Microdialysis of Triamcinolone Acetonide in Rat Muscle

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ABSTRACT: The objective of this study was to compare plasma and muscle concentrations of triamcinolone acetonide (TA) in the rat by microdialysis. Microdialysis experiments were carried out at steady state in rats after an initial I.V. bolus 50 mg/kg of the phosphate ester of TA (TAP) followed by 23 mg/kg/h infusion. *In vivo* recovery was calculated by retrodialysis. The concentration determined at steady state in microdialysate, corrected for recovery, was 2.73 ± 0.42 μg/mL compared to 21.9 ± 2.3 μg/mL in plasma. The pharmacokinetics of TA in plasma was described by an open two-compartment model with a terminal half-life of 2.7 h. The clearance of TA in rats determined by compartmental analysis was 0.94 L/h/kg. The measured microdialysate levels of TA in muscle, corrected for recovery, were comparable to the predicted free drug levels in the peripheral compartment. Protein binding in rat plasma, measured by ultrafiltration, was 90.1%. The microdialysis *in vivo* recovery in muscle was similar to the *in vitro* recovery under stirred conditions. The results show the applicability of microdialysis to measure free tissue concentrations of TA in rats. J Pharm Sci 92:394–397, 2003

Keywords: triamcinolone acetonide; microdialysis; recovery; rat muscle, protein binding

INTRODUCTION

The technique of microdialysis is used to measure the unbound drug concentration in tissues and body fluids *in vivo*. This technique employs a semipermeable dialysis membrane through which water and small molecules can cross. Microdialysis takes place in nonequilibrium conditions, and hence, recovery of the analyte in the dialysate needs to be measured to calculate the free drug concentrations in the tissue. The recovery is usually calculated by retrodialysis and no-net-flux methods. Previous *in vitro* studies have shown that stirring, viscosity of the medium, and protein binding affect the recovery of the analyte.[1–4] *In vivo* microdialysis has been applied to a number of compounds that are mainly hydrophilic. Recently, it has been argued that microdialysis of lipophilic compounds may be difficult to perform.[5–9] The low recovery of lipophilic compounds in the dialysate may be attributed to lower solubility in the hydrophilic perfusate medium, nonspecific binding to the probe, and high protein binding. Triamcinolone acetonide (TA) is a lipophilic corticosteroid with a variety of applications. *In vitro* microdialysis recovery of TA has been reported to be about 60%.[2] The objective of this study was to develop a rat model for measuring the unbound tissue concentrations of TA at steady state by microdialysis.

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MATERIALS AND METHODS

Triamcinolone acetonide dihydrogenphosphate, dipotassium salt [Volon® soluble (TAP)] was obtained from Squibb-Heyden, Munich, Germany. Triamcinolone acetonide (TA), methylprednisolone (MP), phosphoric acid (85%), and ethylcarbamate (Urethane) were purchased from Sigma (St. Louis, MO). Ringer's solution (NaCl (189 mM), KCl (3.9 mM), $CaCl_2$ (3.4 mM); pH 7.2), and the heparinized saline solution were prepared in the laboratory. Methanol and ethyl acetate were of HPLC grade, and ammonium sulfate was of analytical grade. Fresh human plasma was obtained from Shands Hospital, University of Florida. Microdialysis experiments were performed with a flexible custom made microdialysis probe (shaft length 70 mm, membrane length 16 mm, 20 kDa cutoff, CMA/10 A, Stockholm).

After approval by the Institutional Animal Care and Use Committee of the University of Florida (IACUC), anesthetized (methoxyflurane and ethylcarbamate) male Wistar rats (300–450 g, $n = 3$) were tracheally intubated by a rodent ventilator and the body temperature was maintained on an electric heating pad. Catheters were inserted into the carotid artery and the femoral vein of the left leg. The microdialysis probe was inserted into the right hind leg muscle. A small skin incision was made in the right hind leg to expose the muscle tissue. The probe was inserted into the muscle using a needle guide, the guide was then removed, and the probe was secured to the muscle tissue using surgical silk. Ringer's solution was perfused (3 μL/min) through the probe for 30 min, allowing the equilibration followed by perfusion with TA solution (5 μg/mL). Following a 10-min equilibrium period, two dialysate samples (20 min each) were collected to calculate the recovery by retrodialysis *in vivo*. Recovery (R) was calculated as follows:

$$\text{Loss}\,(\%) = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}} \cdot 100$$

and

$$\text{Recovery} = \text{Loss}$$

where C_{in} and C_{out} are the TA concentrations in the perfusate and the dialysate, respectively.

The probe was perfused with Ringer's solution for 20 min before the drug was administered. The perfusion of Ringer's solution for 20 min was determined in pilot studies to be adequate to remove the compound from the dialysis system. The phosphate salt of TA (TAP) was injected (50 mg/kg) as I.V. bolus as loading dose followed by a constant rate infusion over the entire period of experiment (23 mg/kg/h, 2 mL/h).

Blood samples were collected every 30 min over a period of 5 h (300–400 μL) in heparinized tubes and the plasma samples were stored at −18°C until analysis. Microdialysis samples (dialysate) were collected every 20 min and stored at −18°C until analysis.

Protein binding in rat plasma was determined by ultrafiltration. Plasma samples (1 mL) containing 5 μg/mL of TA were centrifuged in the ultrafiltration devices (30 kDa, Amicon) at $1530 \times g$ in a fixed angle rotor at ambient temperature.

The concentration of TA, TAP, and methylprednisolone (MP, internal standard), were determined by HPLC using a Zorbax ODS C18 (150 × 4.6 mm) column and UV detection (254 nm), with the lowest quantitation limit of 1 μg/mL.[10] Rat plasma samples were suitably diluted with blank human plasma to get adequate volume for analysis. The plasma samples (0.5 mL) were analyzed after extraction by ethyl acetate, evaporating the organic layer and reconstituting the residue in the mobile phase before injecting into the HPLC system. The ultrafiltrate was directly injected into the column.

Microdialysis concentrations, after correcting for recovery, and the time corresponding to the midpoint of collection period were combined with plasma levels and simultaneously fitted to a two-compartment model using SCIENTIST® software (Version 2, MicroMath, Salt Lake City, UT) as shown below. Intercompartmental clearances were assumed to be equal and the microdialysate concentrations (corrected for recovery) were assumed to be equal to the unbound tissue concentrations.

$$\frac{dX_c}{dt} = k_0 - k_{12} \cdot X_c - k_{10} \cdot X_c + k_{21} \cdot X_p$$

$$\frac{dX_p}{dt} = k_{12} \cdot X_c - k_{21} \cdot X_p$$

$$C_c = \frac{X_c}{V_c}$$

$$C_{Pu} = \frac{f_{up} \cdot X_p}{V_p}$$

where

$$\frac{V_p}{f_{up}} = \frac{k_{12} \cdot V_c}{k_{21} \cdot f_u}$$

where, X_c and X_p are the amounts of TA in central and peripheral (tissue) compartments, respectively; k_0 is the zero-order infusion rate; k_{12} and k_{21} are the intercompartmental rate constants; k_{10} is the elimination rate constant from the central compartment; C_c and C_p are the concentrations in the central and the peripheral compartments with volumes of V_c and V_p, respectively; C_{pu} is the unbound drug in muscle; and f_u and f_{up} are the fractions unbound in the plasma and peripheral compartment, respectively. The data was fitted using f_u as a parameter in the model.

RESULTS AND DISCUSSION

The objective of the present study was to develop a rat microdialysis model for measuring unbound tissue concentrations of TA. The drug was administered by a constant rate infusion to avoid the problem of time to equilibrium between plasma and tissue compartments following bolus doses. High loading and maintenance doses were administered to obtain measurable levels of TA in both plasma and microdialysis samples. Following I.V. administration, TAP was rapidly hydrolyzed into TA in rats as had been described before.[11]

Recovery of TA by retrodialysis in different rats ranged from 41.1 to 71.8% (median 59.8%). The *in vivo* recovery of TA by retrodialysis (59.8%) was in the same range obtained in the *in vitro* experiments.[2] In the *in vitro* experiments, the retrodialysis recovery of TA was 67.2–72.8% and 55.1–63.8% at room temperature; and 77.7–81.3% and 65.3–68.7 at 37°C under stirred and unstirred conditions, respectively. It is reported in the literature that the microdialysis of lipophilic and highly protein-bound substances is problematic.[5–9] The low recovery of lipophilic compounds in the dialysate may be attributed to lower solubility in the hydrophilic perfusate medium, nonspecific binding to the probe, and high protein binding. TA is a lipophilic compound with a log *p*-value of 2.4, and is poorly soluble in water.[12,13] In the present study, the recovery of TA from muscle into Ringer's solution was consistent (59.8%) and no problems with the recovery or nonspecific binding to the probe were encountered.

Figure 1 shows the concentration-time profile of TA at steady state. The mean plasma concentration over a period of 5 h was 21.9 ± 2.3 μg/mL and the measured microdialysate concentrations corrected for recovery was 2.73 ± 0.42 μg/mL. The pharmacokinetic model adequately fitted the total plasma concentrations. The pharmacokinetic parameters of TA are summarized in Table 1. TA plasma concentrations were compared to unbound concentrations in muscle as measured by microdialysis. In the simultaneous fitting of plasma and recovery corrected microdialysate concentrations, f_u, a model parameter, was 0.093 and was comparable to the unbound fraction of TA in rat plasma determined by ultrafiltration (0.099). Protein binding of TA in rat plasma was different from that in human plasma where percent binding estimates have been reported to be 71, 68, and 81%.[2,14,15] Microdialysate concentrations, corrected for recovery, showed excellent agreement with the

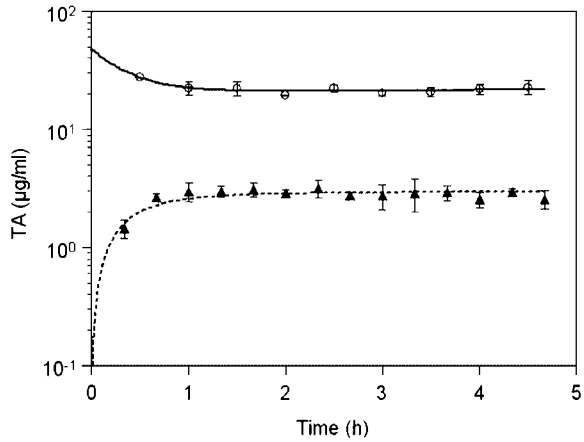

Figure 1. Concentrations of TA (mean ± SD) in plasma and microdialysis samples in rats ($n = 3$) after a loading dose (50 mg/kg) followed by continuous 23 mg/kg/h infusion of TAP (○, total plasma concentration; ▲, microdialysate concentration in muscle, corrected for recovery;—, fitted total plasma profile;– – –, fitted free drug in the peripheral compartment). The error bars indicate the standard deviations.

Table 1. Pharmacokinetic Parameters of Triamcinolone Acetonide in Rat Plasma After i.v. (50 mg/kg bolus + 23 mg/kg/h Infusion) Administration of Triamcinolone Acetonide Phosphate

Parameter	Rat 1	Rat 2	Rat 3	Mean ± SD
V_c (L/kg)	0.684	0.856	1.29	0.944 ± 0.314
CL (L/h/kg)	1.15	0.790	0.872	0.937 ± 0.188
k_{12} (h^{-1})	1.64	1.79	1.59	l1.67 ± 0.102
k_{21} (h^{-1})	1.04	0.640	1.13	0.937 ± 0.261
$T_{½\beta}$ (h)	1.55	3.71	2.87	2.71 ± 1.09
f_u	0.084	0.110	0.085	0.093 ± 0.015

predicted unbound tissue concentrations. Similar comparisons of microdialysate concentrations in tissues with plasma concentrations have been performed.[16,17] Although good correlation between unbound plasma concentration and unbound tissue levels, measured by microdialysis, was observed for piperacillin and tazobactam, unbound muscle and lung concentrations of cefaclor were found to be lower than the free concentrations in plasma. Because a peripheral compartment in a compartment model can not be attributed to a particular physiological space, the correlation observed in this study between measured microdialysate concentrations and unbound plasma concentrations of TA should not be generalized for other drugs.

The clearance of TA in the rat was calculated based on plasma level data. CL was calculated as the ratio of the infusion rate (R_0) to the steady state concentration (C_{ss}) and was found to be 1.06 L/h/kg and was comparable to the model fitted CL (0.937 L/h/kg, Table 1).

The present study addresses the *in vivo* microdialysis of triamcinolone acetonide in rat muscle. The measured microdialysis concentrations of TA, corrected for recovery were comparable to the predicted free drug levels in the muscle and to the unbound plasma concentrations. The study also shows that it is possible to apply microdialysis to the measurement of free tissue levels of lipophilic drugs.

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