Study of the Skin Concentrations after Administration of the Various Phototoxic Drugs

Yoon-Kee Park, Seung Kyung Hann, Moo Yon Cho, Hyung Joo Kim, Sang Chul Shim, Seung Ju Choi and Mi Hong Yun

The skin concentrations of 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4, 5', 8-trimethylpsoralen (TMP) were studied in the guinea pig following oral administration and bathing. The skin concentration of phototoxic drugs after oral administration peaked at 1.5 hours, and the concentration of 8-MOP was 3.5 times greater than that of 5-MOP. The skin concentration of TMP was not detected in our study (limit of sensitivity 5ng/ml). The skin concentrations of phototoxic drugs after bathing decreased in the order of 5-MOP, TMP, and 8-MOP.

Key Words: Skin concentration, 8-MOP, 5-MOP, TMP

Photochemotherapy (PUVA) with phototoxic drugs and UVA has been widely used and has shown a good therapeutic effect in a variety of skin diseases including psoriasis, vitiligo, mycosis fungoides and atopic dermatitis (Parrish et al. 1974; Parrish et al. 1976; Lowe et al. 1979). The phototoxic drugs mainly used in photochemical treatment are 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and 4, 5', 8-trimethylpsoralen (TMP). To achieve the maximum therapeutic effect in photochemotherapy, it is believed that ultraviolet light should be irradiated when the drug level reaches the highest concentration in the skin (Bersaques et al. 1984). Most reports to date are made on the measurement of blood concentration (Siddique et al. 1984; Chakrabarti et al. 1982). The few made on skin concentration are mostly simple reports on the skin level of one drug (Roelandts et al. 1983; Boven et al. 1984). Because the blood concentration of TMP was the only drug measured after bathing (Fischer et al. 1980), an integrated study of the skin levels of various drugs was thought necessary. Therefore, the authors measured the skin concentrations of 8-MOP, 5-MOP and TMP hourly after ingestion or bathing of the above drugs and found the time and level at which the drug concentration was highest in the skin after biometabolism for the purpose of clinical application to patients.

EXPERIMENTAL MATERIALS AND METHOD

Experimental animals
Seventy guinea pigs, 300-500gm in weight, were divided into the following subgroups:
a. Control-ten guinea pigs
b. Oral administration (10mg/kg)
   Group with 8-MOP-10 guinea pigs
   Group with 5-MOP-10 guinea pigs
   Group with TMP-10 guinea pigs
c. Bathing
   Group with 8-MOP-10 guinea pigs
   Group with 5-MOP-10 guinea pigs
   Group with TMP-10 guinea pigs
Experimental drugs

The pure powders of 8-MOP (Sigma Chemical Co., St. Louis, Missouri, USA), 5-MOP (Sigma Chemical Co., St. Louis, Missouri, USA) and TMP (Sigma Chemical Co., St. Louis, Missouri, USA) were used after being dissolved in absolute alcohol and then diluted in saline. As for the internal standards of 8-MOP and TMP, 5-MOP was used and for that of 5-MOP, 8-MOP was used.

Experimental apparatus

The concentration of the drug was measured with high performance liquid chromatography (HPLC; Waters Associates, M-244, Milford, Conn., USA) equipped with a 254nm UV detector and a Zorbax Sil 4.6mm x 15cm column (Dupont Instrument).

Experimental method

Oral administration of phototoxic drugs: The pure powders of the phototoxic drugs, 8-MOP, 5-MOP and TMP, were separately dissolved in absolute alcohol, diluted in saline, and ingested through the mouth with a tube at a dosage of 10mg/kg.

Bathing in phototoxic solution: The pure powders of 8-MOP, 5-MOP and TMP were separately dissolved in absolute alcohol and diluted in saline to a concentration of 10mg/L and the guinea pigs, which were epilated on the back, were bathed for ten minutes.

Skin biopsy: In the group with ingestion of phototoxic drugs, the punch biopsy was performed after removal of the hair on the abdomen in each of the guinea pigs before drug ingestion and at 30 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 5 hours, and 7 hours after drug ingestion. Also, a skin biopsy was done at 0.5 hours, 1 hour and after removal of the horny layer by repetition of attachment and detachment of scotch tape about 30 times to 1 hour after bathing.

Homogenization of the biopsied specimen: After weighing the biopsied specimen, it was cut into thin sections, which were transferred into a test tube and then mixed with 100ul of the internal standards and homogenized with a polytron homogenizer after grinding for 2 minutes.

To the homogenized tissue, 5cc of extraction solution was added and it was mixed well with the rotator. After centrifugation at 3000 × g for 10 minutes, the supernatant alone was taken, and this supernatant was evaporated using nitrogen gas and vacum oven.

Measurement of the drug concentration in the tissue using HPLC: After complete evaporation, 40 μl of dichoromethane was added to the test tube to dissolve the residue. This solution was injected into the HPLC column, and the peak on the HPLC recorder was measured with the integrator.

RESULTS

Oral administration of phototoxic drugs

After oral administration of 8-MOP, 5-MOP and TMP at 10mg/kg, the concentrations of the phototoxic drugs in 1 gram of biopsied tissue at 0.5 hour,

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>8-MOP*</th>
<th>5-MOP*</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>895.00 ± 166.68</td>
<td>100.00 ± 37.45</td>
<td>ND</td>
</tr>
<tr>
<td>1.0</td>
<td>1194.40 ± 384.10</td>
<td>256.20 ± 62.51</td>
<td>ND</td>
</tr>
<tr>
<td>1.5</td>
<td>1441.80 ± 173.05</td>
<td>385.80 ± 55.92</td>
<td>ND</td>
</tr>
<tr>
<td>2.0</td>
<td>1330.80 ± 144.00</td>
<td>285.40 ± 48.13</td>
<td>ND</td>
</tr>
<tr>
<td>2.5</td>
<td>753.00 ± 227.27</td>
<td>264.40 ± 35.50</td>
<td>ND</td>
</tr>
<tr>
<td>3.0</td>
<td>505.60 ± 140.24</td>
<td>204.00 ± 46.17</td>
<td>ND</td>
</tr>
<tr>
<td>5.0</td>
<td>502.60 ± 87.68</td>
<td>50.90 ± 13.32</td>
<td>ND</td>
</tr>
<tr>
<td>7.0</td>
<td>312.20 ± 61.23</td>
<td>66.40 ± 14.86</td>
<td>ND</td>
</tr>
</tbody>
</table>

The values are mean ± SEM (ng/g)
ND: Not detected
* p<0.05, as compared with Wilcoxon signed-rank test
Concentrations of Phototoxic Drugs on Skin

1 hour, 1.5 hours, 2.5 hours, 3 hours, 5 hours and 7 hours for 8-MOP were 895.00 ± 166.68ng, 1,194.40 ± 384.10ng, 1,441.80 ± 173.05ng, 1,130.80 ± 144.00ng, 753.00 ± 27.27ng, 505.60 ± 140.24ng, 502.60 ± 87.68ng and 312.20 ± 61.23ng respectively and for 5-MOP, 100.00 ± 37.45ng, 256.20 ± 62.51ng, 385.80 ± 55.92ng, 285.40 ± 48.13ng, 264.40 ± 35.50ng, 204 ± 46.17ng, 59.90 ± 13.32ng and 66.40 ± 14.86ng respectively (Table 1, Fig. 1.) The highest skin concentrations were achieved for both drugs at 1.5 hours, with the level of 8-MOP approximately 3.5 times higher than that of 5-MOP. The level of TMP could not be measured in this experiment with 5ng sensitivity.

Bathing in Phototoxic Solutions

After bathing for ten minutes in the 10mg/L solutions of 8-MOP, 5-MOP and TMP skin biopsies were made at 0.5 hour and 1 hour; in the other group skin biopsies were done after removing the horny layers by stripping with scotch tape 30 times at one hour. The concentrations of the phototoxic drugs in 1 gram of biopsied tissue were 1,153.82 ± 85.03ng, 538.48 ± 84.21ng, and 406.65 ± 78.93ng for 8-MOP; 4,002.85 ± 675.84ng, 3,111.48 ± 797.73ng, and 2,080.30 ± 501.02ng for 5-MOP; 2,665.94 ± 2,101.41ng, 1,331.36 ± 954.44ng and

![Graph showing skin concentration over time](image)

**Fig. 1.** The concentration of the drug in guinea pig skin in ng/g wet weight after oral administration.

<table>
<thead>
<tr>
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<th>8-MOP*</th>
<th>5-MOP*</th>
<th>TMP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1153.82 ± 85.03</td>
<td>4002.85 ± 675.84</td>
<td>2665.94 ± 2101.41</td>
</tr>
<tr>
<td>1.0</td>
<td>538.48 ± 84.21</td>
<td>3111.48 ± 797.73</td>
<td>1331.36 ± 954.44</td>
</tr>
<tr>
<td>stripping</td>
<td>406.65 ± 78.93</td>
<td>2080.30 ± 501.02</td>
<td>1280.56 ± 1184.44</td>
</tr>
</tbody>
</table>

The values are mean ± SEM (ng/g)

* p<0.05, as compared with Wilcoxon signed-rank test
1,280.56±1,184.44ng for TMP (Table 2, Fig. 2). The concentrations of the drugs in decreasing order were 5-MOP, TMP and 8-MOP.

DISCUSSION

The pharmacologic effect of psoralen was proved scientifically by Lerner (1953) after its first use in vitiligo by El Mofty (1947). Pathak et al. (1960) reported the mechanism of erythema formation by phototoxic drugs and ultraviolet light, and Parrish et al. (1974) made a report on the first systematic photchemotherapy using psoralen in psoriasis and other dermatoses. The principle of photochemotherapy is irradiation of UVA after ingestion of selected phototoxic drugs and to achieve the maximum therapeutic effect, the ultraviolet light should be irradiated when the drug level is highest in the skin. In the past the concentration of the drug was measured with thin layer chromatography (Chakrabarti et al. 1978; Steiner et al. 1978) or gas chromatography (Ehrsson et al. 1977; Schmid et al. 1978), but it has been recently shown that HPLC (high performance liquid chromatography) with a few nanograms sensitivity is more accurate (Ljunggren et al. 1980).

The blood level of 8-MOP is reported to be highest at 2 hours after oral administration (Thune et al. 1977; Kligman et al. 1973), and Kornhauser (1982) has reported that the skin concentration is proportional to the blood concentration.

In this study the maximum skin concentration of 8-MOP, 1,441.80±173.05ng was similar to the highest skin concentration reported by Boven et al. (1984), 1,595.6±420.4ng; but was greater than the skin concentration reported by Warmer et al. (1987), 666±219ng. The reason for the difference in skin concentration is that our study measured concentration of epidermis and dermis but Warmer et al. (1987) measured only epidermal concentration.

The time when the highest skin concentration was achieved was 1.5 hours in this study, but in Warmer's study (1987) it was 2 hours, and Boven (1984) reported his result at various peak times. Kornhauser et al. (1982) reported that the blood level reached the maximum around 2 hours of ingestion and decreased skin concentration as a function of time, and that the skin and blood concentrations were proportional to each other; this means rapid diffusion from blood to tissue of 8-MOP and 5-MOP. Kornhauser et al. (1982) and Warmer et al. (1987) have reported that the concentration of 5-MOP was lower than that of 8-MOP.
In this study the level of 5-MOP was conspicuously lower than that of 8-MOP. If taken in equal amounts, because the concentration of 5-MOP is lower than that of 8-MOP, it can be assumed that the risk of skin cancer and damage to DNA that may be caused by photochemotherapy with 8-MOP may be reduced by the use of 5-MOP.

The reason for the low level of 5-MOP is thought to be the decreased absorption from the G-I tract. It is known that phototoxicity by 5-MOP and 8-MOP is induced by the insertion of 5-MOP and 8-MOP into DNA, through formation of a covalent bond with the pyrimidine base, the monofunctional adduct and bifunctional adduct and finally the DNA cross-link. It is also known that the capability of adduct formation is similar to both 5-MOP and 8-MOP (Kornhauser et al. 1982). Reports have been made that ingestion of TMP is improper for production of photosensitive reactions (Kligman et al. 1973), that the blood concentration of TMP can be measured only after bathing, but not after ingestion (Fisher et al. 1980) and that good therapeutic effects can be achieved with irradiation of UVA after bathing with TMP in patients with psoriasis (Salo et al. 1981). However, contradictory reports were made on the blood concentration of TMP after ingestion. Murata et al. (1970) reported that ingestion of TMP with 200 mg/kg resulted in the skin level of 1 μg/g, but Chakrabarti et al. (1982) said that a much higher blood level, 600 ng/ml, could be detected after oral administration of TMP 30 mg/kg.

However, in this study of 5ng/g sensitivity the concentration could not be detected after oral administration of TMP 10 mg/kg. The reasons for the above result are poor solubility of TMP, a low absorption rate and rapid metabolism. These reasons also apply to the fact that the level of 5-MOP was found to be lower than that of 8-MOP.

It is known that bathing with 8-MOP is therapeutically more effective than oral ingestion of it, but no reports have been made on systemic absorption and skin concentration on bathing with 8-MOP as well as on bathing with 5-MOP. After bathing with TMP and ultraviolet irradiation, a good therapeutic effect was reported in psoriatic patients, and the blood concentrations in 6 out of 45 psoriatic patients were reported to be 1-9 ng/ml (Salo et al. 1981). Fisher et al. (1980) reported that the blood concentration of TMP in two of four patients who bathed in TMP was 2 ng/ml, but no reports on the skin level after bathing were made. The skin concentrations after bathing in solutions dissolved with 10 mg/L descended in the order of 5-MOP, TMP and 8-MOP, and there was much variation between individuals.

The skin concentrations measured after stripping 30 times with scotch tape were not much different from those of non-stripped skin after 1 hour of bathing, which means that a larger amount of the phototoxic drug is absorbed through skin than remains in the stratum corneum. In fact, to determine how the degree of phototoxicity changes in relation to the change of skin concentration, more research must be carried out on phototoxic reactions after UVA irradiation.

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