Journal of Chromatography, 342 (1985) 251—260
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2629

RAPID METHOD FOR THE MEASUREMENT OF METHYLPREDNISOLONE AND ITS HEMISUCCINATE IN PLASMA AND URINE FOLLOWING "PULSE THERAPY" BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

GRAHAM J. LAWSON

Department of Clinical Chemistry, Kent and Sussex Hospital, Tunbridge Wells, Kent TN4 8AT (U.K.)

(First received November 14th, 1984; revised manuscript received March 12th, 1985)

SUMMARY

A rapid method for the measurement of methylprednisolone and its 21-hemisuccinate ester in plasma and urine following high dose pulse therapy is described. The drugs were extracted using Extrelut® columns, eluted with ethyl acetate which was evaporated to dryness and the residue was reconstituted in chromatographic mobile phase. High-performance liquid chromatography was performed on a reversed-phase column using a mobile phase of acetonitrile—acetate buffer with detection at 251 nm. No interference from any drugs or endogenous compounds has been observed. The method has been used to analyse over 200 plasma and 150 urine samples from patients with rheumatoid disease or renal failure who have received high dose methylprednisolone hemisuccinate infusions.

INTRODUCTION

High doses of intravenous steroids have been useful in the treatment of several diseases. Solu-Medrone (Upjohn; methylprednisolone-21-hemisuccinate, MPHS) is a water-soluble ester which is rapidly hydrolysed to the active 21-hydroxycorticosteroid (MP) following parenteral administration [1] and has been used to treat glomerulonephritis, systemic lupus erythematosus, rheumatoid disease, shock and for the reversal of rejection episodes following organ transplantation. Intravenous infusions of MPHS lasting 15—20 min in doses of up to 2 g appear to be associated with a lower incidence of side-

*This work forms part of a thesis to be submitted to the University of Surrey for the degree of Ph.D.

0378-4347/85/$03.30

TABLE I

COMPARISON OF HPLC METHODS USED TO MEASURE MPHS AND/OR MP

Reference	MPHS	MP	Plasma	Urine	Dose	Sample volume (ml)	Acid preservative
7	No	Yes	Yes	No	MP 7 × 4 mg p.o.	2.5	No
10	Yes	Yes	Yes	No	MPHS 125 mg i.m.	0.5	No
8*	No	Yes	Yes	No	MP 20—40 mg p.o.	2.5	No
9	No	Yes	Yes	Yes	MPHS 12—20 mg/kg i.v.	1.0	No
11	Yes	Yes	Yes	No	MPHS 40 mg i.v.	"up to 1"	No
This paper	Yes	Yes	Yes	Yes	MPHS 0.5—2.0 g i.v.	0.5	Yes

*Modified from Garg et al. [7].

effects and complications than the more conventional, and smaller, oral maintenance doses [2].

The pharmacokinetics of such large intravenous doses of MPHS and MP are not well established. It is not always relevant to extrapolate from information on drugs obtained from low-dose to high-dose studies, especially in this case as MP is an analogue of prednisolone which has been shown to exhibit dose dependency [3]. Prednisolone is cleared more rapidly from serum when administered at higher doses. Sudden death following MPHS pulse therapy has been reported recently [4, 5]. This may be due to drug-induced arrhythmias. Monitoring MPHS and MP blood levels may be of use in investigating the aetiology of this complication.

Thin-layer chromatography has been used to measure corticosteroids although this technique lacks the required reproducibility. Radioimmunoassay is very sensitive, but the antibody (which is not available commercially) measures MPHS and MP equally [6]. This renders the method unsuitable for pharmacokinetic studies on the two drugs. High-performance liquid chromatography (HPLC) does possess the necessary selectivity and has been used to measure MP alone in serum [7—9] and urine [9], and MP and MPHS have been measured in serum following intramuscular injection [10] or intravenous infusion [11]. Assael et al. [9] are the only group to have examined blood and urine drug levels following high dose intravenous MPHS but just measured the hydrolysis product, MP using normal phase chromatography. In the present study both MPHS and MP were measured in blood and urine following high-dose MPHS infusion by the method described below.

Extraction	Chromatog. system (time, min)	Limit of detection (nmol/l)	Internal standard	Number of patients
Chloroform	Reversed (8)	80	MP acetate	1
Hexane Ethyl acetate	Reversed (7)	53 (both) 'estimated empirically'	17-OH-Progesterone	1
*	*	<27	*	5
Chloroform	Normal (11)	27	Dexamethasone	14
Methylene chloride	Normal (12)	27	Dexamethasone	1
Extrelut Ethyl acetate	Reversed (5)	MP-267 MPHS-603	11-Deoxy-17-hydroxy-corticosterone	28

HPLC methods available for measuring MP and/or MPHS are presented for comparison in Table I.

MATERIALS AND METHODS

Apparatus

A Varian Model 5010 liquid chromatograph was used with a Varian Model UV-50 variable wavelength detector. A stainless-steel column 100 mm × 5 mm I.D. (Shandon, Cheshire, U.K.) was packed with 5-μm Spherisorb S5 ODS 2 (HPLC Technology, Cheshire, U.K.) using a Shandon column packing instrument. The analytical column was surrounded by a Varian heating block. Samples were injected using a Valco injection valve fitted with a 10-μl loop. Chromatograms were recorded, and results calculated, by a Spectra-Physics SP 4270 Integrator.

Reagents

Acetonitrile (HPLC grade) was obtained from BDH (Poole, U.K.). Ethyl acetate, ammonium acetate, methanol, sulphuric acid and glacial acetic acid were AnalaR grade. 0.05 *M* acetate buffer was prepared by dissolving 3.85 g ammonium acetate in 1 l distilled water, the pH was then adjusted to 3.0 with glacial acetic acid.

Standards

Solu-Medrone, methylprednisolone and 11-desoxy-17-hydrocorticosterone

(internal standard) were gifts from Upjohn (Crawley, U.K.).

Stock standards of MP and internal standard, 1 mg/ml in methanol, were prepared, stored at 4°C and found to be stable over several months. MPHS stock standard, 1 mg/ml in distilled water, was prepared immediately before each assay as degradation to MP starts to occur after 24 h at 4°C. Subsequent dilutions for serum working standards were prepared in Technicon SMA reference serum (human) for the analysis of plasma samples, followed by the addition of 0.1 ml 3 *M* sulphuric acid/ml plasma. Urine working standards were diluted in urine from volunteers not receiving MPHS and adjusted to pH 2.0 with 3 *M* sulphuric acid. The internal standard (I.S.) was diluted to 50 μg/ml in 5% acetic acid.

Sample collection

Blood samples were collected at 0, ½, 1, 1½, 2, 3, 4, 6, 8, 24 h post infusion into heparinised tubes, centrifuged immediately and acidified by adding 0.1 ml 3 *M* sulphuric acid per ml of plasma. Urine samples collected at 0—2, 2—4, 4—8 and 8—24 h post infusion contained 0.5 ml 3 *M* sulphuric acid per 20 ml urine. All samples were stored at −20°C until analysed (usually within one week of collection).

Extraction

Extraction columns were prepared by placing a glass ball (3 mm diameter) into a 5-ml Macrotip (Laboratory, Industrial and Pharmaceuticals, Equipment and Services, West Yorkshire, U.K.) and adding Extrelut® (Merck) to a height of approximately 4 cm. 200 μl I.S. were added to 500 μl standard or test (plasma or urine), vortex mixed and applied to the Extrelut columns. After 10 min the columns were eluted with 5 ml ethyl acetate and the eluates evaporated to dryness under an air stream in a 40°C water bath. The residue was reconstituted in 100 μl mobile phase and 10 μl injected. Macrotips and glass balls were reused after washing, but the Extrelut was discarded.

Chromatography

The optimum mobile phase was found to be acetonitrile—acetate buffer (32.5:67.5, v/v) at a flow-rate of 1.5 ml/min. Chromatography was performed at 30°C with detection at 251 nm.

RESULTS

Fig. 1 shows the separation of MPHS, MP, I.S. and related compounds chromatographed using the described system. Representative chromatograms of patient samples who received MPHS are presented for plasma (Fig. 2a) and urine (Fig. 3a). No interfering peaks were detected in blood and urine samples taken immediately before infusion of MPHS (Figs. 2b and 3b). The following drugs may also be administered to patients receiving MPHS infusions and none was found to produce any chromatographic interference: prednisolone, prednisone, dexamethasone, cortisol, paracetamol, dextropropoxyphene, salicylic acid, oxyphenbutazone, indomethacin, penicillamine, piroxicam,

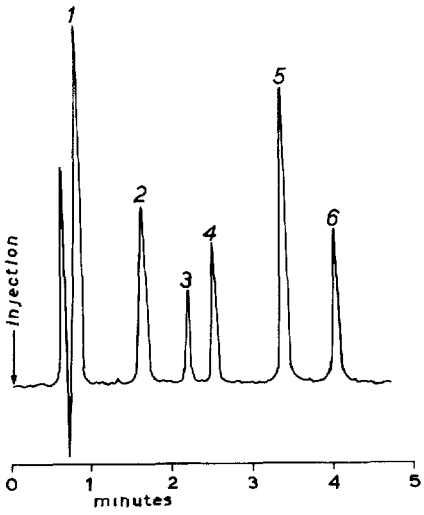

Fig. 1. Separation of cortisol (1), prednisone and prednisolone (2), methylprednisolone (3), dexamethasone (4), 11-desoxy-17-hydroxycorticosterone (5), methylprednisolone-21-hemisuccinate (6).

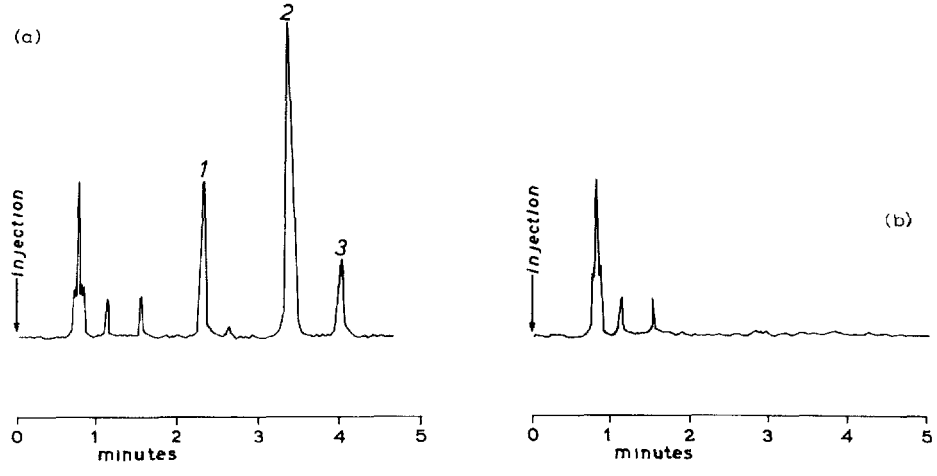

Fig. 2. (a) Chromatogram of a plasma sample from a patient with rheumatoid disease taken 30 min following infusion of 1 g MPHS, MP (1), I.S. (2), MPHS (3). (b) Chromatogram of a plasma sample from a patient taken immediately before infusion of 1 g MPHS.

flurbiprofen, naproxen and fenclofenac. Furthermore, no interference from endogenous compounds or drugs has been observed during the analysis of over 200 plasma and 150 urine samples.

Standard curves for MPHS and MP in serum and urine were linear up to 201 μmol/l and 267 μmol/l, respectively.

Recovery of MPHS and MP from serum and urine is shown in Table II. The recovery of I.S. at the concentration used was found to be 83.9%. These values were calculated by comparing the peak heights of extracted drugs against peak heights of the corresponding unextracted aqueous standards. It is important to

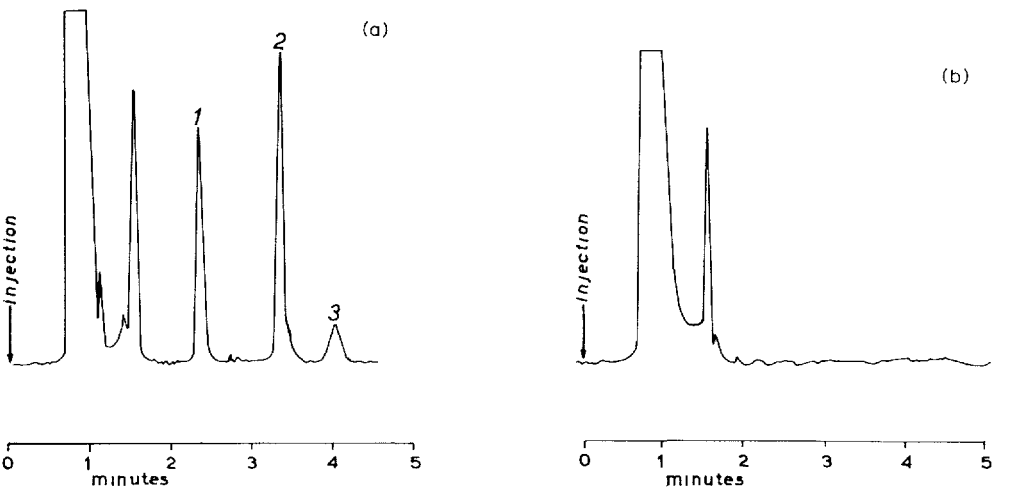

Fig. 3. (a) Chromatogram of a urine sample from a patient with rheumatoid disease collected 2—4 hours following infusion of 1 g MPHS, MP (1), I.S. (2), MPHS (3). (b) Chromatogram of a urine sample from a patient collected immediately before infusion of 1 g MPHS.

TABLE II

RECOVERY OF MPHS AND MP FROM SERUM AND URINE

n = number of samples.

		Amount of drug added (μmol/l)	n	Recovery (%)
Serum	MPHS	10.1	10	52.4
		40.2	10	56.4
		100.5	9	54.9
	MP	13.4	10	62.1
		53.4	10	63.9
		133.5	10	64.7
Urine	MPHS	2.0	9	89.2
		10.1	10	96.8
		100.5	10	92.4
	MP	2.7	10	93.0
		13.4	10	89.3
		133.5	10	83.0

note that the presence of acid in samples had little effect on the extraction efficiency of MP and I.S. but was crucial for MPHS. Without acid, recovery of MPHS from serum fell to less than 10%. The within- and between-batch coefficients of variation for serum and urine are shown in Table III. It was impossible to calculate between-batch variation for MPHS due to its slow degradation to MP over several weeks, despite being stored frozen with added acid. The lowest measurable levels of MPHS and MP were considered to be 603 nmol/l and 267 nmol/l, respectively. These levels represent approximately five times baseline noise.

MPHS and MP were measured in patients with severe renal failure, some of

TABLE III

PRECISION STUDIES FOR SERUM AND URINE

		Amount of drug added (μmol/l)	Within assay coefficient of variation	Between assay coefficient of variation
Serum	MPHS	2.0	13.7 (7*)	—**
		10.1	12.6 (10)	—**
		100.5	4.3 (10)	—**
	MP	2.7	7.6 (6)	15.1 (9)
		13.4	5.6 (10)	8.2 (10)
		133.5	5.7 (10)	8.6 (10)
Urine	MPHS	2.0	0.2 (10)	—**
		10.1	3.9 (9)	—**
		100.5	1.6 (9)	—**
	MP	2.7	9.6 (9)	9.6 (10)
		13.4	1.9 (10)	2.7 (10)
		133.5	1.4 (10)	3.4 (10)

*Number of samples.
**See text.

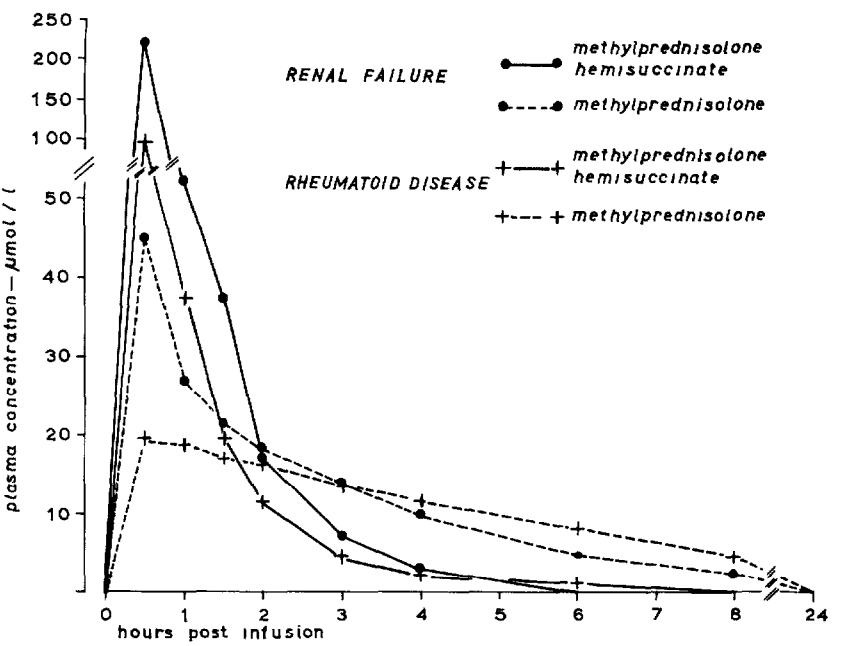

Fig. 4. Clearance of MPHS and MP from the plasma of a patient with rheumatoid disease and another with renal failure following a 1-g infusion of MPHS.

whom had received transplants, and those suffering from rheumatoid disease. Fig. 4 shows a typical example of the clearance of MPHS and MP from plasma following a 1-g infusion of MPHS given to a renal and a rheumatoid patient. Peak plasma levels were found at 30 min for MPHS and 30 min to 1 h for MP,

TABLE IV

MEAN PEAK PLASMA MPHS AND MP LEVELS (μmol/l) FOLLOWING INFUSION OF MPHS

Dose (g)	Rheumatoid disease		Renal failure	
	MPHS	MP	MPHS	MP
0.5	5.6 (3*)	15.5 (3)	67.6 (3)	18.7 (3)
1.0	23.7 (9)	26.7 (9)	189.0 (11)	36.1 (11)
2.0	55.9 (2)	36.6 (2)	—	—

*Number of patients.

TABLE V

MEAN % DOSE EXCRETED IN URINE AS FREE DRUG (MPHS + MP) PER 24 h FOLLOWING INFUSION

Dose (g)	Rheumatoid disease	Renal failure
0.5	22.2 (2*)	—
1.0	21.0 (6)	4.7 (7)
2.0	26.0 (2)	—

*Number of patients.

post-infusion of MPHS in both sets of patients (Table IV). MPHS was cleared more rapidly from plasma than MP; neither drug was detected in plasma 24 h after infusion. The amount of MPHS and MP excreted in the urine as the free drug during the 24 h following infusion is shown in Table V.

DISCUSSION

The method described was found to be selective for MPHS and MP with baseline separation from endogenous compounds and co-administered drugs. Sample preparation was easier and less time-consuming than previously reported methods, which use more classical extraction techniques [7—11]. This, coupled with faster chromatography enabled fifty patient samples to be analysed per day (Table I). The assay was developed for measuring MPHS and MP in biological fluids following "pulse" doses of MPHS. The high blood and urine levels of the drugs which result can easily be measured and therefore a smaller sample volume can be used when compared to other methods. This is important when multiple samples are taken from individual patients for pharmacokinetic studies. If a higher sensitivity is required, this can be achieved by increasing sample volume and using a larger injection loop. The present study is the only one in which MPHS and its hydrolysis product MP are measured in plasma and urine following "pulse" MPHS infusion. The other group to examine blood and urine following pulse therapy only measured MP, using a slower normal-phase chromatographic procedure [9]. However, their results may possibly be misleading since the analyses are based on the assump-

tion that MPHS is stable in human blood in vitro under their conditions of handling and storage (−20° C). In my experience, complete hydrolysis of MPHS to MP takes place after several weeks storage at −20°C, and even when preserved with acid very slow degradation takes place [1]. The findings of the present study also vary with those of Assael et al. [9] regarding the solubility of MPHS in ethyl acetate.

Peak plasma levels of MPHS and MP reflected the dose of MPHS administered. Patients with renal failure had consistently higher peak plasma levels of MPHS and MP than those with rheumatoid disease, which probably reflects the impaired renal function of the former. This could also explain the difference in urinary excretion of the free drug between the two patient groups and, in turn, would mean that there would be more opportunity for metabolism, further decreasing the amount of free drug excreted by renal patients.

However, a reduced clearance implies that the drugs would be detected in the plasma of renal patients for longer than those with rheumatoid disease but this was not found to be the case, even in patients given repeated infusions at 48-h intervals. The unaccounted for drug must have been metabolised, excreted into the urine as a compound at present undetectable or via some other route (e.g. faecal), or taken up into the general lipid depot and excreted at levels below the limit of detection of this assay over several days. Another study has reported less than 10% of the free drug recovered in urine [9] following pulse-dose MPHS. However, it is difficult to draw comparisons with this study as the conditions of sample collection and storage, extraction and chromatography are different. Slaunwhite and Sandberg [12] injected 0.4 mg of radioactively labelled MP intravenously and found 75% of the dose in the urine, of which 2.5% was unconjugated.

The ease and speed of sample preparation and chromatography make this method practical, while bulk purchase of Extrelut and reusable pipette tips for extraction columns contribute to low running costs. It is hoped that this method will help in the introduction of high-dose pulse MPHS infusions as an adjunct to established regimes for treating rheumatoid disease, or possibly as a more efficacious alternative to conventional oral low doses of steroids [13].

ACKNOWLEDGEMENTS

The author thanks Dr E.M. Baylis for help with the preparation of this manuscript, Miss M. Marchant for typing it and Miss J. Graham for the illustrations.

REFERENCES

1 B.D Anderson and V. Taphouse, J. Pharm. Sci., 70 (1981) 181.
2 Anonymous, Lancet, i (1977) 633.
3 W.J. Jusko and J.Q. Rose, Ther. Drug. Monit., 2 (1980) 169.
4 T.S. Bocanegra, M.O. Castaneda, L.R. Espinoza, F.B. Vasey and B.F. Germain, Ann. Intern Med., 95 (1981) 122.
5 R.E. Moses, A. McCormick and W. Nickey, Ann. Intern. Med., 95 (1981) 781.
6 I.A. Williams, E.M. Baylis and J. English, Scand. J. Rheumatol., 10 (1981) 153.

7 D.L. Garg, J.W. Ayres and J.G. Wagner, Res. Commun. Chem. Pathol. Pharmacol., 18 (1977) 137.
8 K.E. Anderson, B. Bengtsson, S.R. Helgesen, B. Lindergard and T. Lindholm, Scand. J. Urol. Nephrol., 14 (1980) 123.
9 B.M. Assael, G. Banfi, A.C. Appiani, A. Edefonti and W.J. Jusko, Eur. J. Clin. Pharmacol., 23 (1982) 429.
10 M.D. Smith, J. Chromatogr., 164 (1979) 129.
11 W.F. Ebling, S.J. Szefler and W.J. Jusko, J. Chromatogr., 305 (1984) 271.
12 W.R. Slaunwhite and A.A. Sandberg, J. Endocrinol. Metab., 21 (1961) 753.
13 I.A. Williams, E.M. Baylis and M.E. Shipley, Lancet, ii (1982) 237.