The Relationship Between 8-Methoxypsoralen Skin and Blood Levels

RIK ROELANDTS, M.D., MAURITS VAN BOVEN, DR.SC., PAUL ADRIAENS, PH.D., FRANS DE SCHRYVER, DR.SC., AND HUGO DEGREEF, M.D.

Departments of Dermatology (RR, HD) and Chemistry (PA, FDS) and the Laboratory of Toxicology (MVB), Katholieke Universiteit Leuven, Leuven, Belgium

A method is described to determine the 8-methoxypsoralen (8-MOP) concentration in vivo in the skin by means of high-performance liquid chromatography (HPLC). Skin and blood samples were taken from 80 rats at specific intervals after oral administration of [3H]8-MOP. The pharmacokinetic results obtained for the skin levels were compared to the blood levels. In addition, liquid scintillation counting (LSC) was done on all the samples to compare the concentrations of 8-MOP plus metabolites to the concentrations of 8-MOP alone.

There was a good correlation between the 8-MOP skin and blood levels. The values obtained with LSC were higher in function of time than the corresponding values obtained by HPLC, which indicates the presence of metabolites in both the skin and the blood. No statistically significant difference in the time of peaking was noted for the skin and blood levels. The blood levels seem to be a good parameter for the 8-MOP skin concentration.

The most suitable time for the UVA (long-wave ultraviolet radiation) exposure seems to be when the UVA sensitivity of the skin is at a maximum so that the exposure time may be as short as possible. However, there can be considerable interindividual differences in maximal UVA sensitivity [1] as well as high interindividual variability in the 8-methoxypsoralen (8-MOP) serum levels [2–12]. In addition, there is a rather high intraindividual variability of 8-MOP plasma levels [13].

A poor response to PUVA (psoralen plus UVA) therapy may be due to low 8-MOP serum levels at the time of the UVA irradiation [8]. Nevertheless, good results have been reported in some cases with very low serum levels [2]. This might be explained by the lack of a positive correlation between the blood and the skin concentrations.

In vitro, the human epidermis is able to bind and concentrate 8-MOP without the influence of UVA [14], which could lead to high epidermal 8-MOP levels and a prolonged rediffusion time of the drug from the skin. This may result in retention of skin photosensitivity at even markedly reduced serum levels. Tissue blister fluid studies suggest that the reduction of 8-MOP concentration is slower in the skin than in the serum [15]. Thus, the 8-MOP plasma concentration does not always seem to be a reliable parameter for the prediction of treatment results [16]. A more reliable parameter would be the 8-MOP skin concentration. Therefore, we set out to develop a method for 8-MOP skin determination and to study the pharmacokinetics of 8-MOP in the skin as compared to the blood levels. We went on to compare these results obtained with high-performance liquid chromatography (HPLC) to the levels obtained by liquid scintillation counting (LSC), measuring not only the 8-MOP but also its metabolites.

MATERIALS AND METHODS

Animals

Eighty shaven female Fsd:WIST outbred rats weighing about 200 g were each orally administered 2 mg of [3H]8-MOP in oil (0.5 ml; 71.20 µCi). At regular intervals (0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 5.0, and 10 h), 10 rats were decapitated to collect blood and skin samples. After adding 5 drops of heparin solution, one blood sample of 0.5 ml was set aside for HPLC and two 0.5-ml blood samples for LSC. The skin samples were taken from the backs of the animals, collected in vials, and weighed. Two samples were set aside for HPLC and 6 for LSC. This was done for each animal.

Drug

An 8-mCi sample of 8-methoxy-[G-3H]psoralen was obtained from Amersham International Ltd, Amersham, U.K. It was purified with HPLC [17] on a 23 × 2.5 cm column of 15-µm silicagel particles in methylene chloride. Elution was carried out with methylene chloride containing 0.2% of methanol at 15 ml/min.

8-MOP Skin and Blood Levels

For each rat, 2 skin samples of about 100 mg each consisting of epidermis, dermis, and subcutis were homogenized and extracted with a mixture of toluene and n-hexane (60:40) following the addition of 100 ng of 8-MOP as the internal standard. The organic phases were separated by centrifugation and transferred into conical tubes. After evaporation of the solvent, the residues were redissolved in 40 µl of methylene chloride and examined by HPLC. A silicagel column (Si60, 7 µm) was used. The mobile phase consisted of a mixture of methylene chloride-hexane: ethanol (100:20:0.1) at 2 ml/min. A UV detector at 254 nm was used for the detection. The concentrations were calculated by means of a calibration graph prepared by adding known amounts of 8-MOP along with 100 ng of internal standard to drug-free skin samples.

The other 6 samples from each rat were used to measure total radioactivity by means of LSC using [*H]n-hexadecane [18] as the internal standard. For each rat, 1 blood sample was used for HPLC and 2 for LSC. The blood samples for HPLC were extracted according to a method we have described elsewhere [19].

RESULTS AND DISCUSSION

The mean values for the 8-MOP skin levels as measured with HPLC and LSC are given in Figs 1 and 2. There was no statistically significant difference between the different mean values 0.5–2.0 h after oral intake, but the differences were significant thereafter (p < 0.025 after 2.5 h; p < 0.01 after 3.5 h; p < 0.005 after 6 h; p < 0.005 after 10 h). The LSC values were about 50% higher at 3.5 h than the HPLC levels. These differences may obviously be explained by the presence of formed metabolites not measured by HPLC.

The 6 skin samples from each rat for LSC were taken from different areas on the back and often showed significant differences in radioactivity, indicating a different 8-MOP concentration depending on the area. This might be because of differences in the fat content of the skin at different places. Indeed, 8-
MOP is liposoluble and has a low degree of solubility in water. This is illustrated by the fact that a fatty meal may enhance the resorption of 8-MOP [20]. It has also been demonstrated by whole-body autoradiography that UVA irradiation can produce an increase in the amount of radioactivity in the subcutis, particularly in the loose connective tissue layers and in the hair follicles [21]. The subcutis accumulation was seen after a high 8-MOP and radiation dose. No higher radioactivity concentrations have been found in the subcutis after UVA exposure using therapeutic doses of 8-MOP [22].

With skin samples from psoriasis patients but under in vitro conditions, a higher label incorporation has been noted in the upper layers of the epidermis after UVA irradiation. Indeed, parakeratotic cells show a greater degree of photobinding than the cells of the basal layer [23].

Figures 1 and 2 also show the mean 8-MOP blood levels as a function of time as determined by HPLC and by LSC. There was no significant difference (p > 0.2) between the different mean values 0.5–2.0 h after oral intake for the 8-MOP as measured by HPLC and by LSC. However, after 2 h the LSC levels were significantly higher than the HPLC values for the 8-MOP alone (p < 0.05 after 2.5 h; p < 0.01 after 3.5 h; p < 0.0005 after 6 h; p < 0.0005 after 10 h). After 2.5 h, the difference was about 25%. In contrast to the skin samples, there was no significant difference between the two samples used for LSC each time.

Because 8-MOP is rapidly metabolized, metabolites appear in the plasma shortly after oral administration. A reason for the relatively long elimination time of the 8-MOP metabolites may be a rediffusion of the radioactivity from the tissues into the blood [24].

Comparison Between 8-MOP Skin and Blood Levels

On the basis of Figs 1 and 2, there seems to be a good correlation between the 8-MOP skin and blood levels as determined by both HPLC and LSC. Only after 1.5 h with LSC and after 2 h with HPLC were significant differences noted between the blood and skin levels (p < 0.05). The fact that the skin levels are then higher than the blood levels might account for the retention of skin photosensitivity at even markedly reduced serum levels. This concurs with the results of other experiments and suggests that the skin may act as a deep compartment [25].

The difference between LSC and HPLC is higher for the skin samples than for the blood samples. This could be attributed to a difference in pharmacokinetics between the skin and the blood for 8-MOP and its metabolites. Another explanation could be significant metabolism in the skin.

Determination of the 8-MOP blood levels seems to be a good parameter for the determination of the skin levels, although our experiments do not allow us to conclude that the time of peaking is exactly the same for both the skin and the blood. However, the similarity of the curves in Figs 1 and 2 suggests that there is not a major difference.

REFERENCES


10. Smyth RD, Van Harken DR, Pfeffer M, Nardella PA, Vasilev M,
Brief pulses of 577-nm radiation have recently been shown to selectively damage superficial cutaneous blood vessels, resulting clinically in purpura. There was a sharp threshold of exposure dose necessary for causing purpura in any given subject, which correlated with histologic evidence of extravasation and specific vascular injury. As a means of studying mechanisms for such damage, heat, cold, pressure, suction, UV radiation, and intradermal epinephrine were used to alter human cutaneous microvascular responses. At high performance liquid chromatography. Journal of Labelled Compounds and Radiopharmaceuticals 19:463–468, 1982.

In Caucasian skin, superficial cutaneous blood vessels are the major sites absorbing 577-nm radiation (green-yellow visible light). When absorbed, this wavelength therefore generates heat specifically within superficial blood vessels, and if delivered in a brief, intense pulse can cause selective damage to cutaneous microvessels. Accordingly, it has recently been shown that single, 3 × 10^{-7} s (300 ns)-duration pulses of 577-nm radiation from a pulsed tunable dye laser selectively damage Caucasian cutaneous microvessels in vivo at exposure doses of 2.0 J/cm² or more [1,2]. Histologically, rupture and hemorrhage of the superficial vascular plexus were noted with associated endothelial and vessel wall necrosis. All vessels to a depth of 0.5 mm were affected. A pattern of acute vasculitis evolved with fibrin, polymorphonuclear leukocytes, and karyorrhexis in the necrotic venular walls. Few or no alterations were apparent at the light microscopic level in dermal fibroblasts, collagen, elastic tissue, or in the overlying epidermis, despite significant vascular damage. The same exposure doses that caused histologically specific effects on blood vessels also caused purpura in the exposed skin. This purpura was an all-or-none response occurring within minutes at most and usually immediately after exposure.

Although it is clear that purpura results from extravasation of blood, it is not clear which of several possible damage mechanisms may be causing this superficial hemorrhage. Possibilities include thermal denaturation, vaporization, and shock wave damage [3–5]. At temperatures less than 100°C, protein denaturation, membrane disintegration, and rapid endothelial and pericyte cell necrosis may weaken the vessel wall enough to cause hemorrhage and purpura. Alternatively, or in addition, intravascular vaporization of erythrocytes or plasma may occur.