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Plasma Concentrations after Bath Treatment and Oral Administration of Trioxsalen

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Received May 10, 1979

Abstract. An analytical method comprising homogenous extraction and determination by gas chromatography mass spectrometry has been developed for the quantitation of trioxsalen in plasma. The limit of sensitivity of the method has been 2 ng/ml of trioxsalen. The method was used to monitor concentrations of trioxsalen in plasma after a 40 mg oral dose or after trioxsalen bath treatment. The concentrations in plasma after oral administration did not exceed 3 ng/ml, while in 2 patients after bath treatment trioxsalen could be determined (2–3 ng/ml). The low plasma concentrations found throw light on the poor UV-sensitizing properties found after oral trioxsalen therapy.

Key words: Trioxsalen; Photochemotherapy; Plasmaconcentrations; Skin absorption

Photochemotherapy implies sensitization of the skin, usually with psoralens, followed by UV-irradiation. This method (PUVA) is increasingly used in the treatment of psoriasis and other dermatoses (3, 4, 6, 12).

The most commonly used sensitizing agent is 8-methoxy-psoralen (8-MOP) given orally (12). Oral trioxsalen is less commonly used but the sensitizing properties of this therapy are not well documented. Local administration with 8-MOP in an ointment or in a bath also gives the skin high UV-sensitivity, but the highest sensitization is achieved with trioxsalen bath treatment (3, 4, 6).

Several studies have been performed on the pharmacokinetics of 8-MOP in human, after both oral and local treatment (7, 11). Studies on the disposition of trioxsalen have only been reported in rats (10).

SUBJECTS AND METHODS

Patients

Blood samples were drawn from 4 patients with psoriasis at different time intervals after taking a trioxsalen bath. The bath was prepared by adding 50 mg trioxsalen (Paul B. Elder Co., Bryan, Ohio) in 0.1 litre of alcohol to 150 litres of 37°C water. After the 15 min bath and drying, the patient was exposed to unfiltered light of the dysprosium solarium (3). Blood samples were drawn before, immediately after and at 30 min after the bath. In another 2 patients, samples were also drawn at 1, 2, 4, 6 and 8 hours after the bath. Two patients were given 40 mg of trioxsalen (Trisoralen; Elder Co, Bryan, Ohio) orally after a breakfast consisting of milk, two slices of bread and butter with marmalade or cheese, and coffee (2). Blood samples were drawn at 0, 1, 2, 3, 4, 6 and 8 hours after the administration. After sampling, the plasma was immediately separated and stored at −18°C until analysis not more than 3 days later.

Analytical procedure

Extraction. Four different extraction procedures were tested.

1. One ml of plasma was, after addition of an internal standard (8-methoxypsoralen 50 ng in 0.1 ml of alcohol) and 1 ml of phosphate buffer 0.1 M, pH 7.4 and water to 5 ml, shaken with 5 ml of distilled toluene for 60 min. After removal of the aqueous phase, the toluene layer was washed for 15 min in order with 2 ml of 0.05 M phosphoric acid and then with 2 ml of 0.1 M phosphate buffer, pH 10. The organic layer was evaporated to dryness and reconstituted in 30 μl of heptane.

2. The composition of the extraction mixture was the same as in 1. The mixture was shaken at 50°C for 20 min and, after centrifugation, the toluene layer was evaporated to dryness and redissolved in heptane.

3. One ml of plasma was, after addition of an internal standard (8-methoxypsoralen 50 ng in 0.1 ml of alcohol) shaken with 5 ml of acetone for 15 min. Four ml of toluene and 5 ml of water were added and the mixture was shaken

for 15 min and centrifugated. The organic layer was evaporated to dryness and re-constituted in 30 μl of heptane.

4. One ml of plasma was, after addition of an internal standard (8-methoxypsoralen 50 ng in 0.1 ml of alcohol) mixed with 3 ml of methanol and 0.5 ml of heptane (5). After 15 min shaking, 5 ml of heptane and 3 ml of water were added and shaken for another 15 min. After centrifugation the organic phase was evaporated and redissolved in heptane as above.

In all methods spiked plasma samples with trioxsalen were treated in parallel.

Gas chromatography—mass spectrometry. An LKB 2091 gas chromatograph mass spectrometer was used and operated at an ionizing energy of 35 eV. The capillary glass column (18 m×0.3 mn inner diameter) was coated with SP 1000 (Ultrasept® Oy Separation Research AB, Turku, Finland) and operated at 235°C. The injector temperature was 245°C. Flow of helium carrier gas was 1.5 ml/min. The mass spectrometer was adjusted to record the ions m/e=228 (M^+, base peak) for trioxsalen and m/e=216 (M^+, base peak) for the internal standard. The sample was introduced in the gas chromatograph by the solvent-less injection technique.

RESULTS

Four methods have been tested for the extraction of trioxsalen from plasma. With these methods, rectilinear standard curves were obtained in the range 2–50 ng/ml of added trioxsalen to plasma. Plasma was also incubated for several days with trioxsalen and after extraction the yield was complete. The limit of sensitivity of the extraction methods was 2–4 ng/ml of trioxsalen.

Only two samples from the patients treated with the trioxsalen bath exceeded the limit of sensitivity of the method. In one patient a plasma concentration of 2 ng/ml of trioxsalen could be registered one hour after bath treatment. In another patient 2.5 ng/ml was seen 2 hours after the bath.

After oral administration of the usual therapeutic dose of 40 mg of trioxsalen the plasma concentration in all samples was below the limit of sensitivity, irrespective of extraction method.

DISCUSSION

Recently, 8-methoxypsoralen was determined from biological samples by electron capture gas chromatography after selective purificatoin (1). As the hydrolysis rate of trioxsalen is low, even in strong alkaline solution, this approach could not be used for purification. Therefore, the more selective technique mass fragmentography was chosen to determine plasma levels of trioxsalen.

Several extraction procedures of the serum sample were used in order to exclude extraction failures of trioxsalen from the sample. The homogenous extraction procedure developed (5) has found use in the extraction of highly lipophilic compounds from biological material. Acetone or methanol was used for homogenization and, after mixing, non-polar solvent and water were added to get separation into two phases. Extraction with toluene at 50°C was also used in order to get a more efficient extraction, for example, of protein-bound trioxsalen. As the yield of trioxsalen incubated with plasma was completed with the extraction methods used, it may be concluded that no extraction failures occurred.

The pharmacokinetics of 8-MOP, also in correlation to its UV-sensitizing effect, has been reported on recently (11). The UV-sensitization parallelled the plasma levels found after an oral dose of 0.5 mg/kg body weight and a maximum plasma level of 8-MOP coincided with maximum light sensitization. A high UV-sensitization has also been demonstrated for 8-MOP after local application to the skin (7). A 1% ointment of 8-MOP applied to half the body surface gave the same absorbed amount as after oral administration of 0.5 mg/kg body weight of the drug.

The UV-sensitization after bath treatment with 8-MOP is considered better than after oral administration. Data on the systemic absorption are not available for bath treatment with 8-MOP. The degree of trioxsalen absorption after oral or bath treatment in man has not been reported. In the present study trioxsalen could not be detected in plasma after oral administration. In rats a very low absorption has been demonstrated after a 200 mg/kg body weight oral administration of trioxsalen (10). In a recent report one urinary metabolite of trioxsalen in man and mouse 4,8-dimethyl'-carboxypsoralen was identified (9). In the mouse an absorption degree of 80% was estimated. Thus absence of trioxsalen from plasma is apparently not only related to poor absorption, but also to rapid biotransformation to non-photosensitizing metabolites. The extremely low plasma concentrations explain the poor UV-sensitization reported after oral trioxsalen (8).

The plasma concentrations of trioxsalen found after bath treatment imply some systemic absorption but a rapid biotransformation means a minimal systemic toxicity of the compound. This, taken together with the extremely good sensitizing proper-

ties, gives the trioxsalen bath treatment a high rank in the choice of type of PUVA treatment.

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Disturbances in the Metabolism of Calcium and Vitamin D in a Case of Sarcoidosis

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Received February 21, 1979

Abstract. A case of subcutaneous nodular sarcoidosis presented low 25-hydroxy-vitamin D levels during the active phase of the disease, while the serum calcium levels were increased. During spontaneous remission both 25-OHD levels and serum calcium levels returned to normal.

Key words: Vitamin D; Serum calcium; Sarcoidosis

Vitamin D is now known to undergo hydroxylation in the liver to 25-hydroxy-vitamin D (25-OHD), which is the main circulating metabolite of the vitamin. A further hydroxylation occurs in the kidneys, where 1,25-dihydroxy-vitamin D (1,25-$(OH)_2$-D) is synthesized. This metabolite is regarded the most potent vitamin D metabolite (2).

The hypercalcaemia of sarcoidosis is well known. A recent study (1) has provided evidence that the cause of the hypercalcaemia in sarcoidosis is the increased quantity of circulating 1,25-dihydroxy-vitamin D.

We have recently seen a patient suffering from sarcoidosis and hypercalcaemia. Remission occurred without any treatment, concomitant with changes in the serum 25-OHD and calcium concentrations.

METHODS

Serum calcium and serum and urinary phosphate and creatinine were analysed on a Greiner GSA II by routine colorimetric methods. Urinary calcium was analysed by atomic absorption spectrophotometry (Perkin Elmer 300). 25-OHD was assayed in duplicate by a competitive protein-binding assay after extraction and purification on Sephadex LH 20 columns (5).

CASE REPORT

A 40-year-old woman was admitted 20th April 1978 with 6–7 subcutaneous noduli of hazel-nut size which were indolent and bluish-red, and localized on the arms, thighs and back. They had developed during the preceding 2 years, following the appearance of an erythema nodosum.