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High-performance liquid chromatography analysis, preliminary pharmacokinetics, metabolism and renal excretion of methylprednisolone with its C6 and C20 hydroxy metabolites in multiple sclerosis patients receiving high-dose pulse therapy

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Abstract

A gradient eluent HPLC analysis in human plasma and urine was developed and validated for methylprednisolone (MP), its prodrug methylprednisolone-21-hemisuccinate (MPS) with the metabolites 6β-hydroxy-6α-methylprednisolone (MPA), 20-hydroxymethylprednisolone (MPC), 6β-hydroxy-20α-hydroxymethylprednisolone (MPB), 6β-hydroxy-20β-hydroxymethylprednisolone (MPE), 20-carboxymethylprednisolone (MPD), methylprednisolone-glucuronide (MPF) and 21-carboxymethylprednisolone (MPX). The column was Cp Spherisorb C8 5 μm, 250 mm×4.6 mm I.D. (Chrompack, Bergen op Zoom, The Netherlands) with a guard column 75 mm×2.1 mm, packed with pellicular reversed-phase. The eluent was a mixture of acetonitrile and 0.067 *M* KH_2PO_4 buffer, pH 4.5. At $t=0$, the eluent consisted of 2% acetonitrile and 98% buffer (v/v). Over the following 35 min the eluent changed linearly until it attained a composition of 50% acetonitrile and 50% buffer (v/v). At 37 min ($t=37$) the eluent was changed over 5 min to the initial composition, followed by equilibration over 3 min. The flow-rate was 1.5 ml/min and UV detection was achieved at 248 nm. Preliminary pharmacokinetic data were obtained from one patient who showed illustrative plasma concentration–time curves and renal excretion-time profiles after a short-lasting infusion (0.5 h) of 1 g of methylprednisolone hemisuccinate. The half-life of prodrug methylprednisolone-21-hemisuccinate (MPS) was 0.3 h, that of metabolite MPX (21-carboxy MP) was 0.4 h and that of the parent drug methylprednisolone (MP) was 1.4 h. The half-lives of the metabolites are almost similar (4 h). The main compounds in the urine are methylprednisolone hemisuccinate (prodrug, 15.0%), methylprednisolone (parent drug, 14.6%), metabolite MPD (20-carboxy, 11.7%), and metabolite MPB (13.2%). The renal clearance values of metabolites MPB, MPC and MPD are approximately 500 ml/min, that of MP is 100 ml/min.

Keywords: Methylprednisolone

1. Introduction

High dose pulsed intravenous infusions or oral

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administrations of methylprednisolone hemisuccinate (MPS) have been used for many years as a treatment of acute relapses or progressive worsening of multiple sclerosis [1–5]. Clinical improvement or failure may be correlated with plasma concentrations and even better brain concentrations of methylprednisolone. A prerogative is the availability of an analytical method which is able to measure pro- and parent drug, with all the known metabolites, as the whole spectrum may enter the brain. The analysis of methylprednisolone (MP) and its prodrug the hemisuccinate (MPS) were described frequently in the literature [6–16]. Defer et al. analyzed elegantly the brain concentration of methylprednisolone after a high intravenous dose and reconstructed a kind of brain-elimination curve by taking one liquor sample of each patient, but at different times [3].

Only 5% of a 1 g intravenous dose was recovered in the urine as intact drug whether the bulk of the drug (i.e. 95%) undergoes hepatic metabolism. This provided a strong rationale to develop and validate a gradient elution method for quantification of MP and its metabolites (Fig. 1). Application of a gradient elution indicated that at least seven metabolites of methylprednisolone could be detected in plasma and urine, isolated from urine and identified [17].

The aim of this study was to validate the HPLC analysis of methylprednisolone and its metabolites in plasma and urine of multiple sclerosis patients after a high intravenous dose for therapeutic treatment.

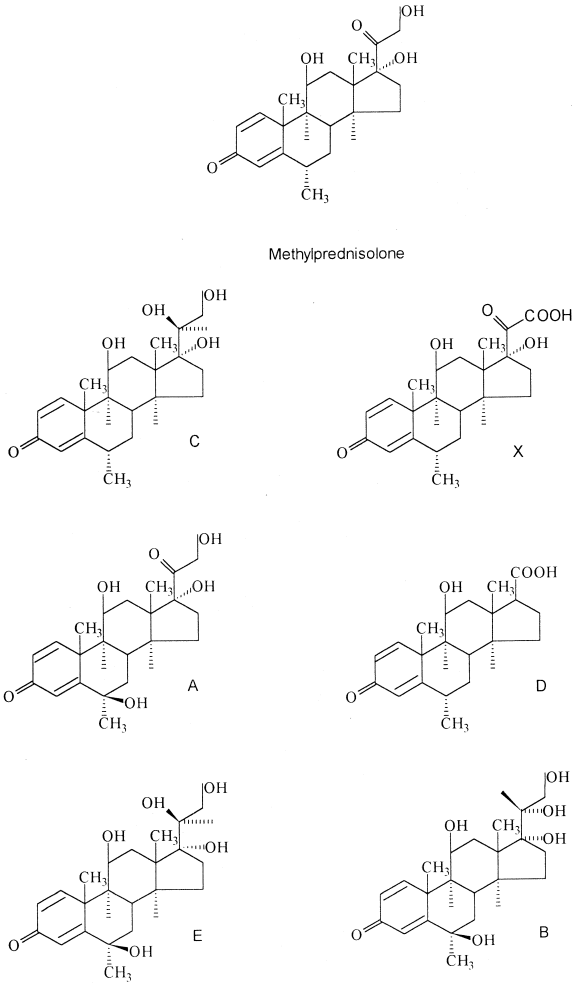

Fig. 1. Structure of methylprednisolone and metabolites.

2. Experimental

2.1. Chemicals

Methylprednisolone (p.a. quality) and methylprednisolone sodium hemisuccinate (p.a. quality; Solumedrol®) were obtained from Upjohn (Ede, The Netherlands) and Sigma (Zwijndrecht, The Netherlands). All other reagents were of p.a. quality and obtained from Merck (Darmstadt, Germany).

Methylprednisolone (11β,17α,21-trihydroxy-6α-methylpregna-1,4-diene-3,20-dione); $C_{22}H_{30}O_5$; MW 374.5; CAS number 83-43-2. Methylprednisolone-21-hemisuccinate; $C_{26}H_{34}O_8$; MW 474.6; CAS number 2921-57-5. Methylprednisolone-21-hemisuccinate sodium; $C_{26}H_{33}NaO_8$; MW 496.5; CAS number 2375-03-3.

The metabolites 6β-hydroxy-6α-methylprednisolone (MPA), 20-hydroxymethylprednisolone (MPC), 6β-hydroxy-20α-hydroxymethylprednisolone (MPB), 6β-hydroxy-20β-hydroxymethylprednisolone (MPE), 20-carboxymethylprednisolone (MPD), methylprednisolone-glucuronide (MPF) and 21-carboxymethylprednisolone (MPX) were isolated and identified as described elsewhere [17].

2.2. Gradient HPLC analysis

The HPLC system consisted of a Marathon autosampler (Separations, H.I. Ambacht, The Netherlands), a Spectra Physics SP 4000 quaternary HPLC pump, a Spectra Physics UV 2000 detector (Thermo Separation Products, Breda, The Netherlands), and a Hitachi D2500 integrator (Merck, Amsterdam, The Netherlands).

The column was Cp Spherisorb C8 5 μm, 250 mm×4.6 mm I.D. (Chrompack, Bergen op Zoom, The Netherlands) with a guard column 75 mm×2.1 mm, packed with pellicular reversed-phase (Chrompack Cat. no. 28653).

The eluent was a mixture of acetonitrile and 0.067 *M* KH_2PO_4 buffer, pH 4.5. At $t=0$, the eluent consisted of 2% acetonitrile and 98% buffer (v/v). Over the following 35 min the eluent changed linearly until it attained a composition of 50% acetonitrile and 50% buffer (v/v). At 37 min ($t=37$) the eluent was changed over 5 min to the initial composition, followed by equilibration over 3 min. The flow-rate was 1.5 ml/min and UV detection was achieved at 248 nm (Fig. 2).

The capacity factors of methylprednisolone and the metabolites are given in Table 1.

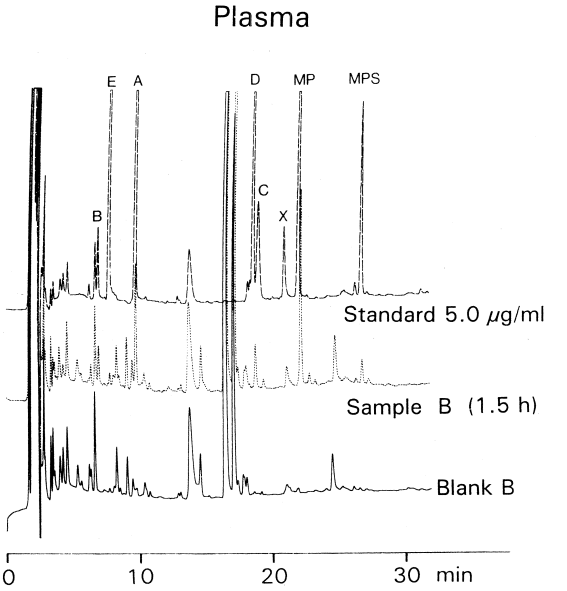

Fig. 2. Chromatograms of methylprednisolone (MP) and metabolites (MPA, -B, -C, -D, -E, -F, -X) in patient plasma sample, in blank plasma and blank plasma spiked with standards. Legend metabolites, see Tables 1 and 2.

Table 1
Retention times (t_r), capacity factors (k'), molecular weight (MW) and UV absorption maximum (nm) of methylprednisolone and metabolites[a]

	t_r (min)	k'	MW	UV_{max} (nm)
Methylprednisolone-21-hemisuccinate (MPS)	32.9	26.4	474	243
Methylprednisolone (MP)	29.0	23.2	374	243
Methylprednisolone X-21-COOH	28.0	22.3	390	243
Methylprednisolone C-20-βOH	26.2	20.8	376	243
Methylprednisolone F-glucuronide	25.2	20.0	548	242
Methylprednisolone D-20-COOH	24.3	19.3	344	241
Methylprednisolone A-C6-βOH	18.6	14.5	390	244
Methylprednisolone E-C6-βOH-20-βOH	16.5	12.8	392	244
Methylprednisolone B-C6-βOH-20-αOH	15.8	12.2	392	245
Hippuric acid	13.0	9.83	179	
Prednisolone	24.0	19.0	360	242
Prednisone	24.2	19.2	358	238
t_0	1.2			

[a] The metabolites 6β-hydroxy-6α-methylprednisolone (MPA), 20-hydroxymethylprednisolone (MPC), 6β-hydroxy-20α-hydroxymethylprednisolone (MPB), 6β-hydroxy-20β-hydroxymethylprednisolone (MPE), 20-carboxymethylprednisolone (MPD), methylprednisolone-glucuronide (MPF) and 21-carboxymethylprednisolone (MPX) were isolated and identified as described elsewhere [17].

2.3. Subjects

From six female patients of the Multiple Sclerosis Centre therapeutically treated with an intravenous dose of 1 g methylprednisolone hemisuccinate/day (Solumedrol®, Upjohn, Ede, The Netherlands), urine portions, blood samples and one liquor sample were collected. The study had the approval of the hospital ethics committee and informed consent was obtained from the patients.

2.4. Sampling

Blood samples were collected via an indwelling venous catheter at the start of the infusion ($t=0$) and 0.5 h (end of infusion), 1.5 h, 1.75 h, 2.25 h, 2.75 h, 3.75 h, 4.75 h, 5.75 h, 8 h, 11 h and 24 h. The infusion lasted 0.5 h.

One liquor sample was obtained of a series of MS patients at different time intervals after infusion with methylprednisolone hemisuccinate in order to reconstruct the liquor/brain concentration–time curve [3].

Urine was collected upon timed voiding (0–3 h; 3–5 h; 5–7 h; 7–9 h, 9–12 h, 12–15 h, 15–24 h) for construction of a renal excretion rate-time profile. The total time of sample collection was 24 h. Three aliquots (7 ml) of each void were stored at −20°C pending analysis.

2.5. Sample treatment

Plasma samples (0.150 ml) were deproteinized with 0.150 ml 0.33 *M* perchloric acid, vortexed for 10 s, centrifuged at 3000 *g* for 5 min, and 50 μl of the supernatant was injected onto the column.

Urine samples were centrifuged at 3000 *g*, the supernatant was diluted 1:10 with 0.02 *M* KH_2PO_4 buffer pH 6.8 and 50 μl was injected onto the column.

Liquor samples were centrifuged at 3000 *g*, the supernatant was diluted 1:1 with 0.02 *M* KH_2PO_4 buffer pH 6.8 and 50 μl was injected onto the column.

2.6. Limits of quantitation

The limits of detection in water and quantitation of methylprednisolone with its metabolites in plasma and urine were determined at a signal-to-noise ratio of 3, and shown in Table 2.

2.7. Recovery

A calibration curve of 5 concentrations in solvent was compared with a calibration curve of the same concentrations in plasma and urine.

2.8. Stock solutions

A stock solution of methylprednisolone and each of the metabolites was prepared in methanol (1 mg/ml). MPF, the glucuronide, was dissolved in water (0.5 mg/ml). Stock solutions of 0.5 ml were stored −20°C and were stable for 6 months.

2.9. Stability

The stability of methylprednisolone and its metabolites in plasma and urine was tested as follows: three samples of 2 ml urine containing the metabolites were brought to pH 5.0, 6.0 and 8.0.

Autosampler: the stability of spiked methylprednisolone and the metabolites in the autosampler in spiked plasma, water and eluent and in the three urine samples was tested over 48 h. Samples were taken every hour and injected onto the column.

2.10. Intra-day and inter-day variations

Intra- and inter-day coefficients of variation of accuracy and precision were obtained with standard plasma and urine samples of known concentrations of the analytes (Tables 4–7).

2.11. Calibration curves

Calibration curves for methylprednisolone and its metabolites in plasma and urine were constructed by spiking blank human plasma and urine samples with known concentrations of the compounds given in Table 3 ($r^2>0.999$).

2.12. Pharmacokinetics

Pharmacokinetic parameters were calculated using

Table 2
Limits of detection and quantitation and recovery of methylprednisolone and metabolites in plasma and urine ($n=6$)[a]

Compound	Detection limit (μg/ml in water)	Quantitation limit (μg/ml in matrix)
Plasma		
Methylprednisolone-21-hemisuccinate (MPS)	0.10	0.119
Methylprednisolone X (MPX)	0.10	0.370
Methylprednisolone (MP)	0.10	0.138
Methylprednisolone A (MPA)	0.20	1.024
Methylprednisolone B (MPB)	0.10	0.138
Methylprednisolone C (MPC)	0.20	1.024
Methylprednisolone D (MPD)	0.20	1.024
Methylprednisolone E (MPE)	0.10	0.138
Methylprednisolone F (MPF)	0.20	1.024
Urine		
Methylprednisolone-21-hemisuccinate (MPS)	0.10	0.119
Methylprednisolone X (MPX)	0.10	0.370
Methylprednisolone (MP)	0.10	0.138
Methylprednisolone A (MPA)	0.20	1.024
Methylprednisolone B (MPB)	0.10	0.138
Methylprednisolone C (MPC)	0.20	1.024
Methylprednisolone D (MPD)	0.20	1.024
Methylprednisolone E (MPE)	0.10	0.138
Methylprednisolone F (MPF)	0.20	1.024
Percentage recovery from plasma ($n=6$)		
Methylprednisolone-21-hemisuccinate (MPS)	101	
Methylprednisolone X (MPX)	102	
Methylprednisolone (MP)	101	
Methylprednisolone A (MPA)	100	
Methylprednisolone B (MPB)	100	
Methylprednisolone C (MPC)	102	
Methylprednisolone D (MPD)	102	
Methylprednisolone E (MPE)	100	
Methylprednisolone F (MPF)	101	

[a] The metabolites 6β-hydroxy-6α-methylprednisolone (MPA), 20-hydroxymethylprednisolone (MPC), 6β-hydroxy-20α-hydroxymethylprednisolone (MPB), 6β-hydroxy-20β-hydroxymethylprednisolone (MPE), C20-carboxymethylprednisolone (MPD), methylprednisolone-glucuronide (MPF) and 21-carboxymethylprednisolone (MPX) were isolated and identified as described elsewhere [17].

both a 2-compartment model and a non-compartmental analysis using the MW/Pharm computer package (Mediware®, Groningen, The Netherlands) [18].

C_{max} was the maximum plasma concentration read from the fitted plasma concentration–time curve ($r^2>0.98$), and t_{max} the time at which C_{max} occurred. The elimination half-life ($t_{1/2\beta}$) values were calculated from $\ln 2/\beta$, where β is calculated by log-linear regression analysis of the terminal log-linear phase. AUC_{0-t} was the area under the plasma concentration–time curve and was calculated using the linear trapezoidal rule, using C_t/β, with C_t being the last measured concentration.

Apparent $t_{1/2\ absorption}$ is the apparent absorption half-life of the metabolites, which is a composite of rates of formation and elimination. $t_{1/2\alpha}$ is the half-life of the fast elimination phase and $t_{1/2\beta}$ that of the terminal elimination phase. Total body clearance (CL) is described as F. Dose/AUC_{0-t} ($F=1$).

$AUC_{0-\infty}$ was the area under the plasma concentration–time curve and was calculated using the linear trapezoidal rule, using C_t/β, with C_t ($t=\infty$, h) being the last and extrapolated concentration. Body

Table 3
Calibration curves for methylprednisolone and its metabolites (urine)[a]

Compound	Concentration (μg/ml)	Curve[b]	Correlation coefficient (r^2)
Methylprednisolone-21-hemisuccinate (MPS)	1.26–21.6	$y=269\cdot x+178$	0.9994
Methylprednisolone X (MPX)	1.43–22.8	$y=51.8\cdot x-77.0$	0.9989
Methylprednisolone (MP)	1.25–20.0	$y=220\cdot x-1.04$	0.9996
Methylprednisolone A (MPA)	1.26–20.2	$y=435\cdot x+44.7$	0.9996
Methylprednisolone B (MPB)	1.25–20.0	$y=512\cdot x+62.5$	0.9999
Methylprednisolone C (MPC)	1.20–19.2	$y=398\cdot x-88.6$	0.9994
Methylprednisolone D (MPD)	1.30–20.8	$y=313\cdot x+140$	0.9981
Methylprednisolone E (MPE)	1.37–22.0	$y=411\cdot x+76.8$	0.9999
Methylprednisolone F (MPF)	1.25–20.0	$y=211\cdot x+131$	0.9964

[a] Legend metabolites, see Tables 1 and 2.
[b] y=peak height (integration units and x=concentration (μg/ml).

clearance (CL) is described as $F\cdot\text{Dose}/AUC_{0-t}$, with the bioavailability $F=1$. $V_d=F\cdot\text{Dose}/C_0$, the volume of distribution in the central compartment ($F=1$). $V_\beta=CL/\beta$, the apparent volume of distribution. $V_{ss}=F\cdot\text{Dose}\cdot AUMC_{0-\infty}/AUC^2_{0-\infty}$, the volume of distribution at steady-state ($F=1$). Mean residence time (MRT)$=AUMC_{0-\infty}/AUC_{0-\infty}$, where $AUMC_{0-\infty}$ is the area under the moment curve from zero to infinity.

Renal clearance of each compound is calculated as mg excreted over the total excretion period divided by the corresponding AUC. Renal excretion rate (μg/min) is calculated as concentration in a urine sample (μg/ml) multiplied with the average urine production over the urine collection period (ml/min).

Table 4
Intra-day coefficient of variation (precision, C.V., %) of spiked methylprednisolone and metabolites in urine ($n=5$)[a]

	Compound and concentration (μg/ml)								
	MP	MPS	MPA	MPB	MPC	MPD	MPE	MPF	MPX
Intra-day variation									
Low concentration									
conc added	1.263	1.260	1.265	1.250	1.200	1.300	1.375	1.250	1.425
conc found	1.129	0.720	1.489	1.199	1.361	1.016	1.393	0.757	1.541
S.D.	0.082	0.046	0.086	0.044	0.110	0.086	0.136	0.011	0.052
%C.V.	6.7	6.4	5.8	3.7	8.1	8.5	9.8	1.5	3.4
accuracy%	3.4	42.9	17.7	4.1	13.4	21.9	1.3	39.4	8.2
Medium concentration									
conc added	5.050	5.040	5.050	5.000	4.800	5.200	5.500	5.000	5.700
conc found	5.490	5.533	5.053	5.246	4.791	5.183	5.348	5.020	5.558
S.D.	0.166	0.243	0.422	0.078	0.298	0.130	0.067	0.032	0.155
%C.V.	3.0	4.4	7.7	1.5	6.2	2.5	1.3	0.6	2.8
accuracy%	8.7	9.8	0.1	4.9	0.2	0.3	2.8	0.4	2.5
High concentration									
conc added	20.290	21.600	20.200	20.000	19.200	20.800	22.000	20.000	22.800
conc found	20.226	21.368	20.363	20.033	19.277	20.443	22.017	19.505	22.725
S.D.	0.104	0.069	0.181	0.084	0.173	0.015	0.084	0.028	0.081
%C.V.	0.5	0.3	0.9	0.4	0.9	0.1	0.4	0.4	0.4
accuracy%	0.3	1.1	0.8	0.2	0.4	1.7	0.1	2.5	0.3

[a] Legend metabolites, see Tables 1 and 2.

Table 5
Inter-day coefficient of variation (precision, C.V., %) of spiked methylprednisolone and metabolites in urine ($n=5$)[a]

	Compound and concentration (μg/ml)								
	MP	MPS	MPA	MPB	MPC	MPD	MPE	MPF	MPX
Inter-day variation									
Low concentration									
conc added	1.263	1.260	1.265	1.250	1.200	1.300	1.375	1.250	1.425
conc found	1.173	0.743	1.455	1.142	1.401	1.044	1.452	0.783	1.522
S.D.	0.029	0.045	0.035	0.108	0.021	0.088	0.044	0.037	0.034
%C.V.	2.5	6.1	2.4	9.5	1.5	8.4	3.0	4.7	2.2
accuracy%	7.6	69.6	13.1	9.4	14.3	24.5	5.32	59.6	6.3
Medium concentration									
conc added	5.050	5.040	5.050	5.000	4.800	5.200	5.500	5.000	5.700
conc found	5.507	5.524	5.138	5.233	4.819	5.177	5.470	5.023	5.680
S.D.	0.159	0.197	0.225	0.137	0.207	0.125	0.108	0.033	0.136
%C.V.	2.9	3.6	5.0	2.6	4.3	2.4	2.0	0.1	2.4
accuracy%	8.3	8.8	1.7	4.5	0.4	0.4	0.6	0.5	0.4
High concentration									
conc added	20.290	21.600	20.200	20.000	19.200	20.800	22.000	20.000	22.800
conc found	20.205	21.364	20.055	20.010	19.049	20.979	22.101	19.816	22.739
S.D.	0.115	0.067	0.406	0.098	0.316	0.327	0.236	0.372	0.091
%C.V.	0.6	0.3	2.0	0.5	1.7	1.6	1.1	1.9	0.4
accuracy%	0.4	1.1	0.7	0.1	0.8	0.0	0.5	0.9	0.3

[a] Legend metabolites see, Tables 1 and 2.

3. Results

3.1. High-performance liquid chromatography

Fig. 2 shows the chromatograms of MP and its metabolites in plasma of a MS patient, a chromatogram of a blank plasma and blank plasma spiked with the standards. All compounds are well separated of each other. Fig. 3 shows similar data of the chromatograms of urine sample of the same patient as in Fig. 2.

Table 1 shows the retention times, capacity factors, molecular weight and UV_{max} values of methylprednisolone, its prodrug and metabolites. The limits of detection and quantitation and recovery values of methylprednisolone and metabolites in plasma and urine are shown in Table 2.

Table 3 shows the calibration curves and corresponding correlation coefficients of methylprednisolone and its metabolites.

Tables 4 and 5 give respectively the intra- and inter-day coefficients of variation of methylprednisolone and metabolites in plasma and urine samples.

3.2. Pharmacokinetics

Fig. 4a shows the plasma concentration–time curves of prodrug methylprednisolone hemisuccinate (MPS), parent drug methylprednisolone (MP) and that of metabolite X.

Fig. 4b shows the plasma concentration–time curves of parent drug methylprednisolone (MP) and those of the metabolites MPA, -C, -B, -E and -D.

The half-lives of prodrug methylprednisolone-21-hemisuccinate (MPS) was 0.3 h, that of metabolite MPX (21-carboxy MP) was 0.4 h and that of the parent drug methylprednisolone (MP) was 1.4 h. The half-lives of the metabolites are almost similar (4 h) and longer than that of the parent drug (1.4 h). The liquor concentrations of methylprednisolone and its metabolites were below the quantitation limit at $t=$ 23 h.

Fig. 5 shows the renal excretion rate-time curves

Table 6
Intra-day coefficient of variation (precision, C.V., %) of spiked methylprednisolone and metabolites in plasma ($n=7$)[a]

	Compound and concentration (μg/ml)								
	MP	MPS	MPA	MPB	MPC	MPD	MPE	MPF	MPX
Intra-day variation									
Low concentration									
conc added	0.633	0.630	0.631	0.675	0.663	0.625	0.644	0.638	0.71
conc found	0.843	0.740	0.738	0.851	0.791	0.686	0.767	0.541	-.-[b]
S.D.	0.049	0.080	0.017	0.019	0.007	0.029	0.011	0.064	-.-
%C.V.	5.8	10.8	2.3	2.9	0.9	4.2	1.4	11.8	-.-
accuracy%	33.2	17.5	16.8	26.1	19.4	9.7	19.2	15.2	-.-
Medium concentration									
conc added	2.56	2.52	2.53	2.70	2.65	2.60	2.58	2.55	2.85
conc found	2.23	2.31	2.55	2.78	2.54	1.89	2.61	2.54	4.05
S.D.	0.066	0.10	0.077	0.077	0.059	0.037	0.078	0.055	0.116
%C.V.	2.9	4.4	3.0	2.8	2.3	2.0	3.0	2.2	2.9
accuracy%	13.0	8.2	0.1	3.1	4.0	27.3	1.2	0.4	42.0
High concentration									
conc added	10.10	10.80	10.10	10.80	10.60	10.40	10.30	10.80	11.40
conc found	11.19	12.448	10.67	11.303	11.81	10.52	10.96	11.87	14.465
S.D.	0.348	0.385	0.364	0.322	0.339	0.372	0.350	0.342	0.445
%C.V.	3.11	3.1	3.4	2.9	2.9	3.5	3.2	2.9	3.1
accuracy%	10.8	15.2	5.6	4.6	11.4	1.1	6.4	9.9	26.8

[a] Legend metabolites, see Tables 1 and 2.
[b] -.- Interference.

Table 7
Inter-day coefficient of variation (precision, C.V., %) of spiked methylprednisolone and metabolites in plasma ($n=7$)[a]

	Compounds and concentration (μg/ml)								
	MP	MPS	MPA	MPB	MPC	MPD	MPE	MPF	MPX
Inter-day variation									
Low concentration									
conc added	1.265	1.260	1.263	1.350	1.325	1.300	1.288	1.265	1.425
conc found	1.372	1.325	1.245	1.305	1.458	1.412	1.276	0.368	1.532
S.D.	0.024	0.057	0.022	0.068	0.082	0.091	0.018	0.044	0.023
%C.V.	1.7	4.3	1.8	5.2	5.6	6.5	1.4	3.3	1.5
accuracy%	8.4	5.2	1.4	3.4	10.0	8.6	0.9	8.2	7.5
Medium concentration									
conc added	5.050	5.400	5.050	5.400	4.300	5.200	5.150	5.400	5.700
conc found	4.837	5.121	5.028	5.458	4.767	5.024	5.150	5.129	5.474
S.D.	0.139	0.254	0.103	0.185	0.252	0.214	0.109	0.247	0.160
%C.V.	2.9	5.0	2.1	3.4	5.3	4.3	2.1	4.8	2.9
accuracy%	4.2	5.2	0.4	1.1	10.1	3.4	0.0	5.0	4.0
High concentration									
conc added	10.100	10.800	10.100	10.800	10.600	10.400	10.300	10.800	11.400
conc found	10.242	10.946	10.131	10.814	10.862	10.615	10.327	10.918	11.650
S.D.	0.070	0.119	0.055	0.092	0.129	0.252	0.053	0.114	0.226
%C.V.	0.7	1.1	2.0	0.9	1.2	2.4	0.5	1.0	1.9
accuracy%	1.4	1.4	0.3	0.1	2.5	2.1	0.3	1.1	2.2

[a] Legend metabolites, see Tables 1 and 2.

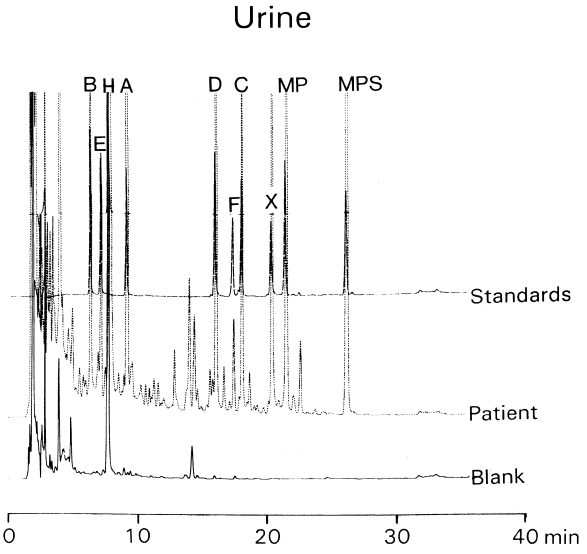

Fig. 3. Chromatograms of methylprednisolone (MP) and metabolites (MPA, -B, -C, -D, -E,-F, -X) in patient urine sample, in blank urine and blank urine spiked with standards. Legend metabolites, see Tables 1 and 2.

of the parent drug methylprednisolone (MP) and those of metabolites MPA, -B, -C, -D, -E and -F in a MS patient after infusion of 1 g MPS. Fig. 6 shows the plasma concentration–time curves and renal excretion rate-time profiles and renal clearance values of MP, and the metabolites MPA and -C.

Table 8 gives the pharmacokinetic parameters of MP and its metabolites in one patient after an infusion of 1 g MP hemisuccinate.

Table 9 shows the renal clearance and excretion values of MP and its metabolites expressed as percentage of the dose. The main compounds in the urine are methylprednisolone hemisuccinate (prodrug, 15.0%), methylprednisolone (parent drug, 14.6%), metabolite MPD (20-carboxy, 11.7%), and metabolite MPB (13.2%). The renal clearance values of metabolites MPB, -C and -D are approximately 500 ml/min, that of MP 100 ml/min.

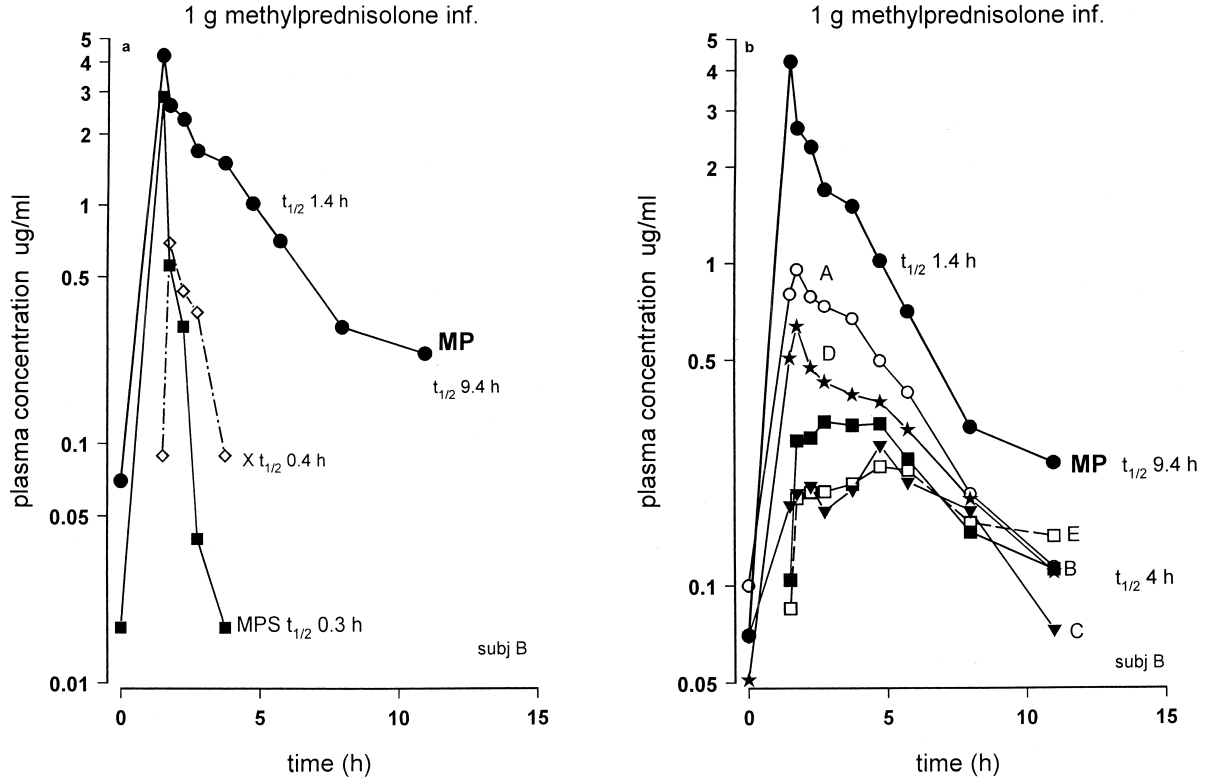

Fig. 4. (a): Plasma concentration–time curves of methylprednisolone (MP), its prodrug 21-hemisuccinate (MPS) and metabolite MPX (21-carboxymethylprednisolone). (b): Plasma concentration–time curves of methylprednisolone (MP), and metabolites MP A–E). Compound F, the glucuronide is not present in plasma. Legend metabolites, see Tables 1 and 2.

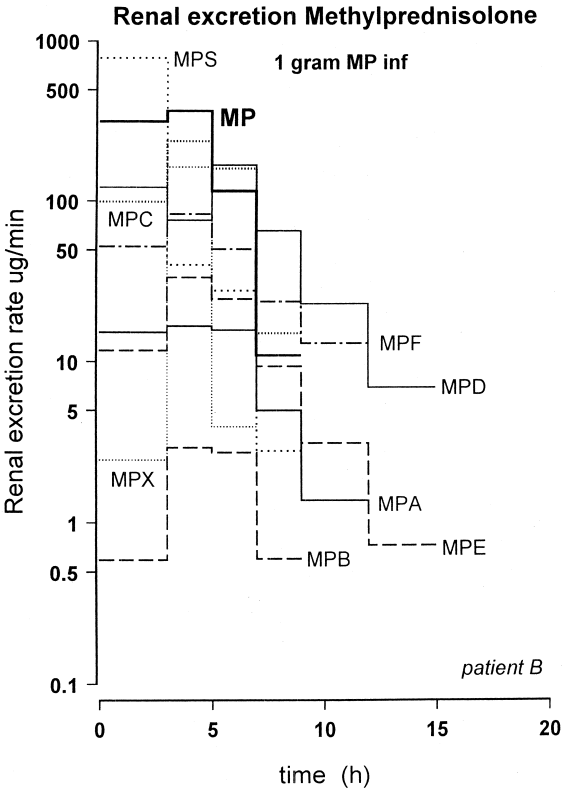

Fig. 5. Renal excretion rate-time profiles of methylprednisolone (MP), prodrug MPS, and each of its metabolites (MPA-X) in one patient after receiving 1 g of MPS intravenously. Legend metabolites, see Tables 1 and 2.

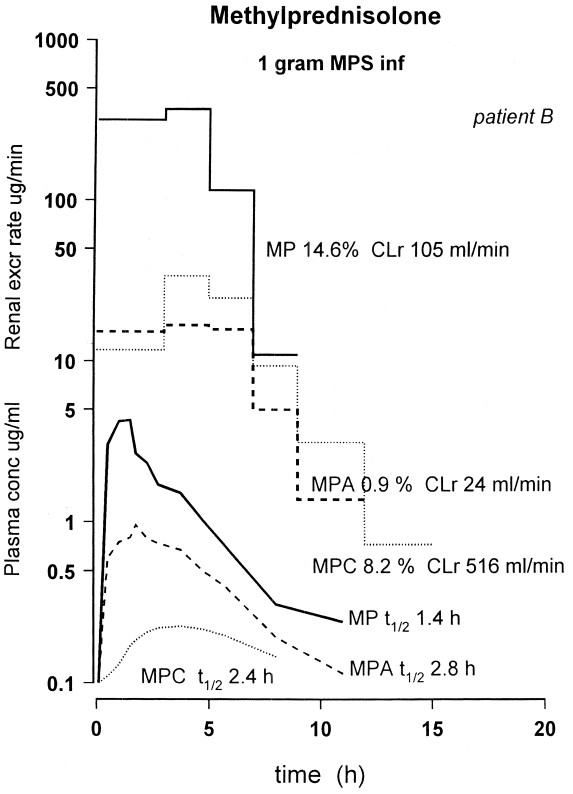

Fig. 6. Plasma concentration–time curves and renal excretion rate-time profiles of methylprednisolone (MP), and metabolites MPA, and MPC, representing metabolites of first metabolic attack, oxidation to 6-βOHMP and reduction of C20 C=O to CHOH in one patient after receiving 1 g of MPS intravenously. Legend metabolites, see Tables 1 and 2.

4. Discussion

We first have isolated the compounds with the most abundant concentration and suggested structural formulae [17]. The metabolites of methylprednisolone were clearly visible when a gradient eluent was used in the HPLC analysis. In this study the validation of the HPLC analysis of methylprednisolone and seven metabolites was performed, plasma concentration–time curves with renal excretion-time profiles obtained, and preliminary pharmacokinetic parameters calculated. For reasons of clarity the results of one patient were chosen to illustrate the validity of the method. The method is relatively simple because apparently the group contribution of the different moieties of the steroid skeleton differed highly to enable a good separation of the compounds. Additionally the applied dose is high, so are the concentrations in plasma and urine. Metabolites MPD and MPX (the carboxylic acids) need a high buffer capacity in the mobile phase otherwise the retention times are not constant and drift to higher values. Therefore a phosphate buffer was chosen over an acetate buffer; the latter buffer can be used for short term analysis in the LC–MS, and preparative HPLC.

4.1. Renal clearance

The renal clearance of methylprednisolone is 100 ml/min, apparently indicating glomerular filtration. The renal clearance values of metabolite MPB, -C and -D are higher than that of creatinine, indicating

Table 8
Pharmacokinetic parameters of methylprednisolone-hemisuccinate and its metabolites in one female patient[a]

	Parent drug and metabolites								Total
	MP-21-S	MP	MPA	MPB	MPC	MPD	MPE	MPX	
Doses (mg)	1000	787							
Doses (mmol)	2.11	2.10	-.-[b]	-.-	-.-	-.-	-.-	-.-	
Parameter									
AUC (mg/l h)	2.92	14.05	4.50	2.16	1.82	3.15	1.82	1.31	
AUC (μmol/l h)	6.16	37.56	11.54	5.51	4.67	9.13	4.64	2.76	81.97
AUC (%total)	7.51	45.82	14.08	6.72	5.70	11.14	5.66	3.37	100
Clearance[c] (l/h)	72.8	43.6	-.-	-.-	-.-	-.-	-.-	-.-	
V_d (l)	32.8	123	-.-	-.-	-.-	-.-	-.-	-.-	
V_{ss} (l)	33.0	250	-.-	-.-	-.-	-.-	-.-	-.-	
V_β (l)	33.5	588	-.-	-.-	-.-	-.-	-.-	-.-	
$t_{1/2\ abs}$ (h)	-.-	0.08	0.56	2.52	2.46	0.40	3.38	0.42	
$t_{1/2\alpha}$ (h)	0.23	1.43	-.-	-.-	-.-	-.-	-.-	-.-	
$t_{1/2\beta}$ (h)	0.32	9.37	2.80	2.52	2.46	3.93	3.38	0.42	
MRT (h)	0.45	5.86	4.85	7.28	7.09	6.23	9.75	2.24	
t_{max} (h)	-.-	0.34	1.63	3.64	3.55	1.45	4.87	1.64	
C_{max} (mg/l)	-.-	5.45	0.86	0.28	0.23	0.54	0.21	0.68	

[a] Plasma concentrations at time $t=23$ h are 0 (below detection limit). MPF, the glucuronide, is not present in plasma. Legend metabolites, see Tables 1 and 2.
[b] -.- Not present.
[c] C1=F·dose/*AUC*, bioavailability $F=1$.

glomerular filtration plus active tubular secretion. The difference in renal clearance between the isomers MPB and -E, therefore may indicate that the low renal clearance of MPE belongs to the structure whose 20-hydroxy group is not available for binding at the tubular secretion mechanism, thus presumably folded over the steroid skeleton, in the β-position.

Table 9
Renal clearance and percentage of the dose excreted of methylprednisolone and its metabolites after a short infusion of 1 g methylprednisolone hemisuccinate in one female patient

Compound	Excretion (% dose)	Renal clearance (ml/min)
MPS	15.0	184
MP	14.6	106
MPA	0.9	24
MPB[a]	13.2	665
MPC	8.2	516
MPD	11.7	471
MPE[a]	0.2	10
MPF-glucuronide	2.5	-.-
MPX	1.7	170
Total	68.0	

[a] B=*E* isomers. Legend metabolites, see Tables 1 and 2.

Oxidation of the 6-position to 6β-hydroxy-6α-methylprednisolone (MPA) appears unfavourable for renal excretion, the renal clearance drops from 100 ml/min to 25 ml/min, apparently due to tubular reabsorption.

4.2. Metabolism

The overall picture of the metabolic pathways of methylprednisolone is apparently simple: reduction of the 20-carbonyl group and oxidation of the C20–C21 side chain, in competition with or additional to the oxidation at the 6-position [17].

Reduction of the 20-carbonyl group results in the stereoisomers 20α-hydroxy, and 20β-hydroxy-methylprednisolone.

5. Conclusion

We developed and validated a relatively simple HPLC analysis with gradient elution for the analysis of methylprednisolone and seven isolated metabolites in human plasma and urine of human patients with

multiple sclerosis. Preliminary pharmacokinetics showed that metabolites in urine are the administered prodrug (15.0%), the parent drug (14.6%), metabolite D (20-carboxy MP, 11.7%) and metabolite B (6β-hydroxy-20α-hydroxymethylprednisolone, 13.2-%). The half-life of prodrug methylprednisolone-21-hemisuccinate (MPS) was 0.3 h, that of metabolite X (21-carboxy MP) was 0.4 h and that of the parent drug methylprednisolone (MP) was 1.4 h. The half-lives of the metabolites are almost similar (4 h) and longer than that of the parent drug (1.4 h).

References

[1] M.P. Barnes, D.E. Bateman, P.G. Cleland, D.J. Dick, T.J. Walls, P.K. Newman, J. Neurol. Neurosurg. Psychiatry 48 (1985) 157.
[2] D.A. Compston, Arch. Neurol. 45 (1988) 669.
[3] G.L. Defer, J. Barré, P. Ledulal, J.P. Tillement, J.D. Degos, Eur. Neurol. 35 (1995) 143.
[4] D.S. Goodin, Neurology 41 (1991) 980.
[5] N.M. Milligan, R. Newcombe, D.A.S. Compston, J. Neurol. Neurosurg. Psychiatry 50 (1987) 511.
[6] M. Alvinerie, P.L. Toutain, J. Chromatogr. 309 (1984) 385.
[7] B.D. Anderson, V. Taphouse, J. Pharm. Sci. 70 (1981) 181.
[8] B.D. Anderson, R.A. Conradi, W.J. Lambert, J. Pharm. Sci. 73 (1984) 604.
[9] W.F. Ebling, S.J. Szefler, W.J. Jusko, J. Chromatogr. 305 (1984) 271.
[10] W.F. Ebling, S.J. Szefler, W.J. Jusko, Drug Metab. Dispos. 13 (1985) 296.
[11] B.M. Frey, F.J. Frey, J. Chromatogr. 229 (1982) 283.
[12] D.B. Haughey, W.J. Jusko, J. Pharm. Sci. 81 (1992) 117.
[13] M. Jonung, S. Björkman, J.J. Idvall, Chromatogr. 415 (1987) 35.
[14] G.J. Lawson, J. Chromatogr. 342 (1985) 251.
[15] J.A. Shah, D.J. Weber, J. Chromatogr. 344 (1985) 41.
[16] M.D. Smith, J. Chromatogr. 164 (1979) 129.
[17] T.B. Vree, C.P.G.W.M. Verwey-van Wissen, A.J. Lagerwerf, A. Swolfs, R.A.A. Maes, R.D. van Ooijen, O.R. van Eikema Hommes, P.J.H. Jongen, J. Chromatogr. B 726 (1999) 157.
[18] J.H. Proost, D.K.W. Meijer, Comput. Biol. Med 22 (1992) 155.