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Toxicology in Vitro 19 (2005) 931–934
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Phototoxicity of bituminous tars—correspondence between results of 3T3 NRU PT, 3D skin model and experimental human data

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Received 30 March 2005; accepted 17 June 2005
Available online 2 August 2005

Abstract

Bituminous tars (Ichthammol and Ichthyol Pale) are widely used in pharmaceutical, veterinary and cosmetic industries for their anti-microbial, anti-inflammatory and anti-pruritic effects. In contrast to coal tar, no phototoxicity of bituminous tars has been reported in man, although both Ichthammol and Ichthyol Pale exhibit UV absorption which is higher and broader for the former. The validated 3T3 NRU phototoxicity test indicated phototoxic potential of both substances. The phototoxicity test in a 3D human skin model (EpiDerm) only confirmed phototoxicity for Ichthammol. Human data on Ichthammol phototoxicity are missing. A photopatch test in human volunteers was performed in order to clarify the discrepancy between the phototoxicity found in the skin model and the absence of reported human phototoxicity. Following 4 h exposure to 5% and 10% aqueous solutions of Ichthammol and Ichthyol Pale the test sites were irradiated with a UVA dose of 5 J/cm^2. Early phototoxic reaction (erythema) within 4–6 h after irradiation was only elicited by Ichthammol and not by Ichthyol Pale. These data correspond well with those from the 3D skin model test and suggest the necessity to employ several test systems for final phototoxicity assessment. In addition to the results obtained in 3T3 NRU PT, further testing on 3D skin models may better reflect bioavailability of a given chemical in the skin, relevant to the situation in humans.

Keywords: Phototoxicity; Bituminous tars; 3T3 NRU phototoxicity test; 3D human skin model; Photopatch test

1. Introduction

The European cosmetic legislation has banned coal tar (Commission Directive 97/45/EC, 1997) and its derivatives from use in cosmetic products due to their mutagenic, carcinogenic and teratogenic effects. Moreover, all coal tar derivatives cause substantial phototoxic reactions in human skin (Kaidbey and Kligman, 1977). Bituminous tars are suggested as alternative substances with a similar spectrum of pharmacological action (Warnecke, 1999). Both Ichthammol and Ichthyol Pale are products of destructive distillation of bitumen separated from shale deposits containing fossilized fish (European Pharmacopeia, 1997). They consist of sulfur (about 10%), ammonium sulfate (5–7%), hydrocarbons, nitrogenous bases, acids, and thiophene derivatives (Rietschel and Fowler, 1995). Bituminous tars are widely used in pharmaceutical, veterinary and cosmetic industries for their anti-microbial, anti-inflammatory and anti-pruritic effects (Fadrhoncová, 1990). In contrast to coal tar, no phototoxicity of bituminous tars has been reported in man.

Abbreviations: 3T3 NRU PT, 3T3 Neutral Red Uptake Phototoxicity Test; PIF, Photoirritation Factor; PII, Primary Irritation Index.

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doi:10.1016/j.tiv.2005.06.013

Phototoxicity is an acute toxic response elicited after the first exposure of skin to certain chemicals and subsequent exposure to light/UV radiation, or a response similarly induced by skin irradiation after the systemic administration of a chemical, i.e. a photosensitizer. Photoirritation is a particular type of phototoxicity covering only those phototoxic acute skin reactions that are induced by chemicals 0–72 h after exposure to light/UV radiation (Spielmann et al., 2000). Erythema, oedema, vesiculation, hyperpigmentation, and desquamation are typical phototoxic skin effects. For coal tars the historical findings are mostly epidermal, describing an immediate wheal followed by a raised red infiltrated lesion peaking at 24–48 h (Kaidbey and Kligman, 1977). Considering the similar pharmacologic effects of bituminous tars, the aim of the study was to assess the phototoxic potential of Ichthammol and Ichthyol Pale. Moreover, the estimation of phototoxicity is an integral part of the required hazard information on cosmetic ingredients (SCCNFP, 2003). A sequential approach was applied, using methods in vitro (validated 3T3 NRU PT and 3D human skin model) and in vivo (photopatch test in human volunteers).

2. Materials and methods

Ichthammol (CAS No. 8029-68-3) and Ichthyol Pale (CAS No. 1340-06-3) were supplied by HERBACOS-BOFARMA, Czech Republic. The reference material for spectrophotometry, coal tar (CAS No. 8007-45-2), was supplied by TAMDA, Czech Republic. Spectral properties were determined by means of Spectrophotometer Varian Cary 1E.

The UV light source used in all of the in vitro and in vivo experiments was a doped mercury–metal halide lamp (SOL 500, Dr. Hönle, Germany) which simulates the spectral distribution of natural sunlight. A spectrum almost devoid of UVB (<320 nm) was achieved by filtering with 50% transmission at a wavelength of 335 nm (Filter H1, Dr. Hönle, Germany). The emitted energy was measured before each experiment with a calibrated UVA meter (Type No. 37, Dr. Hönle, Germany).

The 3T3 Neutral Red Uptake Phototoxicity Test was performed according to INVITTOX Protocol No. 78 (http://ecvam-sis.jrc.it/invittox/static/index.html), using 3T3 Balb/c fibroblasts (L1, ECACC No. 86052701), passage 50–70. For concentration–response analysis Phototox Version 2.0 software (ZEBET, Germany) was employed.

The EpiDerm Skin Phototoxicity Test was conducted according to Liebsch et al. (1999). 3D skin models, EpiDerm EPI-200, were supplied by MatTek, USA. Before dosing the tissues were preincubated in fresh medium for 1 h (37 °C, 5% CO_2). The test materials diluted in distilled water were applied for 24 h in a volume of 50 μl per tissue. One set of tissues was irradiated with a non-toxic dose of 6 J/cm^2 (as measured in the UVA range). One day after the treatment and UVA exposure the cytotoxicity was detected as reduction of mitochondrial conversion of MTT to formazan. The optical density of the formazan extract was determined at 540 nm by means of Spectrophotometer Varian Cary 1E.

The photopatch test in human volunteers (Neumann et al., 2000) was performed on 10 healthy females, age 26–61. Aqueous solutions (10% and 5%) of the test samples were applied in occlusion (Finn Chamber, USA), using saturated filter paper discs (diameter of 10 mm), on both volar forearms. The exposure time was 4 h and immediately after removal of the patch test the irradiation of one forearm followed (at a dose of 5 J/cm^2, as measured in the UVA range). The other non-irradiated forearm served as a control. Test reactions recorded 1 h, 6 h, 24 h and 48 h after irradiation were graded on a four-point scale (0 no erythema, 1 very slight, barely perceptible erythema, 2 well-defined erythema, 3 moderate erythema, 4 severe erythema). The observations were used for calculation of the Primary Irritation Index (PII). PII represents the sum of erythema found in all individuals divided by the number of individuals.

The selection of volunteers and the test method were carried out in compliance with the ethical principles of the Declaration of Helsinki and subsequent revisions (CIOMS, 1993). The study was approved by the Ethical Review Committee of the National Institute of Public Health, Prague.

3. Results

The UV absorption range of the tested chemicals, including coal tar as a reference material, is documented in Fig. 1. Ichthammol exhibits higher and broader UV absorption comparing to Ichthyol Pale.

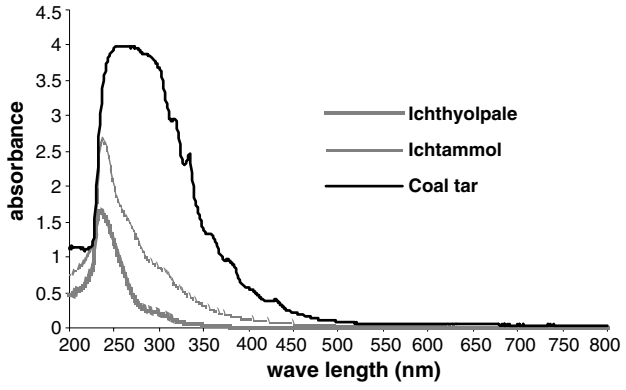

Fig. 1. Spectral properties of Ichthyol Pale, Ichthammol and coal tar.

The 3T3 NRU Phototoxicity Test demonstrated phototoxicity of both substances (Fig. 2). However, the photoirritation factor (PIF) was compelling for Ichthammol (97.3), reaching only a borderline value (5.2) for Ichthyol Pale.

Phototoxicity test in the 3D human skin model (EpiDerm) only confirmed phototoxicity for Ichthammol (Fig. 3a). Ichthyol Pale (Fig. 3b) did not exhibit phototoxicity in the 3D skin model; at none of the concentrations tested did the decrease in viability exceed 30% in the presence of UVA (+UVA) when compared to identical concentrations tested without irradiation (−UVA).

Human data supporting possible phototoxicity of bituminous tars are missing. In order to clarify the discrepancy between the phototoxicity found in the 3T3 NRU PT and the 3D skin model and considering the absence of reported human phototoxicity data, we performed photopatch test in a group of healthy volunteers. Phototoxic reactions were detected solely in the form of skin erythema. The reactions were only elicited by Ichthammol and not by Ichthyol Pale (Fig. 4). The peak of the phototoxic skin reactions was reported between 1 and 6 h after irradiation. The erythema was transient, lasting up to a maximum of 72 h after irradiation.

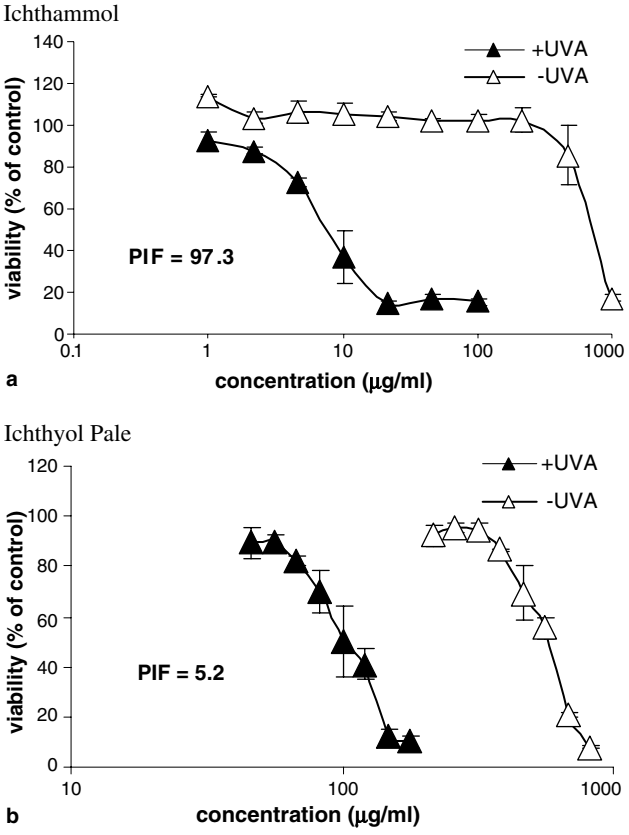

Fig. 2. Phototoxicity of bituminous tars in 3T3 NRU Phototoxicity Test. Each point represents the mean of 12 wells ± SD. (a) Ichthammol, (b) Ichthyol Pale.

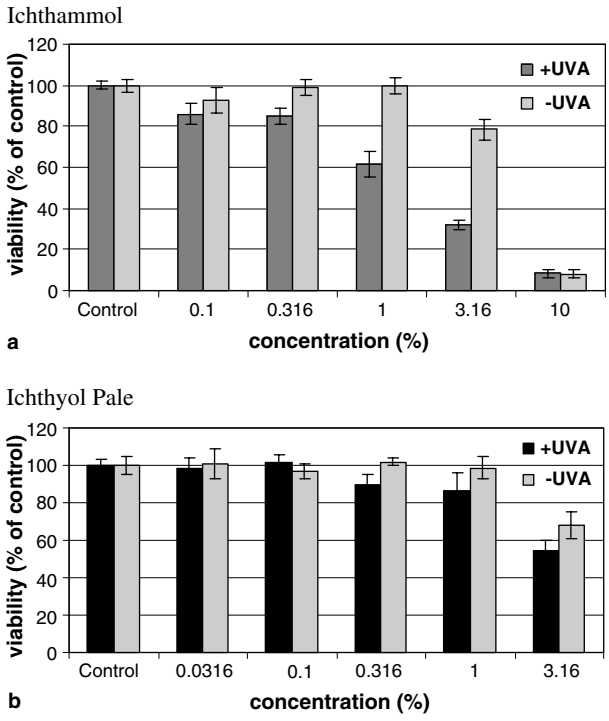

Fig. 3. Phototoxicity of bituminous tars in EpiDerm Skin Phototoxicity Test. Each column represents the mean viability of 4 tissues ± SD. (a) Ichthammol, (b) Ichthyol Pale.

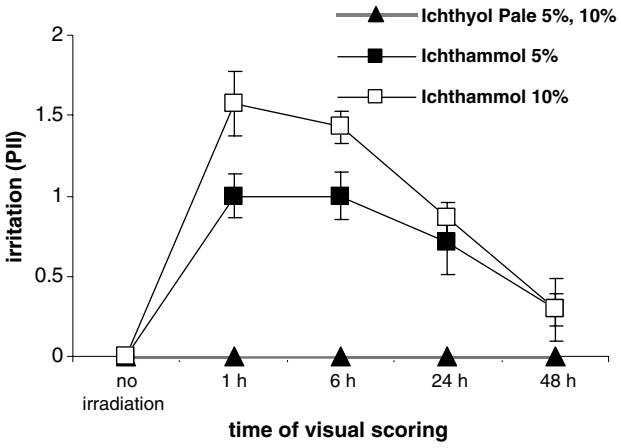

Fig. 4. Ichthammol and Ichthyol Pale induced photoirritation in a group of volunteers. Each point represents the mean erythema grade for 10 volunteers ± SD.

4. Discussion

The 3T3 NRU Phototoxicity Test is a robust and valid method for phototoxicity hazard identification. The phototoxic potential was proved for both Ichthammol and Ichthyol Pale, reflecting their UV absorption properties. Higher UV absorption and substantially higher PIF were recorded for Ichthammol. Nevertheless, the 3T3 NRU PT result itself without additional information on skin penetration has only limited value in

the case of substances applied on the skin. The actual phototoxic reaction is elicited in humans only if both the chemical and radiation reach the target organ, i.e. the lower skin layers.

Regarding the skin, the 3D human skin models are a more suitable alternative approach to mimic the in vivo situation in man, as they better reflect bioavailability of the phototoxic substance in the skin (Liebsch et al., 1999). In the 3D skin models the phototoxicity was elicited only by Ichthammol, while Ichthyol Pale did not exhibit any phototoxic effect. The borderline positive result in the 3T3 NRU PT for Ichthyol Pale (PIF = 5.2) was not confirmed using the 3D skin models. The 3D skin models provided clear distinction between dermally applied Ichthammol and Ichthyol Pale in terms of their phototoxic potential, since reflecting their bioavailability in the skin.

In order to clarify the situation in man, considering the broad clinical use of both substances in pharmaceutical and cosmetic industries, human data were generated using a photopatch test in a limited group of healthy volunteers. The results of the clinical test were in agreement with the data obtained in the 3D skin model assay with the phototoxicity being demonstrated only for Ichthammol.

The overall conclusion from our study, supported by similar findings of other studies (Jones et al., 2003), is that even a substance with intrinsic potential for photoactivation and a positive phototoxicity result in the 3T3 NRU PT may not necessarily represent a hazard to human skin. The 3T3 NRU PT is a highly advantageous method for screening large numbers of substances for their phototoxic hazard. However, this validated method is based on the use of monolayer cell culture and its results may not be relevant when chemicals are applied topically to the skin at lower concentrations. In case of foreseen dermal application, the 3D skin models represent a more relevant system, reflecting to a certain extent also the parameters of skin penetration and barrier function of stratum corneum. Particularly in the case of beneficial substances of great interest to man, this complementary testing simulating real exposure conditions should be considered.

The sequential testing strategy, as was suggested in the OECD Guideline for the testing of acute dermal irritation/corrosion (OECD, 2002), may provide more relevant information on substances intended for dermal use also in terms of their phototoxicity potential.

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