Who Decided We Should Measure Plasma?

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Worldwide PDM Pharmacokinetics Dynamics & Metabolism

Shakespeare meets bioanalysis – The Merchant of Venice

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Merchant of Venice – a metaphor for the banking crisis and analysis of blood

Antonio borrows money from Shylock – guaranteed by 1 pound of flesh cut nearest the heart

Turns out to be toxic debt

Bassiano offers to pay back double the debt

Metaphor for Government bail out

Shylock refuses – want his 'pound of flesh'

Akin to payment of banking bonuses

'If you prick us, do we not bleed?'

NB **NOT** If you prick us, do we not plasma?

Contract does not allow the spilling of 1 drop of blood

NB **NOT** the spilling of 1 drop of plasma?

Is blood is the most appropriate matrix to measure?

Pharmacokinetic parameters are generally based on blood

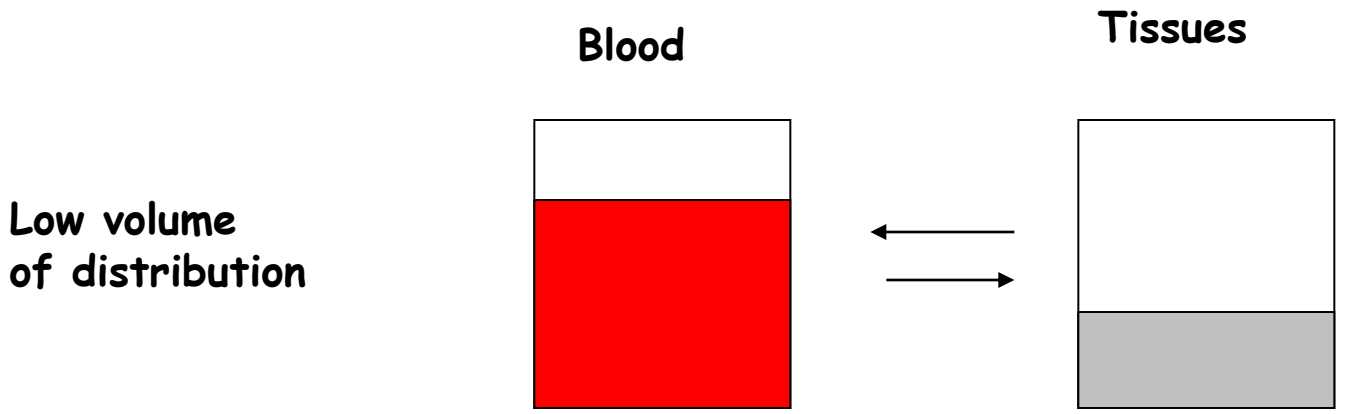
Well Stirred Model

$$Cl = \frac{Q \; fu \; Clint_{un}}{(fu \; Clint_{un}) + Q}$$

Where Q is hepatic blood flow

To enter the CNS a drug must cross the blood brain barrier

Some drugs tend to distribute into blood volume (Volume of Distribution ca 0.2L/kg)



All pharmacokinetic parameters must be related back to blood

So why do we measure plasma?

Largely historical – goes back to early days of PK and bioanalysis

HPLC-UV assays
Large volumes of matrix required for sensitivity
Generally liquid-liquid extraction

What happens if you liquid-liquid extract 1ml of blood?

Dirty extracts were not acceptable to most mass spectrometrists

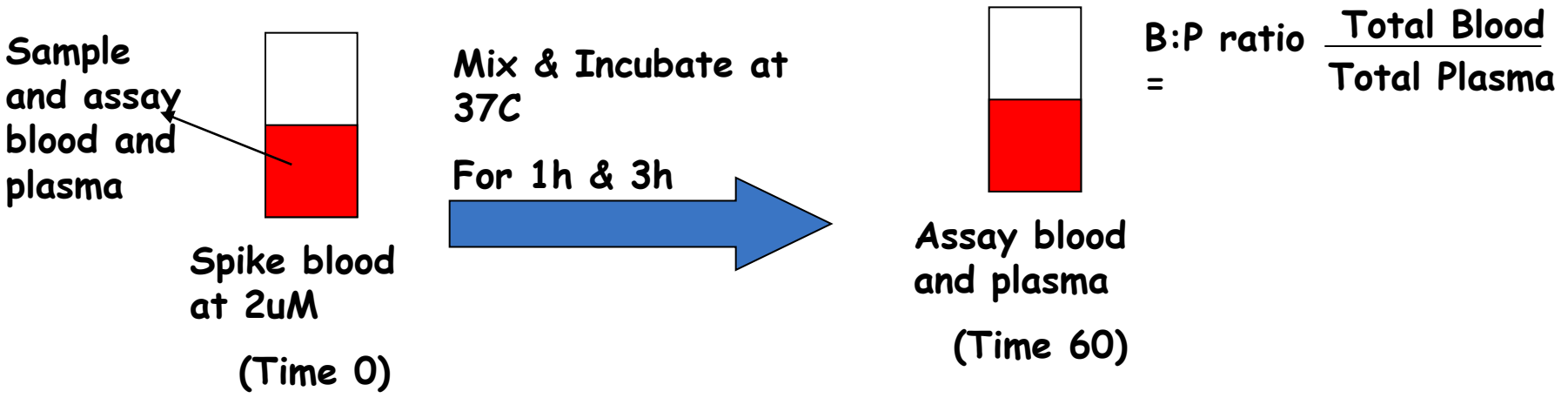
Plasma samples became the accepted norm for all PK studies

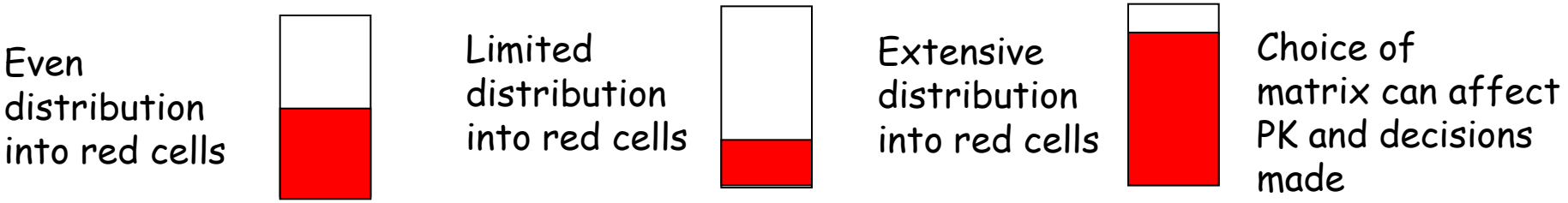
How do we relate plasma to blood concentration?

If you measure plasma, you need to make sure you relate the concentration back to blood concentration

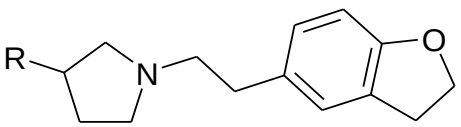
The Blood To Plasma Assay

Methodology





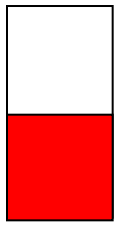
Experiences with a Lipophilic Basic Compound



Lipophilic and basic molecule

Plasma protein binding is 96%

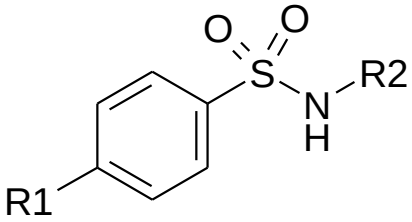
Compound does distributes evenly into red cells
(B:P ratio = 1)
(due to moderate ppb and high membrane permeation)



Plasma PK	Blood PK
Clp = 59ml/min/kg	Clb = 59ml/min/kg
Q = 70ml/min/kg	Q = 70ml/min/kg
Oral Bioavailability = 22%	Oral Bioavailability = 22%
Est. oral absorption = 100%	Est. oral absorption = 100%

Pharmacokinetics based on plasma and blood are identical
Either matrix is appropriate to measure

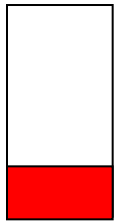
Experiences with an Acid Compound



R2 substituent means NH is acidic (pKa approx 4)

Compound shows high affinity for basic lysines of albumen

Plasma protein binding is >99%

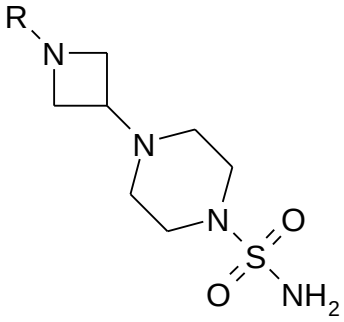


Compound does not distribute into red cells (B:P ratio = 0.66)
(due to high ppb and low membrane permeation)

Plasma PK	Blood PK
Clp = 44ml/min/kg	Clb = 67ml/min/kg
Q = 70ml/min/kg	Q = 70ml/min/kg
Oral Bioavailability = 13%	Oral Bioavailability = 13%
Est. oral absorption = 35%	Est. oral absorption = 100%

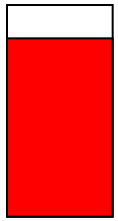
Based on plasma, we have an absorption and a clearance problem
Based on blood, we have only a clearance problem

Experiences with a Basic Sulphonamide Compound



Sulphonamide substituents are know to extensively distribute into red cells

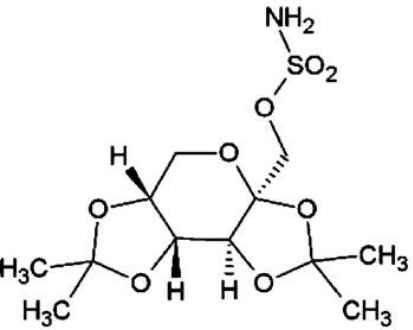
Compound distributes extensively into red cells (B:P ratio = 9 : 1)



Plasma PK	Blood PK
Clp = ~20ml/min/kg	Clb = 2.3ml/min/kg
Q = 20ml/min/kg	Q = 20ml/min/kg
Oral Bioavailability = 7%	Oral Bioavailability = 7%
Est. oral absorption = 100%	Est. oral absorption = 10%

Plasma PK suggests hepatic blood flow clearance drives oral bioavailability
Blood PK shows that oral absorption is the issue
Matrix determines the response to the issue

Topiramate

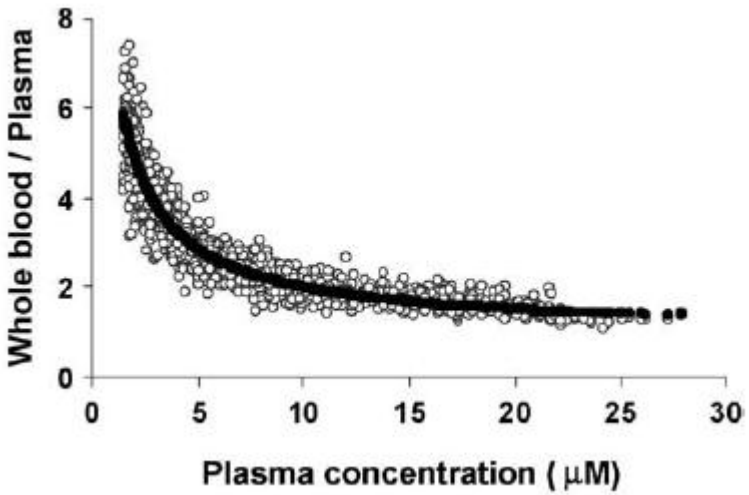


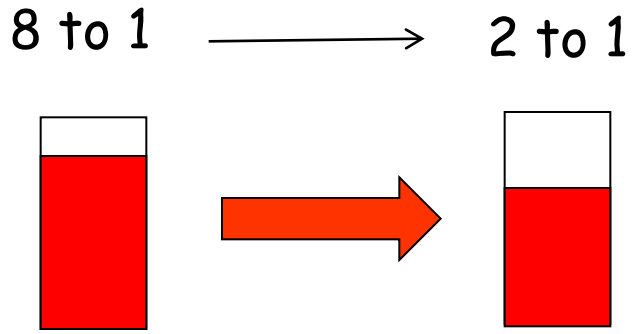
c – Topiramate (TPR)

Topiramate is an antiepiletic carbonic anhydrase inhibitor

Sulphonamide drives significant red blood cell partitioning

Partitioning is concentration dependent





Oral Plasma Clearance is 1.5 L/h at all doses

Oral Blood Clearance is 0.2, 0.3 and 0.5 L/h at 100, 200 and 400mg Respectively Blood concentrations determine exposure

Shank *et al* Epilepsy Research (2005) **63** pp103-112

Be careful when you measure plasma

Shylock tells us that blood not plasma circulates around the body

Pharmacokinetic parameters are always related back to blood

Most drug tend to distribute evenly between plasma and blood cells

Tempting to assume that all compounds in the discovery phase do so

A significant proportion of drugs do not distribute evenly

When distribution is uneven, assumption of even partitioning can lead to signficant errors

If using plasma, need to have an assay to determine blood to plasma

If you can measure blood….

….why wouldn't you?

Bring on the Dried Blood Spots!!