Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse

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Summary

The current performance of analytical techniques used for drug control in horses lead the Regulatory Authorities to decide whether trace levels of drugs legitimately used for therapeutic medication should or should not be reported. Here, we propose a well-ordered and nonexperimental pharmacokinetic/pharmacodynamic approach for the determination of irrelevant drug plasma (IPC) and urine concentrations (IUC). The published plasma clearance is used to transform an effective (marketed) dose into an effective concentration (EPC). EPC is transformed into an IPC by applying a safety factor (SF). This method is based on several assumptions (e.g. drug effects reversibly driven by plasma concentration, linearity of drug disposition). The suitability of the computed IPC and IUC can be checked by calculating the residual amount of drug at IPC and computing a minimal drug withdrawal time. It is concluded that controlling the drug effect (using drug or any analyte concentration as a marker) rather than the drug exposure will be more demanding and also makes urine a less than ideal matrix.

Introduction

Difficulties with the findings of analytical techniques, currently used in drug control in horses, have been encountered when very low levels of drugs employed in the treatment of racehorses have been detected on the day of racing (Houghton 1994; Tobin 1995). In such situations, the regulatory authorities have to decide whether trace levels should be reported (i.e. considered as a potential threat to the integrity of competition) or ignored (i.e. considered as biologically irrelevant and of no concern).

Two approaches can be adopted to take a decision. The first consists of reporting any detected drug, which is operationally equivalent to fixing the reporting level at the level of detection (LOD) of the selected analytical technique. Here the aim is to control any drug exposure; this is mandatory for prohibited substances or masking agents for which the most sensitive analytical technique should be used.

A second approach consists of reporting only concentrations that are above a selected cut-off value, in order to control competition fairness without preventing the possibility of providing proper veterinary care. It has to be based not only on appropriate pharmacokinetic and pharmacodynamic data (i.e. biological data) but also on the selection (implicit or explicit) of safety factors which should reflect what is considered as acceptable or unacceptable by the regulatory authorities.

The objective of the present report is to outline a general and nonexperimental approach for the determination of irrelevant drug plasma concentrations (IPC) and irrelevant drug urine concentrations (IUC) from data in the literature (meta-analysis) in terms of pharmacological and/or clinical effect.

Definitions

For the purpose of this article, the irrelevant plasma drug concentration (IPC, i.e. drug or metabolite) and irrelevant urine drug concentration (IUC, i.e. drug or metabolite) are defined as plasma or urine concentrations which guarantee the absence of any relevant drug effect and for which there will be no regulatory action. Relevant or irrelevant drug effects are qualified later (see section on safety factor).

Assumptions in computing IPC and IUC

The proposed estimation of IPC relies on 3 basic assumptions:

(i) The drug effects are driven reversibly by plasma concentrations and a relationship exists between the overall drug exposure and drug effect. The total area under the plasma concentration vs. time curve (AUC) is used as an index of drug exposure.

(ii) In horses, the currently used (approved) dosage regimens for the drugs under investigation are appropriate, i.e. they correspond to an efficacious dose which does not greatly exceed the ED50 (i.e. dose giving half-maximal effect).

(iii) The drug disposition is linear (neither dose- nor time-dependent) within the range of concentrations under consideration and plasma clearance is a parameter (not a variable) relating the recommended dose to plasma concentrations (for further explanations on linearity, see Gibaldi and Perrier 1982).

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Computational steps to estimate IPC and IUC

Step 1: computation of an effective plasma concentration (EPC)

For any drug, calculation of the average EPC over the dosing interval corresponding to the standard dosage regimen is given by equation 1:

\[
EPC = \frac{\text{standard dose (per dosing interval)} \times F}{\text{plasma clearance (per dosing interval)}}
\]  

(1)

In equation 1, standard dose is the generally recommended dose per dosing interval, plasma clearance is the pharmacokinetic parameter which expresses the body’s capacity to eliminate the drug, F is the bioavailability factor lying between 0 and 1. The EPC will be calculated using i.v. data only so that \( F = 1 \), as the EPC is the same whatever the route of drug administration. Therefore, equation 1 can be reduced to equation 2:

\[
EPC = \frac{\text{standard dose (per dosing interval)}}{\text{plasma clearance (per dosing interval)}}
\]  

(2)

Step 2: computation of irrelevant plasma concentration (IPC)

An irrelevant plasma concentration (IPC) can be deduced from EPC by applying a safety factor (SF) to EPC (equation 3):

\[
IPC = \frac{EPC}{SF}
\]  

(3)

The selection of an SF is mainly a regulatory decision (see Discussion).

Step 3: determination of irrelevant urine concentration (IUC)

An irrelevant urine concentration (IUC) can be derived from IPC using equation 4:

\[
IUC = IPC \times R_{ss}
\]  

(4)

In equation 4, \( R_{ss} \) is the steady-state urine to plasma concentration ratio.

Step 4: checking the appropriateness of IPC and IUC

After computing IPC and IUC, the suitability of the proposed figures can be checked by computing the amount of drug remaining in the body when the plasma concentration is equal to IPC and by calculating the shortest possible withdrawal time (WT) of the drug, i.e. the time after i.v. administration during which the drug can be detected at concentrations higher to IPC or IUC.

The residual amount (RA) of drug in the body when the plasma concentration is equal to IPC is given by equation 5:

\[
RA = IPC \times V_{area}
\]  

(5)

Where \( V_{area} \) is the volume of distribution calculated by the area method. This RA can then be compared to the recommended dosage regimen and should be lower than a given percentage of the recommended dose (e.g. 1%).

Estimation of the drug WT after i.v. administration can be obtained with the following equation (6):

\[
WT = 1.44 \times \frac{\text{selected half-life}}{\text{IPC}} \times \text{Log intercept of selected half-life}
\]  

(6)

where the selected half-life is the half-life which encompasses the calculated IPC (generally the terminal half-life) and the intercept of the selected half-life is the plasma concentration at time zero when the (terminal) phase of interest begins to decay.

Supplementary considerations for computing and checking IPC and IUC

The computation of EPC (equation 1) requires the selection of a dose. For many drugs, there is only a standard dose but for others (e.g. tranquillisers) different dose levels can be used, depending on the desired effect. For those drugs, the lowest recommended dose should be selected when computing an EPC. Alternatively, the dose corresponding to the effect most relevant to doping control and for which the drug has the greatest potency should be selected.

When very different values of plasma clearance are reported in the literature, the highest clearance should be selected to ensure a conservative approach. When similar clearances are reported, mean or median can be determined. For treatments involving multiple drug administrations, clearance (generally expressed as l/kg/h or ml/kg/min) should be expressed for the recommended dosing interval (e.g. l/kg/24 h). For treatments involving a single dose administration, the period should be selected case by case taking into account a conservative estimate of the possible duration of drug action.

The computation of IPC (equation 3) requires the selection of a safety factor (SF). The selection of safety factors (SF) is a conjointed policy and a scientific decision (see Discussion). A default SF of 500 (i.e. 10 x 50) can be proposed, with 50 to transform an effective plasma concentration close to EC50 into an ineffective one and 10 to take interindividual variability into account (Calabresse 1983) (Fig 1).

In Figure 1, \( E_{max}, ED_{50}, h \) are parameters. For drug A, \( h = 1 \) and the equation follows the classical \( E_{max} \) model. For drugs B and C, \( h = 0.5 \) or 2, respectively. Visual inspection of the curves shows that the effect corresponding to a dose equal to \( ED_{50}/50 \), i.e. a dose of 0.2, is highly dependent on the \( h \) value. For \( h = 1 \), the effect is equal to 2% \( E_{max} \), for \( h = 0.5 \) and \( h = 2 \), the effects are 12.4 and 0.04% \( E_{max} \), respectively (see Fig 1 inset). The figure suggests that for drugs with a shape factor below 1, a SF higher than 500 should be selected. Conversely, a lower SF can be used for drugs in which the shape factor is higher than 1.

The determination of IUC requires knowing the urine to plasma concentration ratio, i.e. \( R_{ss} \). \( R_{ss} \) is seldom reported in scientific literature and has to be approximated from published raw data (or from published figures).

For calculating an amount of drug in the body, \( V_{area} \) is the volume of distribution when the computation is performed for a single dose administration and \( V_{ss} \) (steady state volume of distribution) is the appropriate volume term when the computation is performed for steady state conditions, but these numerical values are close enough for either to be usable for assessing the suitability of a computed IPC.
The regular dosage Time (h) evaluated the effect of PBZ at a low plasma PBZ plasma clearance has been (1994) using a PK/PD approach in ED 50 (1996) or Step 1 - selection of the recommended dosage: and IUC for phenylbutazone An example: computational steps for the determination of an IPC ‘encompasses the IPC (Fig 2) and, for many drugs, this should be the half times are theoretically the same for plasma and urine, and if IPC is lower than the plasma LOQ, WT should also be calculated using IUC and urine data. The phenylbutazone (PBZ) case provides a good illustration of the proposed method (see next section).

An example: computational steps for the determination of an IPC and IUC for phenylbutazone

Step 1 - selection of the recommended dosage: The regular dosage regimen for phenylbutazone (PBZ) is 4.4 mg/kg/24 h (maintenance dose), but an 8.8 mg/kg/24 h dose is also recommended as an i.v. loading dose; the 4.4 mg/kg/24 h dose has been selected as the standard dose.

It is also desirable, when checking the appropriateness of IPC and IUC, to have a rough estimate of the withdrawal time (WT) required to reach the IPC after administering a standard dose. This should be obtained from i.v. data, because these provide information on the actual drug WT (irrespective of the pharmaceutical formulation) and the WT obtained in this way should be the shortest possible. In contrast, the data obtained by extravascular route is influenced by the bioavailability factors, some being due to the pharmaceutical formulation and not to the drug (e.g. a slow process of absorption leading to a prolonged terminal half-life).

In equation 6, the selected half-life should be the half-life that encompasses the IPC (Fig 2) and, for many drugs, this should be the ‘terminal half-life’. However, for some drugs the IPC will be lower than the level of quantification (LOQ) of the current analytical method and the computation of withdrawal time using the last observed plasma half-life could be misleading, because there is no guarantee that this is the terminal half-life encompassing the IPC. If the reported urine terminal half-life is longer than the terminal plasma half-life (often the case) despite the fact that the terminal half-times are theoretically the same for plasma and urine, and if IPC is lower than the plasma LOQ, WT should also be calculated using IUC and urine data. The phenylbutazone (PBZ) case provides a good illustration of the proposed method (see next section).

Fig 1: Selection of a safety factor (SF) and shape factor of the dose-effect relationship model. This figure shows the shape of the dose-effect relationship for 3 hypothetical drugs (A,B,C) simulated with a Hill model of the form:

\[ \text{Effect} = \frac{\text{Emax} \times \text{Dose}^h}{\text{ED}_{50} + \text{Dose}^h} \]

\[ \text{Emax} = \text{maximal possible effect (fixed at 100); } \text{ED}_{50} = \text{dose producing half Emax; } h = \text{shape factor; Dose = simulated dose. For full explanation, see text.} \]

\[ C(\mu g/ml) = 100 \exp(-t) + 30 \exp(-0.2t) + 3 \exp(-0.04t) \]

The LOQ is 5 µg/ml and only the 2 first phases are quantitatively measurable. If only the data above LOQ are used, a bi-exponential equation has to be selected to fit the data which leads to an estimated WT of 21.4 h, whereas it is actually 29.5 h.

Step 2 - computation of EPC: PBZ plasma clearance has been measured by many authors; mean reported values range from 16.3 to 41.3 ml/kg/h (Soma et al. 1983; Lees et al. 1987; Mills et al. 1996). The most conservative value is obtained by taking the highest clearance value, i.e. 41.3 ml/kg/h (Mills et al. 1996) or 991 ml/kg/24 h. Using equation 7:

\[ \text{EPC} = 4400 \mu g/kg/24 h = 4.44 \mu g/ml \]

\[ 991 \text{ ml/kg/24 h} \]

This EPC is consistent with the EC_{50}, which was directly determined by Toutain et al. (1994) using a PK/PD approach in horses subjected to experimental arthritis (EC_{50} from 1.5 to 4.3 µg/ml depending on the pharmacodynamic endpoint). Lees et al. (1987) reported that the inhibitory action of PBZ on thromboxane B2 formation was still 50% 24 h after an i.v. PBZ administration (4.4 mg/kg), giving an EC_{50} of about 2 µg/ml. In addition, in this experiment, it was necessary to wait 48 h to reach a not effective plasma PBZ concentration (i.e. about 0.1 µg/ml). Dreveremo et al. (1994) evaluated the effect of PBZ at a low plasma concentration by studying locomotion in horses with low grade distal forelimb lameness by means of a quantitative kinetic method. They concluded that PBZ may engender a significant change in the locomotor pattern of horses with low grade lameness and that the effect may last longer than 48 h after PBZ administration (2.5 mg/kg b.i.d.). The authors concluded that the PBZ effect lasted for plasma concentrations lower than 2 µg/ml (their LOQ) and it was concluded from this clinical trial that a plasma concentration of about 0.1 µg/ml still has some therapeutic meaning. The IPC should, therefore, be lower than 100 ng/ml. Finally, these results taken together suggest that the EPC of PBZ is around 1 µg/ml and that the IPC should be lower than 100 ng/ml.

For several drugs, not only the drug but also its primary metabolite(s) can participate in the overall drug effect. This is the case for oxyphenbutazone (OPBZ), a primary metabolite of PBZ in horses. Therefore, the question is whether this active metabolite should be taken into account when computing an EPC for PBZ.

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The answer is negative, as the calculated EPC is solely the observed plasma PBZ concentration for which effects are expected (i.e. taking into account the action of all the active metabolites), and not the actual PBZ concentration at the receptor level required to trigger a given effect. In other words, with the proposed approach, PBZ is the ‘marker analyte’ of the PBZ response and the proposed EPC value has no mechanistic value.

**Step 3 - computation of an IPC for PBZ:** Using equation 8 and the default value for SF (500) for illustration:

$$\text{IPC} = \frac{4440 \text{ ng/ml}}{500} = 8.88 \text{ ng/ml} \quad (8)$$

rounded to give an IPC = 10 ng/ml, which is much lower than the 5 µg/ml threshold fixed in North America (ARCI) (Tobin et al. 1999).

**Step 4 - determination of IUC for PBZ:** Soma et al. (1983) published plasma and urine PBZ concentrations after i.v. and oral PBZ administration. They demonstrated an excellent correlation between the decrease in urinary and plasma concentrations after oral (r = 0.95) and i.v. (r = 0.99) administration. In addition, after oral administration (8.8 mg/kg every 24 h for 8 consecutive days) the urinary concentrations at 24, 48, 72, 96 and 120 h, i.e. 24 h after each administration, were about 4–6 µg/ml, i.e. of the same order of magnitude as the plasma concentration and suggesting a urine to plasma ratio (Rss) of about 1.

Similar results are reported in the monograph compiled by Canada’s Agriculture and Agri-food Equine drug evaluation programme (Anon 1994), and Gerring et al. (1981) reported a urine to plasma concentration ratio of 0.2 to 1.

Using equation 9 and fixing Rss at 1:

$$\text{IUC} = 10 \text{ ng/ml} \times 1 = 10 \text{ ng/ml} \quad (9)$$

It is noteworthy that the computed IUC is lower than the LOD (about 50 ng/ml) reported in the literature; and that the urinary concentration of PBZ can be modified dramatically by the urinary pH. It was shown in postrace urine from horses racing in Kentucky that urinary PBZ concentrations can be multiplied by 200 when the pH increases from 4 to 8.5 (Houston et al. 1985). As urinary pH does not influence plasma clearance, it is probable that a PBZ IUC of 10 ng/ml is much more conservative for a horse with a basic urine pH than for one with an acid urine pH (see Discussion).

**Step 5 - computation of residual PBZ amount for IPC:** The residual amount (RA) of PBZ can be calculated from IPC and V area (see equation 5). Several authors have published V area for PBZ with figures ranging from 0.152 l/kg (Soma et al. 1983) to 0.37 l/kg (Toutain et al. 1994). Using equation 10:

$$\text{RA} = 10 \text{ µg/l} \times 0.37 \text{ (or 0.15) l/kg} = 3.7 \text{ (or 1.5) µg/kg} \quad (10)$$

This residual amount is lower than 1% of the recommended dose (4400 µg/kg).

The minimal PBZ withdrawal time (WT) can be computed using the mean parameters obtained by Mills et al. (1996) (their Table 1) for a dose of 8.8 mg/kg by i.v. route:

$$C \text{ (µg/ml)} = 80 e^{-18.55t} + 33 e^{-0.14t}$$
By solving this equation for IPC, different WT can be estimated for different single doses, namely 58 h for an i.v. dose of 8.8 mg/kg and 53 h for a 4.4 mg/kg dose. Similar results are obtained with the simplified equation 6, in which the intercept Yz is equal to 33 µg/ml and the terminal half-life equals 4.95 h.

Using the equation proposed by Lees et al. (1987) for a dose of 4.4 mg/kg:

$$C (\mu g/ml) = 34.5 e^{-5.9t(h)} + 29.76 e^{-0.115t(h)}$$

The estimated WT required to reach the IPC is 75.5 h for an 8.8 mg/kg dose and 69.5 h for a 4.4 mg/kg dose.

These figures assumed that the reported terminal half-life (4.95 h from Mills et al. [1996] and 6 h from Lees et al. [1987]) would be the true terminal half-life of PBZ, i.e. the plasma half-life which would be measured if the plasma concentrations had been measured over 3 days. There is some evidence that this is not the case and that there is a supplementary phase that cannot be detected accurately from the plasma disposition curve as the LOQ is too high. A terminal half-life of about 8 h can be calculated graphically from the urine data published by the Canadian Agriculture and Agrifood Equine drug evaluation programme (Anon 1994). If we accept that the terminal half-life in urine is theoretically equal to the terminal half-life in plasma (Gibaldi and Perrier 1982), then the true terminal plasma half-life for PBZ is 8 h, rather than 4 or 6 h. Therefore, a minimal WT slightly longer than 70 h should be expected after i.v. administration of PBZ at the recommended dosage regimen. This conclusion fits the experimental data, a delay of about 80 h being obtained graphically from figures published (Anon 1994) for i.v. PBZ administration of 2.0 g.

The issue of active and inactive metabolites in IPC and IUC calculation

The analyte(s) selected to calculate the IPC or IUC can be the drug itself or its active moiety (e.g. dipyrone, which is transformed within a few minutes into the active 4-methylaminoantipyrine or 4-AMM), and this is the most desirable situation. However, the drug or its active moiety may not be eliminated in the urine in sufficient amounts, thereby making appropriate monitoring impossible, and an inactive metabolite has to be selected. A relevant question in the context of this present approach is whether an inactive metabolite can be used to calculate IPC.

As we are concerned with both IPC and IUC control, it must be ensured that this inactive (or weakly active) metabolite is an authentic tracer of the overall drug activity. For this, the ratio of the plasma or urinary marker (metabolite) to the plasma active principle (drug, active moiety) must be known, and be more or less constant over the period of the IPC or IUC control.

For an analyte to be a marker (tracer) of the active principle (hereafter named ‘tracee’), the slopes of the terminal phase of the tracer and of the tracee should be the same as this guarantees a constant ratio between them. The conditions for this are 2-fold: (i) the intrinsic half-life of the marker (i.e. the half-life which will be obtained after administering the tracer by i.v. route) should be shorter than or equal to the half-life of the tracee; (ii) the system is in pseudo-distribution equilibrium (after a single dose administration) or in steady state conditions (after a multiple dose administration). This explains the fact that inactive metabolites cannot always be used if the purpose of drug control is to ensure a lack of relevant effect (Figs 3, 4).

For example, in Figure 3, plasma concentrations for the drug
and its 2 metabolites, M1 and M2, were simulated for a multiple dose administration (every 12 h for 6 days). Visual inspection of the figures shows that the drug and M1 rapidly reach the steady-state conditions, i.e. that the same plasma concentration profile is repeated after each administration. In contrast, the steady-state conditions are not totally achieved after 6 days for M2. This is due to the fact that the time to reach steady-state conditions is governed by the terminal half-life of each analyte, which is much higher for M2 than M1. This simulation shows that the M2/drug ratio can be different at the beginning of the drug administration up to the attainment of steady state conditions and that a high M2 concentration can be measured whereas drug concentration (and effect) are very low. In this kind of situation, M2 is not an appropriate analyte for a drug control aimed at the control of a noneffect level. Instead, using M2 will be operationally equivalent to controlling drug exposure.

In Figure 4, the effect was simulated using a classical Emax equation with drug plasma concentrations as the driving concentration. This figure shows that there is a perfect relationship between plasma drug concentrations and effect, i.e. when plasma drug concentrations decrease (after i.v. administration) the drug effect decreases. The relationship between M1 plasma concentrations and effect is different and exhibits a hysteresis phenomenon, i.e. after drug administration, M1 concentrations increased to reach a maximum concentration (80) and then began to decrease to 0. It can be seen that for a given M1 concentration (e.g. 40) there are two possible effects, one nearly total and the other of about 30. For M2, a plasma concentration of about 15 can correspond to either a nearly full effect or to a lack of effect. This figure shows that a snapshot inactive metabolite concentration cannot predict univocally a drug effect. Similarly, a hysteresis phenomenon can also exist between the plasma drug concentration itself and the effect.

Discussion

A drug control can be based on one of 2 lines of reasoning: control of drug exposure or control of the absence of a (relevant) drug effect. Any analyte (drug, active or inactive metabolite) can be used for monitoring drug exposure and sensitivity will depend on analytical performance. This approach is sound for prohibited substances which have no place in horse racing. On the other hand, the control of drug exposure for therapeutic medications raises the problem of ‘trace’ concentrations and, due to the permanent improvement of analytical techniques, some drugs used in horses can still be detected long after their effect has totally vanished. Under these conditions, the drug control programme can become a limiting factor in the use of drugs for proper veterinary care of racehorses.

Here we present a standardised approach for the treatment of data that has already been published. It is by definition a nonexperimental approach, which differs from the one proposed by Tobin et al. (1999) in which a dose-effect relationship is established experimentally, then a dose without effect and finally the corresponding plasma concentration is measured. The advantage of the present approach is that it is inexpensive, because it consists of performing a meta-analysis on already existing data. Its main limit is that it can only be applied to drugs for which the effects are driven by plasma concentration, i.e. drugs that act systemically. It cannot be applied to drugs with presystemic effect (i.e. which act before reaching the general circulation). This is the case for local anaesthetics or any drug which can be administered by local route (e.g. glucocorticoids). The approach outlined by Tobin et al. (1999) is recommended for these classes of drug. The 2 approaches are compared in Figure 5, together with their domains of application.

In the present (nonexperimental) approach, either a plasma drug or active metabolite concentration drives the effect at the biophase level, i.e. is the independent variable with respect to the concentration-effect relationship. The approved (marketed) dose is considered as the pivotal dose giving a relevant overall clinical response which, divided by plasma clearance, gives an effective plasma concentration (EPC). A safety factor (SF) is used to transform EPC into an irrelevant plasma concentration (IPC). The advantages of this method are its cheapness, the fact that it is based on clinical response rather than on pharmacodynamics (surrogate effect) and its independence from route of administration (providing its action is systemic) and from formulation. The limits of the approach are apparent with drugs which can be administered locally (biophase exposure preceding plasma exposure), drugs for which the effect lasts much longer than their actual presence in the body (anabolic), the need to obtain a quantitative measurement of plasma or urine analytes and the depreciation of urine as a matrix for doping control, and of any inactive metabolite if the aim is to control the presence or absence of relevant effect. In the approach of Tobin et al. (1999) (experimental), the plasma drug (metabolite) concentration does not drive the effect but is conceptualised as a marker of a presystemic biophase drug exposure to the highest no effect dose (HNED). The HNED has to be determined experimentally using a critical pharmacological effect (rather than an overall clinical response) by performing a dose-effect relationship. An analyte marker (drug, metabolite) is then used to establish a ‘no effect point’ or no effect level (NOEL), i.e. a cut-off plasma or urine concentration corresponding to the HNED. As quoted by Tobin et al. (1999), the limit of this approach is that ‘if the drug is administered by different routes of administration, or repeated doses, or a different formulation or with another therapeutic rational, then the analytical pharmacological data base reported (here) may not necessarily be applicable to the specific regulatory circumstances’.

The present approach relies on several assumptions, the central one being that drug effects are reversibly driven by the plasma concentration profile and the present method cannot be used for drugs (e.g. steroids) whose effects persist long after the drug has disappeared.

The action of some drugs is related more to the achievement of a minimal plasma concentration than to overall exposure (e.g. anti-arrhythmics, anaesthetics), leading to an all-or-nothing type of response rather than a graded dose-response. The present approach is not recommended for such drugs, because an AUC fraction (more or less important and which is dose-dependent), does not participate in their action.

The present approach requires selecting a pivotal dose to compute an EPC and we suggest choosing the approved dosage regimen (e.g. as indicated in the marketing authorisation). Therefore, the clinical response expected by clinicians rather than selected pharmacodynamic effects is used as efficacy endpoint, which guarantees the relevance of EPC. One difficulty may arise for drugs which have obtained a marketing authorisation with too high a dosage thanks to a good safety margin. This is probably the case for acepromazine in horses, which is marketed in France at 500 µg/kg and at 130–260 µg/kg in the UK but has proved
effective at a much lower dose (Freestone et al. 1991; Wood et al. 1992). To deal with this situation, the lowest effective dose can be selected to compute the EPC or, alternatively, a more conservative SF can be selected.

The plasma clearance needs to be known for the computation of EPC and is known, in horses, for many drugs. If the drug disposition is linear, clearance is a genuine parameter allowing transformation of an efficacious dose into an overall average efficacious plasma concentration. It should be noted that an efficacious plasma concentration is a drug (i.e. molecule) property that is independent of the route of administration or formulation. EPC can also be obtained tentatively from an extravascular route of administration by dividing the measured AUC by the recommended dosing interval. The advantage of computing an EPC using clearance is that clearance is a species parameter reflecting only the drug (molecule) property, whereas an AUC obtained by extravascular administration is also influenced by the route of administration, formulation etc. AUC is therefore less attractive when international harmonisation is required.

The computation of EPC also requires determination of a time interval corresponding to the duration of a relevant effect. For drugs with a marketing authorisation for an approved dosing interval, this dosing interval (often 24 h) will be the best to consider. In contrast, for drugs used in a single dose administration (e.g. tranquiliser), it is necessary to evaluate the duration of the action in order to express clearance for a relevant duration of time.

To transform an EPC into an IPC an a priori safety factor has to be selected. The selection of an SF is a policy and a scientific decision. It is the responsibility of the regulatory authorities to determine what is acceptable (or unacceptable) in terms of drug effect. The magnitude of the SF also involves, for example, the reliability of the data, the nature of the effect, the possibility of large interindividual variability between the population subgroups, the existence of different effects for which a drug has different potencies and the shape of the dose-effect relationship. A default value of 500 would take into account the interindividual variability and transform an efficacious plasma concentration into an irrelevant one. The interindividual factor (10) is relatively large and can be reduced if the interindividual plasma clearance has been documented from population kinetics. The 50 factor to convert EPC to IPC holds if the selected dose is close to the ED₅₀ and if the dose-effect relationship obeys the Emax model. If these 2 conditions are fulfilled, the residual effect is about 2% Emax. If the selected dose is much higher than the ED₅₀, it will be necessary to increase this factor. Similarly, the SF should be increased if the dose-effect relationship obeys a Hill model with a slope factor lower than 1 (Fig 1). The slope factor is known for some drugs in the horse, e.g. for NSAIDs the slope factor for PBZ was higher than 1 (Toutain et al. 1994) whereas, in human medicine, the slope factor is considered to be lower than 1 for betablockers (Campbell 1990).

It is interesting to note that fixing SF at infinity is operationally equivalent to controlling drug exposure, as any detected drug will be reported; and fixing SF at 1 is operationally equivalent to accepting drugs at therapeutic levels, showing that the present approach is very general. For PBZ, the threshold has been fixed at 5 µg/ml in North America (ARCI) (Tobin et al. 1999) which is precisely the EPC reported here. In this case, an SF equal to 1 has been implicitly applied.

The drug control is generally undertaken using urine as a matrix rather than plasma. However, it should be kept in mind that only plasma concentration is governing the effect and, in the framework of the present approach, urine becomes a surrogate matrix for plasma. As plasma concentration is also the driving concentration for urine excretion rate, a relationship exists between plasma and urine drug concentrations, but the urine to plasma ratio is not a robust factor. After a single dose administration, it is not a parameter but a variable, with values ranging between 0 (just after drug administration) and a theoretically constant value (Rss) in pseudoequilibrium conditions. For a multiple drug administration, the urine to plasma ratio is permanently changing until steady state conditions are reached, when Rss can be constant over a more or less large fraction of the dosing interval. In addition, Rss can be influenced by water diuresis (urine volume) and some physiological factors, such as urine pH (Houston et al. 1985). For all these reasons, urine is a less than ideal matrix for controlling a no effect drug level and changing the drug control policy could make urine much less attractive (appropriate) than for controlling drug exposure.

Another consequence of the proposed approach could be the necessity to abandon many metabolites currently used for drug control. If the purpose of drug control is to control exposure (e.g. cocaine, masking agents), any analytes (drug, active or inactive moiety) can be used for monitoring. In contrast, if the purpose is to control an IPC or IUC for a drug recognised as a therapeutic agent in horse, the recourse to an inactive metabolite can become debatable, because measuring a snapshot inactive metabolite concentration can be very misleading, as explained in Figures 3 and 4.

The minimal WT of a drug should be checked to make sure that IPC and IUC are acceptable. It should be remembered that the WT is not a pivotal issue for regulatory authorities. Given the IPC, it will be the responsibility of the drug company to estimate the WT of their commercial preparations for their selected dosage regimen and route of administration. In this paper, WT is computed (without any statistical protection) in order to assess the acceptability of the proposed IPC and IUC.

It should be understood that the concept of detection time (Tobin et al. 1999) or WT is fundamentally different from that of IPC (or threshold). A threshold is a drug parameter, whereas detection time is a variable influenced by many factors unrelated to the drug properties, such as dose, route of administration, pharmaceutical formulation or LOD.

In conclusion, this paper proposes a well-ordered and nonexperimental approach on the basis of pharmacokinetics and clinical data for the determination of what are the appropriate analytical performances, for drug control, on the day of racing. Its purpose is to discriminate between concentrations of drugs which could threaten the integrity of racing, and irrelevant concentrations which can be detected using operational analytical tools. The present approach holds only for drugs acting systemically. It is also emphasised that such a change to drug control policy could make urine a much less appropriate matrix than plasma for the control of drug exposure and that a snapshot inactive metabolite measurement (urine or plasma) cannot be used unequivocally to control a drug effect. Finally, controlling drug effect will require not only an LOD to check presence or absence of an analyte, but also quantification of the analyte in plasma and urine.
References


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