Spatial distribution of 8-methoxypsoralen penetration into human skin after systemic or topical administration

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Aims Photochemotherapy employing psoralens combined with UVA irradiation (PUVA) is a standard therapy for a variety of dermatoses. Psoralens can be administered orally or topically in the form of bath or cream preparations. Recommendations for the time of UVA irradiation are mainly based on the time course of minimal phototoxic doses. However, the time course and depth of skin penetration of psoralens is not well characterized.

Methods We assessed the time course of 8-MOP concentrations in horizontal epidermal and dermal skin sections in 10 patients undergoing oral ($n = 3$), cream ($n = 4$) and bath ($n = 3$) PUVA therapy. Punch biopsies (4 mm) were taken from 'healthy' skin sites. A highly sensitive LC-MS-MS method was employed for 8-MOP analysis.

Results Epidermal concentrations following cream or bath were highest at the end of the application period (time zero) when irradiation is performed. At this time, 8-MOP cream provided significantly higher epidermal concentrations (mean $\pm$ s.e. mean 128.0 ± 22.6 pg mm⁻³; 95% CI: 77.6, 178.4) than oral 8-MOP (27.0 ± 25.3 pg mm⁻³; 95% CI: 29.3, 83.3 at 1 h; $P = 0.025$). Conversely, concentrations in the papillary dermis were significantly higher with oral 8-MOP (20.2 ± 3.1 and 16.2 ± 2.2 pg mm⁻³ at 1 and 2 h, respectively) than with 8-MOP cream (7.1 ± 2.8 and 8.4 ± 2.0 pg mm⁻³ time zero and 0.5 h, respectively; $P = 0.020$ and 0.045 , respectively) or bath (8.8 ± 3.1 and 7.7 ± 2.2 pg mm⁻³; $P = 0.050$ and 0.039 , respectively). The observed time courses of 8-MOP concentrations correspond to time courses of photosensitivity found previously with the different treatment modalities.

Conclusions The higher epidermal 8-MOP concentrations found after topical 8-MOP may explain the lower UVA doses needed with the topical route. These results suggest that topical 8-MOP may be superior in patients where the pathology is localized in the epidermis. In sclerosing diseases, which mainly affect the dermis oral PUVA might be advantageous because dermal concentrations are highest with this route of administration.

Keywords: 8-methoxypsoralen penetration, photochemotherapy, spatial distribution

Introduction

Photochemotherapy with psoralen and its derivatives combined with long-wave ultraviolet (UV) irradiation (PUVA) is a standard phototherapy in the treatment of a

variety of dermatoses [1]. Psoralens are frequently administered orally because systemic PUVA therapy is cheap and easy to perform. However, it is now well established that oral administration of psoralen derivatives is associated with a wide range of side-effects and a potential long-term carcinogenic risk [2, 3]. To reduce side-effects topical PUVA therapy has been developed. The psoralen is applied either in bath water or as a cream preparation [4–8]. The clinical efficacy of topical PUVA has been shown to be comparable with that of oral PUVA therapy [7, 9]. Therapeutic protocols for systemic and topical PUVA therapy are mainly based on results of minimal

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phototoxic dose (MPD) testing, and in the case of oral PUVA, also on the determination of psoralen plasma concentrations. In some studies a good correlation was found between plasma concentrations and the therapeutic response [10, 11]. However, this was not confirmed unequivocally in other studies [12]. Since plasma concentrations following oral 8-MOP reach maximum at about 2 h after drug intake, UVA irradiation is usually performed at this time [13]. Data concerning concentrations of psoralens in human skin, however, are scarce. In particular, the time course and depth of skin penetration following topical dosing and the extent and rate of skin distribution following oral administration are largely unknown [14–16]. Therefore we assessed the time course of 8-MOP concentrations in thin skin layers ranging from the epidermis to capillary dermis following oral, bath and cream administration of 8-MOP.

Methods

Subjects and drug administration

Ten patients (7 male, 3 female, age 42–68 years) who were undergoing PUVA therapy for the treatment of psoriasis were included in the study after giving written informed consent. The study was approved by the Ethics Committee of the University of Frankfurt and conducted in accordance to the Declaration of Helsinki (Edinburgh, 2000).

Patients received 8-MOP either orally ($n = 3$) or were treated with 8-MOP bath ($n = 3$) or cream ($n = 4$). The sample size was estimated on the basis of tissue concentrations (mean and s.d.) of 8-MOP obtained in a previous microdialysis study [17]. Oral 8-MOP (0.6 mg kg^{-1} bodyweight, Meladinine®, Galderma, Freiburg, Germany) was administered 2 h before UVA irradiation. 4 mm punch biopsies were taken at 60, 120 and 180 min after drug intake from nonlesional skin. 8-MOP cream (0.001% 8-MOP) was prepared as previously described [18]. It was applied on nonlesional skin of the back ($50\text{--}60 \text{ mg cm}^{-2}$) for 30 min. UVA irradiation was performed directly after removing the cream with soft tissue. PUVA bath delivery ($0.5 \text{ mg 8-MOP l}^{-1}$, Meladinine® solution, Galderma, Freiburg, Germany) was performed for 20 min at 37°C according to standard protocols [7]. Following cream or bath application 4 mm punch biopsies were taken at the end of the application of 8-MOP cream or bathing time (time zero) and at 30 and 60 min thereafter.

Preparation of skin samples

All skin biopsies were stored immediately at -80°C . The frozen biopsies were sliced in parallel to the skin surface

with a cryomicrotome (Kryostat 1720; Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) [19]. The first five $50 \mu\text{m}$ slices are localized within the epidermis and the subsequent seven $250 \mu\text{m}$ sections correspond to the papillary dermis [20].

Analysis of 8-MOP concentrations in skin sections

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was employed for the determination of 8-MOP concentration in skin sections. For the extraction of tissue homogenates, a liquid-liquid extraction method with trimethoxypsoralen (TMP) as internal standard was employed as previously described [17].

Calibration standards were prepared in water/acetonitrile (2:1) and analysed at the beginning of each sequence. For control of interassay variation, spiked quality control samples in water/acetonitrile were measured in each run in randomized order among the samples. The correlation coefficient for all measured sequences was 0.99. The intraday and interday coefficients of variation were less than 10%. The lower limit of quantification was 0.05 ng ml^{-1} .

Statistics analysis

Data are presented as the mean $\pm$ s.e. mean. SPSS 10.0 was used for statistical comparisons. To assess whether there were significant differences of epidermal or dermal 8-MOP concentrations following cream, bath or oral administration at the various times, mean epidermal (sections 1–5) and dermal concentrations (sections 6–12) of individual patients were submitted to univariate analysis of variance (ANOVA) with the between subject factor 'mode of administration'. Separate ANOVAs were performed for epidermis and dermis and the various times. Subsequently, a Dunnett *post hoc* analysis *vs* oral treatment (cream *vs* oral and bath *vs* oral) was performed. α was set at 0.05.

Results

Concentrations of 8-MOP in the respective skin sections are shown in Figure 1. At the first time point (Figure 1a: directly after the topical application period or 1 h after oral drug intake), 8-MOP concentrations in the upper skin layers that comprise the epidermis were significantly higher after 8-MOP cream than after oral 8-MOP ($P = 0.03$, 95% CI for the difference: 14.1, 187.9 pg mm^{-3}). Differences between 8-MOP bath and oral 8-MOP were not statistically significant ($P = 0.6$, 95% CI for the difference: -61.9 , 121.4). The mean epidermal and dermal concentrations are shown in Table 1.

Figure 1 8-MOP concentrations in skin sections following oral (0.6 mg kg^{-1} , $\blacktriangle$), cream (0.001% , $50\text{--}60 \text{ mg cm}^{-2}$, $\bullet$) and bath (0.5 mg l^{-1} , $\circ$) administration of 8-MOP at different times (a, b, c). Four or three patients were treated in each group. Data are the mean $\pm$ s.e. mean. The first five $50 \mu\text{m}$ sections belong to the epidermis, the next seven $250 \mu\text{m}$ sections refer to papillary dermis. a: directly at the end of the cream or bath application; 1 h after oral drug intake. b: 30 min after the end of the cream or bath application; 2 h after oral drug intake. c: 60 min after the end of the cream or bath application; 3 h after oral drug intake.

Table 1 Mean $\pm$ s.e. mean (95% confidence interval) concentrations of 8-MOP in epidermis (section 1–5) and dermis (section 6–12).

8-MOP concentrations (pg mm^{-3})	8-MOP administration		
	Cream	Bath	Oral
Epidermis (Time 1)	$128.0 \pm 22.6^*$ (77.6, 178.4)	56.7 ± 25.3 (0.4, 113.1)	27.0 ± 25.3 (–29.3, 83.3)
Dermis (Time 1)	$7.1 \pm 2.8^*$ (0.9, 13.3)	$8.8 \pm 3.1^*$ (1.8, 15.7)	20.0 ± 3.1 (13.1, 27.0)
Epidermis (Time 2)	58.2 ± 11.5 (32.4, 83.9)	39.6 ± 12.9 (10.8, 68.3)	30.0 ± 12.9 (1.8, 58.8)
Dermis (Time 2)	$8.4 \pm 2.0^*$ (4.0, 12.8)	$7.7 \pm 2.2^*$ (2.8, 12.6)	16.2 ± 2.2 (11.2, 21.1)
Epidermis (Time 3)	58.6 ± 8.4 (39.8, 77.4)	42.4 ± 9.4 (21.4, 63.5)	28.5 ± 9.4 (7.5, 49.6)
Dermis (Time 3)	5.8 ± 2.6 (10.1, 21.7)	8.1 ± 2.9 (1.6, 14.5)	12.9 ± 2.9 (6.5, 19.3)

Time 1: directly after cream or bath application time or 1 h after oral administration. Time 2: 0.5 h after the end of cream or bath, 2 h after oral administration. Time 3: 1 h after the end of cream or bath, 3 h after oral administration. *Statistically significant mean difference *vs* oral administration with $P < 0.05$.

In contrast to epidermal concentrations, oral administration provided higher dermal 8-MOP concentrations than those achieved with 8-MOP cream ($P = 0.02$ and 0.05 for 1 and 2 h, respectively; Figure 1a, b). Oral 8-MOP also yielded higher dermal concentrations than 8-MOP bath ($P = 0.05$ and 0.04 for 1 and 2 h, respectively, Figure 1a, b).

After topical application, highest 8-MOP concentrations were found in the topmost layer of the skin. They declined sharply with the section depth (Figure 1a, b). Concentrations in the topmost layer were higher following 8-MOP cream than after 8-MOP bath (most prominent directly after the application period; Figure 1a). Following oral 8-MOP, epidermal and dermal concentrations were similar and remained relatively constant between 1 h and 3 h after drug intake (Figure 1a–1c).

Discussion

It has been shown previously that UVA radiation dosage can be reduced considerably when 8-MOP is administered topically compared with oral ingestion of the drug [7, 9, 21]. Thus, it has been hypothesized that the concentrations of the photosensitizing psoralen in human epidermis are higher following topical application than after oral administration. We show in the present study that 8-MOP rapidly penetrates into human epidermis and dermis following topical application. This is in line with previous *in vitro* studies where radioactively labelled 8-MOP was shown to penetrate into epidermal and dermal skin layers following exposure of the skin to a watery solution or aqueous wool-wax alcohol ointment of 8-MOP [22, 23]. Using microdialysis, we have recently shown that skin tissue concentrations following topical 8-MOP application are considerably higher than those obtained by the systemic route [17]. In the present study, the highest epidermal concentrations were found at time zero, i.e. directly at end of the topical application

period. This corresponds well with clinical data showing that photosensitivity following topical application is maximal during the first half hour and then declines rapidly [6]. Since the penetration of 8-MOP into epidermis and dermis was similar with 8-MOP bath and cream, both topical formulations may be expected to provide similar therapeutic responses. This is supported by pharmacodynamic studies showing that minimal phototoxic doses are comparable with both formulations [6]. The advantages of cream PUVA therapy are low cost and the therapy is easy to organize and especially suitable for patients with localized dermatoses. However, topical 8-MOP application with cream may be less homogeneous than with 8-MOP bath which theoretically could be associated with an increased risk of burning.

8-MOP concentrations in the epidermis were considerably higher following topical application than after oral administration of the drug. This may explain why the minimal phototoxic doses are lower after topical application. Conversely, oral administration of 8-MOP provided higher dermal concentrations than those achieved with 8-MOP cream or bath. With the oral route, 8-MOP concentrations were similar in both the dermis and epidermis and remained relatively constant during the observation period of 1 h to 3 h after drug intake, thus spanning the usual irradiation time (2 h after oral drug administration). However, it is important to note that biopsies were taken from 'healthy' skin sites. The amount and rate of skin penetration following topical application may be altered when 8-MOP is applied onto inflamed or thickened skin regions [26].

Since topical PUVA ensures that high 8-MOP concentrations are achieved in the epidermis while concentrations in blood are low [27], topical PUVA should be considered first line therapy for dermatoses, e.g. psoriasis or lichen planus where the target site is in the upper skin. However, if PUVA therapy is used for the treatment of sclerosing diseases, systemic PUVA may be superior, since the pathological processes occur in the dermis in these diseases where higher 8-MOP concentrations are achieved with oral administration of the drug.

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