The quantitative analysis of 8-MOP in human plasma by HPLC-UV

N. FINCHAM, M.W. GREAVES, C.N. HENSBY & D. VELLA BRIFFA

Professorial Unit, Institute of Dermatology, Homerton Grove, London E9 6BX.

The use of 8-methoxypsoralen (8-MOP) in combination with long wavelength (360 nm) ultraviolet irradiation (PUVA chemotherapy) for the long-term treatment of psoriasis is under worldwide clinical evaluation. However, only minimal information is available on the circulating and tissue levels of 8-MOP achieved following therapeutic dosage. To overcome this problem we have developed a quantitative analysis for 8-MOP in human plasma based on reversed phase high pressure liquid chromatography with ultraviolet detection (HPLC-UV).

Human blood (10 ml) is equilibrated with lithium heparin and the plasma removed by centrifugation. Aliquots (0.5 ml) in triplicate are equilibrated with a solution containing 1.0 ml distilled water, 50 µl of 1 N KOH and 50 µl of methanolic trimethylpsoralen (TMP) solution (5 µg/ml). After 15 min the samples are extracted twice with 2.5 ml of redistilled ethyl acetate and the pooled organic phase is taken to dryness under vacuum at 40°C. The organic residues are redissolved in methanol (100 µl) and aliquots (20 µl) injected into the HPLC. The instrument (two Waters 6000 A pumps, a 660 solvent programmer and U6K loop injector) is used to pump a methanol–water solution (70:30 v/v) at 3 ml/min through a 250 mm × 3.9 mm (i.d.) octadecylsilane column. The UV detector (Cecil 272 variable wavelength UV monitor) used is equipped with a 10 µl flow cell (1 cm path length) and records of the absorbance monitors are recorded on a Servoscribe 600 potentiometric recorder. The limit of detection is 10 ng/ml of 8-MOP added to human plasma and the effective range 0 to 100 ng of 8-MOP per ml of plasma.

The plasma concentrations of 8-MOP found 2 h after therapeutic doses of 8-MOP (0.6 mg/kg body weight to the nearest 10 mg) range from 17 to 383 ng/ml in males (125.8 ± 18.1; $n = 23$, mean ± s.e. mean) and from 64 to 254 ng/ml in females (147.3 ± 13.5; $n = 15$, mean ± s.e. mean).

The method has also been modified for the analysis of 8-MOP in two proprietary tablet preparations of 8-MOP nominally containing 10 mg per tablet. The values obtained for tablet brands A and B were 8.76 ± 1.00 (mean ± s.d.; $n = 169$, range 3.09 to 10.58) and 9.24 ± 0.69 (range 8.19 to 11.67, $n = 30$) mg of 8-MOP per tablet, respectively. They reveal a greater than expected inconsistency in the 8-MOP content of tablets from the same batch and thus pose major problems for their use in the therapeutic treatment of psoriasis in PUVA chemotherapy.

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Electrophysiological effects of imipramine in guinea pig-myocardium

P. GARCIA DE JALON, S.M. RODRIGUEZ & J. TAMARGO

Department of Pharmacology, School of Medicine, Universidad Complutense, Madrid - 3 (Spain)

Cardiovascular complications following the administration of tricyclic antidepressant drugs have been observed both in cases of accidental overdosage as well as during chronic therapy (Editorial, 1971). However, just a few papers refer to the electrophysiological effects of these compounds on isolated cardiac fibres. The present study was undertaken to determine the effects of imipramine (IMI) on the electrophysiological properties of ventricular muscle fibres.

Right ventricular guinea pig papillary muscles were perfused with warmed (34°C) and oxygenated Tyrode solution and stimulated at a basal rate of 1 Hz. Intracellular action potentials were recorded with glass microelectrodes filled with 3 M KCl solution. Preparations were exposed to each concentration of IMI (0.1, 1, 5, 10, 15, 20 and 25 mg/l) for 30 minutes. Spontaneous activity was induced by adding 0.2 mM $BaCl_2$ to the normal Tyrode solution. Ca action potentials were elicited by adding isoprenaline (0.2 mg/l) to high K (27 mM) Tyrode solution. IMI in concentrations between 1 and 25 mg/l caused a significant decrease ($P < 0.05$) in amplitude, overshoot and peak maximum rate of phase 0 depolarization (dv/dt). No change was observed in the resting membrane potential. Perhaps the most significant finding was the effect on the action potential duration. IMI (0.5–25 mg/l) shortened phase 2 and accelerated the initial portion of repolarization. Thus, phase 3 occurred earlier, resulting in a significant decrease in the duration when measured at the 50% level of repolarization. On the other hand, it prolonged the terminal portion of phase 3 in such a way that the duration of the action potential at the 90% level of repolarization was not different from control values. At 5 mg/l, IMI suppressed the pacemaker activity elicited by Ba ions in ventricular fibers. At 5–10 mg/l, decreased the rate of rise, amplitude and duration of the Ca-mediated action potentials. In terms of changes in ion conductance these results can be explained by a reduction in the conductance for Na and Ca ions.

References

EDITORIAL (1971). Cardiovascular complications of tricyclic antidepressants. *N.Z. med. J.*, **74**, 390–391.