

Hemolysis of Blood Samples has no Significant Impact on the Results of Pharmacokinetic Data

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Abstract

Purpose: This study examined whether hemolyzed blood samples affect the pharmacokinetic (PK) profile.

Methods: A validated LC-MS/MS method was used to analyze both hemolyzed and non-hemolyzed plasma samples obtained from healthy volunteers to whom Clopidogrel, Methylprednisolone and Ropinirole were administered orally in three independent bioequivalence (BE) studies.

Results: The drug concentrations of hemolyzed and non-hemolyzed plasma samples, were, respectively: clopidogrel (n=12) 862.57 ± 860.16 (ng/mL) and 920.61 ± 959.14 (ng/mL); Methylprednisolone (n=10) 155.21 ± 33.60 (ng/mL) and 160.01 ± 29.9 (ng/mL); Ropinirole (n=16) 1322.87 ± 392.96 (ng/mL) and 1151.42 ± 299.91 (ng/mL). The drug concentrations between hemolyzed and non-hemolyzed plasma samples did not yield a significant difference (P>0.05).

Conclusions: The measurable plasma concentrations of the test drugs were not significantly different from those of normal non-hemolyzed plasma samples, suggesting that there was no impact on the accuracy of PK profile of the three test drugs.

Keywords: Hemolysis; Pharmacokinetics; Bioequivalence; LC-MS/MS

Introduction

Hemolysis is a common phenomenon during blood collection. The phenomenon refers to the breakage of the membrane of erythrocytes, which causes the release of hemoglobin and other internal components into the surrounding fluid of cells. Hemolysis is visually detected by showing pink to red in serum or plasma sample during or after the sample process. There are two main types of hemolysis: in-vivo and in-vitro hemolysis. In-vivo hemolysis occurs as a result of pathological conditions, such as autoimmune hemolytic anemia or transfusion reaction. In-vitro hemolysis commonly occurs during blood collections or blood sample processes [1,2].

The quality of blood samples can impact the accuracy of medical laboratory test results [3], especially potassium values, because the free hemoglobin increases the amount of potassium in the serum. The increases or decreases in other laboratory test values caused by in-vitro hemolysis are based on a concentration gradient between cells and plasma. The hemoglobin and other intracellular components that leak into the surrounding fluid induce false elevations of some analyses or dilution effects. Besides hemoglobin, erythrocytes contain several structural proteins, enzymes, lipids, and carbohydrates, which may also interact or compete with the assay reagents. The hemolyzed blood samples may lead to an overestimation of aspartate aminotransferase (AST), creatinine, creatine kinase (CK), iron, lactate dehydrogenase (LDH), lipase, magnesium, phosphorus, potassium and urea, whereas values of albumin, alkaline phosphatase (ALP), bilirubin, chloride, γ -glutamyltransferase (γ -GT), glucose and sodium may be substantially decreased by dilution effects [4-7].

During a Bioequivalence/Pharmacokinetics (BE/PK) study, serial-time-point collections of blood samples are required. A range of several hundred to several thousand blood samples may be required in a given BE/PK study for analyses of test drug concentrations through LC/MS/MS method. Non-hemolyzed and good quality blood samples

are always preferred even it is not clear whether hemolyzed samples would affect the PK profile. Therefore, we picked one time point and analyzed the data collected from three pilot BE studies with Clopidogrel, Methylprednisolone and Ropinirole, respectively to explore whether hemolyzed blood samples could impact the PK profiles of the three drugs.

Materials and Methods

Subjects

All three pilot BE studies with Clopidogrel, Methylprednisolone and Ropinirole, respectively were conducted at the Clinical Research Center, First Affiliated Teaching Hospital of Zhengzhou University. All study subjects were healthy Chinese male and female volunteers (male is 24 and female is 14). The study protocols had been approved by the Institutional Review Board (IRB) (IRB00007169) of the Hospital. Before enrollment, all subjects signed an IRB approved Informed Consent Form (ICF) and underwent clinical screening, including a physical examination and medical laboratory tests. Total 12, 10 and 16 health volunteers were enrolled into the pilot BE studies of Clopidogrel, Methylprednisolone and Ropinirole, respectively.

Study drugs and design

Single doses of Clopidogrel bisulfate 75 mg tablet, Ropinirole

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Received June 04, 2012; Accepted July 23, 2012; Published July 26, 2012

Citation: Zhao J, Kan Q, Wen J, Li Y, Sheng Y, et al. (2012) Hemolysis of Blood Samples has no Significant Impact on the Results of Pharmacokinetic Data. J Bioequiv Availab 4: 082-085. doi:10.4172/jbb.1000117

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2 mg tablet, and Methylprednisolone 4 × 4 mg tablets, respectively, were studied in their respective study. Each of three studies was a randomized, open-label, single-dose, two-way-crossover study under fasting condition to assess the bioequivalence of reference (R) and test (T) formulations of the tested drug in healthy adult male and female subjects. Each subject was randomized to one of two treatment sequences (R, T) or (T, R) according to a pre-generated randomization schedule. The content of the meals, meal times, intakes of liquid, activities during in-house confinements were standardized for all subjects.

In the Clopidogrel study, serial blood samples for PK analysis were obtained at time 0 (within 30 minutes pre-dose) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, hours post-dose. In the study of Ropinirole, serial blood samples were obtained at time 0 (within 30 minutes pre-dose) and at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36 and 48 hours post-dose. In the study Methylprednisolone, serial blood samples for PK analysis were obtained on day 1 at time 0 (within 30 minutes pre-dose) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12, and 24 hours post-dose.

Samples processing and hemolyzed blood samples preparation

The study protocol and applicable standard operation procedures (SOPs) were followed. Whole blood samples were collected from enrolled subjects in accordance with above individual study sampling schedule. One time point obtained from the blood samples during the PK profile in each BE study were used for hemolyzed investigation. The blood sample obtained was divided into two tubes with about equal amount. One tube was processed appropriately to isolate plasma based on SOP (Standard Operation Procedures) for BE study, and the other was prepared for hemolyzed blood sample. A straw was used to pull in and out the whole blood sample for several times before centrifuge, then the visible hemolysis following centrifugation was defined as the presence of free hemoglobin (>100 mg/L) [8] in plasma based on Figure 1.

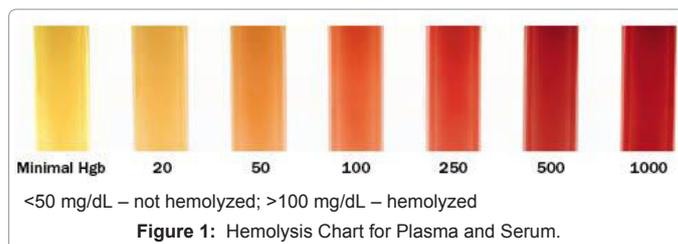
Blood samples were collected into vacutainers containing K₂EDTA (1 × 7 mL) and stored on ice prior to processing, which was begun within 30 minutes of collection. Plasma was separated by centrifugation at a speed of 3500 rpm for 10 minutes at 4°C. Equal aliquots were transferred to 2 clearly labeled tubes and stored in a freezer at approximately -70°C. One plasma sample was the primary assay sample and the second was severed as the backup sample.

Drug concentration measurement

The LC-MS/MS quantitation methods were developed and validated for Clopidogrel, Ropinirole, and Methylprednisolone, respectively, at Frontage Laboratories, (Shanghai) Ltd. These validated methods were employed for determination of the drug concentrations in the human plasma samples generated from the studies. All statistical analyses were performed using SPSS software version 10.0.

Results

The quality assurance (QA) verified drug concentrations are shown in Tables 1, 2, and 3 for Clopidogrel, Methylprednisolone and Ropinirole, respectively. The first period at 1 hour post-dosing of Clopidogrel plasma concentration was 862.57 ± 860.16 (ng/mL) for hemolyzed plasma and 920.61 ± 959.14 (ng/mL) for non-hemolyzed plasma. The second period at 1 hour post dosing of clopidogrel plasma concentration was 895.61 ± 590.47 (ng/mL) for hemolyzed plasma and 941.60 ± 601.91 (ng/mL) for non-hemolyzed plasma (Table 1).



	n	Period 1		Period 2	
		Conc(ng/mL)	log conc	Conc(mg/mL)	log conc
Hemolyzed sample	12	862.57 ± 860.16	2.77 ± 0.38	895.61 ± 590.47	2.81 ± 0.42
Non-hemolyzed sample	12	920.61 ± 959.14	2.77 ± 0.39	941.60 ± 601.91	2.84 ± 0.41
t		0.1560	0.1002	0.1889	0.1759
P		0.8774	0.9211	0.8519	0.8619

Clopidogrel plasma concentration at 1 hour post dosing during the first period and second period Clopidogrel pilot BE study

Table 1: Clopidogrel Concentrations (ng/mL).

The first period at 2.5 hours post-dosing of Methylprednisolone plasma concentration was 155.21 ± 33.60 (ng/mL) for hemolyzed plasma and 160.01 ± 29.9 (ng/mL) for non-hemolyzed plasma. The second period at 2.5 hours post-dosing of Methylprednisolone plasma concentration was 160.01 ± 29.99 (ng/mL) for hemolyzed plasma and 127.40 ± 41.61 (ng/mL) for non-hemolyzed plasma (Table 2).

The first period at 4 hours post-dosing of Ropinirole plasma concentration was 1322.87 ± 392.96 (ng/mL) for hemolyzed plasma and 1151.42 ± 299.91 (ng/mL) for non-hemolyzed plasma. The second period at 4 hours post-dosing of Ropinirole plasma concentration was 1146.30 ± 249.89 (ng/mL) for hemolyzed plasma and 1220.01 ± 196.67 (ng/mL) for non-hemolyzed plasma (Table 3).

The differences of drug concentrations between the two different samples (hemolyzed and non-hemolyzed) for each drug were considered significant if p ≤ 0.05. Based on the results shown in the tables, there were no significant differences (p>0.05) in drug concentrations between the hemolyzed and non-hemolyzed samples for Clopidogrel, Methylprednisolone, respectively. In summary, hemolysis of blood samples has no significant impact on the results of the above three bioequivalence studies in healthy volunteers.

Discussion

During collections, processes and transports of PK samples in pharmacokinetics or bioequivalence (BE) clinical studies, hemolysis could occur due to many reasons, including improper venipuncture processes, sample processes, and sample transports. Improper venipuncture procedures include difficulty to find veins, improper venipuncture sites, multiple times of venipuncture at the same site, prolongation of tourniquet time, incomplete drying of the venipuncture site after cleansing with alcohol, too slow or too fast blood flow, and use of a small-bore needle [1,2]. Improper sample processes include too warm or too cold blood sample storage, delay to centrifugation, and improper centrifugation rate and time [3]. Improper sample transports include vigorous mixing or shaking of the blood samples, inadequate time for clot which can result in fibrin formation in serums [3].

Clopidogrel is a thienopyridine class antiplatelet agent, and is used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease [9]. Clopidogrel has an absolute S configuration at carbon 7 and its chemical structure is shown in Figure 2 [9]. This study showed that the hemolyzed blood samples did not significantly impact the PK profile of Clopidogrel in healthy volunteers.

Ropinirole is a non-ergoline dopamine agonist and is used in the treatment of Parkinson's disease [10] and its chemical structure is shown in Figure 3. The metabolic pathway for Ropinirole in human is predominantly through CYP1A2 in the liver by forming two metabolites. In this study, the hemolysis of blood samples did not affect the PK profile of Ropinirole in healthy volunteers.

Methylprednisolone is a synthetic glucocorticoid and is widely used for anti-inflammatory effects [11] and its chemical structure is shown in Figure 4. In this study it showed that the hemolyzed blood samples did not significantly impact the PK profile of Methylprednisolone in healthy volunteers.

The reason the above three drugs were chosen for this study is their variety. Since hemolysis of the blood sample didn't affect any of PK profiles of these drugs, the finding can be extrapolated to other drugs. However, when additional studies are conducted that analyze the effect of hemolyzed blood samples on the PK profile of a wider variety of drugs, more information will be provided that extend these findings.

Hemolysis of blood samples is a common problem encountered during medical practice and PK/BE study. It leads to inaccurate medical laboratory results and often necessitates a repeat experiment. For a BE study, serial blood samples need to be collected on dosing day and should extend to at least 3 terminal elimination half-lives beyond the time to peak concentration (T_{max}). The PK profile has always been a concern if hemolyzed blood samples were obtained since research has not been conclusive on the effect of hemolyzed blood samples on

	Period 1			Period 2	
	n	Conc(ng/mL)	log conc	Conc(ng/mL)	log conc
Hemolyzed sample	16	1322.87 ± 392.96	3.11 ± 0.12	1146.30 ± 249.89	3.05 ± 0.10
Non-hemolyzed sample	16	1151.42 ± 299.91	3.05 ± 0.11	1220.01 ± 196.67	3.07 ± 0.11
t		1.387	1.436	0.7602	0.6609
P		0.1756	0.1614	0.4531	0.5137

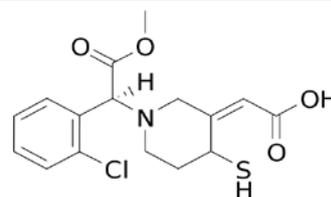
Ropinirole plasma concentration at 4 hour post dosing during the first period and second period Ropinirole pilot BE study

Table 2: Ropinirole Concentrations (ng/mL).

	Period 1			Period 2	
	n	Conc(ng/mL)	log conc	Conc(ng/mL)	log conc
Hemolyzed sample	10	155.21 ± 33.60	2.18 ± 0.09	160.01 ± 29.99	2.20 ± 0.08
Non-hemolyzed sample	10	160.01 ± 29.9	2.20 ± 0.08	127.40 ± 41.61	2.07 ± 0.23
t		0.3371	0.3754	2.011	1.704
P		0.74	0.7117	0.0596	0.1055

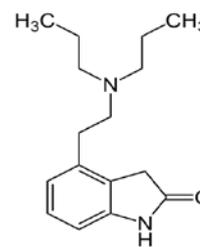
Methylprednisolone plasma concentration at 2.5 hour post dosing during the first period and second period Methylprednisolone pilot BE study

Table 3: Methylprednisolone Concentrations (ng/mL).



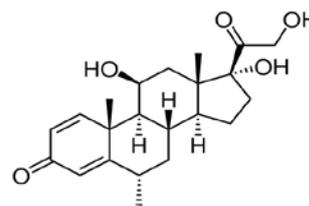
(+)-(S)-methyl 2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl) acetate

Figure 2: Clopidogrel Chemical Structure.



4-[2-(dipropylamino) ethyl]-1,3-dihydro-2H-indol-2-one

Figure 3: Ropinirole Chemical Structure.



Pregna-1,4-diene-3,20-dione, 11,17,21-trihydroxy-6-methyl-, (6a,11b)-. 11b,17,21-Trihydroxy-6a-methylpregna-1,4-diene-3,20-dione [83-43-2].

Figure 4: Methylprednisolone Chemical Structure.

PK profile. However, it remains that hemolyzed plasma samples can influence some medical laboratory test results even if it does not affect PK profiles.

Acknowledgements

We would like to thank James E. Barrett, Ph.D. (Professor and Chair, Department of Pharmacology and Physiology, Director, Program in Drug Discovery and Development, Drexel University College of Medicine, 245 North 15th Street, Philadelphia, PA 19102-1192) for his serious editing and critical review this paper.

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