Red Blood Cell (RBC) Partitioning

Determine distribution of drug candidates between plasma and RBCs

Over 99% of the total cellular space in human blood is made up of red blood cells (RBCs) to which drugs can bind or become sequestered by passive diffusion. This can significantly impact absorption and distribution and determine whether a drug reaches its target and how much of it is available to exert its therapeutic effect.

Certain compounds are preferentially sequestered into RBCs and therefore have a blood-to-plasma ratio that is greater than 1. For example, chloroquine has a high potential for binding to RBCs and its blood-to-plasma ratio is higher than 2[1]. Other compounds, such as metoprolol, do not sequester into RBCs and have a blood-to-plasma ratio of approximately 1. Some other drugs, for example naproxen, are found predominantly in plasma with blood-to-plasma ratios lower than 1[2]. These examples illustrate that an assumption that the drug concentration found in plasma is representative of the concentration found in whole blood can be inaccurate.

This is important because pharmacokinetic (PK) parameters are usually determined by measuring drug concentration in plasma and not whole blood. As a result, PK parameters determined using only plasma may be misleading and potentially result in data misinterpretation and an over- or underestimation of clearance.

It is therefore important to experimentally determine the blood-to-plasma distribution ratio to better understand PK data. To account for RBC binding, the blood-to-plasma ratio can then be included in some calculations to improve predictions of *in vivo* drug distribution and drug-drug interactions.

BioIVT offers RBC partitioning assays to determine the potential for a drug candidate to bind to RBCs. The study design quantifies the blood-to-plasma ratio by measuring drug concentration in whole blood and plasma in human and various animal species.

Study Design

Element	Feature	Standard
Design	Pooled whole blood from three human donors, mixed gender or pooled whole blood from three animals of chosen species (male) Species: human, rat, mouse, dog, monkey (additional species available upon request)	✔
	Drug candidate concentrations	7
	Analysis by LC-MS/MS incl. method development	✔
Deliverables	Stability (%), distribution to RBCs (%), blood-to-plasma ratio	✔
	Data Summary Reports	✔

Methodological Considerations and Test Systems

RBC partitioning experiments start by treating whole blood with the drug candidate followed by a centrifugation step to separate the plasma. The amount of the drug candidate in the plasma is then compared to the amount in whole blood.

Study Deliverables

BioIVT's RBC partitioning study reports include a tabular summary of the stability, distribution to RBCs, and blood-to-plasma ratio for all species evaluated as illustrated in Table 1 on page 2.

In vivo RBC Partitioning

In collaboration with research partners, BioIVT offers *in vivo* RBC partitioning evaluation using whole blood from animals treated with radiolabeled compounds.

[1] Hinderling PH. Pharmacol Rev. 1997 Sep;49(3):279-95. Erratum in: Pharmacol Rev 2000 Sep;52(3):473..
[2] Berry LM, Li C, and Zhao Z (2011) Drug Metab Dispos 39:2103-2116.

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Table 1. RBC partitioning data showing % stability, distribution to blood cells, and blood-to-plasma ratio.

Test article (µM)	Matrix	Time	Species	Mean area ratio ± SD	% Stability	% Distribution to blood cells	Blood-to-plasma ratio
0.2	Blood	0	Rat	0.229 ± 0.015	NA	40.0	0.986
		60		0.201 ± 0.012	87.9	55.7	1.33
	Plasma	0		0.232 ± 0.011	NA		
		60		0.151 ± 0.004			
1	Blood	0		1.13 ± 0.02	NA	44.7	1.07
		60		0.874 ± 0.027	77.5	55.2	1.32
	Plasma	0		1.05 ± 0.06	NA		
		60		0.663 ± 0.024			
20	Blood	0		13.8 ± 0.5	NA	34.4	0.900
		60		13.5 ± 0.4	97.8	59.1	1.45
	Plasma	0		15.3 ± 0.9	NA		
		60		9.33 ± 0.3			
0.2	Blood	0	Human	0.181 ± 0.009	NA	39.2	0.944
		60		0.155 ± 0.007	85.6	41.9	0.987
	Plasma	0		0.192 ± 0.009	NA		
		60		0.157 ± 0.005			
1	Blood	0		0.843 ± 0.023	NA	39.5	0.949
		60		0.757 ± 0.035	89.8	47.4	1.09
	Plasma	0		0.889 ± 0.048	NA		
		60		0.693 ± 0.015			
20	Blood	0		14.2 ± 0.1	NA	49.2	1.13
		60		12.3 ± 0.3	86.6	52.1	1.20
	Plasma	0		12.5 ± 0.1	NA		
		60		10.2 ± 0.4			

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