

Plasma clearance

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Plasma (total, systemic...) clearance is determined by all the individual metabolizing/eliminating organ clearances and involves mainly liver and kidney clearances. Plasma clearance (a volume per time, i.e. a flow) expresses the overall ability of the body to eliminate a drug by scaling the drug elimination rate (amount per time) by the corresponding plasma concentration level. The interpretation of plasma clearance and inter-species comparisons are made easier by computing the overall body extraction ratio (from 0 to 1), which is the ratio of the body clearance divided by cardiac output. Plasma clearance is the most important pharmacokinetic parameter because it is the only one which controls the overall drug exposure (for a given bioavailability) and it is the parameter which allows computation of the dosage required to maintain an average steady-state plasma concentration.

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INTRODUCTION

Plasma clearance is the most important of all pharmacokinetic (PK) parameters. It is almost systematically reported in PK papers but is only rarely actually used and almost never interpreted.

This review aims to explain what clearance is, why plasma clearance is the most important PK parameter, how to compute it, how to provide a physiological interpretation to its numerical value in domestic species, and what its main applications are in veterinary pharmacology. An authoritative review on this topic has been written by Wilkinson (1987). The descriptions in veterinary text books by Baggot (1977) and Riviere (1999) also provide good accounts.

THE TERMINOLOGY 'PLASMA CLEARANCE': A PRELIMINARY COMMENT

Plasma clearance is the term generally used, because plasma is the matrix most frequently used to measure circulating drug concentrations. When blood concentration is quantified, a blood clearance is computed. Blood and plasma clearances have the same numerical value as long as whole blood and plasma concentrations are equal. Whenever blood and plasma concentrations are different, attention should be paid to selection of the most appropriate matrix to evaluate the body capacity to eliminate a drug, and on the physiological interpretation of the calculated clearance (*vide infra*).

The plasma clearance measuring the overall ability of the living organism to eliminate a drug, is also termed total, systemic

or body clearance. Unless stated otherwise, we will use all these terms as synonymous.

WHY PLASMA CLEARANCE IS THE MOST IMPORTANT PK PARAMETER

Plasma clearance is the most important PK parameter because it is one of the three determinants of a dosage rate. Figure 1 gives a pictorial view of the position of plasma clearance in relation to the dosing rate.

Equation 1 expresses formally the relationship between a dosing rate, the plasma clearance, the therapeutic plasma concentration in steady-state conditions and the systemic bioavailability:

$$\begin{aligned} \text{Dosing rate} \\ = \frac{\text{Plasma clearance} \times \text{therapeutic plasma concentration}}{\text{Bioavailability}}, \end{aligned} \quad (1)$$

where 'Dosing rate' is the dose per dosing interval (amount/time), 'Clearance' is the plasma clearance expressed for the given dosing interval (e.g. per day), 'therapeutic concentration' is the average effective target plasma concentration needed to obtain the desired clinical response, and 'Bioavailability' is a factor between 0 and 1. Bioavailability \times Dose expresses the actual amount of drug that reaches the systemic circulation when a formulation is administered via extravascular route.

Inspection of Eqn 1 shows that a dose is not a genuine drug parameter but rather a PK/PD variable, because it is influenced

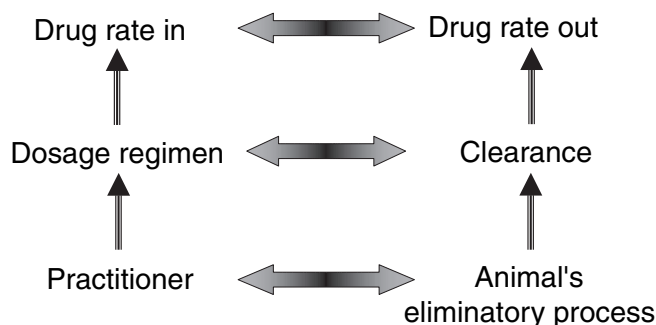


Fig. 1. A fundamental relationship in steady-state conditions. Under steady-state conditions (for a multiple dosage regimen), the drug rate in should be equal to the drug rate out. This guarantees maintenance of a constant plasma (blood) drug concentration which in turn guarantees maintenance of the expected (steady-state) effect. Drug is considered by the animal as a foreign compound and is removed by the animal's eliminatory processes with variable efficiency. Clearance is the parameter which expresses the ability of the animal to eliminate a drug. Thus, the dosage regimen (which is under the clinician's control) should be proportional to clearance.

by a PK parameter (plasma clearance), a PK variable (bioavailability for a particular route of administration and a given formulation), and a pharmacodynamic (PD) parameter (target therapeutic concentration) which reflects the *in vivo* drug potency (for more explanation see Toutain, 2002).

WHAT IS PLASMA CLEARANCE?

Plasma clearance is often defined as the plasma volume which is totally cleared of drug per unit of time. Unfortunately, this is not a formal definition of plasma clearance, but rather an operational statement that expresses what is being observed regardless of how it may be interpreted. To be clearly understood, plasma clearance, like any clearance (*in vitro*, *in vivo*, total, for a given organ...) should be formally defined as the ratio of two terms: a rate of drug elimination and the corresponding reference concentration (i.e. the driving concentration which 'supplies' the eliminatory processes) (Fig. 2). Therefore, any clearance is given by the following general relationship:

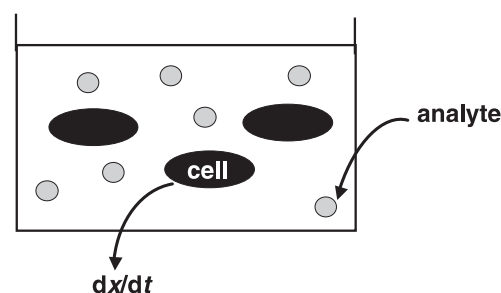
$$\text{Clearance} = \frac{\text{Rate of drug elimination}}{\text{Driving concentration}} \quad (2)$$

and in a more compact form:

$$\text{Clearance} = (dx/dt)/C(t), \quad (3)$$

where dx/dt (amount per time) is the rate of drug elimination at a given time t and $C(t)$, the corresponding driving concentration. For plasma clearance, the rate of drug elimination is the total rate of drug body elimination, whilst the driving concentration is the plasma concentration. Therefore, plasma clearance is defined by the following equation:

$$\text{Plasma clearance} = \frac{\text{Total (body) rate of drug elimination}}{\text{Plasma concentration}}. \quad (4)$$



$$\text{Intrinsic clearance} = \frac{dx/dt}{\text{driving concentration}} = \frac{V_{\max}}{K_m + C}$$

Fig. 2. Clearance: introduction of the concept using a single *in vitro* system. Assume an *in vitro* system in which there is an eliminatory mechanism [here cells suspended in a bathing drug (dot) solution]. Drug concentration in the solution is the driving concentration which supplies an unlimited amount of drug to cells. Here the drug concentration is considered as a constant because the amount of drug eliminated per unit of time (dx/dt), i.e. uptake by the cells is negligible in relation to the total drug amount in the beaker. The definition of clearance for this *in vitro* system is:

$$\begin{aligned} \text{Clearance of the system} &= \frac{dx/dt}{\text{driving concentration}} = \frac{\text{mass/time}}{\text{mass/volume}} \\ &= \frac{\text{volume}}{\text{time}} = \text{flow}. \end{aligned}$$

As there is a nonlimitation of the drug supply to the clearing organs, this clearance is termed 'intrinsic clearance' of the system (Cl_{int}). Cl_{int} is a measure of cellular drug removal, and is related to the Michaelis–Menten kinetic parameter according to the equation: $Cl_{int} = V_{\max}/(K_M + C)$, where V_{\max} is the maximal capacity of the system to eliminate the drug and K_M , the concentration corresponding to $V_{\max}/2$. For the majority of drugs, K_M (a concentration which is a measure of drug affinity for the clearing system (e.g. enzymes)), is much higher than C , and Cl_{int} (V_{\max}/K_M) is a constant (parameter). The dimensional analysis of the expression of clearance indicates that Cl_{int} is numerically equal to the (virtual) volume of the system that is totally cleared of drug by unit of time i.e. a flow.

The rationale for scaling (or normalizing) the total rate of drug body elimination by the corresponding plasma concentration is to express the ability of the body to eliminate a drug by a genuine parameter (a constant), rather than by an excretion rate which is a concentration-dependent variable for a drug following a first-order elimination process. If drug clearance does not obey a first-order elimination process, clearance is no longer a parameter but instead becomes a concentration-dependent variable.

Rearrangement of Eqn 4 allows computation of the amount of drug eliminated for a given snapshot plasma concentration:

$$\begin{aligned} \text{Rate of drug eliminated at time } t & \\ &= \text{Plasma clearance} \times \text{plasma concentration at time } t. \end{aligned} \quad (5)$$

Thus, plasma clearance corresponds to the proportionality constant linked to the rate of drug elimination and a given plasma concentration. The numerical value of body clearance depends on the measured matrix concentration [blood, total

plasma or unbound (free) plasma concentration], and the following relationship holds:

$$\begin{aligned} \text{Rate of drug elimination} &= Cl_{\text{blood}} \times C_{\text{blood}} = Cl_{\text{plasma}} \times C_{\text{plasma}} \\ &= Cl_{\text{free}} \times C_{\text{free}}, \end{aligned} \tag{6}$$

where Cl_{blood} , Cl_{plasma} and Cl_{free} are the clearances corresponding to the total concentration in blood, total concentration in plasma, and free concentration in plasma. Free plasma clearance can be the relevant parameter to estimate when the binding of the drug to circulating proteins is nonlinear, the total plasma clearance being a concentration-dependent variable, whereas the unbound plasma clearance is a parameter. This is the case for inhibitors of the angiotensin-converting enzyme such as enalaprilat and benazeprilat (Toutain *et al.*, 2000). Blood clearance can be the relevant parameter to estimate for the best physiological interpretation when blood and plasma concentrations are different (*vide infra*).

HOW TO EVALUATE PLASMA CLEARANCE

The most frequent technique for estimating plasma (total) clearance (Cl_{tot}) consists of administering a single dose of the drug by the intravenous (i.v.) route and measuring sequentially plasma concentration until drug concentration in plasma is less

than the limit of quantification. Then, the total clearance is derived from:

$$Cl_{\text{tot}} = \text{Dose}/AUC, \tag{7}$$

where 'Dose' is the administered dose and AUC , the area under the plasma concentration vs. time curve. AUC may be directly evaluated using a trapezoidal rule with or without extrapolation to infinity, or alternatively, by integrating the multiexponential function describing the drug disposition (Fig. 3). Whatever the method used, attention must be paid to the units of AUC which are often wrongly reported. For plasma concentrations measured in $\mu\text{g}/\text{mL}$ and for time measured in hours, AUC unit is $\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$ (or $\mu\text{g}\cdot\text{h}/\text{mL}$) not $\mu\text{g}/\text{h}\cdot\text{mL}$!

Equation 7 is easily deduced from the definition of the clearance (Eqn 3). After rearrangement of Eqn 3, the following relationship holds:

$$dx = Cl \times C(t) \times dt. \tag{8}$$

Integrating both sides in relation to time from $t = 0$ to infinity, we obtain:

$$\int_0^\infty dx = \int_0^\infty Cl \times C(t) \times dt. \tag{9}$$

When clearance is a parameter, then:

$$\int_0^\infty dx = Cl \int_0^\infty C(t) \times dt \tag{10}$$

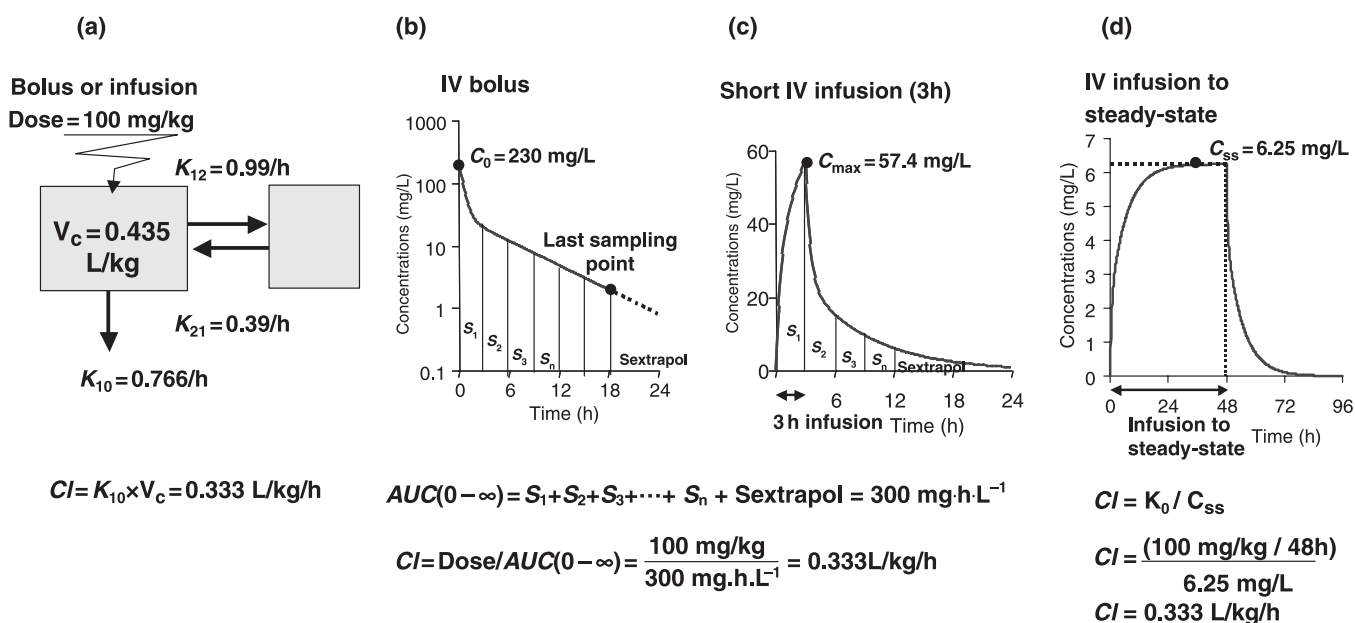


Fig. 3. Estimation of plasma clearance: bolus vs. infusion study. Plasma clearance can be evaluated using different modalities of the i.v. administration namely, a bolus, short infusion, or infusion to reach steady-state conditions. This figure shows these approaches for a total administered dose of 100 mg/kg. (a) Parameters of a bi-compartmental model used to simulate data. (b) The i.v. bolus is the most convenient modality of administration. Clearance can easily be obtained either by the noncompartmental approach ($\text{Dose}/AUC_{0-\infty}$) or after modelling ($Cl = K_{10} \times V_c$). (c) If for safety reasons an instantaneous bolus administration is dangerous, an alternative is a relatively short infusion (e.g.: 3 h allowing a reduction in the maximum plasma concentration (57.4 vs. 230 mg/L for the short infusion and the bolus administration respectively). Here also, the clearance can be obtained either by modelling or by a noncompartmental approach. (d) The last strategy consists of achieving a steady-state condition with a 48-h infusion (here infusion of 10 times the terminal half-life, which is of 4.62 h). Using a snapshot plasma concentration during the steady-state, clearance (0.333 L/kg/h) can be obtained with $\text{dose}/\text{infusion time} = 2.08 \text{ mg/h/kg}$ divided by C_{ss} (6.25 mg/L).

and therefore

$$Cl = \frac{\int_0^{\infty} dx}{\int_0^{\infty} C(t) \times dt} \quad (11)$$

because the term $\int_0^{\infty} dx$ is equal to the total amount of drug ultimately eliminated, i.e. to the administered dose after i.v. and $\int_0^{\infty} C(t) \times dt$ is the total AUC, therefore, Eqn 11 is equal to Eqn 7.

An alternative to Eqn 7 is to measure plasma clearance in steady-state conditions after an intravenous infusion:

$$Cl_{tot} = K_0 / C_{ss}, \quad (12)$$

where K_0 is the infusion rate and C_{ss} , the plasma concentration at equilibrium (Fig. 3). Equation 12 is a direct application of Eqn 3, K_0 , at equilibrium, being by definition, equal to the overall excretion rate. An advantage of intravenous infusion is avoidance of possible side-effects from high plasma concentrations obtained after a single i.v. bolus administration. In addition, plasma clearance at equilibrium can be theoretically obtained using a single snapshot plasma concentration. The limit of the steady-state approach is to ascertain achievement of equilibrium, which can be difficult for drugs having a long terminal half-life.

Regardless of the approach selected, the total dose considered in Eqn 7, or the infusion rate in Eqn 12 should give the amount of drug which actually gains access to the systemic (arterial) circulation. If a fraction of the i.v. dose is destroyed by a first-pass pulmonary effect before reaching the arterial circulation (*vide infra*), or if the administered agent is a pro-drug (e.g. an ester for corticosteroids) that requires metabolic transformation (e.g. hydrolysis), estimation of total clearance by Eqn 7 or 12 can be very misleading. Figure 4 illustrates the example of methylprednisolone, for which clearance has been calculated by injection of either methylprednisolone itself or methylprednisolone sodium succinate.

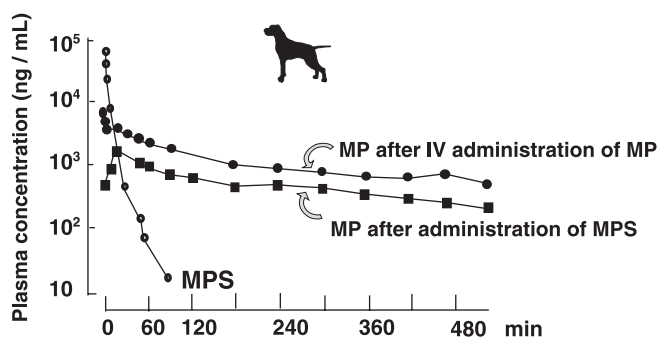


Fig. 4. Plasma clearance of methylprednisolone in the dog. Methylprednisolone (MP) is a nonhydro-soluble steroid which was administered by the i.v. route using a hydro-soluble salt of a succinate ester (methylprednisolone sodium succinate, MPS). After an i.v. administration of MPS, its concentration decreases rapidly giving the active moiety, i.e. MP. Using the MP plasma concentration profile to estimate the MP clearance would lead to gross overestimation of the true MP clearance, because only 44% of the MPS is transformed into MP. The true clearance of MP was obtained by administered MP itself by i.v. route (Toutain *et al.*, 1986).

HOW TO EVALUATE PLASMA CLEARANCE WHEN IT IS NOT POSSIBLE TO ADMINISTER THE DRUG BY THE I.V. ROUTE

In some circumstances, the drug cannot be administered by the i.v. route but the estimation of a plasma (total) clearance (Cl_{tot}) may still be possible if the drug is totally eliminated by a route which is experimentally measurable (e.g. urine, faeces...). Indeed, in most instances, total plasma clearance is the sum of organ clearances, typically:

$$Cl_{tot} = Cl_{renal} + Cl_{liver} + Cl_{other}, \quad (13)$$

where Cl_{renal} is the renal clearance, Cl_{liver} is the hepatic clearance and Cl_{other} represents collectively all other clearance mechanisms.

Equation 13 expresses that clearances by the liver and the kidneys are additive. Generally, Cl_{other} is negligible and the plasma clearance is often the sum of renal and hepatic clearance. If Cl_{liver} is nil, Eqn 13 reduces to:

$$Cl_{tot} = Cl_{renal}. \quad (14)$$

In this situation, the plasma clearance can be measured by injecting the drug by any route of administration (actual bioavailability is not an issue), and providing that the total amount of drug eliminated in urine is actually known (not the administered dose), the following relationship holds (Eqn 15):

$$Cl_{tot} = Cl_{renal} = \frac{\text{Total amount of drug eliminated in urine}}{AUC}, \quad (15)$$

where AUC is as defined for Eqn 7. The difference between Eqn 7 (Dose/AUC) and Eqn 15 is that the former uses the nominal administered dose, whereas the latter uses a measured eliminated drug amount. Both equations can be used to measure a renal clearance, Eqn 7 being valid only if the drug is given intravenously and is (almost) totally eliminated by the kidney. A clinical application is the measurement of the glomerular filtration rate using as probes, compounds such as iohexol, creatinine or inulin...

In the same circumstance (i.e. when an excretion rate is directly and sequentially measurable), clearance can be evaluated by dividing the average excretion rate ($\Delta x / \Delta t$) by the plasma drug concentration at the time corresponding to the midpoint of the excreta collection period. Indeed, Eqn 3 indicates that clearance is the slope of the regression line linking the drug excretion rate to the plasma concentration. The advantage of this method is that it takes account of possible nonlinearity (Fig. 5).

THE PHYSIOLOGICAL INTERPRETATION OF PLASMA (BLOOD) CLEARANCE

Is a given computed plasma clearance a high, medium or low body clearance? This very relevant question is seldom addressed in veterinary papers because the answer is not straightforward. For instance, a plasma clearance of 20 mL/kg/min should be

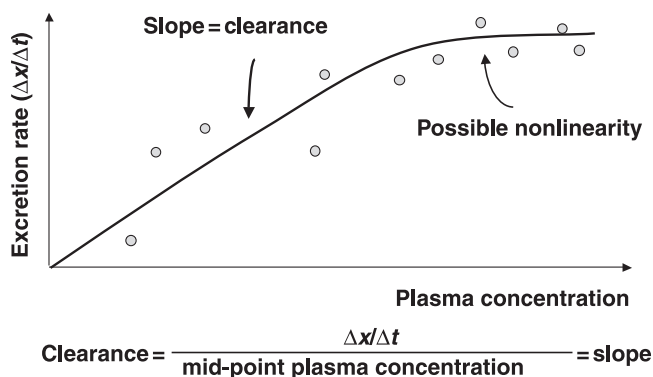


Fig. 5. Estimation of a clearance from experimental measurement of the excretion rate. Clearance can be computed from the ratio of the excretion rate (urinary, faecal, milk...) to the drug concentration in plasma (see Eqn 3). In practice, clearance is estimated by plotting excretion rate vs. drug concentration in plasma at the times corresponding to the mid-points of the matrix (urine, milk, faeces...) collection periods. The slope of the excretion rate vs. plasma concentration plot is equal to the clearance. If the drug is totally eliminated by the investigated matrix, the computed clearance is numerically equal to the plasma clearance. An advantage of this method is that it recognizes possible nonlinearity (e.g. for high plasma concentrations for a saturable elimination process).

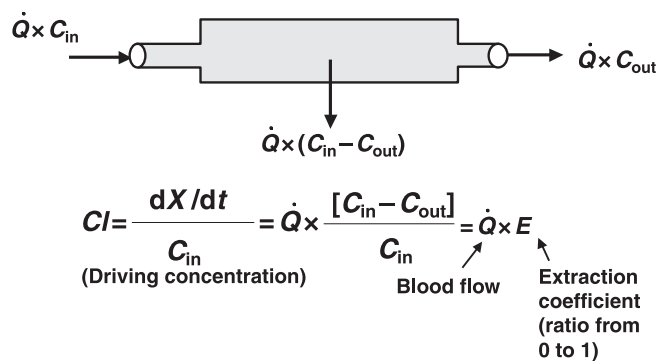


Fig. 6. Minimal model for an *in vivo* clearance. The grey box represents an organ or a system (several organs) capable of eliminating a drug. The organ is perfused with a blood flow (\dot{Q}). The input drug concentration is C_{in} . This is the driving blood concentration. The output concentration is C_{out} . If some drug has been eliminated during passage through the system, then $C_{out} < C_{in}$. The total amount of drug which is eliminated per unit of time can be obtained by mass balance consideration. It is given by the difference between the rate of drug presentation by arterial blood ($\dot{Q} \times C_{in}$) and the rate of the drug leaving the organ in the (venous) blood, ($\dot{Q} \times C_{out}$) i.e. $\dot{Q}(C_{in} - C_{out})$, which is the rate of drug extraction. Applying to this simple system the general definition of clearance (see Eqn 2 in the text), allows expression of the clearance of the system as:

$$Cl = \frac{\dot{Q}(C_{in} - C_{out})}{C_{in}}$$

The ratio $(C_{in} - C_{out})/C_{in}$ is termed the extraction ratio (E), therefore, any *in vivo* clearance can be expressed as the product of a blood flow (\dot{Q}) and an extraction ratio (E), a figure without dimension and with a numerical value between 0 and 1; hence a clearance is numerically equal to the volume of the entering blood from which drug appears to be totally extracted.

considered as high in cattle and horses, but rather low in rodents.

The physiological interpretation of plasma clearance needs to have recourse to a model of body clearance. In Fig. 6 the general model of an *in vivo* clearance is explained, and Eqn 16 shows that *in vivo* clearance is the product of a blood flow (\dot{Q}) and an extraction ratio (E):

$$Cl = \dot{Q} \times E, \tag{16}$$

where E is a numerical value between 0 and 1 that can be regarded as the percentage (from 0 to 100%) of the drug which is cleared during a single passage through the clearing organ. Equation 16 corresponds to the minimal physiological model of a clearance and it is not to be confused with Eqn 3 (the definition), and Eqn 7 (the equation for its estimation).

Considering the body as the whole system clearing the drug, Eqn 16 can be used to model the body clearance with cardiac output. Hence,

$$Cl_{body} = \text{Cardiac output} \times E_{body}. \tag{17}$$

Figure 7 gives supplementary information on derivation and assumption for Eqn 17. Inspection of Eqn 17 shows that body clearance is a hybrid parameter involving cardiac output (which

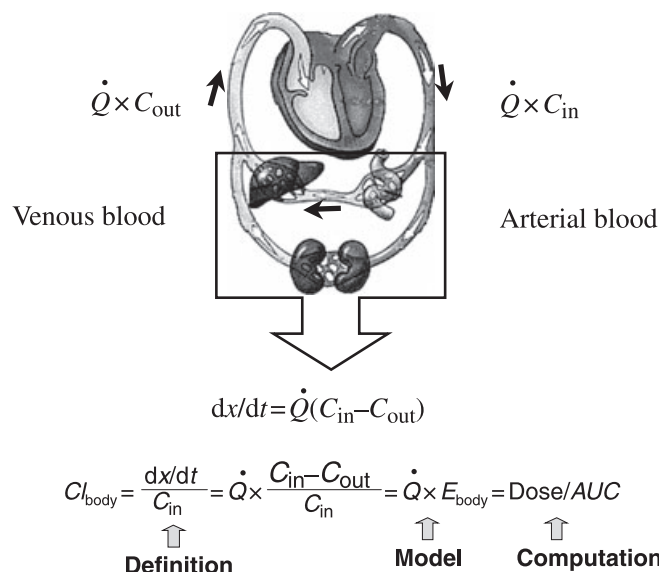


Fig. 7. A minimal physiological model for total body clearance. The entire body is considered as the drug reservoir. In mammals, kidney and liver are generally the two main organs for drug metabolism and excretion. Kidney and liver are the two parallel components of the clearing system. As a whole, the clearing organs are perfused by a blood flow equal to cardiac output (\dot{Q}). The model assumes that no drug is eliminated by lung (not represented) and that blood concentration is equal to plasma concentration. Under these conditions Eqn 13 applies ($Cl_{tot} = Cl_{renal} + Cl_{hepatic} + Cl_{others}$). By applying Eqn 4 to the system, with C_{in} being the arterial blood concentration (driving concentration) and C_{out} (the venous blood concentration), it can easily be demonstrated that $Cl_{tot} = \dot{Q} \times E_{body}$ with E_{body} being the overall body extraction ratio.

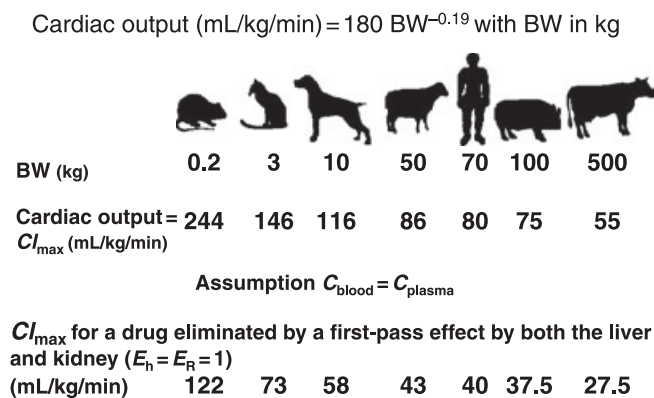


Fig. 8. Maximal blood clearance in different domestic species. According to the minimal model of blood clearance, (i.e. $Cl = \text{cardiac output} \times \text{extraction ratio}$) the maximal possible blood clearance is equal to the cardiac output when the overall extraction ratio is equal to 1. If a higher clearance is computed, attention should be paid to possible errors or misconceptions (e.g. blood concentrations much higher than plasma concentrations). If the hepatic (E_h) and renal (E_R) extraction ratios are equal to 1 (i.e. if there is a first-pass effect for both liver and kidney), the body clearance should be equal to about half the cardiac output because blood flows for liver and kidney are approximately equal to 50% of the cardiac output.

is a specific species property) and E , the overall body extraction ratio being directly related to the properties of the drug under investigation. Thus, the interpretation of body clearance consists of computation and interpretation of E_{body} , which can be regarded as the percentage of drug being cleared by the entire body during a single passage through the different clearing organs contributing to the body clearance:

$$E_{\text{body}} = \frac{\text{Body clearance}}{\text{Cardiac output}} \quad (18)$$

Cardiac outputs are known for different species or can easily be computed using the following allometric relationship:

$$\text{Cardiac output (mL/kg/min)} = 180 \times \text{Body weight (kg)}^{-0.19} \quad (19)$$

It should be noted that as cardiac output is a blood flow and not a plasma flow, the interpretation of body clearance using Eqn 19 assumes that plasma clearance and blood clearance are equal (or at least not too different), i.e. the blood and plasma concentrations are equal or similar (*vide infra*).

The clearance model described by Eqn 17 also indicates that body clearance cannot be higher than the cardiac output (Fig. 8). For a drug exhibiting an extraction ratio equal to 1 (i.e. a total first-pass effect) for both liver and kidney, the expected E_{body} is equal to about 0.5 due to the fact that the sum of liver and kidney blood flows represents about half of the cardiac output.

If after i.v. administration (e.g. into the jugular vein), body clearance is higher or of the same order of magnitude than the cardiac output (55 mL/kg/min in horse) as reported for acepromazine (46 mL/kg/min) (Marroum *et al.*, 1994) or

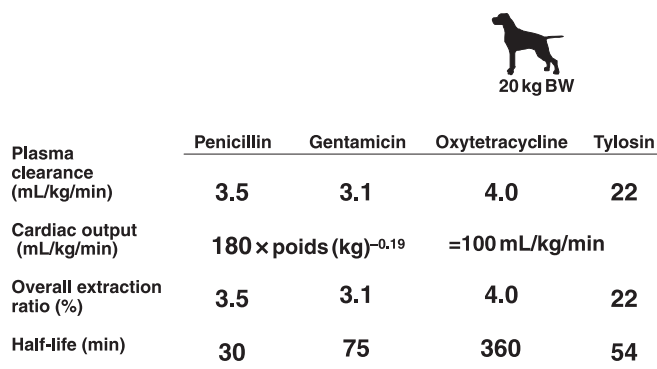


Fig. 9. Interpretation of plasma clearance for four antibiotics in the dog. Typical values of plasma clearance and body overall extraction ratio for four antibiotics in the dog are reported. Penicillin, gentamicin and oxytetracycline while having the same plasma clearance also have the same overall extraction ratio (about 3–4%). In contrast, tylosin has an extraction ratio fivefold higher, indicating that the ability of the body to eliminate tylosin in the dog is fivefold higher than for the three other antibiotics. For the same antibiotics, the plasma half-lives are reported and a totally different picture appears between these antibiotics, oxytetracycline having the longest terminal half-life despite a similar clearance to penicillin and gentamicin. This is due to the fact that terminal half-life is a hybrid parameter also influenced by the extent of drug distribution, which is much larger for oxytetracycline than for penicillin.

pentoxifylline (51 mL/kg/min) (Crisman *et al.*, 1993) in the horse, consideration should be given to a possible pulmonary first-pass effect. Indeed, if the lung is able to eliminate a drug with an extraction ratio higher than 0.5, the body clearance might be greater than the cardiac output because only 50% of the administered dose will be actually available to the systemic circulation (see Chiou, 1982 for further explanation). For PgF 2α in sheep, the lung extraction ratio is about 0.9, indicating that 90% of PgF 2α is eliminated after a single lung passage (Bonnin *et al.*, 1999). Other possible explanations for a very high body clearance are a direct metabolism of the drug in blood.

Figure 9 shows how to interpret body clearance for four antibiotics in dogs. This figure draws attention to the fact that body clearance and terminal half-life do not convey the same information on drug elimination. Very different terminal half-lives can be observed for drugs having the same body clearances, because terminal half-life is also influenced by drug distribution. Terminal half-life is said to be a hybrid parameter (influenced by both clearance and distribution), and it will be extensively discussed in our companion paper on terminal half-life. Thus, half-life is not an appropriate parameter to assess the intrinsic body capacity to eliminate a drug. For instance, in the horse it was shown for gentamicin in nephrotoxic conditions that terminal half-life was unchanged (192 min vs. 204 min) although plasma clearance was decreased by 40% (Riviere *et al.*, 1983).

The next step in interpreting plasma clearance involves interpreting the body extraction ratio *per se* by having recourse to some breakpoint values for E_{body} . Figure 10 explains how to

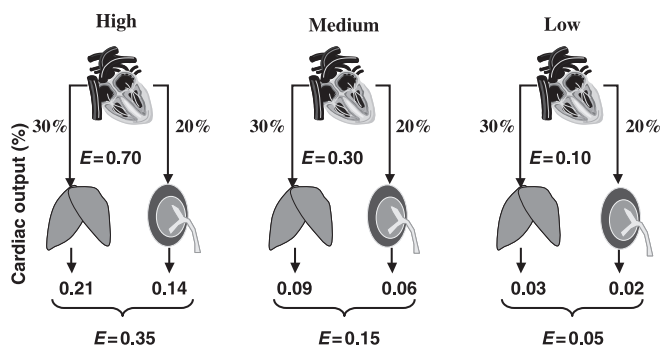


Fig. 10. Breakpoint values for high, medium and low overall body extraction ratio (E). A high, medium, or low overall body E can be calculated bearing in mind that plasma (body) clearance is given by the relation: $Cl_{\text{body}} = Cl_{\text{renal}} + Cl_{\text{hepatic}} + Cl_{\text{other}}$. The kidney and liver are the two most important contributors to drug elimination, with Cl_{other} usually negligible. For liver and kidney, E can be considered high if $E > 0.70$, medium if $E = 0.30$, and rather low if $E < 0.10$ (see Rowland & Tozer, 1995 for explanation). Considering that kidney (20%) and liver (30%) blood flow represent about 50% of cardiac output, overall E should be considered high if above 0.35 (0.70×0.50), medium if around 0.15 (0.30×0.50), and low if around 0.05 (0.10×0.50).

Table 1. Breakpoint values to classify drugs as having high, medium and low blood clearance (mL/kg/min) in different species (see Fig. 10 for further information)

	Rat	Cat	Dog	Sheep	Man	Pig	Cattle/horse
BW (kg)	0.2	3.0	20	50	70	100	500
High ($E = 0.35$)	85	51	41	30	28	26	19
Medium ($E = 0.15$)	37	22	17.4	13	12	11.3	8.25
Low ($E = 0.05$)	12.2	7.3	5.8	6.2	5.8	5.4	3.6

E : extraction ratio between 0 and 1.

compute these breakpoints. Practically speaking, an E_{body} of 0.05 or lower is generally desirable to develop a drug for oral administration with a not too high dosage regimen (*vide infra*). Table 1 gives the breakpoint values for high, medium and low clearances in domestic species.

APPLICATION OF PLASMA CLEARANCE FOR COMPUTATION OF A DOSE

There are many practical uses for plasma clearance, the most relevant application being computing a dose (therapeutic, toxic...) using Eqn 1. For this, the target (therapeutic, toxic...) average plasma concentration must be known (from the literature, from a PK/PD trial, extrapolated from an *in vitro* assay for example for antibiotics, etc.). For phenylbutazone in the horse, therapeutic concentration was directly estimated by PK/PD modelling (3.6 $\mu\text{g/mL}$) (Toutain *et al.*, 1994), and considering the plasma clearance (41.3 mL/kg/h), the daily dosage regimen by the i.v. route should be 3.6 mg/kg/day, which is consistent with the recommended dosage regimen. Usually, the plasma clearance is

known but this is generally not the case for the plasma concentration of interest (therapeutic, toxic...); compilation of therapeutic and toxic plasma concentrations in man for several hundreds of drugs is given by Schulz and Schmoldt (1997).

For antibiotics, the drug potency is often assessed experimentally by measuring the *in vitro* minimum inhibitory concentration (MIC) for a given pathogen. In addition, surrogate indices to predict antibiotic efficacy have been proposed such as the area under the inhibitory curve (AUC) for quinolones (Lees & Shojaee Aliabadi, 2002; Toutain *et al.*, 2002).

Considering the MIC of a given pathogen (e.g. MIC_{50} or MIC_{90}) and a breakpoint value for AUC, the dose for a quinolone (or any antibiotic for which AUC is the relevant surrogate index of efficacy) can be computed with the following relationship:

$$\text{Dose (per day)} = \frac{AUC \text{ (h)} \times MIC \text{ (}\mu\text{g/mL)} \times Cl \text{ (per hour)}}{fu \times F}, \quad (20)$$

where AUC (or AUC/MIC) is the targeted endpoint in hours (e.g. 125 h) measured for 24 h, the MIC is for the targeted pathogen, Cl the plasma (total) clearance per hour (not per day), fu the free fraction of drug in plasma (from 0 to 1) and F the bioavailability factor (from 0 to 1). In Eqn 20, AUC/24 h may be regarded as the desired multiplicative factor for the targeted MIC.

Equation 20 can be simplified by ignoring fu when the free fraction is dominant (e.g. for aminoglycosides) and also F for i.v. route ($F = 1$). Conversely, for drugs extensively bound in plasma, fu should be taken into account (Hyatt *et al.*, 1995). For further explanation concerning Eqn 20 and its units, see Bousquet-Melou *et al.* (2002), Toutain *et al.* (2002) and Toutain (2003).

Estimation of a dose from *in vitro* data can be carried out for any drug for which an efficacious concentration has been obtained on the basis of an *in vitro* (or *ex vivo*) assay. For example, using a membrane feeding system, the IC_{99} of lufenuron (a compound for control of flea infestation in dog and cat) for *Ctenocephalides felis* was found to be from 50 to 100 ng/mL. Assuming a lufenuron blood clearance in the cat of about 0.56 L/kg/day, (MacKichan & Hink, 1993) Eqn 1 predicts a minimal lufenuron dose of 0.028–0.056 mg/kg/day (or 5–10 mg/kg per 6 months), which matches the experimentally determined dose of subcutaneously injected lufenuron in cats.

When estimating a dose from *in vitro* drug potency, it should be remembered that *in vitro* drug concentrations are generally free drug concentrations (Toutain & Bousquet-Melou, 2002).

THE USE OF PLASMA CLEARANCE TO ESTIMATE PLASMA THERAPEUTIC AND TOXIC CONCENTRATIONS

The order of magnitude of effective or toxic plasma concentration is required in order to interpret a snapshot plasma concentration in terms of drug efficacy and/or toxicity. If the effective (or toxic) dose is reasonably known for a drug in a given species, the effective plasma concentration (EPC) can easily be estimated by rearranging Eqn 1:

Table 2. Dose (mg/kg), dosing interval (h) and plasma clearance (mL/kg/h) considered to compute the effective (therapeutic) plasma concentrations (EPC) (ng/mL) for a selection of drugs used in horse (Toutain & Lassourd, 2002b)

Drugs	Dose (mg/kg)	Dosing interval (h)	Clearance (mL/kg/h)	EPC (ng/mL)
Acepromazine	0.100	24	2600	1.6
Bromhexine	0.200	24	3200	2.6
Butorphanol	0.100	5	450	44
Caffeine	5.000	24	35	5952
Carprofen	0.700	24	8.7	3352
Clenbuterol	0.0008	12	350	0.19
Codeine	0.600	24	800	31
Detomidine	0.020	24	400	2.1
Dexamethasone	0.020	24	480	1.7
Dipyron	5.000	24	300	694
DMSO	1000	24	80	520833
Eltenac	0.500	24	74	282
Flunixin meglumine	1.100	24	60	764
Furosemide	1.000	8	500	250
Glycopyrrolate	0.004	24	1000	0.17
Guaiphenesin	100	24	300	13889
Heptaminol	10	24	1245	335
Holdenine	2.000	24	4300	19
Ibuprofen	10	24	164	2541
Isoxsuprine	0.600	24	2600	9.6
Ketamine	2.200	24	1500	61
Ketoprofen	2.200	24	300	306
Meclofenamic	2.200	24	120	764
Meperidine	1.0	5	1100	182
Methadone	0.100	5	680	29
Methylprednisolone	0.400	24	1000	17
Morphine	0.100	5	500	40
Naproxen	10	24	32	13021
Omeprazole	0.250	24	600	17
Pentazocine	0.300	5	1700	35
Phenylbutazone	4.400	24	41.3	4439
Quinidine	5	24	330	631
Salicylate	20	24	120	6944
Triamcinolone	0.020	24	486	1.7
Vedapofen	1.000	24	70	595
Xylazine	1.100	24	1200	38

$$\text{EPC} = \text{Effective dose rate/plasma clearance}, \quad (21)$$

where EPC is the average plasma concentration over the dosing interval corresponding to the administration of an efficacious dose. EPC also reflects the drug potency. This approach was subsequently used to determine irrelevant plasma concentration for doping control in the horse (Toutain & Lassourd, 2002a). Table 2 gives the EPC for a selection of drugs in the horse.

THE USE OF PLASMA CLEARANCE TO EXTRAPOLATE A DOSE FROM ONE SPECIES TO ANOTHER

When the therapeutic plasma concentration is unknown in a given species (e.g. for minor species), a dose can be tentatively

extrapolated from another species (e.g. a major species). One of the best ways to extrapolate a dose from one species to another is to assume that the same overall body exposure (*AUC*) will produce the same effect in both species (that drug potency is species-independent). The following relation therefore holds:

$$AUC_{\text{species1}} = AUC_{\text{species2}} = \frac{\text{Dose}_{\text{species1}}}{Cl_{\text{species1}}} = \frac{\text{Dose}_{\text{species2}}}{Cl_{\text{species2}}}, \quad (22)$$

where Cl_{species1} and Cl_{species2} are plasma clearances for species 1 and 2, respectively.

Therefore, in order to estimate the unknown dose for species 2 from an efficacious dose in species 1, the following equation can be applied:

$$\text{Dose}_{\text{species2}} = \frac{\text{Dose}_{\text{species1}} \times Cl_{\text{species2}}}{Cl_{\text{species1}}}. \quad (23)$$

A refinement of Eqn 23 emerges when introducing a bioavailability factor, *F*, for extra-vascular administration. If plasma protein binding differs widely between the two species, the equation will also need to include an allowance for free fraction, *fu*, as it is only the free concentration that is responsible for the ultimate effect:

$$\text{Dose}_{\text{species2}} = \frac{\text{Dose}_{\text{species1}} \times fu_1 \times Cl_{\text{species2}}}{fu_2 \times Cl_{\text{species1}}}, \quad (24)$$

where fu_1 and fu_2 are free fractions for species 1 and 2, respectively.

To illustrate the use of Eqn 24, the most likely dose for morphine in the dog and horse can be calculated from the dose recommended in humans, assuming equivalent drug potency between species. The recommended dose in humans is 10 mg *in toto* (i.e. about 0.17 mg/kg), and plasma clearance is reported to be 14.7 mL/kg/min (Stanski *et al.*, 1978). In the dog, the morphine plasma clearance is higher (85 mL/kg/min) (Barnhart *et al.*, 2000), which leads to an estimated dose of about 1 mg/kg for the dog. In contrast, the morphine plasma clearance in the horse is rather low (8.64 mL/kg/min) (Combie *et al.*, 1983), which implies that a lower dose is needed in horses (0.1 mg/kg). No correction for drug binding to plasma protein is required as the extent of plasma binding is similar across all three species (Baggot & Davis, 1973).

TOTAL CLEARANCE AND DOSAGE REGIMEN ADAPTATION

Equation 13 indicates that total clearance is the sum of liver and kidney clearances, other clearing mechanisms often being negligible. When measuring simultaneously the total body clearance and renal clearance (with Eqn 15) the hepatic clearance can be determined by the difference, i.e.:

$$Cl_{\text{hepatic}} = Cl_{\text{tot}} - Cl_{\text{renal}}. \quad (25)$$

By comparing the numerical value of Cl_{hepatic} and Cl_{renal} , relevant information is obtained to predict the potential needs for dosage adjustment in animals having liver or kidney

disorders. Practically speaking, if the renal clearance mainly contributes to the total clearance (e.g. more than 50% of total body clearance), a significant drug overexposure can be expected in case of renal insufficiency (e.g. renal function is <50% of physiological value), and marketing drug companies should investigate the influence of kidney function on the kinetics of such drugs in animal species prone to renal insufficiency.

PLASMA CLEARANCE DURING EARLY DEVELOPMENT OF A NEW DRUG ENTITY IN ORDER TO DOCUMENT THE FEASIBILITY OF AN ORAL ROUTE OF ADMINISTRATION

The plasma clearance concept can be useful in early drug development in order to address some basic questions such as: can the drug be successfully administered as an oral dosage form or conversely, will the drug undergo an extensive hepatic first-pass effect leading to a low and variable systemic bioavailability?

The question can be answered from a single i.v. trial by measuring both total and renal clearance and thus allowing the evaluation by difference of a non-renal clearance, which in the first instance can be attributed to liver clearance.

The liver clearance, as all clearances, is the product of the hepatic blood flow (\dot{Q}_{hepatic}) and the hepatic extraction ratio (E_{hepatic}), i.e.

$$Cl_{\text{hepatic}} = \dot{Q}_{\text{hepatic}} \times E_{\text{hepatic}} \quad (26)$$

and

$$E_{\text{hepatic}} = Cl_{\text{hepatic}} / \dot{Q}_{\text{hepatic}}. \quad (27)$$

As the extraction ratio can be regarded as the percentage of drug which is irreversibly removed during a single (first) passage, the maximal possible bioavailability for a drug cleared by the liver should be:

$$F_{\text{max}} = 1 - E_{\text{hepatic}} = 1 - \frac{Cl_{\text{hepatic}}}{\dot{Q}_{\text{hepatic}}}. \quad (28)$$

As \dot{Q}_{hepatic} is known (about 30% of the cardiac output), the F_{max} can be assessed easily by evaluating only Cl_{hepatic} , which in turn requires knowledge of both Cl_{tot} and Cl_{renal} (Eqn 15). The computed F_{max} is the real oral bioavailability if the drug is totally absorbed by the digestive tract, i.e. if the loss of drug is only due to the hepatic first-pass effect. Using this approach, the low oral bioavailability of some drugs can easily be predicted. For example, in the dog, propranolol is totally absorbed after oral administration, and is completely eliminated by a high hepatic clearance (41 mL/kg/min). This explains the relatively low measured bioavailability despite total absorption ($16 \pm 12\%$) (Bai *et al.*, 1985). Such a low systemic bioavailability was expected because the hepatic clearance of propranolol is of the same order of magnitude as hepatic blood flow.

BLOOD VS. PLASMA CLEARANCE

The whole blood, rather than plasma, flows throughout the circulating system, and whole blood rather than plasma is generally the more appropriate (but not the most convenient) reference liquid to measure and from which interpret the body clearance. For the sake of simplicity, we have assumed in this review that blood and plasma are equivalent matrices, but this is not true if blood and plasma concentrations are different. For example, if total blood concentration is much higher than plasma concentration, interpretation of plasma clearance in terms of blood flow can be very misleading, and evaluation of blood clearance is preferred. Blood clearance can be directly evaluated by measuring blood concentration vs. time profiles (which is not very convenient but more and more used with analysis by mass spectrometry) or alternatively, by measuring plasma concentration vs. time profiles, and transforming the plasma clearance to the blood clearance using the haematocrit value (H) and the blood-to-plasma (B/P) partition ratio (which can be computed *in vitro* and is assumed to be constant). Equation 29 allows transformation of a plasma clearance into a blood clearance when a rapid equilibrium exists between plasma and red blood cells:

$$Cl_{\text{blood}} = \frac{Cl_{\text{plasma}}}{B/P} = \frac{Cl_{\text{plasma}}}{1 + H \left(\frac{C_{\text{rbc}}}{C_{\text{plasma}}} - 1 \right)}, \quad (29)$$

where B/P is the experimental blood-to-plasma concentration ratio for that drug, H is the haematocrit, C_{rbc} is the red blood cells concentration and C_{plasma} , the plasma concentration.

For a drug totally excluded from the RBCs ($C_{\text{rbc}} = 0$), Eqn 29 becomes:

$$Cl_{\text{blood}} = \frac{Cl_{\text{plasma}}}{1 - H}. \quad (30)$$

Considering a haematocrit value of 0.4, Cl_{blood} is equal to about $1.66 \times Cl_{\text{plasma}}$, so that plasma clearance underestimates blood clearance. Therefore, for a drug almost wholly excluded from red blood cells, the maximum possible error when using plasma clearance as a surrogate of blood clearance is no greater than 40%.

In contrast, when a drug accumulates in RBC (e.g. pimobendan, chloroquine, cyclosporin, acetazolamide...), Cl_{plasma} may considerably overestimate Cl_{blood} , making the interpretation of plasma clearance incorrect in terms of blood flow. That is why knowledge of the B/P ratio is required very early in drug development, as this will determine whether the appropriate matrix for PK studies is blood or plasma.

The B/P ratio varies between a value of $(1 - H)$ for drugs highly bound to plasma protein or/and poorly partitioned between RBC and plasma, to a possibly large value when drug has a high affinity for RBC (e.g. affinity for haemoglobin...). When B/P is higher than 1, it can be useful to know if the partitioning/departitioning of the drug between plasma and RBC is rapid or not with regard to the transit time through the clearing organ (liver and kidney). Indeed, the presence of drug in RBC is of relevance for PK interpretation only if the exchange between RBC and plasma is sufficiently rapid to participate in the drug clearance process. In other words, if

during blood transit throughout the liver (about 10 sec), the kidney cortex (about 2 sec) or the renal medulla (about 30 sec), a drug located in RBC (e.g. the fraction bound to the RBC membrane) can immediately return to plasma to re-equilibrate with plasma which is stripped of the drug during the transit time, the blood concentration and blood clearance are relevant. In contrast, if the departitioning is so sufficiently slow that drug removal from the plasma does not lead to significant re-equilibration during organ transit, plasma concentration becomes the relevant concentration, because then, RBC should be regarded as a peripheral (circulating) tissue compartment and not a central one. This is the case for para-aminohippuric acid (PAH), which is used to assess kidney circulation. PAH undergoes a first-pass effect during its passage throughout the kidney, but only PAH located in plasma is involved. Thus, PAH plasma clearance measures an effective plasma flow and not a blood flow. For further explanation see Hinderling (1997) and Wilkinson (1987).

ARTERIAL VERSUS VENOUS BLOOD SAMPLING: THE QUESTION OF THE JUGULAR VEIN

In veterinary medicine, blood administration and sampling are often carried out at the venous level, which can lead to two possible drawbacks. The first is related to drug administration and to a possible pulmonary first-pass effect, which can reduce the available (systemic) dose by a factor equal to the pulmonary extraction ratio (*vide supra*). The second difficulty is related to blood sampling from venous vessels and the possibility that the venous plasma concentration may differ from that of arterial plasma. Indeed, for clearance computation, plasma concentration measured at the venous level is assumed to be a surrogate of the simultaneous plasma concentration at the arterial level. This is true only if there is no significant metabolic site between the aorta and the sampling site. This assumption is reasonable when the blood is sampled on the arm in man because the venous blood has only crossed muscles, but it is more debatable for jugular blood which is more often sampled in animals as the blood has crossed the entire head (brain, possible saliva elimination in ruminants, etc.)

CONCLUSION

Total body clearance is the most important PK parameter and needs to be evaluated *in vivo* for any drug through an i.v. study. The main use of clearance is to compute or adapt a dosing rate. When interpreted in physiological terms, it provides an understanding of some basic features of drug disposition.

REFERENCES

Baggot, J.D. (1977) *Principles of Drug Disposition in Domestic Animals: the Basis of Veterinary Clinical Pharmacology*. W.B. Saunders, Philadelphia, PA.

- Baggot, J.D. & Davis, L.E. (1973) Species differences in plasma protein binding of morphine and codeine. *American Journal of Veterinary Research*, **34**, 571–574.
- Bai, S.A., Walle, U.K. & Walle, T. (1985) Influence of food on the intravenous and oral dose kinetics of propranolol in the dog. *Journal of Pharmacokinetics and Biopharmaceutics*, **13**, 229–241.
- Barnhart, M.D., Hubbell, J.A., Muir, W.W., Sams, R.A. & Bednarski, R.M. (2000) Pharmacokinetics, pharmacodynamics, and analgesic effects of morphine after rectal, intramuscular, and intravenous administration in dogs. *American Journal of Veterinary Research*, **61**, 24–28.
- Bonnin, P., Huynh, L., L'Haridon, R., Chene, N. & Martal, J. (1999) Transport of uterine PGF₂ alpha to the ovaries by systemic circulation and local lymphovenous-arterial diffusion during luteolysis in sheep. *Journal of Reproduction and Fertility*, **116**, 199–210.
- Bousquet-Melou, A., Bernard, S., Schneider, M. & Toutain, P.L. (2002) Pharmacokinetics of marbofloxacin in horses. *Equine Veterinary Journal*, **34**, 366–372.
- Chiou, W.L. (1982) The physiological significance of total body clearance in pharmacokinetic studies. *Journal of Clinical and Hospital Pharmacy*, **7**, 25–30.
- Combie, J.D., Nugent, T.E. & Tobin, T. (1983) Pharmacokinetics and protein binding of morphine in horses. *American Journal of Veterinary Research*, **44**, 870–874.
- Crisman, M.V., Wilcke, J.R., Correll, L.S. & Irby, M.H. (1993) Pharmacokinetic disposition of intravenous and oral pentoxifylline in horses. *Journal of Veterinary Pharmacology and Therapeutics*, **16**, 23–31.
- Hinderling, P.H. (1997) Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. *Pharmacological Reviews*, **49**, 279–295.
- Hyatt, J.M., McKinnon, P.S., Zimmer, G.S. & Schentag, J.J. (1995) The importance of pharmacokinetic/pharmacodynamic surrogate markers to outcome. Focus on antibacterial agents. *Clinical Pharmacokinetics*, **28**, 143–160.
- Lees, P. & Shojae Aliabadi, F. (2002) Rational dosing of antimicrobial drugs: animals versus humans. *International Journal of Antimicrobial Agents*, **19**, 269–284.
- MacKichan, J.J. & Hink, W.F. (1993) High-performance liquid chromatographic determination of CGA-184699 (Lufenuron) in dog and cat blood. *Journal of Liquid Chromatography*, **16**, 2595–2604.
- Marroum, P.J., Webb, A.I., Aeschbacher, G. & Curry, S.H. (1994) Pharmacokinetics and pharmacodynamics of acepromazine in horses. *American Journal of Veterinary Research*, **55**, 1428–1433.
- Riviere, J.E. (1999) *Comparative Pharmacokinetics. Principles, Techniques and Applications*. Iowa State University Press/AMES, Raleigh, NC.
- Riviere, J.E., Coppoc, G.L., Hinsman, E.J., Carlton, W.W. & Traver, D.S. (1983) Species dependent gentamicin pharmacokinetics and nephrotoxicity in the young horse. *Fundamental and Applied Toxicology*, **3**, 448–457.
- Rowland, M. & Tozer, T.N. (1995) *Clinical Pharmacokinetics. Concepts and Applications*, 3rd edn. Williams and Wilkins, London.
- Schulz, M. & Schmoldt, A. (1997) Therapeutic and toxic blood concentrations of more than 500 drugs. *Pharmazie*, **52**, 895–911.
- Stanski, D.R., Greenblatt, D.J. & Lowenstein, E. (1978) Kinetics of intravenous and intramuscular morphine. *Clinical Pharmacology and Therapeutics*, **24**, 52–59.
- Toutain, P.L. (2002) Pharmacokinetics/pharmacodynamics integration in drug development and dosage regimen optimization for veterinary medicine. *AAPS PharmSci*, **4**, article 38 (<http://www.aapspharmsci.org/scientificjournals/pharmsci/journal/ps040438.htm>).
- Toutain, P.L. (2003) Antibiotic Treatment of Animals – a Different Approach to Rational Dosing. *The Veterinary Journal*, **165**, 98–100.

- Toutain, P.L. & Bousquet-Melou, A. (2002) Free drug fraction versus free drug concentration: a frequent matter of confusion. *Journal of Veterinary Pharmacology and Therapeutics*, **25**, 460–463.
- Toutain, P.L. & Lassourd, V. (2002a) Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse. *Equine Veterinary Journal*, **34**, 242–249.
- Toutain, P.L. & Lassourd, V. (2002b) Pharmacokinetics/pharmacodynamic assessment of irrelevant drug concentrations in horse plasma or urine for a selection of drugs. In *14th International Conference of Racing Analysts and Veterinarians*. Eds Hill, D. & Hill, W. pp. 19–27. R & W Publication Ltd, Orlando, FL.
- Toutain, P.L., Koritz, G.D., Fayolle, P.M. & Alvinerie, M. (1986) Pharmacokinetics of methylprednisolone, methylprednisolone sodium succinate, and methylprednisolone acetate in dogs. *Journal of Pharmaceutical Sciences*, **75**, 251–255.
- Toutain, P.L., Autefage, A., Legrand, C. & Alvinerie, M. (1994) Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling. *Journal of Veterinary Pharmacology and Therapeutics*, **17**, 459–469.
- Toutain, P.L., Lefebvre, H.P. & King, J.N. (2000) Benazeprilat disposition and effect in dogs revisited with a pharmacokinetic/pharmacodynamic modeling approach. *Journal of Pharmacology and Experimental Therapeutics*, **292**, 1087–1093.
- Toutain, P.L., del Castillo, J.R.E. & Bousquet-Mélou, A. (2002) The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Research in Veterinary Science*, **73**, 105–114.
- Wilkinson, G.R. (1987) Clearance approaches in pharmacology. *Pharmacological Reviews*, **39**, 1–47.