.

Halogenated salicylanilides and related compounds frequently cause persistent light reactors. Epstein (3) described how approximately 25% of the patients with photocontact reactions induced by the halogenated salicylanilides were persistent light reactors. In an attempt to elucidate the mechanism of the reaction, several hypotheses have been proposed. Willis & Kligman (9) presented evidence suggesting that the persistent light reaction could

result from the retention of small amounts of the photosensitizer in the skin for exceptionally long periods. However, the question why these chemicals remain for such a long period is still unresolved.

In contrast to photocontact allergy, persistent reaction in contact allergy is not known with certainty, although both allergic contact and photocontact dermatitis develop via a similar mechanism. However, there is a difference in the character of the responsible allergen between two types of reactions: that is to say, the allergens of contact dermatitis are haptens and those of photocontact dermatitis are prohaptens.

In the present study, we demonstrated that non-irradiated TCSA (prohaptens) remained longer than irradiated TCSA (haptens) in the photosensitized guinea pigs. The sensitized subjects develop an inflammatory reaction at the irradiated site to which TCSA has been applied and photodecomposition products of TCSA may be carried rapidly from the site. On the other hand, TCSA itself might not be rejected by such an allergic process unless the TCSA-treated site is exposed to light. However, it seems probable that with an insufficient dose of radiation only a part of the TCSA is transformed into hapten and the remaining amounts of TCSA are still deposited in the skin. In fact, Willis & Kligman (9) could identify ethanol-extractable TCSA, but not a photodecomposition product, spectrophotometrically in human dermatitic tissues long after a single application.

In conclusion, we assumed that TCSA could remain in the skin for a long time, since it is a prohaptens, and not a hapten.

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