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Technical Note

Oral Bioavailability of Triamcinolone Tablets and a Triamcinolone Diacetate Suspension

Günther Hochhaus,[1,4] Martin Pörtner,[2] Jürgen Barth,[3] Helmut Möllmann,[3] and Peter Rohdewald[2]

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INTRODUCTION

Triamcinolone tablets and a suspension of triamcinolone diacetate are both listed in the USP (1), and identical doses of both preparations are used for the treatment of glucocorticoid sensitive diseases (2), under the assumption that the pharmacodynamic and pharmacokinetic properties of triamcinolone and triamcinolone diacetate are similar.

Employing receptor binding studies in human synovial tissue, we have recently demonstrated the lack of pharmacodynamic activity of intact triamcinolone diacetate (3). Hence, triamcinolone diacetate should be classified as a prodrug. Differences in the physicochemical properties of the drug and prodrug are likely to influence the absorption process and, consequently, the pharmacokinetic profile of the drug (4).

In this study, we compare the pharmacokinetic profiles of triamcinolone after oral administration of triamcinolone diacetate suspension and triamcinolone tablets.

MATERIALS AND METHODS

Materials. The following materials were obtained from the sources indicated: (carboxymethyl)trimethylammonium chloride hydrazide (Girard T reagent) from Fluka Feinchemikalien, Neu-Ulm (FRG); gamma-globulin solution (Beriglobin) from Behringwerke (Marburg, FRG); and 6,7-^{3}H-triamcinolone acetonide from New England Nuclear (Boston, MA). The radiochemical purity was determined by thin-layer chromatography. The triamcinolone antiserum was kindly provided by Drs. Haack and Vescei (Heidelberg, FRG). All other chemicals were of analytical grade.

Subjects. Two groups of subjects were included in the study. Group 1 received triamcinolone tablets and consisted of 10 healthy volunteers with an average age of 32 years (range, 25–48 years) and an average weight of 76 kg (range, 53–113 kg). Pharmacokinetic data for this group have been published elsewhere (5). Group 2 received triamcinolone diacetate suspension and consisted of five subjects with an average age of 57 years (range, 32–76 years) and an average weight of 72 kg (range, 68–90 kg). Study protocols for both groups were otherwise identical and studies were performed over the same time period and in the same hospital without a common reference treatment.

Drug Administration and Sample Collection. At 8 AM, fasting subjects received two tablets of 8 mg triamcinolone (group 1) or 16 mg crystalline triamcinolone diacetate suspended in 150 ml water (group 2) under identical conditions. Blood samples were taken immediately before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5 (only group 1), 6, 8, 11.5 (12 hr for group 1), and 24 hr after dosing. Blood was collected in EDTA-coated tubes and centrifuged immediately, and the plasma was stored at −20°C until assayed.

Buffers and Solutions. Borate buffer (pH 8) was 6.2 g/liter boric acid, 7.45 g/liter potassium borate adjusted to pH 8 with 0.1 *N* NaOH; Sörensen buffer was 3.67 m*M* monopotassium phosphate, 63 m*M* disodium phosphate in distilled water (pH 8.0); Girard reagent T solution was a solution of 10 g Girard reagent T in 81 g methanol and 9 g glacial acid; and lysozyme/borate buffer was a 1% solution of lysozyme in borate buffer. Gamma-globulin/borate buffer was a 1% dilution of gamma-globulin (Beriglobin) in borate buffer. The dextran-coated charcoal suspension was 1.5% Norit A, 0.1% dextran T 70 in gamma-globulin/borate buffer. The antiserum solution employed in the RIA was prepared with lysozyme/borate buffer.

Sample Extraction and RIA. Plasma samples (0.1–0.5 ml) were mixed with 1500 dpm of ^{3}H-triamcinolone acetonide (dissolved in 2.0 ml of a 0.2% aqueous solution of ethylene glycol). The mixture was extracted with a 2.0-ml portion of ethyl acetate for 15 min, and the organic phase obtained after centrifugation was evaporated under nitrogen. Endogenous glucocorticoids that interfere with the radioimmunological determination were inactivated according to the method of Loo *et al.* (7) by incubation with 1 ml of Girard reagent T for 90 min at room temperature. The reaction was stopped by the addition of 0.4 ml of Sörensen buffer, and the

[1] College of Pharmacy, Box J 494, University of Florida, Gainesville, Florida 32610.
[2] Institut für Pharmazeutische Chemie, Westfälische Wilhelms Universität, Münster, FRG.
[3] Medizinische Universitätsklinik und Poliklinik "Bergmannsheil" der Ruhruniversität Bochum, FRG.
[4] To whom correspondence should be addressed.

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reaction mixture was again extracted with 2.0 ml of ethyl acetate. After evaporation the residue was reconstituted in 0.4 ml Sörensen buffer and used for the radioimmunological determination. The radioactivity in 0.1 ml of this solution was determined for the calculation of the overall extraction rate. The concentration of triamcinolone was determined by radioimmunoassay as described earlier (5,6). Briefly, 0.1 ml of ^{3}H-triamcinolone acetonide (9000 dpm) and 0.1 ml of extracted plasma samples (obtained as described above) were mixed with 0.5 ml of the antiserum dilution and incubated overnight at 4°C. The titer of the antiserum was determined directly before the experiment, because the antiserum dilutions were unstable at 4°C (5). Unbound tracer was removed by the addition of charcoal and the radioactivity of the supernatant was determined. For the preparation of the calibration curve, triamcinolone standard solutions prepared in ethylene glycol solution (0.2–100 ng/ml) were treated identically. Blank plasma as well as samples obtained at time point 0 min contained immunoreactive material equivalent to about 2 ng/ml triamcinolone (2% of maximum peak concentrations). This activity is most likely not related to nonderivatized cortisol, as identical results for spiked plasma samples containing varying amounts of hydrocortisone revealed. In contrast to previous studies (5), the pharmacokinetic analysis was performed with data corrected for this blank activity.

Testing for Triamcinolone Esters in Plasma. The presence of triamcinolone mono- and diacetate was investigated in samples obtained after i.m. administration of 40 mg triamcinolone diacetate. Plasma samples after i.m. administration of triamcinolone diacetate were obtained from 15 patients as described earlier (6). Samples obtained after 0.1, 1.5, 2, and 12 hr were pooled so that for all sampling time points, 50 ml of pooled plasma was available. These plasma samples contained triamcinolone at concentrations of more than 10 ng/ml as determined by RIA. Fifty milliliters of the pooled plasma was diluted with 50 ml of distilled water. Portions of 20 ml were applied to prepacked silica columns (Extrelut) and incubated with stationary phase for 20 min, followed by elution with 50 ml of dichloromethane. The five eluates obtained for each specified time point were combined and concentrated using a Rotavapor, dissolved in 5 ml of dichloromethane, transferred into a smaller vial, and evaporated under nitrogen. The residue was dissolved in chromatographic mobile phase (0.1 ml). Twenty microliters was analyzed by reversed-phase HPLC on a 10-μm octadecylsilane column (Macherey Nagel, Dueren, FRG) employing a mixture of water, acetonitrile, methanol, and glacial acid (60:20:20:0.2, v:v) as mobile phase. A flow rate of 1.5 ml/min and UV detection at 254 nm were used. Chromatographic separation of triamcinolone and its mono- and diester is achieved under these conditions. Pooled plasma spiked with known amounts of triamcinolone diacetate (3, 5, 10, and 20 ng/ml) was processed identically and served as a standard. The detection limit (relative standarad deviation ± 30%) was 3 ng/ml for triamcinolone diacetate.

Data Analysis. Pharmacokinetic analysis of plasma concentrations (Cp) was performed individually for both groups using the program Rstrip (Micromath, Salt Lake City, Utah). The plasma concentration–time curve after oral dosing was best described by a one-compartment model with first-order absorption:

$$C_p = \frac{fDk_a}{V_d\,(k_a - k_e)}\,(e^{-k_e t} - e^{-k_a t})$$

where D is the dose, f is the fraction absorbed, V_d is the volume of distribution, and k_a and k_e are the rate constants for absorption and elimination, respectively. The area under the curve (AUC_∞) was determined by integration of the fitted plasma concentration–time profile. Clearance (Cl_{tot}) was calculated from the dose without considering the fraction absorbed by

$$Cl_{tot}/f = D/AUC_\infty$$

The volume of distribution was calculated by

$$V_d/f = D/(AUC_\infty\, k_e)$$

Small differences between these results and those published earlier for group 1 (5) are related to the fact that the earlier study did not correct for immunoreactive material found in blank plasma. Statistical analysis was performed using an unpaired t test. Differences were considered to be statistically significant for $P < 0.05$. Identical results were obtained employing the nonparametric Wilcoxon test.

RESULTS AND DISCUSSION

A RIA was employed for the determination of triamcinolone because more specific assays with sufficient sensitivity are not available. The antiserum employed showed strong cross-reactivity to triamcinolone diacetate and triamcinolone monocinolone monoacetate (85%; results not shown). In order to validate the RIA procedure, we measured triamcinolone, and the corresponding mono- and diesters, by HPLC in plasma samples taken after i.m. administration of 40 mg triamcinolone acetate. Average triamcinolone plasma levels in these samples were higher than 10 ng/ml, while triamcinolone diacetate or the monoester was nondetectable by the HPLC assay, with a limit of detection for triamcino-

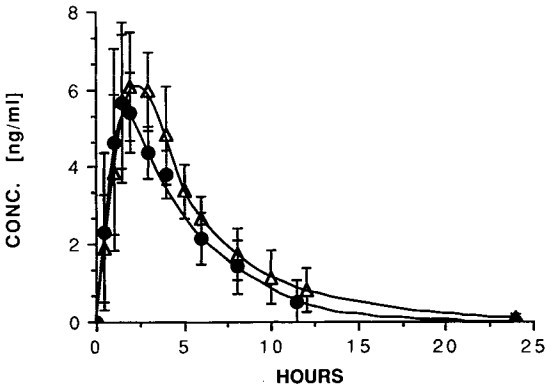

Fig. 1. Standardized triamcinolone plasma concentration–time profiles after oral administration of 16 mg triamcinolone tablets (△) and 16 mg triamcinolone diacetate suspension (●). Concentrations were standardized to 1 mg triamcinolone for both dosage forms.

Table I. Pharmacokinetic Parameters for Triamcinolone Diacetate Suspension (16 mg) and Triamcinolone Tablets (16 mg)

Parameter	16 mg triamcinolone diacetate, oral	16 mg triamcinolone, oral[a]	P^b
$fDk_a [V_d (k_a - k_e)]$	10.97 ± 0.6[c]	18.23 ± 10.01[c]	0.13
k_a (1/hr)	1.09 ± 0.48	0.88 ± 0.65	0.49
k_e (1/hr)	0.284 ± 0.066	0.26 ± 0.09	0.56
C_{max} (ng/ml)	5.33 ± 1.55[c]	5.23 ± 0.84[c]	0.91
	69.84 ± 20.3[d]	83.9 ± 23.5[d]	0.22
t_{max} (hr)	1.86 ± 0.47	2.24 ± 0.78	0.25
Cl_{tot}/f (L/hr)	34.4 ± 10.6[e]	28.6 ± 5.6	0.285
V_d/f (L)	115.2 ± 10[e]	119.7 ± 33.14	0.67
AUC_∞ (ng · hr/ml)	32.7 ± 9.9[c]	36.0 ± 6.2[c]	0.51
	426 ± 158[d]	576 ± 100[d]	0.065
Half-life (hr)	2.7	2.8	

[a] Original data were taken from Ref. 5 and reanalyzed as described under Materials and Methods.
[b] Probability factor of *t*-test analysis.
[c] Data were standardized to 1 mg triamcinolone.
[d] Data are not standardized and based on a dose of 16 mg.
[e] Amount of triamcinolone (13.1 mg) corresponding to 16 mg triamcinolone diacetate was used for the calculation.

lone diacetate of 3 ng/ml. Assuming this limit of detection as the highest possible concentration of mono- and diacetate, we can conclude that the free alcohol represents the main fraction of immunoreactive material. Hence, from a clinical point of view, the RIA is adequate for measuring the relevant pharmacodynamic species.

The plasma concentration–time curves after oral administration of 16 mg triamcinolone diacetate suspension and 16 mg triamcinolone tablets (data taken from Ref. 5) are shown after standardization to 1 mg triamcinolone (Fig. 1) for both dosage forms. No significant differences in the resulting pharmacokinetic parameters with or without standardization to 1 mg triamcinolone (Table I) were observed. Although results after standardization show generally higher *P* values (Table I), the lack of significant differences among the nonstandardized data does not argue for the use of equimolar doses of triamcinolone diacetate and triamcinolone. The lack of significant differences for the AUC_∞ (although close to significance) and t_{max} was surprising because both parameters are thought to be sensitive to differences in the dissolution rate, solubility, and lipophilicity of the drug. Since triamcinolone diacetate is quite stable in artificial gastric and intestinal juice (only 30% of the ester is cleaved during an average intestinal and gastric passage of 4 hr; data not shown), our results cannot be explained by a rapid gastrointestinal cleavage of the prodrug.

Triamcinolone diacetate suspension is commercially available and listed as a syrup in the United States Pharmacopeia (1). It is given at the same dose (mg) as triamcinolone tablets. We found that differences in the physiochemical properties of both dosage forms are not reflected in the triamcinolone plasma profiles. Hence, our results justify identical dosing with both preparations.

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