Novel Method to Determine Bioequivalence of Complex Drugs

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Abstract

An analytical challenge for complex drug bioequivalence is accurate measurement of encapsulated and unencapsulated drug (1). To overcome this analytical challenge, a novel ultrafiltration drug release method utilizing stable isotope tracers has been developed. Stable isotopically-labeled active pharmaceutical ingredient (API) is spiked into plasma containing the complex drug formulation (2). The isotopically-labeled API equilibrates with plasma protein and formulation components identical to the normoisotopic API released from the complex formulation. Therefore, the ultrafilterable fraction of the isotope-labeled API represents a reliable measure of free normoisotopic API fraction in plasma, and can be used to calculate encapsulated and unencapsulated API fractions. To demonstrate the utility of the stable isotope tracer method, we performed an in vitro drug release study in rat plasma, and in vivo bioequivalence study in rats, comparing Janssen's Doxil® and Sun Pharma's Doxorubicin HCl Liposome generic. Using the stable isotope tracer method, a comparable ~2% of both the liposomal doxorubicin (DXR) preparations were released in rat plasma over a 6h period. A parallel design bioequivalence study in rats demonstrated similar encapsulated and unencapsulated drug pharmacokinetic (PK) profiles for both liposomal preparations. Statistical analysis determined the formulations to be bioequivalent on the basis of T_{max}, encapsulated C_{max}, AUC_{all} and AUC_{inf}, and unencapsulated C_{max}, but not unencapsulated AUC_{all}. Notably, the estimated unencapsulated drug profiles in this study differed greatly from previously published rat bioequivalence studies utilizing solid phase extraction methods, with regard to unencapsulated drug concentrations, encapsulated to unencapsulated drug concentration ratio, and unencapsulated drug terminal half-life.

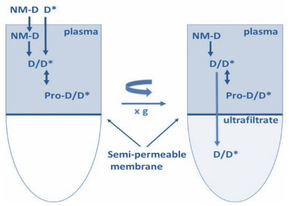

Fig. 1. Drug release assay using a stable isotope tracer ultrafiltration method. The stable isotopically labeled drug (D*) is spiked into nanomedicine (NM-D) in plasma. D* behaves identically to normoisotopic drug (D) with regard to protein binding (Pro-D/D*). After protein binding equilibrium is reached, the plasma sample is transferred to an ultrafiltration device and the filtrate is separated by centrifugation. The stable isotope tracer free fraction, represented as the ultrafilterable fraction, can be used to calculate protein bound, unencapsulated and encapsulated drug fractions, according to equations (i), (ii) and (iii), respectively:

i. $\%Bound = \frac{[Reservoir\ D^*] - [Ultrafilterable\ D^*]) * 100}{[Reservoir\ D^*]}$

ii. $[Unencapsulated\ D] = \frac{[Ultrafilterable\ D]}{(1-(\%Bound\ D^*/100))}$

iii. [Encapsulated D] = [Total D] – [Released D]

Design and methods

In Vitro Drug Release in SD Rat Plasma

Blood pooled from 8 male Sprague Dawley rats was collected in K_2EDTA tubes, spun at 2500xg to collect plasma, and HEPES buffer added at 50 µL/2 mL plasma to control pH. Plasma samples in glass vials were spiked with liposomal formulations at final concentration of 0.5 µg/mL, 1 µg/mL, and 5 µg/mL DXR in triplicate, and then spiked with 0.1 µg/mL DXR_C13 (stable isotope). Samples were then incubated at 37°C with agitation, and at time points 10 min, 2 h and 6 h, 25 µL of the sample was collected in Eppendorf tubes for analysis and 150 µL of the sample was transferred to a prewarmed ultrafiltration tube. The ultrafiltration tube was spun for 10 min at 12000xg at 37°C, and 50 µL of filtrate collected in Eppendorf tubes for analysis. 200 µL ACN/0.1% formic acid containing 25 ng/mL aclarubicin (ISTD) was added to plasma and filtrate samples, freeze-thawed to precipitate proteins, centrifuged, and supernatants dried in a vacuum centrifuge and the resulting residue reconstituted in 150 µL 25% ACN/0.1% formic acid. Samples were then analyzed on a Thermo Fisher Q Exactive OrbiTrap, using matrix matched standard curves and controls.

Animal Study Design

A parallel design bioequivalence study was conducted in double jugular catheterized 15-week-old male Spargue Dawley rats (approx. weight of 400 grams, Charles River Laboratories, Raleigh, N.C.). Rats were treated intravenously by left catheter with 5 mg DXR/5 mL/kg of Janssen's Doxil® or Sun Pharma's Doxorubicin HCl Liposome generic (8/treatment group). Blood samples (400 µL) were collected in K_2EDTA tubes by the right jugular catheter at 0.25, 0.5, 1, 4, 8, 24, 48 and 96 h. Blood was spun at 2500xg for 10 min and plasma collected in a glass vial. DXR_C13 stable isotope was spiked into the plasma at approximately 50 ng/mL. Samples were vortexed and incubated for 10 min at 37°C with agitation. 25 µL plasma was removed to Eppendorf tubes containing 200 µL ACN/0.1% formic acid with 25 ng/mL Aclarubicin (ISTD). The remainder of plasma was transferred to a prewarmed 10 kDa MWCO filter tube and spun at 12,000xg for 10 min at 37°C. 50 µL of the filtrate was transferred to an Eppendorf tube containing 200 µL ACN/0.1% formic acid with 25 ng/mL aclarubicin (ISTD). Samples were frozen at -80°C until analysis. Samples were then thawed, centrifuged, and supernatants dried in a vacuum centrifuge and the resulting residue reconstituted in 150 µL 25% ACN/0.1% formic acid. Samples were then analyzed on a Thermo Fisher Q Exactive OrbiTrap, using matrix matched standard curves and controls.

Validated Q-OrbiTrap Analysis of DXR

Mobile Phase A: H_2O with 0.1% Formic Acid
Mobile Phase B: Acetonitrile with 0.1% Formic Acid
Column: Zorbax SB C18, 2.1 x 100 mm, 1.8 µm
Gradient: Hold at 25% B for 3 min, linear increase from 25% - 80% B in 4 min, linear increase to 95% B in 0.5 min, hold at 95% B for 0.5 min, linear decrease from 95% - 25% B in 0.1 min, column regeneration time of 4 min
ESI Positive mode, PRM 544.18 → 130.08, 379.07, 397.08 amu
Plasma LLOQ = 10 ng/mL , Protein free plasma LLOQ = 100 pg/mL

Noncompartmental Pharmacokinetic Analysis

Noncompartmental pharmacokinetic parameters were determined using Phoenix WinNonlin version 6.3 software (Pharsight Corporation, Mountain View, CA): the area under the time concentration curve (AUC_{inf}) was calculated using the linear trapezoidal rule with extrapolation to time infinity; terminal half-life ($t_{1/2}$) was calculated from 0.693/slope of the terminal elimination phase; the C_{max} term is the maximum concentration; the T_{max} term is the time of maximum concentration.

Statistics

In vitro data were evaluated by a two-sided Student's t-test, with α=0.05 . In Vivo PK parameters were evaluated by two one-sided t-tests, with α=0.05 and Θ=0.2, to determine the 90% CI of the geometric mean of log transformed T/R ratio.

Results

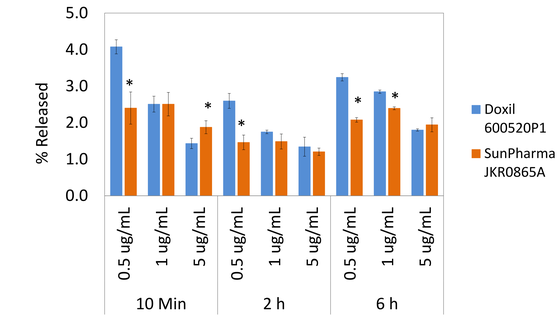

Fig. 2. DXR release in rat plasma at 37°C. Displayed is the % DXR release at each concentration and time point, for the Sun Pharma and Doxil formulations (Mean + SD, *N*=3). (*p<0.05, Students t-test)

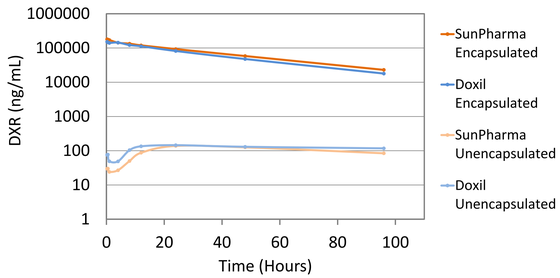

Fig. 3. DXR pharmacokinetics in SD rats. Displayed are the average encapsulated and unencapsulated DXR concentrations for each formulation expressed as ng/mL at each time point (*N*=8).

		Unencapsulated			Encapsulated			
	Animal	T_{max} h	C_{max} ng/mL	AUC_{all} ng*h/mL	T_{max} h	C_{max} ng/mL	AUC_{all} ng*h/mL	AUC_{inf} ng*h/mL
Sun Pharma	1	48	171	12522	1	166940	4341721	4541653
	3	24	117	8871	0.25	185303	7569868	9067173
	5	24	224	9827	1	173156	6894633	8199833
	7	48	157	11167	0.5	191985	8056557	10190358
	9	24	104	8011	0.25	202248	7194422	8911107
	11	24	115	7863	0.5	191702	7198496	8403677
	13	48	125	8427	0.25	195679	5739068	6339516
	15	24	241	14367	1	180606	6758153	7585164
	AVG	33	157	10132	0.59	185952	6719115	7904810
	SD	12	52	2359	0.35	11855	1171877	1764188
Doxil	17	48	155	11556	0.25	156683	5883992	7031063
	19	48	139	10770	4	145434	5641037	6337460
	21	48	139	10770	4	172599	6080370	7044728
	23	96	273	16159	4	145738	6324194	7617542
	25	24	201	12397	0.5	163420	5645537	6109138
	27	12	198	13022	0.25	153541	5015026	5083284
	29	12	163	12182	0.5	180756	5643580	6575046
	31	0.25	218	13410	0.25	169214	6626650	8017609
	AVG	36	186	12533	1.72	160923	5857548	6726984
	SD	31	46	1751	1.89	12804	493830	918891

Table 1. Individual animal pharmacokinetic parameters. Individual PK parameters are presented for the Sun Pharma and Doxil, encapsulated and unencapsualted, DXR plasma profiles: area under the time concentration curve extrapolated to infinity (AUC_{inf}); area under the time concentration curve all time points (AUC_{all}); time of maximum concentration (T_{max}); maximum concentration (C_{max}).

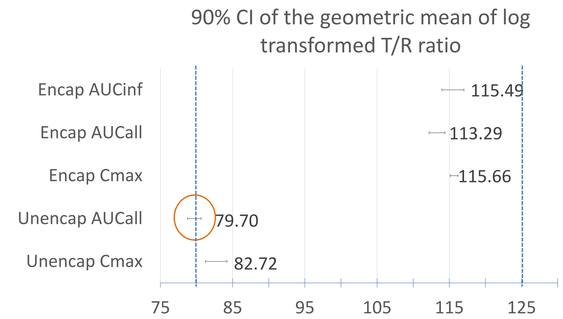

Fig. 4. TOST bioequivalence analysis. All PK parameters found to be equivalent, with 90%CI of the test (Sun Pharma)/reference (Doxil) ratio within 80-125% by TOST, except for unencapsulated AUCall (orange circle).

Summary

- A similar ~2% in vitro drug release was estimated for both formulations, with some instances of statistically significant differences.
- All PK parameters were found to be equivalent, except for unencapsulated AUC_{all}.
- Important differences were observed for this stable isotope tracer method study compared to a previous literature SPE method study in rats (3):
  - Unencapsulated drug concentrations were much lower for the stable isotope study, resulting in encapsulated/unencapsulated drug ratios of ~800 compared to ~60 for the previous SPE method study.
  - The terminal slope of the unencapsulated profile did not parallel the encapsulated profile, as it did for the previous SPE method study.
  - T_{max} is much later for the stable isotope study at ~33 h, compared to ~4 h for the SPE study.

References

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