Title
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Characterization of Cutaneous Phototoxicity Induced by Topical Alpha-Terthienyl and Ultraviolet A Radiation*

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Alpha-terthienyl (α-T), a phototoxic thiophene compound isolated from marigolds (*Tagetes* species), affects cell membranes and does not appear to induce cytogenetic damage. This study was undertaken to investigate topical delivery of α-T and characterize its cutaneous phototoxicity in combination with long-wave UV radiation (UVA) in comparison with locally (intradermal) administered α-T.

Percutaneous penetration (PC) of 0.1% and 1% α-T in a 3% Azone gel vehicle was studied in guinea pig skin in vitro and quantitated by UV fluorescence microscopy. Dose-dependent PC of epidermis, adnexae, and superficial dermis was demonstrated in vitro. Alpha-terthienyl (0.1% and 1%) in this vehicle was applied topically in vivo and irradiated with 30 J/cm^2 UVA at intervals of 10 min–24 h. Maximum sensitization was achieved with irradiation 1 h following drug application. The clinical response was dose-dependent consisting of erythema, edema, crusting, erosion, and inhibition of hair growth and was observed 72 h to 7 days postirradiation. A comparable dose-dependent phototoxic response was observed when 5–500 μg α-T were injected intradermally and irradiated with UVA. These results indicated that low-dose topical α-T in a nonirritating vehicle can rapidly produce cutaneous photosensitization. Topical α-T/UVA may provide a selective and safer alternative approach for the photochemotherapy of psoriasis and other cutaneous diseases. *J Invest Dermatol 87:354–357, 1986*

Drugs now used for photochemotherapy of psoriasis are weak photosensitizers (tars), or are carcinogenic (psoralens). Other classes of drugs that would have a photodynamic action on cells without involving DNA structure and function might be useful for psoriasis without future carcinogenic potential. Hematoporphyrin derivative (HPD) is a drug that has been used in the photochemotherapy of various malignant neoplasms [1]. It may avoid the carcinogenic and mutagenic potential of psoralen but it produces a prolonged photosensitivity which makes its clinical use difficult.

Various photosensitizing polyacetylenes and acetylenic thiophenes are synthesized by many plant species including members of the Asteraceae (Compositae) family. Alpha-terthienyl (α-T) (Fig 1) is a phototoxic thiophene compound which has been isolated from many of these plants, including marigolds (*Tagetes* species) [2]. Alpha-terthienyl plus UVA has demonstrated phototoxicity toward several prokaryotic and eukaryotic organisms including human skin in vivo [3]. The mechanism of action appears to involve generation of singlet oxygen with resultant disruption of cell membranes and does not appear to interact with DNA structure or function. With these properties, photoactivation of α-T with UVA would be a potential candidate for therapy of psoriasis and other photoresponsive diseases such as mycosis fungoides and atopic dermatitis.

In this study we have investigated topical delivery of α-T to characterize its cutaneous phototoxicity in combination with UVA in comparison with locally (intradermal) administered α-T.

MATERIALS AND METHODS

Alpha-terthienyl was chemically synthesized [4], then purified using high-performance liquid chromatography (HPLC) and characterized by UV, infrared and mass spectroscopy, and stored in the dark. The compound has an absorbance maximum at 353 nm and fluoresces blue under UVA or UVB radiation. Topical formulations were prepared by reconstituting α-T in the selected vehicle in the dark. Vehicles were commercially obtained. Azone (1-dodecylazacycloheptan-2-one) was from Nelson Research, Irvine, California; isopropanol, propylene glycol, dimethylsulfoxide, acetone, and n-decylmethylsulfoxide were from Mallinckrodt, Inc.

In Vitro Percutaneous Penetration Full-thickness skin excised from the backs of guinea pigs (Charles River Co.) was used for these studies. Neet, a commercial hair depilatory preparation (Whitehall Laboratories), was used as directed to depilate the backs of the guinea pigs prior to excision. Alpha-terthienyl at concentrations of 0.1% and 1.0% in 3% Azone gel was applied to the epidermal surface as a single application or as 3 applications

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Abbreviations:
α-T: alpha-terthienyl
HPD: hematoporphyrin derivative
PC: percutaneous penetration
TPA: 12-O-tetradecanoyl-phorbol-13-acetate

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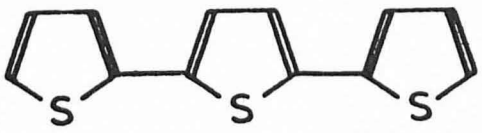

Figure 1. Alpha-terthienyl.

during a 12-h period (0.015 ml/cm^2/application). Forty-eight hours after the first application of α-T, skin specimens were frozen. Cryostat sections of the full-thickness skin specimens were examined for α-T fluorescence with a fluorescence microscope (excitation, BP 515-560, mirror FT 580, LP 590).

In Vivo Animal Studies Mature female white guinea pigs were housed individually and had free access to food and water. They were kept under natural light and dark conditions until administration of the α-T, at which time they were placed in the dark. Neet was used as directed to depilate the backs of the guinea pigs prior to the administration of α-T.

Radiation Source The UVA radiation source was provided by a bank of Derma Control F73T12 UVA (National Biological Co.) lamps (peak emission level 360–365 nm). The intensity measured by a IL442 light meter (International Light) was 5.5 mW/cm^2 at the treatment sites. A dose of 30 J/cm^2 represented an exposure time of 91 min. During exposures, animals were lightly anesthetized with xylazine and ketamine.

Alpha-Terthienyl Administration Alpha-terthienyl in quantities of 0.05–500 μg was administered intradermally in 0.1 ml dimethylsulfoxide:normal saline (4:1) 3 h before irradiation. Various topical α-T preparations or vehicle control solutions (0.05 ml) were applied to the back skin (3 cm^2 area) one or more times before irradiation. Both the upper and lower back was used in these studies, with treatment and control sites localized to similar anatomic regions.

12-*O*-Tetradecanoyl-phorbol-13-acetate Administration 12-*O*-Tetradecanoyl-phorbol-13-acetate (TPA) (Consolidated Midland Corporation) was reconstituted in acetone to make a 1 mg/ml solution. Twelve microliters (20 nmol TPA) were applied to the dorsal aspect of each ear. After 21 h, topical α-T preparations or vehicle control solution (0.15 ml) were applied to the dorsal aspect of each ear 3 h before irradiation.

Erythema Evaluation Photosensitization by α-T as manifested by delayed erythema was evaluated at various time intervals after irradiation using the following scale: 0, no reaction; 1+, minimal erythema with sharp borders; 2+, more pronounced, bright erythema without edema; 3+, marked erythema with edema; 4+, violaceous erythema with vesiculation.

Autoradiographic Studies Autoradiographic techniques were used to study the effects of topical α-T, TPA, and UVA on epidermal DNA synthesis in vivo. At various times after radiation exposure, animals were injected intradermally into separate treated sites with 0.1 ml containing 10 μCi of [methyl-^{3}H]thymidine (25 Ci/mmol, Amersham). One hour after isotope administration the injected sites were punch biopsied (4 mm), the biopsies fixed in Mirsky's solution and prepared histologically for autoradiography [5]. The slides were coated with Kodak NTB-2 liquid nuclear track emulsion for 6 weeks, developed, and stained with hematoxylin and eosin. The labeling index was determined as a measure of DNA synthesis by counting the number of labeled basal cells per 1000 interfollicular basal cells.

RESULTS

In Vitro Percutaneous Penetration Percutaneous penetration of α-T was obtained with a 3% Azone gel vehicle. Maximum tissue fluorescence indicating effective penetration through epidermis, adnexae, and superficial dermis was achieved with 3 applications of 1% α-T (Fig 2). Fewer applications of drug and/or lower concentrations showed a corresponding decrease in fluorescence intensity.

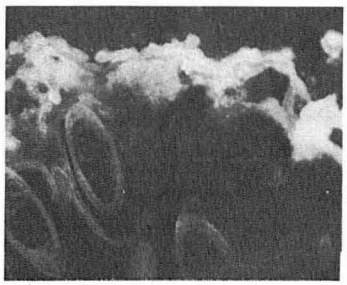

Figure 2. Ultraviolet fluorescence photomicrograph of α-T-treated guinea pig skin in vitro demonstrating penetration of α-T into epidermis, superficial dermis, and adnexae (× 250).

In Vivo Phototoxicity Cutaneous phototoxicity in vivo was obtained with topical application of 1% α-T in 3% Azone gel and in vehicles containing propylene glycol:water:Azone (57:38:5) with 2,5% n-decylmethyl sulfoxide or isopropanol:water:Azone (45:45:10). However, a strong primary irritant effect was seen clinically with the 2 latter vehicles. There was enhanced cutaneous phototoxicity with multiple applications (3 applications during the 24 h prior to irradiation) vs a single application. The optimal interval between single drug application and irradiation was between 1–6 h to achieve maximum photosensitization. Clinical changes included mild erythema and inhibition of hair regrowth during the first 72 h after irradiation, with increasing erythema and edema progressing to erosion and crusting at 1 week. Clinical changes remained localized to the α-T-treated sites. Histopathologic changes at 72 h after irradiation paralleled macroscopic changes with crusting, subepidermal and intraepidermal edema and vesiculation, presence of "sunburn" cells in the epidermis, and epidermal erosion ranging to complete epidermal degeneration (Fig 3).

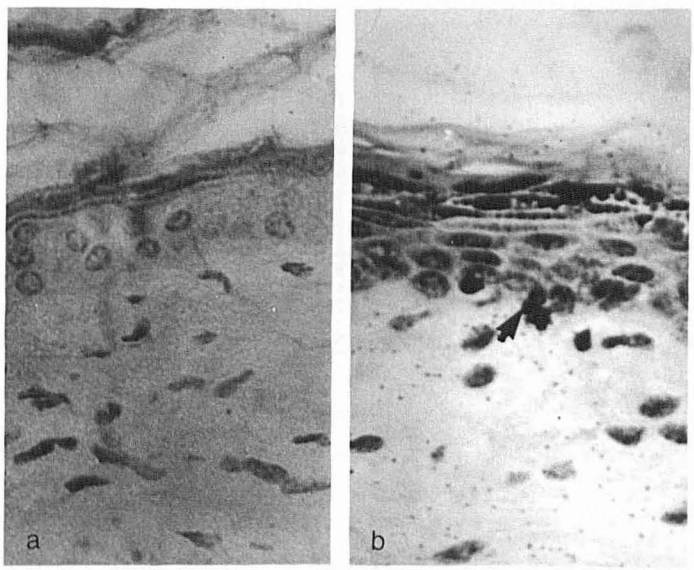

Figure 3. Autoradiographs of normal guinea pig skin treated with (*a*) 1% α-T in 3% Azone gel or (*b*) vehicle only; followed by UVA irradiation (30 J/cm^2). Skin biopsied 72 h postirradiation. Labeling index: (*a*) 0%; (*b*) 8.7 ± 2.8%. Arrow denotes labeled cell in (*b*) (× 400). Note crusting, subepidermal and intraepidermal edema and vesiculation, "sunburn" cells in the epidermis, and epidermal erosion in (*a*) indicative of phototoxic response.

Clinical changes in hyperproliferative TPA-treated guinea pig skin treated with topical 1% α-T plus UVA were not seen until 1 week after irradiation when erythema, erosion, edema, and crusting were noted. A prominent acanthosis was observed in histologic sections of TPA-treated skin which was absent in TPA-treated skin that was then treated with 1% α-T plus UVA (Fig 4).

Autoradiographic results from both normal (Figs 3, 5) and hyperproliferative TPA-treated (Figs 4, 6) guinea pig skin treated with topical 1% α-T plus UVA demonstrated a selective inhibition of epidermal synthesis without affecting DNA synthesis in the structures in the superficial dermis. Although there was no clinical evidence of irritation with the Azone gel vehicle, there was a significant stimulation of epidermal DNA synthesis 24 h after topical vehicle application which returned to baseline by 48–72 h.

A dose-dependent clinical response with similar histopathologic changes was seen after intradermal injection of 5–500 μg α-T followed by UVA. Doses of α-T less than 5 μg produced no significant effect.

DISCUSSION

Records detailing the use of several species of *Tagetes* for medicinal purposes date back many centuries [6]. *Tagetes* has been used topically in Asia, Europe, and South America for various skin conditions. *Eclipta alba,* a related species which contains α-T, has been used for the treatment of vitiligo, dermatophytosis, and other chronic skin diseases [3].

Alpha-terthienyl has demonstrated phototoxicity toward various bacteria and yeasts [2,6–11]. Nematicidal activity of α-T in combination with UVA has also been demonstrated [4,12–14]. Topical preparations of 1% α-T in petrolatum and in ethanol have also produced phototoxicity in human skin [3,15].

The mechanism of action of the cytotoxicity induced by α-T appears to result from the intracellular formation of singlet oxygen (a short-lived, highly reactive state of the oxygen molecule) when cells containing α-T are exposed to UVA radiation [8,9,13,16–19]. MacRae et al [20] found that α-T/UVA did not induce sister chromatid exchange or chromosomal aberrations in cultured Syrian hamster cells, in contrast to 8-methoxypsoralen/UVA. Downum et al [9] found no evidence of DNA damage using recombination deficient mutants of *Escherichia coli* K12 irradiated in the presence of α-T.

Effective penetration of α-T through the epidermis and superficial dermis using Azone vehicle systems has been demonstrated in the present investigation. These studies show that low-dose topical α-T in a nonirritating vehicle can produce dose-dependent cutaneous photosensitization with UVA comparable to that of locally (intradermal) administered α-T. A phototoxic response is also obtained by topical α-T and UVA using the hyperproliferative TPA-treated guinea pig ear model. Phototoxicity is accompanied by a corresponding inhibition of epidermal DNA synthesis

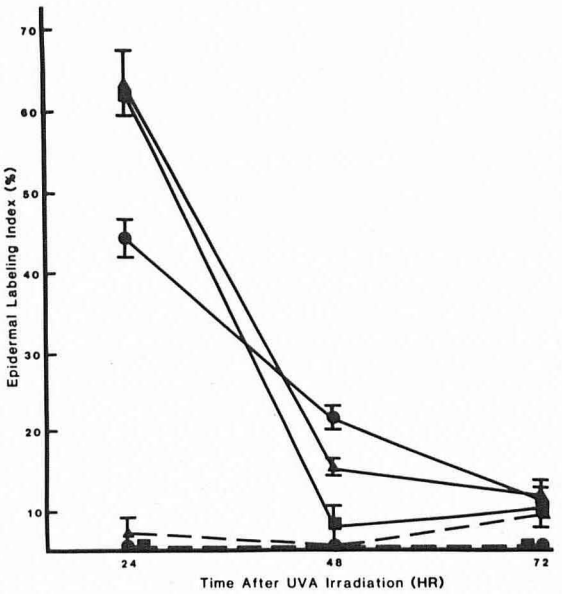

Figure 5. Effect of time interval between α-T application and UVA irradiation (30 J/cm²) on epidermal DNA synthesis in normal guinea pig skin. Time interval: *triangle,* 10 min; *circle,* 1 h; *square,* 6 h. 3% Azone Gel: (——) vehicle control; (----) 1% α-T.

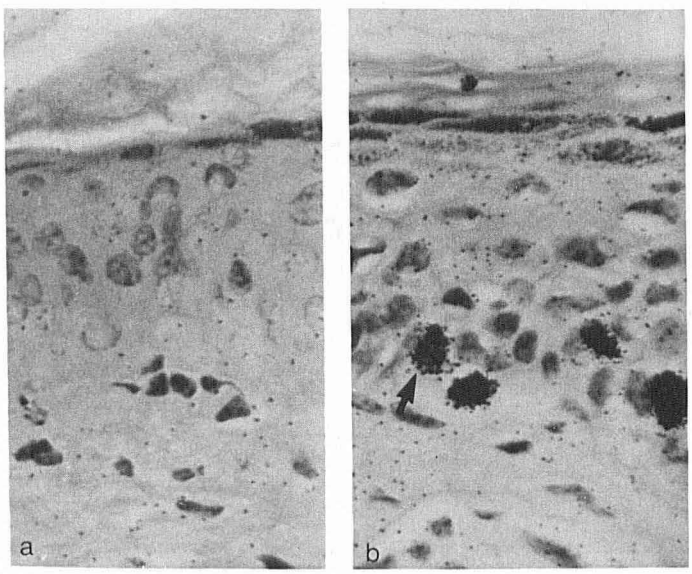

Figure 4. Autoradiographs of TPA-treated (hyperproliferative) guinea pig ear skin with (*a*) 1% α-T in 3% Azone gel or (*b*) vehicle only; followed by UVA irradiation (30 J/cm²). Skin biopsied 72 h postirradiation. Labeling index: (*a*) 0%; (*b*) 17.6 ± 1.8%. *Arrow* denotes labeled cell in (*b*) (× 400). Note prominent acanthosis in (*b*) indicative of hyperproliferative state which is not seen in (*a*).

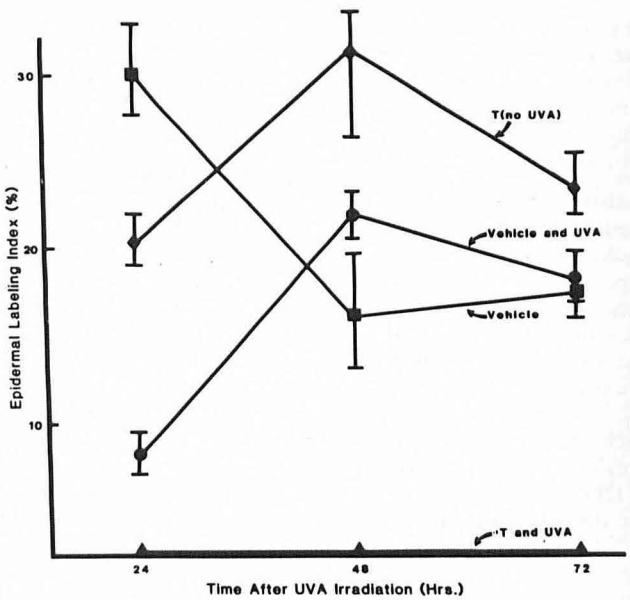

Figure 6. Effect of topical 1% α − T in 3% Azone gel vehicle and UVA irradiation (30 J/cm²) on epidermal DNA synthesis in TPA-treated (hyperproliferative) guinea pig skin.

in both normal and hyperproliferative skin models. The cytotoxic effect of photoactivated α-T does not appear to involve direct interaction with DNA structure, thus reducing the potential for carcinogenesis and mutagenesis. Topical α-T and UVA irradiation may therefore provide a selective and safer alternative approach for the photochemotherapy of psoriasis and other photoresponsive cutaneous diseases.

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REFERENCES

1. Dahiman A, Wile AG, Burns RG, Mason R, Johnson FM, Berns MW: Laser photoradiation therapy of cancer. Cancer Res 43:430–434, 1983
2. Chan GFQ, Towers GHN, Mitchell JC: Ultraviolet-mediated antibiotic activity of thiophene compounds of *Tagetes*. Phytochemistry 14:2295–2296, 1975
3. Towers GHN, Arnason T, Wat C-K, Graham EA, Lam J, Mitchell JC: Phototoxic polyacetylenes and their thiophene derivatives (effects of human skin). Contact Dermatitis 5:140–144, 1979
4. Wat C-K, Prasad SK, Graham EA, Partington S, Arnason T, Towers GHN: Photosensitization of invertebrates by natural polyacetylenes. Biochemical Systematics and Ecology. 9:59–62, 1981
5. Weinstein GD: Autoradiographic studies of turnover time and protein synthesis in pig epidermis. J Invest Dermatol 44:413–419, 1965
6. Neher RT: The ethnobotany of *Tagetes*. Economic Botany 22:317–325, 1968
7. Arnason T, Chan GFQ, Wat C-K, Downum K, Towers GHN: Oxygen requirement for near-UV mediated cytotoxicity of α-terthienyl to *Escherichia coli* and *Saccharomyces cerevisiae*. Photochem Photobiol 33:821–824, 1981
8. Downum KR, Hancock REW, Towers GHN: Photodynamic action on *Escherichia coli* of natural acetylenic thiophenes, particularly 5-(buten-1-ynyl)-1,1′-bitheinyl. Photobiochem Photobiophys 8:8–15, 1983
9. Downum KR, Hancock REW, Towers GHN: Mode of action of α-terthienyl on *Escherichia coli*: evidence for a photodynamic effect on membranes. Photochem Photobiol 36:517–523, 1982
10. Arnason T, Swain T, Wat C-K, Graham EA, Partington S, Towers GHN: Mosquito larvicidal activity of polyacetylenes from species in the *Asteraceae*. Biochemical Systematics and Ecology.9:63–68, 1981
11. Arnason T, Stein JR, Graham E, Wat C-K, Towers GHN: Phototoxicity to selected marine and freshwater algae of polyacetylenes from species in the *Asteraceae*. Canadian Journal of Botany. 59:54–58, 1981
12. Gommers FJ, Geerlings JWG: Lethal effect of near ultraviolet light on *Pratylenchus penetrans* from roots of *Tagetes*. Nematologica 19:389–393, 1973
13. Bakker J, Gommers FJ, Nieuwenhuis I, Wynberg H: Photoactivation of the nematicidal compound α-terthienyl from roots of marigolds (*Tagetes* species). A possible singlet oxygen role. J Biol Chem 254:1841–1844, 1979
14. Gommers FJ, Bakker J, Smits L: Effects of singlet oxygen generated by the nematicidal compound α-terthienyl from *Tagetes* on the nematode *Aphelenchus avenae*. Nematologica 26:369–376, 1980
15. Chan GFQ, Prihoda M, Towers GHN, Mitchell JC: Phototoxicity evoked by alpha-terthienyl. Contact Dermatitis 3:215–216, 1977
16. MacRae WD, Irwin DAJ, Bisalputra T, Towers GHN: Membrane lesions in human erythrocytes induced by the naturally occurring compounds α-terthienyl and phenylheptatriyne. Photobiochem Photobiophys 1:309–318, 1980
17. Wat C-K, MacRae WD, Yamamoto E, Towers GHN, Lam J: Phototoxic effects of naturally occurring polyacetylenes and α-terthienyl on human erythrocytes. Photochem Photobiol 32:167–172, 1980
18. Yamamoto E, Wat C-K, MacRae WD, Towers GHN: Photoinactivation of human erythrocyte enzymes by α-terthienyl and phenylheptatriyne, naturally occurring compounds in the Asteraceae. FEBS Lett 107:134–136, 1979
19. Reyftmann JP, Kagan J, Santus R, Morliere P: Excited state properties of α-terthienyl and related molecules. Photochem Photobiol 41:1–7, 1985
20. MacRae WD, Chan GFQ, Wat C-K, Towers GHN, Lam J: Examination of naturally occurring polyacetylenes and α-terthienyl for their ability to induce cytogenetic damage. Experientia 36:1096–1097, 1980