The Pharmaceutical Journal of Sri Lanka

Volume 5, Issue 1 2015

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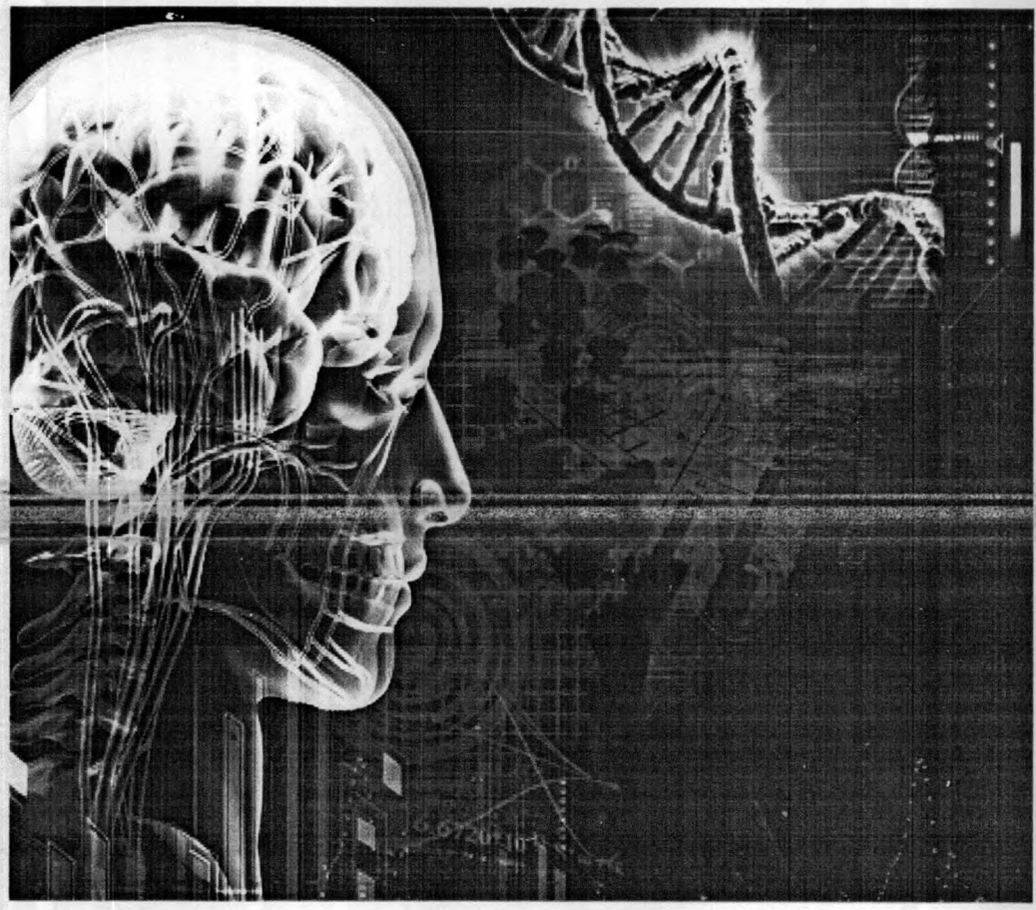

The official journal of the

Pharmaceutical Society of Sri Lanka

Pharmaceutical Journal of Sri Lanka 2015

Erythrocyte uptake of drugs and its impact on volume of distribution (V_D) determinations

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Abstract

In most volume of distribution (V_D) determinations the drug partitioned in to erythrocytes (C_{ery}) occupying 45% of blood volume is disregarded. The V_D determinations can be erroneous on two accounts. The first is the indiscriminate reference to plasma (C_p), whole blood (C_b) or serum (C_s) concentrations. The second is when C_{ery} values are not considered in calculations. Isolated erythrocytes were incubated in plasma water (C_{pw}) represented by physiological saline drug solutions, the C_{pw}, C_{ery} and C_b values were experimentally determined *in vitro*. Aberrations to the V_D determinations are demonstrated using both theoretically and practically determined values of C_{pw}, C_{ery} and C_b. Widely varying V_D values 125 L to 2.55 L resulted when C_p data alone is used while the values differed marginally from 4.56 L to 5.53 L when C_b values were used for two setting using same amount of drug.

Key words: Volume of distribution, Erythrocyte drug concentration C_{ery},

Introduction

The present study highlights the repercussions of indiscriminate use of drug blood concentration (C_b), plasma concentration (C_p) and serum concentration (C_s). A plasma determination is sometimes referred to as blood concentration. The erythrocyte partitioned drug has so far evaded receiving due recognition[1]. This identifies a fourth concentration parameter, which is the erythrocyte concentration of drugs (C_{ery}). This parameter is occasionally mentioned in the literature[2]. The C_{ery} values are sometimes over five times higher than the $C_p$values[3].

Isolated erythrocytes were incubated *in vitro* setting with doxycycline[4], chloramphenicol, rifampicin, oxytetracycline and chloroquine solutions of known strength. The C_{ery} and C_{pw} values were determined using standard curves ('Determination of Uptake of Selected Drugs by Red Blood Corpuscles, B. Sc. Pharmacy, Department of Chemistry,

University of Colombo, 2009 and Influence of Drugs Partitioned in to Red Blood Corpuscles on Volume of Distribution Determinations, University of Sri Jayewardenepura, B. Pharm program 2015).

The results were treated to demonstrate variations in volume of distribution values with and without taking into account C_{rbc} values. Similar variations were also demonstrated for situations where C_b, C_p or C_s is used indiscriminately. The volume of distribution studies are understood with the aid of compartment models[5].

Materials and methods

Model theoretical V_D calculations based on plasma (C_p), serum (C_s) or whole blood (C_b) concentrations:

The symbol C_{pw} is for *in vitro* studies without using blood. The total blood volume of an adult is considered to be 4.5 L. Since 55 % by volume of blood constitute plasma, the volume of plasma approximates 2.5 L.

A 25 mg of a drug X was administered by IV bolus to a subject resulting in an immediate plasma concentration of (25 X 1000)/(2.5 X 1000)= 10 µg/ml, prior to drug partitioning. The figures 1a - e for 100 ml of blood depict the five situations required for the demonstration of model calculations. Lowering effects on drug concentrations C_p and C_{ery} due to 40% drug partitioning in to tissues are shown in Figure 1e.

The volumes of packed erythrocytes: plasma is 45: 55 and calculations must account for this numerical unevenness.

Figure 1a shows a theoretical situation for a 100 ml blood sample immediately after a bolus injection of 25 mg of drug X before any partitioning. The blood and plasma are fully separated. This setup provides for an unaffected initial plasma drug concentration C_p.

Figure 1b shows the changes in Figure1a after the drug has partitioned in to erythrocytes. The concentration of the drug in erythrocytes (C_{ery}) and in plasma water (C_p) can be determined.

Figure 1c shows a clear 100 ml plasma sample collected from supernatant after centrifugation, which had an initial drug concentration of 6µg/ ml as in Figure 1b. A fraction of drug is bound to plasma protein. The separated serum now has a lower drug concentration of 4µg/ml. Here serum drug concentration C_s can be determined.

Figure 1d is similar to figure 1b but with lysed erythrocytes releasing the drug throughout the 100 ml sample. This gives the whole blood drug concentration C_b calculated as follows.

Total drug in Figure 1b is calculated as follows. The amount of drug in plasma fraction = 6 µg/ml X 55 ml plasma = 330µg.The amount in erythrocyte fraction = 4.888 X 45 = 220 µg. Therefore the total drug in Figure 1d following erythrocyte lysis is 330 + 220 = 550 µg. The 550 µg quantity of the drug is distributed throughout the 100 ml sample. Therefore the resulting C_b is 550µg/ 100 ml = 5.5µg/ml.

Figure 1e is similar to Figure 1b but assumed that 40% of the drug has diffused in to tissues.

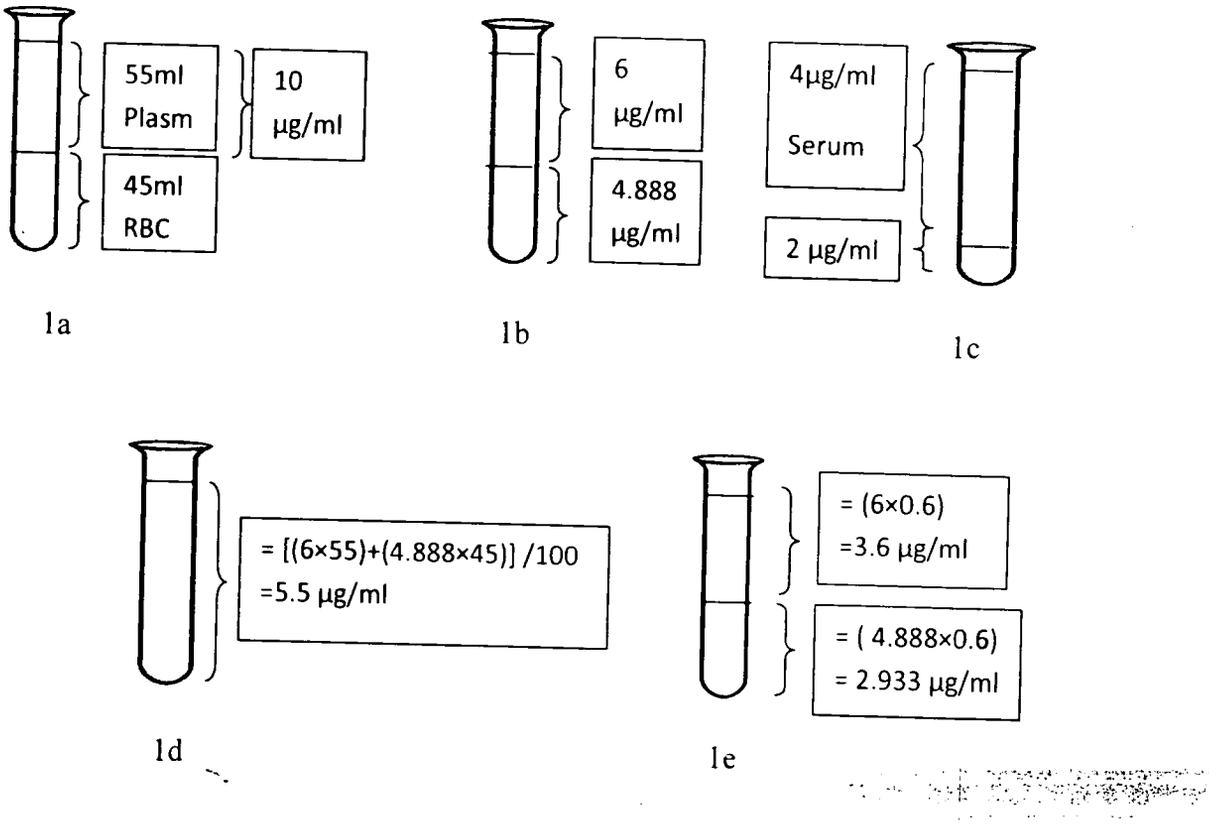

Figure 1: Partitioning of 25mg IV bolus. 1a; Drug in plasma immediately after injection. 1b; A fraction of drug partitioned in to erythrocytes. 1c; Precipitation of plasma proteins with bound drug from plasma fraction in 1b. 1d; Lysed erythrocytes releasing drug. 1e; Drug partitioning as in 1b when 40% drug has diffused in to tissues.

Demonstration of aberrations in V_D values using model calculations:

According to Figures 1a – d several drug concentration factors can be identified in different samples of same blood. They are C_p 6 µg/ml (Fig. 1b), C_{ery} 4.888 µg/ml (Fig. 1b), C_s 4 µg/ml (Fig.1c), $C_{protein}$ 2 µg/ml (Fig.1c) and C_b 5.5 µg/ml (Fig. 1d). In understanding V_D studies properly blood should be considered to have several sub-compartments[6].

According to Figure 1b applying C_p, V_D = (25 X 1000)/6 = 4167 ml. In Figure 1c applying C_s, V_D = (25 X 1000)/4 = 6250 ml. In Figure 1d applying C_b, V_D = (25 X 1000)/5.5 = 4545 ml. It is proposed that the correct V_D is 4545 ml based on C_b in Figure 1d. The difference between the highest and

the lowest V_D values amount to 6250 – 4167 = 2083 ml or 2.1 L.

Theoretical demonstration of anomalies of C_P based V_D determinations when C_{ery} is neglected:

An IV dose of 250 mg results in a 100 µg/ml (C_P) as explained earlier under subsection '**Model theoretical V_D.....**'.Take an extreme example where 98µg/ml of a drug has partitioned in to erythrocytes (C_{ery}) and only 2µg/ml is left in plasma (C_P) and also the reverse situation where 2µg/ml for C_{ery} and 98µg/ml for C_p. The V_D calculations based on the above concentration values will be as follows.

The V_D based on 2µg/ml (C_P): V_D = (250 X 1000)/2 = 125000 ml or 125 L. (Eqn. A)

The V_D based on 98µg/ml (C_P): V_D = (250 X 1000)/98 = 2551 ml or 2.55 L. (Eqn. B)

Whole blood drug concentration C_b can be calculated as shown below based on Figure 1d.

For the first set of data,

Amount of drug in plasma = 2µg/ml X 55 ml = 110 µg

Amount of drug in erythrocytes = 98µg/ml X 45 ml = 4410 µg

Total drug in 100 ml = 4520µg

Therefore C_b = 45.2µg/ml and the V_D based on C_b= (250 X 1000)/ 45.2 or 5.531L (Eqn. C)

For the second set of reversed data:

Amount of drug in plasma = 98µg/ml X 55 ml = 5390 µg

Amount of drug in erythrocytes = 2 µg/ml X 45 ml = 90 µg

Total drug in 100 ml = 5480µg

Therefore C_b = 54.8µg/ml and the V_D based on C_b = (250 X 1000)/ 54.8 or 4.562L (Eqn. D)

When considering 40% tissue drug diffusion, the values for plasma and erythrocytes can be expressed as $C_p \times 0.6$ (µg/ml) and $C_{ery} \times 0.6$ (µg/ml) respectively (Fig.1e).

Selected standard graphs for Doxycycline, Chloramphenicol and Rifampicin are shown in Figures 2, 3 and 4. Zones of inhibition for supernatant and erythrocytes following incubation in drug solutions are shown in Figure 5. Drug concentrations partitioned in to erythrocytes and remained in the supernatant are shown in Table 1.

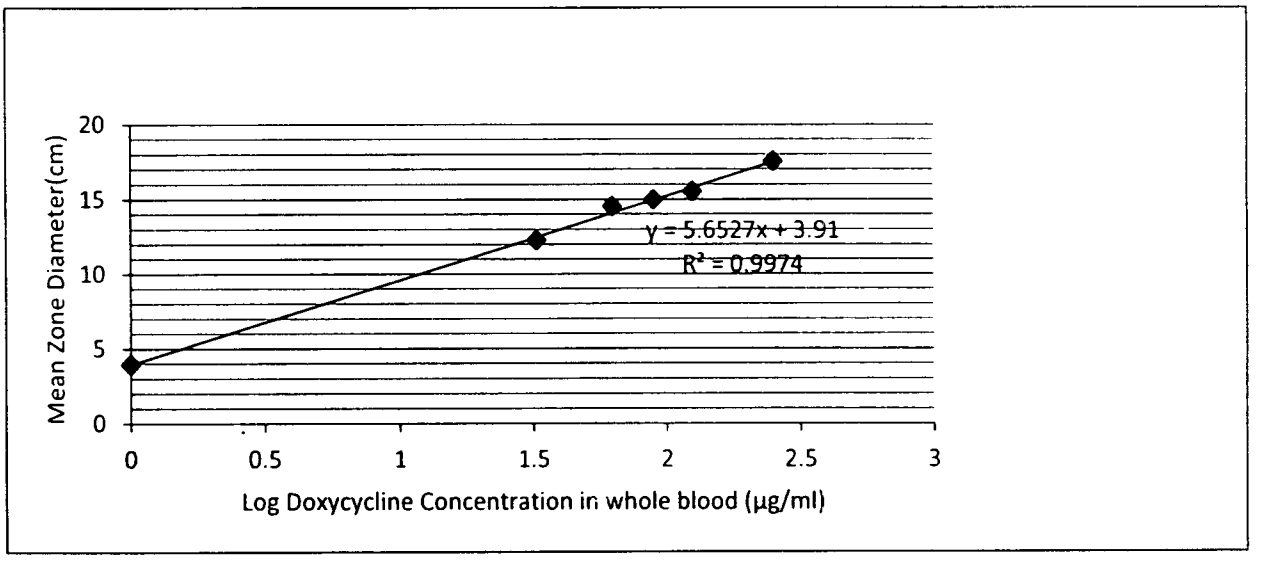

Figure 2: Standard curve for Doxycycline hydrochloride in whole blood.

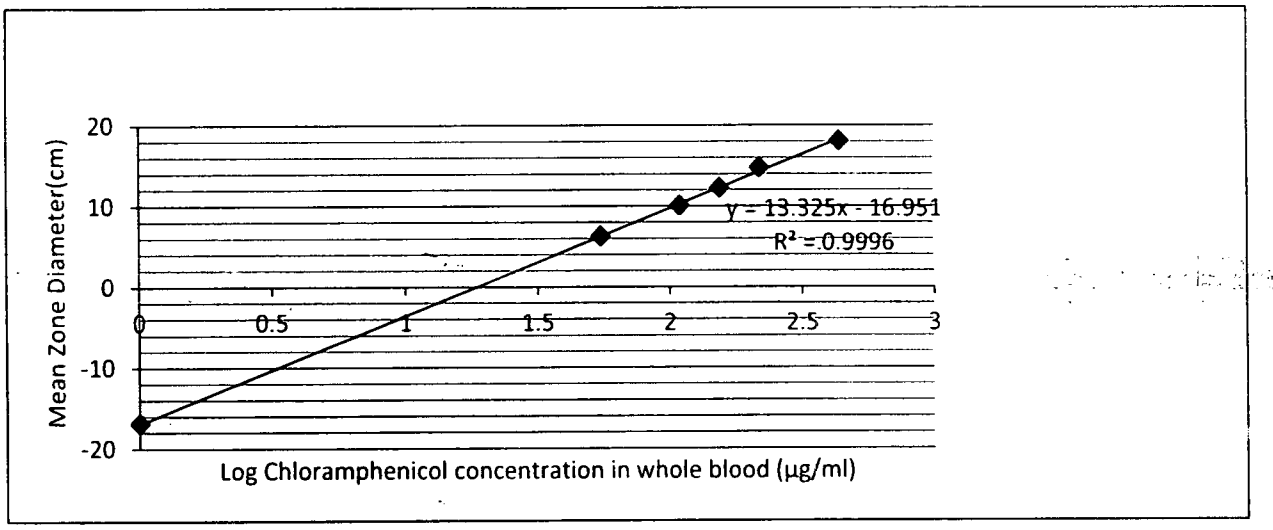

Figure 3: Standard curve for Chloramphenicol in whole blood.

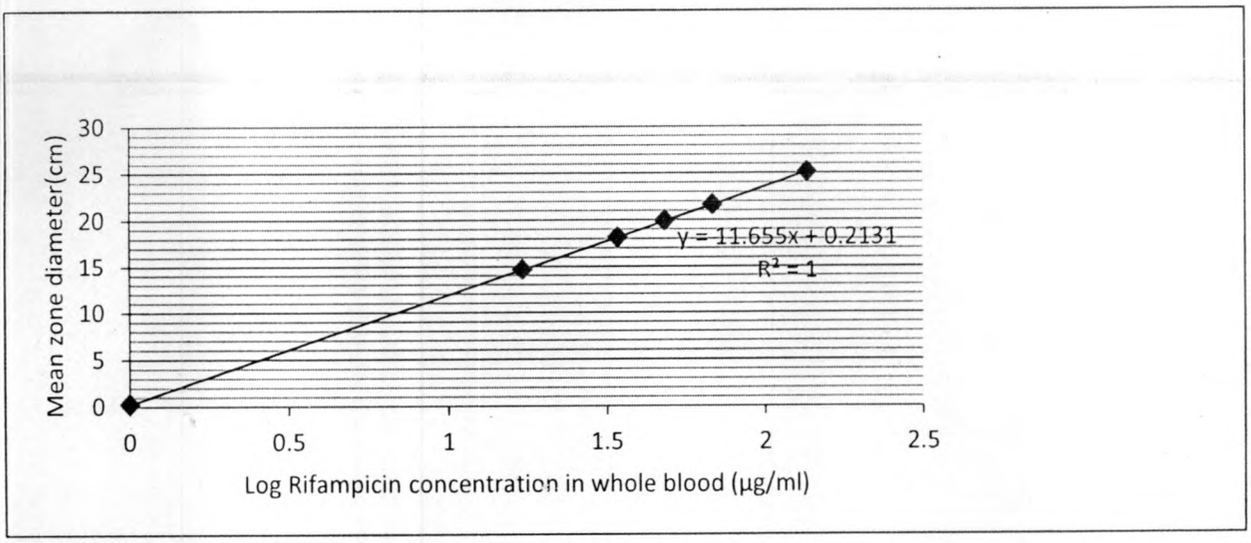

Figure 4: Standard curve for Rifampicin in whole blood

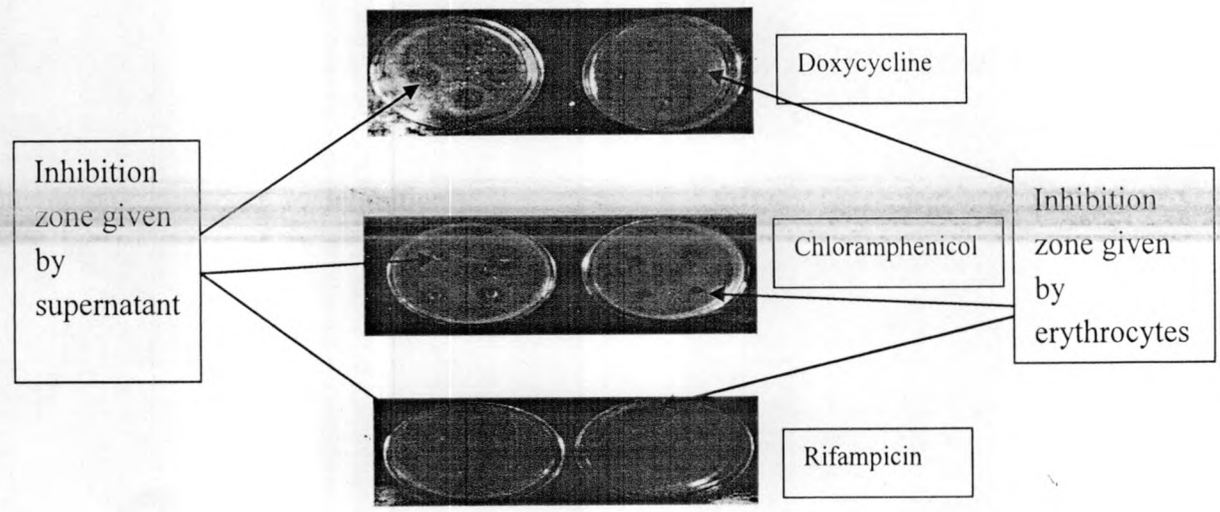

Figure 5: Inhibition zones given by erythrocytes and supernatant

Results

Calculations for percentage difference of V_D values based on C_{Pw}, C_{ery} and C_B given by the third dilution for chloramphenicol, 109.3µg /ml (Table 1) based on Figure 1b are shown below. Values are in same units for 40% tissue diffusion (remaining fraction 0.6) rare given in parenthesis.

Drug in supernatant after partitioning (C_{Pw}) = 49.3 µg/ml (29.58)

Drug in erythrocytes after partitioning (C_{ery}) = 56.8µg/ml (34.08)

Drug in whole blood (C_B) = [(56.8×2000) + (49.3×2500)]/4500

= 52.63 µg/ml (31.58)

Total amount of drug in the body (D_B) = (109.3×2.5×1000)µg

= 273 250 µg (273 250, no change)

V_D based on plasma concentration = (D_B/ C_P) = (273 250 µg/ 49.3 µg/ml) = 5543 ml (9238)

V_D based on erythrocyte concentration = (D_B / C_{ery}) = (273 250 µg/ 56.8 µg/ml) = 4811 ml (8018)

V_D based on whole blood concentration = (D_B/ C_B) = (273 250 µg/ 52.63 µg/ml) = 5192 ml (8653)

Maximum variance among three V_{Ds} = 5543 ml - 4811 ml = 733 ml (1020)

Drug in whole blood (C_B) if C_{ery} and C_P values are reversed

= [(49.3×2000) + (56.8×2500)]/4500 = 53.47 µg/ml (32.08)

Table 1: Drug concentrations partitioned in to erythrocytes and remained in supernatant

Drug	Standard dilution series µg/ml	Plasma drug concentration C_p µg/ml	%	Erythrocyte drug concentration C_{ery} µg/ml	%
Doxycycline	250	233.18	93.27	30.78	12.31
	125	114.34	91.47	14.66	11.73
	62.5	61.11	95.74	4.32	6.85
	32.25	30.03	94.37	3.5	10.89
Chloramphenicol	437.1	210.7	48.2	222.2	50.84
	218.6	109.3	50.0	113.2	51.78
	109.3	49.3	45.11	56.8	51.97
	54.7	ND	ND	ND	ND
Rifampicin	136.9	74.1	54.13	63.4	46.31
	68.5	34.3	50.07	31.2	45.55
	34.3	20.5	59.77	15.8	46.06
	17.2	8.1	47.09	8.4	48.84
Oxytetracycline	50.0	21.26	42.52	28.24	56.48
	100.0	38.13	38.13	63.20	63.2
Chloroquine phosphate	8.0	4.56	57.0	3.44	43.0
	12.0	5.68	47.33	6.32	52.67

V_D based on whole blood concentration = (D_B/ C_B) = (273 250 µg/ 53. 47 µg/ml) = 5110 ml

No drastic difference in V_D values (5192 ml and 5110 ml) even when C_P and C_{ery} values are changed.

Discussion

On an average adult blood volume of 4.5 L the erythrocytes occupy 45% equivalent to 2.0 L. The drug in erythrocytes is not represented in the formula $D_B = V_D . C_P$. Most studies avoid C_b determinations due to the presence of hemaglobin from lysed erythrocytes. The regular formula for volume of distribution determinations should be $V_D = D_B / C_b$. This formula is mainly applicable for an IV administered drug that follows one compartment model. Drug partitioned into erythrocytes practically acts like a portion that has been spilt out of the compartment model studies. In this event all the determinations that follow are flawed.

There is a problem with protein binding $C_{protein}$ (Fig.1c). In the case of the anticoagulant warfarin, protein binding is as much as 99% of the drug. The V_D values using C_P yield unrealistic results unless the analytical procedure extracts the bound drug as well. The degree of saturation of binding sites, the intensity of binding forces, type of bonds, functional groups of amino acids involved, types of plasma proteins involved, the polarity of extracting solvent system can all affect the extraction of the drug from the protein complex as against the free drug.

Equations A and B show that although the same amount of drug remains within the vascular system a drastic difference of over 40 times in the V_D values when C_P values are used in the calculations. Equations C and D show that results from C_b values will remain nearly equal with only about 10% difference. Under C and D, the C_{ery} fraction which has not diffused in to tissues is accounted reflecting the amount of drug held within the vascular system.

For all intent V_D informs us how the drug is distributed in the body. Accordingly, a drug that accumulates in tissues draw most of the drug out of vascular system leading to a low C_P and a large $V_D$as per formula $V_D = D_B/ C_b$. Both the fractions of drug represented in plasma C_P and in erythrocytes C_{rbc} are intravascular based. These two fractions do not account for the drug distributed in to tissues which the V_D intends to reflect. Now a low C_P and a large V_D as described above will still appear to hold good even when C_{rbc} is five times the C_P as in the case of chloroquine infected with Plasmodia. It is a distortion of the real situation with respect to biopharmaceutical interpretation of disposition of a drug. There is not so much drug in tissues as it appears. In such a situation the use of whole blood drug concentration C_b which is a larger value than C_P will better reflect a comparatively lower V_D as most of the drug is accumulated in the erythrocytes.

The accurate formula for volume of distribution determinations should be $V_D = D_B/ C_b$ in which C_b accounts for drug in plasma, plasma protein bound drug and the

drug in erythrocytes[7]. In other words all of C_P, $C_{protein}$ and C_{rbc} should be lumped together using the weighted average as their volumes are not equal.

Conclusion

The amount of drug partitioned in to erythrocytes and remained in plasma varied between large percentages as indicated in Table 1. There appear to be an increasing trend in the erythrocyte partitioning when the concentration of the drug was increased. Chloramphenicol, Rifampicin and Oxytetracycline shows significant amount of erythrocyte partitioning. It indicates a potentially deleterious effect on volume of distribution determinations if C_{ery} values are ignored. When C_b values are used no such differences occur as demonstrated by equations C and D. The determination of volume of distribution using only the plasma drug concentration could be misleading. The erythrocyte partitioning should to be taken into consideration to arrive at C_b for realistic V_D determinations.

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