Pharmacokinetic and pharmacodynamic assessment of bioavailability for two prodrugs of methylprednisolone

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Aims The aim of this study was to establish whether pharmacokinetic differences between two pro-drugs of methylprednisolone (MP) are likely to be of clinical significance.

Methods This study was a single-blind, randomized, crossover design comparing the bioequivalence of MP released from the pro-drugs Promedrol (MP suleptanate) and Solu-Medrol (MP succinate) after a single 250 mg (MP equivalent) intramuscular injection to 20 healthy male volunteers. Bioequivalence was assessed by conventional pharmacokinetic analysis, by measuring pharmacodynamic responses plus a novel approach using pharmacokinetic/pharmacodynamic modeling. The main measure of pharmacodynamic response was whole blood histamine (WBH), a measure of basophil numbers.

Results The MP C_{max} was less for MP suleptanate due to a longer absorption half-life of the prodrug from the intramuscular injection site. The bioavailability of MP was equivalent when based on AUC with a MP suleptanate median 108% of the MP succinate value (90% CI: 102–114%). For C_{max} the MP suleptanate median was 81% of the MP succinate value (90% CI: 75–88%). The t_{max} for MP from MP suleptanate was delayed relative to MP succinate. The median difference was 200% (90% non-parametric CI: 141–283%). The area under the WBH effect-time curve (AUEC) and the maximum response (E_{max}) were found to be equivalent (90% CI: 98–113% and 93–109% respectively). The maximum changes in other white blood cell counts, blood glucose concentration and the parameters of the pharmacodynamic sigmoid Emax model (EC_{50}, E_{max} and γ) were also not significantly different between prodrugs.

Conclusions MP suleptanate is an acceptable pharmaceutical alternative to MP succinate. The use of both pharmacokinetic and pharmacodynamic response data together gives greater confidence in the conclusions compared with those based only on conventional pharmacokinetic bioequivalence analysis.

Keywords: methylprednisolone, suleptanate, succinate, Solu-medrol, Promedrol, intramuscular, bioavailability, bioequivalence, pharmacodynamics, modeling, histamine

Introduction

The assessment of bioequivalence by using pharmacokinetic and statistical analysis is the accepted method of demonstrating therapeutic equivalence for different formulations of the same drug. This approach obviates the need to conduct clinical efficacy trials in order to demonstrate that a new formulation of an established drug is safe and effective. However, there are circumstances when the strict criteria of pharmacokinetic bioequivalence may not be readily met, in this case there is a need to establish whether the differences between the two formulations are likely to be of clinical significance. The approach used in this study was to assess comparative bioavailability in terms of conventional pharmacokinetics (PK) and in addition to use pharmacodynamics (PD) responses plus a novel approach using pharmacokinetic/pharmacodynamic (PK/PD) modeling. The PD and PK/PD data were interpreted together with the PK data, the aim being to establish whether any pharmacokinetic differences between two pro-drugs of methylprednisolone are likely to be of clinical significance.

Medrol (6-α-methylprednisolone) has been marketed as an injectable corticosteroid in the form of the sodium succinate ester (Solu-Medrol, Solu-Medrone) for more than 20 years. MP succinate is not stable in solution and is therefore marketed as a freeze-dried product that requires reconstitution resulting in inconvenience, waste, loss of time in preparation and added cost. Promedrol (methylprednisolone suleptanate: 21-{8-(2-sulphoethyl) methyl amino}-8-oxo-octanoate, sodium salt) is being developed by the Upjohn Company as a pharmaceutical alternative to MP succinate and will be available as a ready to inject solution. This study addresses the question of whether MP suleptanate is bioequivalent to MP succinate following a single intramuscular administration to healthy volunteers. This assessment was conducted using three approaches: (i) pharmacokinetically, i.e. with respect to the release of methyl-

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prednisolone, (ii) by the comparison of methylprednisolone dependent pharmacodynamic responses and (iii) by comparing the parameters of a pharmacokinetic/pharmacodynamic model.

Both MP suleptanate and MP succinate are prodrug esters that are converted to the active agent, methylprednisolone, by the action of esterase enzymes *in vivo*. Studies in animals and man have shown that the pharmacokinetics of methylprednisolone released from MP suleptanate are similar to those of MP succinate [1–4]. However, the rate at which the esterases release methylprednisolone from MP suleptanate is faster than for MP succinate [3].

Apart from establishing the safety of the Promedrol suleptanate moiety, the critical issue is whether the rate and extent of methylprednisolone bioavailability is sufficiently similar for the two produgs that no clinically significant differences in efficacy or safety results. MP suleptanate and MP succinate are not different formulations of the same chemical entity but chemically different prodrugs of methylprednisolone with dissimilar kinetic parameters. Consequently it was likely that bioequivalence in terms of rate of methylprednisolone appearance in plasma may not be found and therefore a conventional bioequivalence comparison of the two prodrugs alone may not be appropriate. For this reason the prodrug pharmacodynamic responses were measured, in addition to the pharmacokinetics, with the aim of evaluating the pharmacodynamic response-time relationship and the methylprednisolone concentration-effect relationship, thereby determining the extent to which the pharmacokinetic differences could be of clinical relevance.

Glucocorticoids play a central role in regulating a number of physiological processes and the biological effects of these are widespread [5]. In this study, whole blood histamine (WBH) was measured at the same times as the drug levels. WBH is a measure of basophil numbers and corticosteroids such as methylprednisolone inhibit the migration of basophils [6]. A dose dependent decline in circulating basophil numbers occurs following dosing with methylprednisolone providing an index of pharmacodynamic response [7, 8]. Using WBH levels the Emax (maximum effect) and AUEC (area under the effect curve) parameters were compared to examine whether the two prodrugs elicit equivalent responses. A sigmoid Emax pharmacodynamic model [9] linked to the pharmacokinetic model via an effect compartment was used to calculate the PK/PD parameters for each prodrug, these parameters were analysed statistically in the same way as the model independent pharmacokinetic and pharmacodynamic parameters. The data for blood glucose and various blood cell counts were also analysed for significant differences between the treatments.

Methods

Study design

This study was a single-blind, randomized, crossover design in 20 male volunteers comparing the pharmacokinetic and pharmacodynamic parameters of methylprednisolone released from the prodrugs MP suleptanate and MP succinate. The study was reviewed and approved by the Bronson Methodist Hospital Human Use Committee, 252

East Lovell Street, Kalamazoo, MI 49007 on 10th September, 1992. All volunteers were provided with information about the study before they agreed to sign an informed consent form.

The subjects reported to the clinic by 19.00 h the day prior to the start of the study (day −1). All subjects fasted from 23.00 h the night of admission until 12.00 h on day 1, drug dosing was at 08.00 h on day 1. Water was allowed *ad libitum* throughout the study. The subjects avoided the consumption of alcohol for 24 h preceding drug administration and for the duration of the study session. During the study period subjects were given a low-fat diet due to the potential for plasma lipids to interfere with the drug assays.

Drug dosing

The 20 volunteers were randomly allocated to two experimental groups. Each group received MP succinate and MP suleptanate as a methylprednisolone equivalent dose of 250 mg in a random sequence, separated by a 2 week wash-out period. Equal numbers of subjects were allocated to each treatment sequence. Two single intramuscular injections were administered into the upper, outer quadrant of the buttocks using a 1.5 inch needle. An evaluation of the site was made to assure that the injection was administered into muscle.

Promedrol (Upjohn SA Pharmaceutical, Rijksweg 12, B-2870, Puurs, Belgium) was supplied as 180 mg ml^{-1} solution (100 mg ml^{-1} methylprednisolone equivalents) and administered as a methylprednisolone equivalent dose of 250 mg. Two 125 mg injections (1.25 ml volume per injection) were made simultaneously, one injection into each buttock for a total volume of 2.5 ml.

Solu-Medrol (The Upjohn Company, 7000 Portage Rd, Kalamazoo, MI 49001, USA) was supplied as the currently marketed formulation and the dose given using the standard solution strength of 62.5 mg ml^{-1} (methylprednisolone equivalents) and administered as a methylprednisolone equivalent dose of 250 mg. Two 125 mg injections (2.0 ml^{-1} volume per injection) were made simultaneously, one injection into each buttock for a total volume of 4.0 ml^{-1}.

Schedule for blood and urine sampling

Blood was drawn 15 min pre-dose and at the following intervals after dosing; 5, 10, 20 and 45 min and 1.5, 3, 6, 8, 12, 18, 24, and 36 h. For the drug assays 7 ml of the blood was placed in a chilled NaF/EDTA tube and centrifuged in a refrigerated centrifuge as soon as possible (not later than 30 min) and the plasma harvested. Immediately after centrifugation the plasma samples were placed in a freezer at −70°C. For the whole blood histamine assays 3 ml of blood was drawn into a 3 ml heparinized or EDTA tube.

Sequential urine collections were made over the following time periods relative to the dose; −8 to 0. 0 to 1, 1 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 18, 18 to 24 and 24 to 36 h. The weight of each volunteer's urine output was measured at the end of each collection period and a 20 ml aliquot was frozen for the assay of MP succinate and MP suleptanate. To ensure the stability of MP suleptanate and MP succinate

on storage 0.20 ml of 1.0 M HClO$_4$ or 2.0 ml of an aqueous solution of 15 mg ml^{-1} Na$_2$EDTA were added to the aliquots for MP succinate and MP suleptanate respectively. The samples were stored prior to analysis at $-70°$C.

Analysis of plasma and urine samples for methylprednisolone, MP suleptanate and MP succinate

Concentrations of methylprednisolone, MP suleptanate and MP succinate in human plasma and urine were measured by h.p.l.c. with u.v. detection [10]. Brief details of the analytical methods are as follows: Plasma and urine samples (0.5 ml) were spiked with internal standard (fluorome-thalone), diluted with 0.5 ml phosphate buffer, and plasma samples processed with solid phase extraction CH cartridges prior to h.p.l.c. separation, u.v. detection at 254 nm was used. The coefficient of variation for the assays for the high and low ends of the calibration range respectively were as follows: *Plasma* MP 3.1–8.5%, MP succinate 7.9–6.0%, MP suleptanate 5.6–6.9%, assay range MP 0.04–2 μg ml^{-1}, MP succinate and MP suleptanate 0.2–4.0 μg ml^{-1}. *Urine* MP succinate 3.5–5.9%, MP suleptanate 3.2–2.9%, assay range 0.6–80 μg ml^{-1}.

Biochemical and haematological measurements

Whole blood histamine was determined on samples collected at the same times as those for the drug assays, a commercial radioimmunoassay method was used (Histamine r.i.a. kit, Catalog number 1302, AMAC Inc). Blood glucose measurements were made pre-dose and at 2, 4, 8, 24 and 36 h post dose. Total and differential white cell counts were made pre-dose and at 4, 8, 24 and 36 h post dose. These were made by standard clinical biochemistry and haematology methods.

Data analysis

Pharmacokinetic and statistical software The plasma concentration *vs* time data were analyzed using the pharmacokinetic software Siphar v 4.0b, SIMED, 9–11 Rue G Enesco, 94008, Creteil, Cedex, France and SAS v 6.04 (SAS Institute INC, SAS Campus Drive, Cary, NC 27513). SAS v 6.04 was also used to calculate the derived parameters not produced by Siphar, the cumulative excretion data and the statistical analysis.

Pharmacokinetic parameters for methylprednisolone Model independent analysis was used for this data. For each subject the total area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule up to the last data point AUC(0,t) and extrapolated from the last data point (C_t) to infinity from AUC(t,∞) = C_t/λ_z. C_{max} was the maximum observed plasma concentration and t_{max} was the time of this observation. The terminal half-life ($t_{1/2,z}$) was calculated from $t_{1/2,z} = \ln2/\lambda_z$. The terminal elimination rate constant λ_z was obtained from non-linear weighted least squares regression with a weighting of $1/y^2$.

Pharmacokinetic parameters for MP suleptanate and MP succinate For each subject a two exponential model with

first order input and first order elimination was fitted to the plasma data. Initial estimates of the model parameters were made by an automatic peeling algorithm. These were refined using non-linear weighted least squares regression, with a weighting of $1/y^2$. The adequacy of the fit of the model to the data was determined by the residual sum of squares, Akaike information criteria, the coefficient of variation of the parameter estimates and examination of the residuals. For all subjects the two exponential model described the data well.

The following pharmacokinetic parameters: AUC, C_{max}, t_{max} were calculated as described above. The input (absorption) half-life ($t_{1/2,\lambda1}$) and rate constant (λ_1) together with the elimination half-life ($t_{1/2,\lambda2}$) and rate constant (λ_2) were obtained from the fitted two exponential model. The total plasma clearance (CL) was estimated from: CL = dose/AUC (Where dose = 450 mg for MP suleptanate and 331 mg for MP succinate.) The volume of distribution at steady state (V_{ss}) was calculated using the mean residence time (MRT = (AUC/AUMC) $- 1/k_a$) as follows: V_{ss} = CL.MRT.

The total amount of drug excreted unchanged in the urine (Ae) was calculated directly from the urine concentration of the drug multiplied by the volume voided for each collection interval and summed for all intervals. The fraction of the dose excreted unchanged in the urine (fe) was calculated as follows: fe = Ae$_{0-\infty}$/dose. The renal clearance (CL$_R$) was estimated by: CL$_R$ = CL.

Pharmacodynamic parameters

The changes in blood basophil count following a single 250 mg dose of MP suleptanate and MP succinate were assessed by comparing the whole blood histamine (WBH) concentration-time data. The following parameters were calculated: AUEC (area under the effect curve) this was the area under the histamine concentration time curve from the time of dosing to 36 h post dosing. The histamine concentration decreased with time before returning to near pre-dose values at 36 h post dose. To calculate the AUEC(0,36 h) for this data the maximum concentration for each profile was noted, each value was then subtracted from this, which had the effect of inverting the profile. The AUEC for these profiles were then calculated by the linear trapezoidal rule. The t_{max} was the time of the maximum effect, this was the nadir for the blood concentration of histamine. The Emax was maximum observed pharmacological effect, this was the baseline WBH (pre-dose) minus the observed minimum WBH concentration.

Integrated pharmacokinetic and pharmacodynamic analysis for whole blood histamine

A sigmoid Emax pharmacodynamic model linked to the pharmacokinetic model via an effect compartment [9] was used to calculate the parameters for each prodrug using the pharmacokinetic software Siphar v 4.0b. Initially the pharmacokinetic parameters for MP were obtained using a single compartment model with first order input and monoexponential elimination. Plots of methylprednisolone plasma concentration *vs* WBH showed clockwise hysteresis therefore the pharmacokinetic model included an effect

compartment and the parameter k_{e0} (rate constant out of the effect compartment) was calculated in Siphar. The following parameters of the pharmacodynamic model

$$E = \frac{E_{max} \cdot C^\gamma}{EC_{50}^\gamma + C^\gamma}$$

were calculated: EC_{50} (the effect compartment MP concentration that produces 50% of the maximum effect), Emax (the maximum decrease in WBH) and γ (the slope factor for the sigmoid curve). These parameters were analysed statistically in the same way as the model independent pharmacokinetic and pharmacodynamic parameters.

Statistical analysis and bioequivalence

The pharmacokinetic and pharmacodynamic parameters were analysed for significant differences between the treatments for methylprednisolone C_{max}, t_{max}, AUC; histamine AUEC, Emax and t_{max} and the PK/PD model parameters. The normal and log-transformed parameters were analyzed by ANOVA for effect of prodrug, subject, time period and group sequence. The data were also examined for evidence of a normal distribution of values before and after log-transformation using the Procedure UNIVARIATE in SAS. The mean and median parameter values (normal and log-transformed) 90% conventional confidence intervals were also calculated. Bioequivalence criteria [11] were evaluated as follows: methylprednisolone mean parameter value, for MP suleptanate: the 90% confidence interval to be within 80–120% (80–125% for log-transformed data) of the methylprednisolone mean parameter value, for MP succinate. A non-parametric confidence interval [12] was used for t_{max} and also for γ since this parameter appeared to be neither normally or log normally distributed. A paired *t*-test was performed on the data for glucose, total white cell count, lymphocytes and neutrophils. This comparison was made between the treatments for each time-point for each subjects paired change from baseline responses ($P < 0.05$).

Results

Demography of subjects

A total of 20 male subjects took part in the study ranging in age from 18 to 45 years (mean$= 27 \pm 9$ years). The overall weight ranged from 59.2 to 109.3 kg (mean weight 83.1 ± 11.2 kg).

Pharmacokinetics and bioequivalence for methylprednisolone

The plasma concentration–time data for methylprednisolone are shown in Figure 1a and the pharmacokinetic parameters calculated from these data are shown in Table 1.

The bioavailability of methylprednisolone from a single 250 mg (methylprednisolone equivalent) intramuscular dose of MP suleptanate was compared with MP succinate by comparing the 90% confidence intervals for the parameters AUC, C_{max} and t_{max}. The results of the ANOVA comparing the parameters for the two prodrugs are shown in Table 1. The confidence intervals are expressed as percentages of the

reference median value, where the MP succinate values were used as the reference. There were no differences in the conclusions regarding the similarity of the bioequivalence parameters, whether confidence intervals were calculated for normal or log-transformed data. In addition there were no significant sequence or period effects found in the analysis of variance results.

In terms of the extent of bioavailability (AUC) the two prodrugs were judged to be bioequivalent with a MP suleptanate median AUC of 108% relative to the 100% for the MP succinate reference median (90% confidence interval: 102–114%, log-transformed data). For the rate of bioavailability (C_{max}) the MP suleptanate median C_{max} was less than the MP succinate median C_{max} 81% relative to the 100% for the MP succinate reference median (90% confidence interval: 75–88%, log-transformed data). The t_{max} for methylprednisolone from MP suleptanate was delayed relative to MP succinate. The values were compared using non-parametric confidence intervals. The median difference was 200% (90% confidence interval 141–283%)

The small but significant difference in the observed elimination half-life for methylprednisolone between the two pro-drugs (Table 1) is probably caused by the longer elimination half-life of MP suleptanate (Table 2, Figure 1b).

Plasma and urinary pharmacokinetics of MP succinate and MP suleptanate

The plasma concentration-time data for MP succinate and MP suleptanate are presented in Figure 1b and the calculated pharmacokinetic parameters in Table 2. Following a single intramuscular injection MP succinate had a higher C_{max}, smaller volume of distribution, a shorter elimination half-life and a higher renal clearance than MP suleptanate (Table 2). The total clearance was very similar for the two prodrugs (Table 2). However, the major difference between the two pro-drugs was the rate of absorption from the intramuscular injection site. The half-life of the absorption process ($t_{1/2} = \ln2/\lambda_1$) for MP suleptanate was slower (0.31 h) than for MP succinate (0.14 h).

Comparative pharmacodynamic responses for MP suleptanate and MP succinate

Figure 2a shows the pharmacodynamic response as assessed by whole blood histamine changes over the 36 h period post dosing. The pharmacodynamic parameters calculated from these data are shown in Table 3. The basophil count pharmacodynamic response arising from a single 250 mg (methylprednisolone equivalent) intramuscular dose of MP suleptanate and MP succinate were compared by calculating the 90% confidence intervals for the parameters AUEC, Emax and t_{max} for the whole blood histamine. The values are expressed as percentages of the reference mean or median value, where MP succinate values were used as the reference.

For both AUEC and Emax there was no significant difference in the response as assessed by ANOVA ($P < 0.05$) for normal or log transformed data. The analysis based on confidence intervals gave similar results:

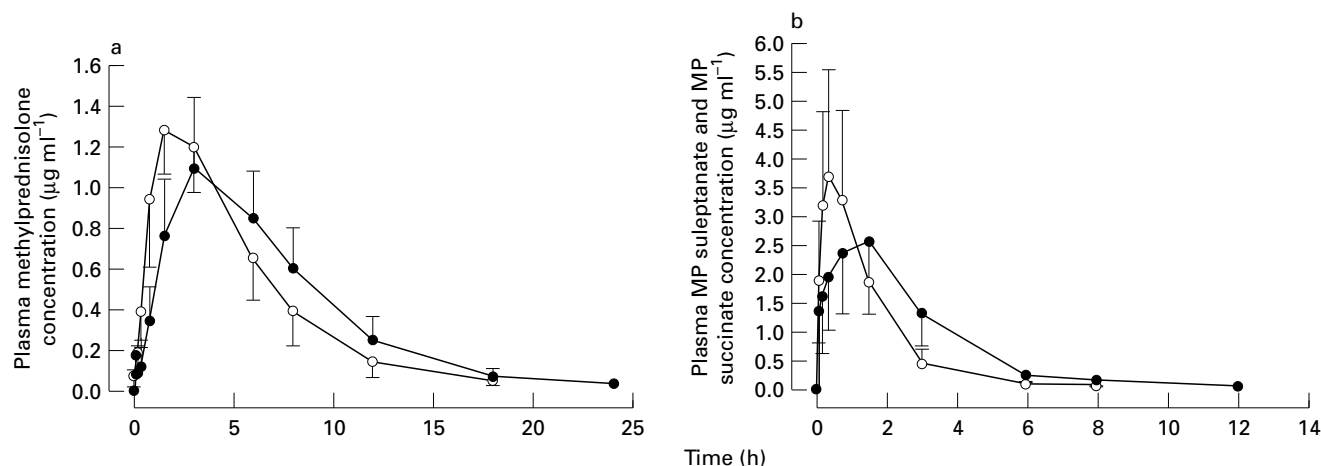For the histamine data the AUEC values were equivalent

Figure 1 Methylprednisolone (a), MP suleptanate and MP succinate (b) plasma concentrations after a single i.m. dose of 250 mg methylprednisolone equivalents of MP suleptanate (●) and MP succinate (○) to healthy male volunteers. Values are mean ± s.d.

Table 1 Pharmacokinetic parameters for methylprednisolone in healthy male volunteers after a single 250 mg methylprednisolone equivalent, i.m. dose of MP suleptanate and MP succinate.

Pharmacokinetic parameter	n	MP suleptanate (methyl-prednisolone) (means ± s.d.)	MP succinate (methyl-prednisolone) (means ± s.d.)	ANOVA P=	90% confidence intervals
AUC (μg ml^{-1} h)	20	9.26 ± 2.40	8.55 ± 2.09	0.0440	102–114%
C_{max} (μg ml^{-1})	20	1.12 ± 0.35	1.34 ± 0.19	0.0002	75–88%
t_{max} (h)	20	3.60 ± 1.23	1.91 ± 0.86	0.0001	141–283%★
$t_{1/2,z}$ (h)	20	2.96 ± 0.64	2.36 ± 0.45	0.0001	—

ANOVA P value = probability of significant difference between treatments for log transformed parameter values. ★ = Nonparametric confidence intervals.

Table 2 Pharmacokinetic parameters for MP suleptanate and MP succinate in healthy male volunteers after a single 250 mg methylprednisolone equivalent, i.m. dose of MP suleptanate and MP succinate.

Pharmacokinetic parameter	n	MP suleptanate (means ± s.d.)	MP succinate (means ± s.d.)
Dose[1] (mg)		450	331
AUC (μg ml^{-1} h)	20	9.22 ± 3.15	6.77 ± 2.41
C_{max} (μg ml^{-1})	20	3.24 ± 2.34	3.93 ± 1.76
t_{max} (h)	20	1.67 ± 0.61	0.47 ± 0.32
$t_{1/2\lambda_2}$ (h)	20	1.57 ± 0.48	0.82 ± 0.29
λ_2 (h^{-1})	20	0.48 ± 0.15	0.97 ± 0.41
λ_1 (h^{-1})	20	2.26 ± 1.05	4.98 ± 2.66
V_{ss} (l)	20	135 ± 45	70.1 ± 23.1
CL (ml min^{-1})	20	871 ± 191	874 ± 186
CL_R (ml min^{-1})	20	33.1 ± 12.7	95.5 ± 25.9
Ae (mg)	20	16.9 ± 4.60	36.0 ± 5.0
Fe (%)	20	3.76 ± 1.02	10.9 ± 1.50

[1]Dose is expressed in terms of the sodium succinate salt (Solu-Medrol) or suleptanate salt (Promedol) of methylprednisolone.

with a slightly higher value for MP suleptanate as follows: MP suleptanate median AUEC of 105% relative to the 100% for the MP succinate reference median (90% confidence interval: 98–113%, log-transformed data). The histamine data Emax was slightly less for MP succinate as follows: MP suleptanate median Emax of 104% relative to the 100% for the MP succinate reference median (90% confidence interval: 93–109%, log-transformed data); the

t_{max} for the WBH nadir was later for MP suleptanate relative to MP succinate. The values were compared using non-parametric confidence intervals. For t_{max} the median difference was 123% (90% confidence interval 100–123%).

The other changes in haematological and biochemical parameters after MP suleptanate or MP succinate were those expected after steroid administration [5]. For example significant increases in total white blood cell (WBC) count occurred at time-points between 4 h and 36 h (Figure 2b) largely reflecting changes in neutrophil count (Figure 2c). Decreases in eosinophil, monocyte counts (not shown) and lymphocytes (Figure 2d) were also seen. The changes were similar after administration of MP succinate and MP suleptanate. Increases in serum glucose, a characteristic of steroid action [5], were also seen after administration of either pro-drug (Figure 3), these increases were statistically significant, although the maximum increase was not significantly different between the two prodrugs. The blood cell count changes for neutrophils and lymphocytes were also not significantly different, between the two prodrugs, at any of the time-points measured. The increase in total white cell count (Figure 2b) was only significantly different between the two prodrugs at 36 h post-dose, but the difference is unlikely to be of clinical significance.

Integrated pharmacokinetic and pharmacodynamic analysis for whole blood histamine

The sigmoid Emax model described that WBH data very well for both pro-drugs, the adequacy of the fit of the
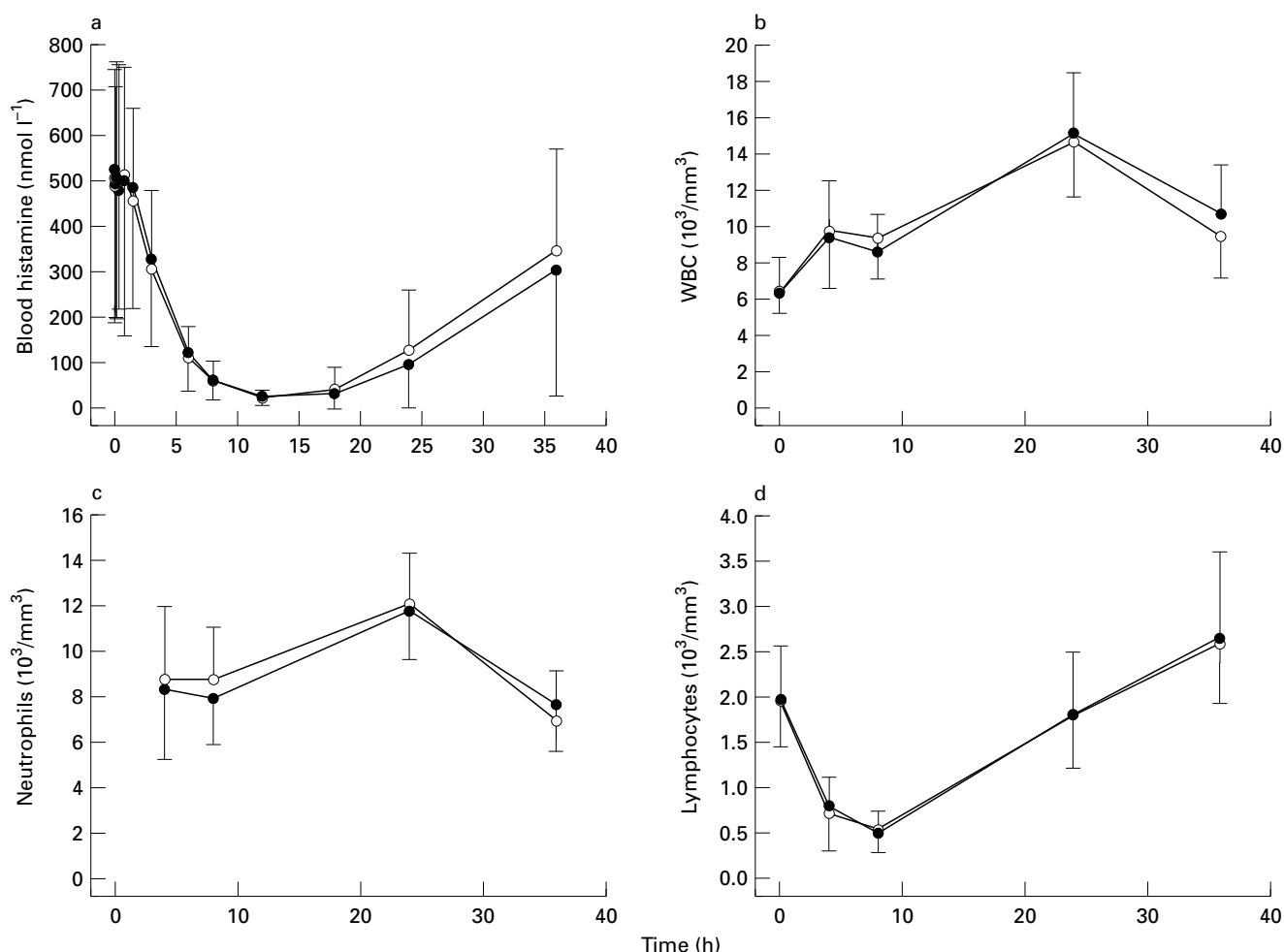
Figure 2 Blood histamine concentrations (a), total white cell count (b), neutrophil count (c) and lymphocyte count (d) after a single i.m. dose of 250 mg methylprednisolone equivalents of MP suleptanate (●) and MP succinate (○) to healthy male volunteers. Values are mean ± s.d.

Table 3 Pharmacodynamic parameters for whole blood histamine in healthy male volunteers after a single 250 mg methylprednisolone equivalent, i.m. dose of MP suleptanate & MP succinate.

Pharmacodynamic parameter	n	MP suleptanate (means ± s.d)	MP succinate (means ± s.d.)	ANOVA P	90% confidence intervals
[a]AUEC (nmol l^{-1} h)	20	14797 ± 7706	13855 ± 6042	0.2166	98–113%★
Emax (nmol l^{-1})	20	−504.7 ± 319.5	−481.8 ± 231.7	0.8907	93–109%★
E_0 (nmol l^{-1})	20	527.4 ± 339.1	504.0 ± 243.1	0.9688	—
t_{max} (h)	20	15.6 ± 3.0	13.0 ± 2.7	0.0008	100–123%★

ANOVA *P* value = probability of significant difference between treatments for log transformed parameter values. ★ = Nonparametric confidence intervals.
[a]AUEC = area under the effect-time curve for whole blood histamine concentration 0–36 h.

model to the data was determined by the residual sum of squares, Akaike information criteria, the coefficient of variation of the parameter estimates and examination of the residuals. Figure 4 shows a typical PK model fit to the MP data and PK/PD model fit to the WBH data for subject number 20 following the MP suleptanate dose.

For EC_{50}, Emax and γ there was no significant difference in the response between prodrugs as assessed by ANOVA ($P < 0.05$) for normal or log transformed data. The analysis based on confidence intervals gave similar results:

For the WBH data the EC_{50}, Emax and γ values were equivalent as follows: MP suleptanate median EC_{50} 103%

relative to the 100% for the MP succinate reference median (90% confidence interval: 89–118%, log-transformed data). The Emax values; MP suleptanate median Emax of 93% relative to the 100% for the MP succinate reference median (90% confidence interval: 85–101%, log-transformed data). The γ values: MP suleptanate median of 98% relative to the 100% for the MP succinate reference median (90% confidence interval: 84–102%, non-parametric confidence intervals). The k_{e0} values were significantly higher for MP suleptanate as follows: MP suleptanate median k_{e0} of 139% relative to the 100% for the MP succinate reference median (90% confidence interval: 126–154%, log-transformed data).
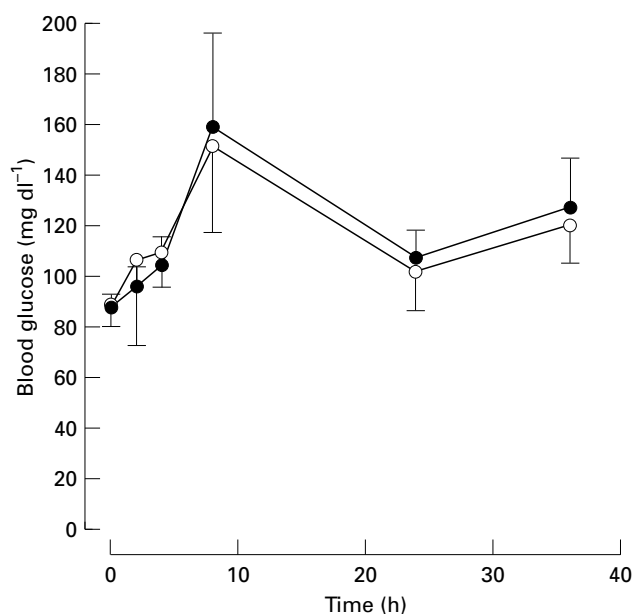
Figure 3 Mean ± s.d. blood glucose after a single i.m. dose of 250 mg methylprednisolone equivalents of MP suleptanate (●) and MP succinate (○) in healthy male volunteers.

Discussion

Comparative bioavailability of MP suleptanate and MP succinate

After a single intramuscular 250 mg methylprednisolone equivalent dose, methylprednisolone released from MP suleptanate had an equivalent extent of bioavailability (AUC) to that of methylprednisolone released from MP succinate. This result reflects the similar extent of conversion of the pro-drugs to methylprednisolone by metabolic action of the esterase enzymes in the liver and other tissues. The small difference in the AUC values of 7.7% was largely accounted for by the difference in the renal elimination (fe %) of the two pro-drugs of 7.1%.

The measures of rate of bioavailability (C_{max} and t_{max}) were not as close, the 90% confidence intervals for MP suleptanate were outside the 80–125% range used by most regulatory authorities. Methylprednisolone from MP suleptanate was available more slowly and attained the maximum concentration at a later time compared to MP succinate. This observation is likely to be a result of a slower absorption of MP suleptanate from the intramuscular injection site. Factors that may have contributed to this are: differences in lipophilicity, the higher molecular weight of MP suleptanate
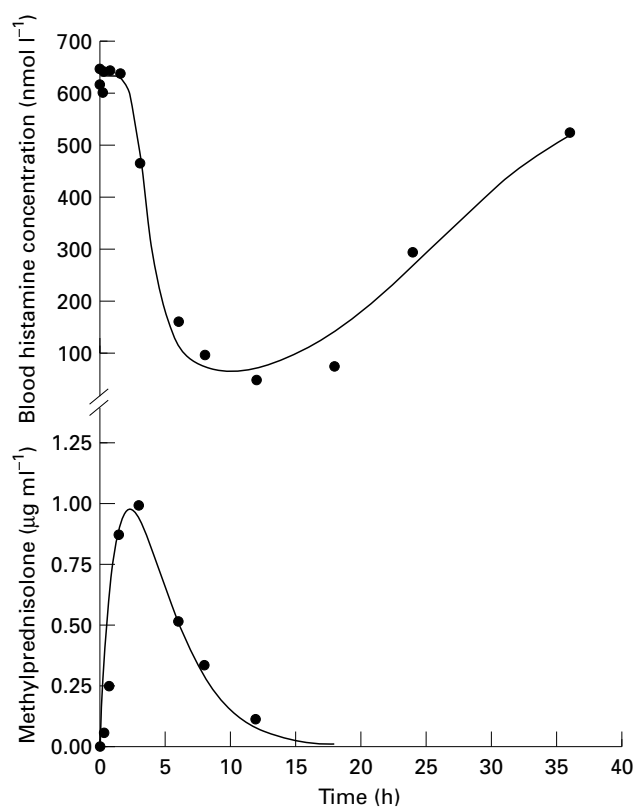

Figure 4 PK model fit to methylprednisolone data and PK/PD model fit to whole blood histamine data for subject number 20 following a single i.m. dose of 250 mg methylprednisolone equivalents of MP suleptanate. Upper graph PK/PD model fit (—), ● whole blood histamine data; lower graph PK model fit (—), ● MP data.

(MW 674) compared with MP succinate (MW 497), differences in the volume, pH and concentration of the injected dose solution. This is in contrast to the previous unpublished intravenous comparison of MP suleptanate and MP succinate where MP suleptanate produced higher C_{max} values for methylprednisolone than MP succinate, in this case the extent of bioavailability was largely determined by the faster rate of metabolic conversion of MP suleptanate to methylprednisolone.

Comparative pharmacodynamic responses for MP suleptanate and MP succinate

The pharmacodynamic measurements were made to assess whether differences in methylprednisolone bioavailability would produce a corresponding difference in pharmacodyn-

Table 4 Parameters of the pharmacodynamic sigmoid Emax model for whole blood histamine and the pharmacokinetic link model in healthy male volunteers after a single 250 mg methylprednisolone equivalent, i.m. dose of MP suleptanate and MP succinate.

Model parameter	n	MP suleptanate (means ± s.d)	MP succinate (means ± s.d.)	ANOVA P	90% confidence intervals
EC_{50} (μg ml^{-1})	20	0.112 ± 0.012	0.113 ± 0.038	0.7326	89–118%
E_{max} (nmol l^{-1})	20	−521.6 ± 310.3	−561.0 ± 295.9	0.1207	85–101%
γ	20	3.40 ± 0.99	4.96 ± 3.34	0.0645	84–102%★
k_{e0} (h^{-1})	20	0.044 ± 0.012	0.032 ± 0.009	0.0001	126–154%

ANOVA P value = probability of significant difference between treatments for log transformed parameter values. ★ = Nonparametric confidence intervals.

amic response. The extent of MP suleptanate conversion to methylprednisolone was marginally greater producing a greater AUC, but this was not significantly different. This finding was reflected in the lack of a significant difference in AUEC for WBH (based on ANOVA and 90% confidence intervals) for the two prodrugs. The pharmacodynamic measure AUEC is subject to the same problems that AUC suffers from, i.e. it has no information about the maximum intensity or shape of the response curve. Therefore AUEC should always be considered together with Emax and t_{max}. The maximum response (Emax) for the WBH measurements was also not significantly different between the two prodrugs. This observation was supported by a lack of significant differences between the prodrugs for the changes in the other pharmacodynamic measurements based on white cell counts and blood glucose. The EC_{50} values were also almost identical for the two prodrugs (Table 4) indicating that MP suleptanate has no apparent intrinsic activity.

Integrated pharmacokinetic and pharmacodynamic analysis for whole blood histamine

The sigmoid Emax model has been compared with other pharmacodynamic models for describing WBH data [7, 8], these authors found that a direct suppression model was a suitable alternative model. The principal advantage with the direct suppression model is that it is better able to simultaneously describe data from a range of doses of methylprednisolone [7]. The sigmoid Emax model, linked to an effect compartment, also fitted the data well and has been used by others [13] for similar examples. One objection to this model is that k_{e0} may not be physiologically appropriate, since the hysteresis in the plasma concentration–effect curve could be due to the migration rate of the basophils. However, good fits for the sigmoid E_{max} model were found for all subjects (Figure 4).

There was a significant difference of 6 h (k_{e0}, half-life) in the parameter of the pharmacokinetic link model (Table 4). This was greater than expected based on differences in the pharmacokinetic parameters for MP or the prodrugs (Tables 1 and 2). The k_{e0} parameter accounts for the temporal differences in drug levels in plasma and the PD effects. Although the plasma concentration profiles for MP are different for the two prodrugs (C_{max}, t_{max} and observed terminal half-life) the corresponding value of k_{e0} should be a function of methylprednisolone pharmacokinetics and not vary significantly between the prodrugs. However, in this example the temporal differences in the MP plasma profiles for the two prodrugs is due to them being different chemical entities and the resulting different input profile for MP may have physiological consequences for the development of the pharmacodynamic effects. Therefore, the parameter k_{e0} in this example may differ because it represents a delay due to physiological events and not pharmacokinetic equilibria. This is more plausible since MP should attain rapid equilibrium with an effect site compared with the on-set on maximum PD effects (13–15 h, Table 3)

A one compartmet model with first order input and first order elimination described the MP PK data very well (Figure 4). More complex models have been explored but were found to offer no advantage: PK/PD modeling with simultaneous fitting of prodrug (including input of prodrug from the IM injection site and elimination of intact prodrug via the urine), MP and WBH data was undertaken using ADAPT II. This model failed to describe the MP data more precisely and were overparameterised in the input phase leading to poor PK parameter estimates for MP. However, these more complex MP inputs did not modify the temporal differences for MP between the prodrugs or the disparate estimates of k_{e0}.

Conclusions

The extent (AUC) of methylprednisolone release from MP suleptanate was found to be equivalent to MP succinate when given as a single 250 mg methylprednisolone equivalent dose via the intramuscular route. The rate (C_{max} and t_{max}) of methylprednisolone bioavailability was slower for MP suleptanate due to a longer absorption half-life from the intramuscular injection site.

The model independent pharmacodynamic measures of WBH showed no significant differences in the extent of response (AUEC) or the maximum response (E_{max}). This was confirmed by the pharmacokinetic-pharmacodynamic model. Furthermore this model predicts that at plasma concentrations of methylprednisolone above the EC_{50} value even quite large changes in C_{max} would not result in a significantly greater pharmacodynamic response. In this study a 250 mg (MP equivalent) i.m. dose of MP suleptanate and MP succinate produced significant differences in C_{max} but this was not translated into a significant difference in Emax. This supports the concept that for MP suleptanate and MP succinate the pharmacodynamic parameters should be taken into account when assessing bioequivalence rather than reaching conclusions based on pharmacokinetic data alone. This was further supported by a lack of a significant difference between the prodrugs in the maximum pharmacodynamic responses seen for glucose and various blood cell count changes.

MP suleptanate appears to be a suitable pharmaceutical alternative to MP succinate when given as an intramuscular injection and a pharmacodynamic or PK/PD approach is a useful technique for investigating this type of comparative bioavailability issue.

References

1 Ozawa N, Yamazaki S, Chiba K, *et al*. Studies of the pharmacokinetics of methylprednisolone suleptanate (U–67590A) 1, Blood level profile distribution excretion and reabsorption of tritiated or carbon-14 U–67590A in rats after a single intravenous dose. *Pharmacometrics* 1993; **45**: 487–505.

2 Ozawa N, Yamazaki S, Chiba K, *et al*. Studies of the pharmacokinetics of methylprednisolone suleptanate (U–67590A) 3, plasma concentration and excretion of tritiated or carbon-14 U–67590A in dogs after a single intravenous dose. *Pharmacometrics* 1993; **45**: 519–528.

3 Weber DJ, Vanderlugt JT, Olanoff LS, Shah JA. Comparative pharmacokinetics of methylprednisolone and its prodrugs

following intravenous doses of methylprednisolone sodium succinate MPSS and methylprednisolone suleptanate MPSP in humans. *Clin Pharmacol Ther* 1988; **43**: 189.

4 Vanderlugt JT, Weber DJ, Metzler CM, Olanoff LS. Comparisons of single and multiple dose pharmacokinetics of methylprednisolone sodium succinate and methylprednisolone suleptanate. *Clin Pharmacol Ther* 1989; **45**: 148.

5 Cupps TR, Fauci AS. Corticosteroid-mediated immunoregulation in man. *Immunol Rev* 1982; **65**: 133–155.

6 Saavedra-Delgado AP, Mathews KP, Pan PM, Kay DR, Muilenberg ML. Dose reponse studies of the suppression of whole blood histamine and basophil counts by prednisolone. *J Allergy Clin Immunol* 1980; **66**: 464–471.

7 Kong AN, Ludwig EA, Slaughter RL, *et al*. Pharmacokinetics and pharmacodynamic modeling of direct suppression effects of methylprednisolone on serum cortisol and blood histamine in human subjects. *Clin Pharmacol Ther* 1989; **46**: 616–628.

8 Wald JA, Salazar DE, Cheng H, Jusko JW. Two compartment basophil cell trafficking model for methylprednisolone pharmacodynamics. *J Pharmacokinet Biopharm* 1991; **19**: 521–536.

9 Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J. Simultaneous modeling of phamacokinetics and pharmacodynamics: Applications to d-tubocurarine. *Clin Pharmacol Ther* 1979; **25**: 358–371.

10 Shah JA, Weber DJ, Bothwell BE. High-performance liquid chromatographic determination of methylprednisolone and methylprednisolone-21-(8-(methyl-(2-sulfoethyl)amino)-8-oxo-octanoate) sodium salt in human plasma. *J Chromatogr Biomed Appl* 1987; **414**: 1–10.

11 Pidgen AW. Statistical aspects of bioequivalence-A review. *Xenobiotica* 1992; **22**: 881–893.

12 Hauschke D, Steinijans VW, Diletti E. A distribution-free procedure for the statistical analysis of bioequivalence studies *Int J Clin Pharm Ther Tox* 1992; **30** (Suppl.).

13 Ooesterhuis B, Tenberge IJM, Schellekens PTA, Koopmans RP, Van Boxtel CJ. Prednisolone concentration-effect relations in humans and the influence of plasma hydrocortisone. *J Pharmacol Exp Ther* 1986; **239**: 919–926.