XENOTECH

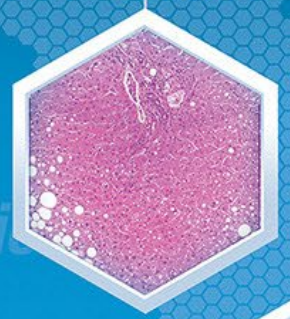
A BioIVT Company

25 Years

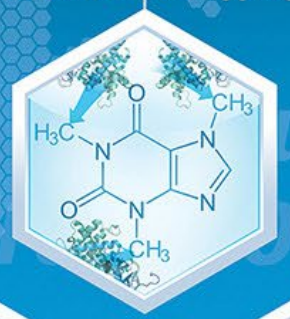
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

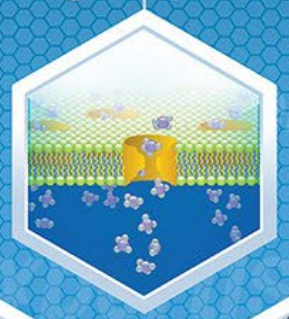


Reaction Phenotyping

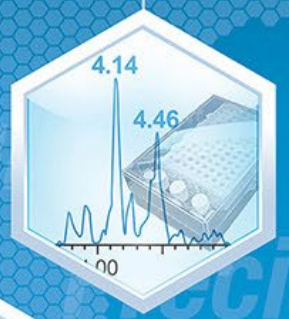


Enzyme Induction & Inhibition

Drug Transporters



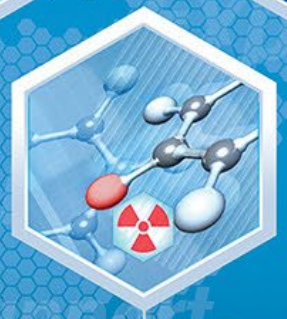
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

Red Blood Cell (RBC) Partitioning Studies

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Research Scientist,
Program Oversight

Presentation Outline

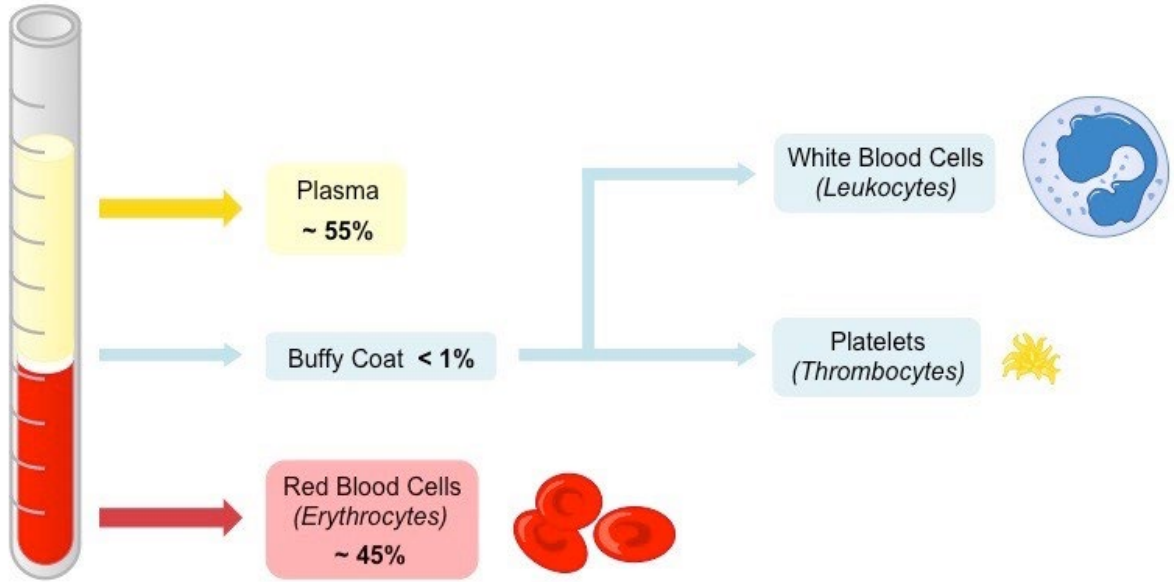
- What is red blood cell (RBC) partitioning?
- Why is measuring RBC partitioning important?
- RBC partitioning study design
- Data example from qualification study

Interactive Webinar Questions for Audience

If you have conducted an RBC partitioning study before, was there a particular trigger for it?

Composition of blood

- The average adult has roughly 5-6 L of blood
- Red blood cells- ~45% (~ 99% of the cellular volume)
- Plasma- ~55%
- White blood cells and platelets- <1%



bioninja.com

What is RBC partitioning?

- Certain compounds will preferentially sequester into red blood cells over plasma
- By measuring the blood to plasma ratio (R_b), we are able to determine the amount of compound in each compartment of the blood
- Knowing the R_b is an important parameter that can help determine whole blood pharmacokinetics
- Failure to take RBC partitioning into account can lead to an over estimation of intrinsic clearance

Why measuring RBC partitioning important?

Use known or predicted clinical PK parameters to estimate:

Maximum unbound hepatic inlet concentration

$$Plasma\ I_{in,max,u} = fu_p \left(Plasma\ I_{max} + \frac{\left(\frac{F_a \cdot F_g \cdot k_a \cdot Dose}{Q_h} \right)}{R_b} \right)$$

$I_{max} = C_{max}\ in\ \mu M$

$Dose\ is\ in\ \mu mol$

fu_p = the fraction of unbound drug in plasma (with a lower limit of 0.01

$k_a = 0.1\ min^{-1}\ (rapid\ absorption)$

$F_a = fraction\ absorbed\ (default\ assumption: F_a = 1)$

$F_g = fraction\ escaping\ gut\ metabolism\ (default\ assumption: F_g = 1)$

$Q_H = hepatic\ blood\ flow = \ 1.6\ L/min$

$R_b = blood - to - plasma\ concentration\ ratio$

This equation is an expanded form of the equation to calculate "[I]h" in Figure 7 of the final 2020 FDA in vitro DDI guidance.

Adapted from Parkinson A (2019). *Drug Metab Dispos* 47:779.

Why measuring RBC partitioning important?

- Traditional pharmacokinetic parameters are measured using plasma not whole blood
- Certain compounds have a high affinity for red blood cells and will have a large blood to plasma ratio
- A high affinity for red blood cells can lead to an overestimation of blood clearance if only the plasma is analyzed
- For these compounds with high blood to plasma ratios, the concentration in whole blood will be more accurate for pharmacokinetic studies

The effects of RBC partitioning

- Compounds can enter RBCs in several ways
  - Lipophilic organic compounds dissolve in the lipid bilayer membrane and enter RBCs
  - Small hydrophilic compounds can enter by diffusion (Hinderling et al. 1997)

- Non-ionized or basic drugs have a R_b close to 1, meaning they distribute evenly between blood and plasma (Halifax et al. 2010)

- Acidic or zwitterionic drugs are excluded from erythrocytes and have R_b values of roughly 0.55 (Halifax et al. 2010)

- The RBC partitioning assay gives us a method to determine the extent a compound is sequestered into red blood cells by measuring the blood to plasma ratio

The effects of RBC partitioning

- Compounds with a high affinity for red blood cells have a large blood to plasma ratio (R_b)
  - Cloroquine has a blood to plasma ratio of greater than 2 (Funmilayo et al. 1989, Hinderling et al. 1997)
- Compounds with a high affinity for plasma have a blood to plasma ratio (R_b) of ≤ 1
  - Metoprolol was a blood to plasma ratio of ~1 (Berry et al. 2011)
- Doripenem has an R_b value of 0.5 (high distribution to the plasma), thus calculated exposure using plasma is almost twice that of calculated exposure using blood (EMA evaluation of Doripenem 2008)
- Butorphanol has an R_b value of almost 2 (high distribution to the blood), thus calculated exposure using blood is almost twice that of calculated exposure using plasma (Paixao et al. 2010)

Tacrolimus is highly bound to RBCs

- Tacrolimus is highly bound to erythrocytes (R_b of 15) and its volume of distribution (Vd) and hepatic clearance vary greatly depending on blood or plasma concentrations

	Volume of distribution	Hepatic clearance
Blood	1.0- 1.5 L/kg	2.1- 6.3 L/h
Plasma	30 L/kg	42- 378 L/h

- When using plasma concentration, the hepatic clearance exceeds hepatic blood flow (97 L/h) and hepatic plasma flow (53 L/h)
- Using plasma overestimates both Vd and hepatic clearance

Wallemacq and Verbeeck 2001
Parkinson 2019

Other drugs highly bound to RBCs

- Some diuretic drugs (chlorthalidone, dorzolamide and methazolamide) bind with high affinity to carbonic anhydrase and due to this binding they do not move freely from erythrocytes to the plasma

- These compounds have high R_b ranging from 30 to 240

- In these cases, using the concentration in the plasma is a more relevant to their ability to cause drug-drug interactions

Hinderling et al. 1997
Parkinson 2019

Interactive Webinar Questions for Audience

When in the developmental process do you conduct RBC partitioning studies?

What knowledge can be gained from RBC partitioning?

- A rational choice of appropriate biological fluid, either whole blood, plasma, or serum, for assay
- Physiologically meaningful referencing of pharmacokinetic parameters of drugs to concentrations in either whole blood, plasma, or serum
- Effective screening of drugs whose biophase resides within the RBCs, thereby enabling the study of the effects of drugs on RBCs

Adapted from Hinderling et al. 1997

XT RBC study design

- Studies conducted using whole blood containing the anticoagulant K_2EDTA from five species
  - mouse, rat, dog and monkey (pool of three or more, male)
  - human (pool of 3 or more, mixed gender
- Three test article concentrations incubated at 37 ± 2 °C for two time points (e.g., 0 and 60 min)
- A high affinity control (chloroquine) and a low affinity control (metoprolol) are included to show competency of the test system

XT RBC study design

- An aliquot of whole blood is removed and the remaining blood is centrifuged to isolate the plasma
- At the end of each time point- whole blood and isolated plasma are mixed with stop reagent containing internal standard then centrifuged a second time
- Supernatants are transferred for analysis of whole blood and isolated plasma by LC-MS/MS, test article estimated by analyte/internal standard area ratio (no calibration curve)

XT RBC calculations

- Stability, distribution to the blood cells and blood to plasma ratio are calculated

$$\text{Stability (\%)} = \frac{\text{Concentration}_{\text{Incubated blood sample}}}{\text{Concentration}_{\text{zero-minute blood sample}}} \times 100$$

$$\text{Distribution to blood cells (\%)} = \left(1 - \frac{\text{Concentration in plasma} \times (100 - \text{Ht})}{\text{Concentration in blood} \times 100}\right) \times 100$$

Ht: Hematocrit value (integer; percent of total blood volume)

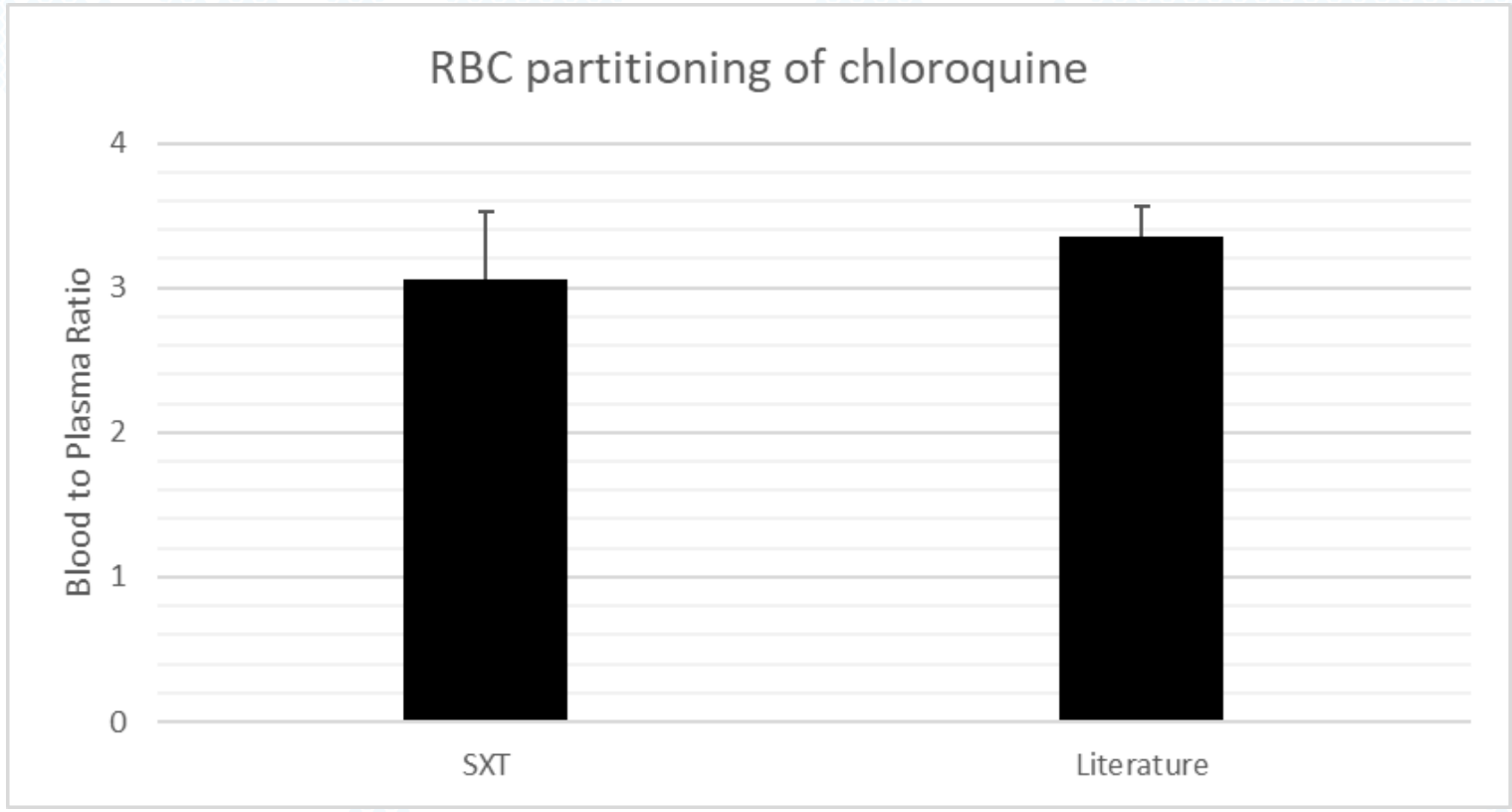
$$\text{Blood to plasma ratio} = \frac{\text{Concentration in blood } (\mu M)}{\text{Concentration in plasma } (\mu M)}$$

XT RBC qualification results

Chloroquine (high affinity control)			
Species	**Stability (%)**	**Distribution (%)**	**Blood to plasma ratio**
Mouse	100 ± 6	82.7 ± 2.8	3.40 ± 0.62
Rat	105 ± 7	81.0 ± 0.9	2.82 ± 0.13
Dog	102 ± 4	91.4 ± 1.0	6.22 ± 0.78
Monkey	96.2 ± 5.3	82.2 ± 2.4	3.05 ± 0.53
Human	89.7 ± 6.3	81.7 ± 3.8	3.06 ± 0.46

Values are an average of 3 or 4 individual trials rounded to three significant figures with standard deviation rounded to the same degree of accuracy

Comparison of human blood to plasma ratio



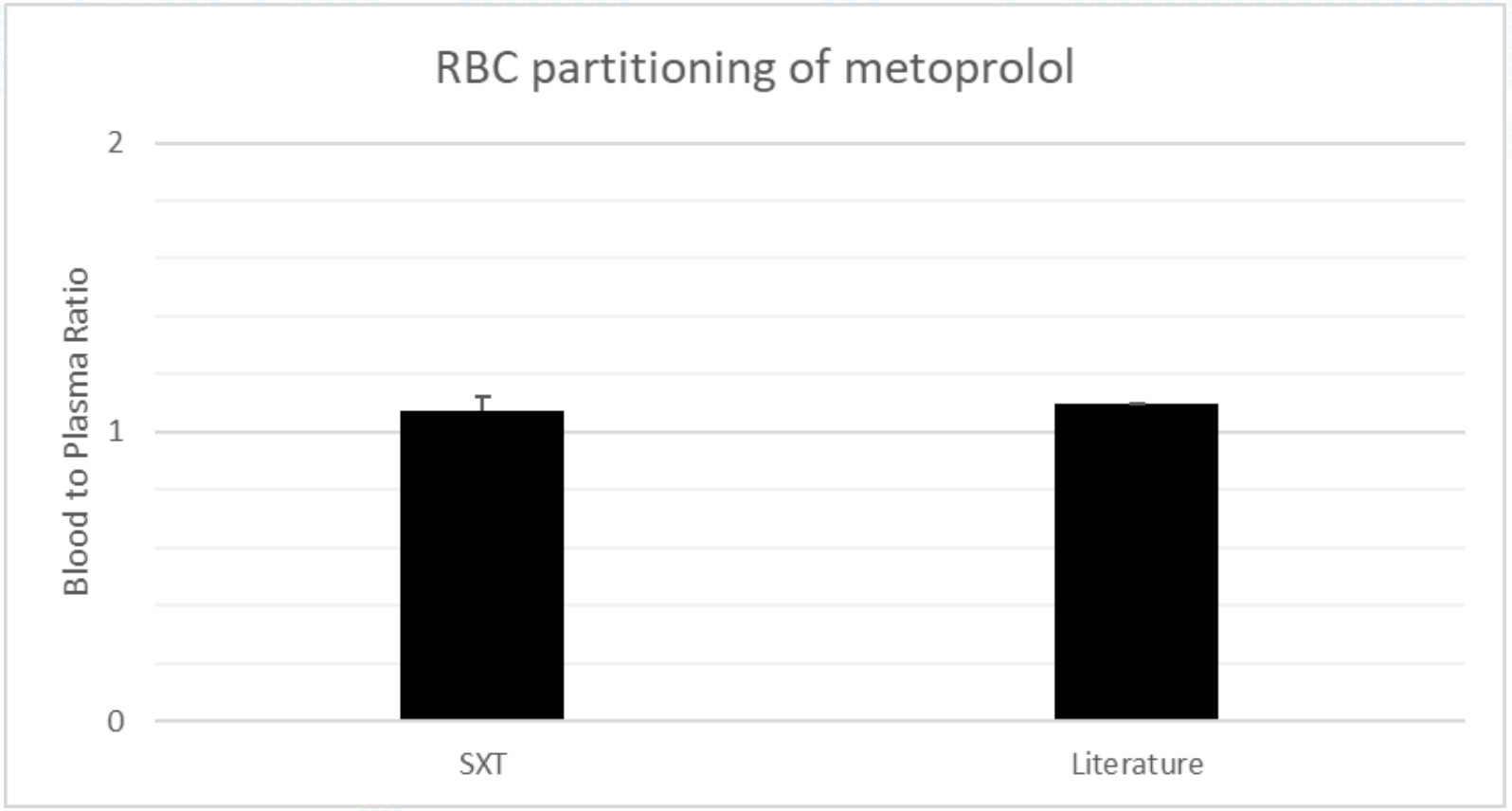
Literature value from Hinderling et al. 1997

XT RBC qualification results

Metoprolol (low affinity control)			
Species	**Stability (%)**	**Distribution (%)**	**Blood to plasma ratio**
Mouse	99.5 ± 3.8	44.2 ± 4.3	1.03 ± 0.08
Rat	104 ± 2	53.4 ± 1.3	1.15 ± 0.03
Dog	105 ± 3.4	53.1 ± 1.5	1.12 ± 0.04
Monkey	98.6 ± 5.3	50.2 ± 0.9	1.07 ± 0.01
Human	92.4 ± 4.1	49.1 ± 5.0	1.07 ± 0.05

Values are an average of 3 or 4 individual trials rounded to three significant figures with standard deviation rounded to the same degree of accuracy

Comparison of human blood to plasma ratio



Literature value from Berry et al. 2011

Interactive Webinar Questions for Audience

After what have you learned during this webinar would you be more likely to conduct RBC partitioning at the same time as plasma protein binding (PPB) ?

Summary

- Certain compounds will preferentially sequester into red blood cells over plasma

- Knowing the extent of RBC partitioning can provide valuable information on which fluid (blood or plasma) to choose for downstream assays

Thank you!

What questions can we answer?