ORIGINAL ARTICLE

Futility of current urine salbutamol doping control

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AIMS

Salbutamol is used in the management of obstructive bronchospasm, including that of some elite athletes. It is claimed that high salbutamol (oral) doses may also have an anabolic effect. Therefore, inhalation of salbutamol is restricted by the World Anti-Doping Agency (WADA) to a maximal daily dose. Urine is tested for violations, but recent cases have resulted in a debate regarding the validity of this approach. It was our aim to determine whether current approaches are sufficiently able to differentiate approved usage from violations.

METHODS

We extracted pharmacokinetic parameters from literature for salbutamol and its sulphated metabolite. From these parameters, a semi-physiological pharmacokinetic model of inhaled and orally administered salbutamol was synthesized, validated against literature data, and used to perform clinical trial simulations (n = 1000) of possible urine concentrations over time resulting from WADA-allowed and oral unacceptable dosages.

RESULTS

The synthesized model was able to predict the literature data well. Simulations showed a very large range of salbutamol concentrations, with a significant portion of virtual subjects (15.4%) exceeding the WADA threshold limit of 1000 ng ml^{-1} at 1 h post-dose.

CONCLUSIONS

The observed large variability in urine concentrations indicates that determining the administered dose from a single untimed urine sample is not feasible. The current threshold inadvertently leads to incorrect assumptions of violation, whereas many violations will go unnoticed, especially when samples are taken long after drug administration. These issues, combined with the dubious assertion of its anabolic effect, leads us to conclude that the large effort involved in testing should be reconsidered.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Inhaled salbutamol is accepted according to WADA guidelines within restricted doses. Urine is tested for violations of these restrictions, possibly resulting in punitive action.
- Inhaled and oral salbutamol administration follows complex pharmacokinetic profiles, including multiple ADME processes contributing to large variability in urine concentrations.
- This variability makes it doubtful that one can determine dose from a (single) urine concentration. WADA's assumptions of its appropriateness are insufficiently supported by the literature and general theory of drug disposition.

WHAT THIS STUDY ADDS

- Simulations of salbutamol urine concentrations resulting from acceptable salbutamol usage (after inhaled therapeutic doses) in 1000 virtual subjects show that 1 h post-dose, concentrations surpass the threshold of 1000 ng ml^{-1} in 15.4% of subjects. Conversely, unacceptable oral doses fall below the threshold within 48 h after the last dose.
- Our results show that single urine measurements do not allow estimation of the applied dose. The applied cutoff value of 1000 ng ml^{-1} is insufficient to differentiate between acceptable and unacceptable salbutamol use. Moreover, it is impossible to determine a cutoff value for this purpose with the current procedures.
- A semi-physiological pharmacokinetic model was built using literature data. The possibility of the model to reasonably predict for several scenarios shows the power of model synthesis using prior literature data, allowing the evaluation of virtual scenarios using readily existing data.

Introduction

Recently one of the most successful male cyclists of the last decade, Chris Froome, came into disrepute due to news of a potential doping violation. Doping control revealed a salbutamol (albuterol in the United States) concentration exceeding the allowed limit of 1000 ng ml^{-1} in a urine sample provided by the British rider during the Vuelta a España of 2017 [1]. The WADA prohibited list indicates that salbutamol use is allowed in inhaled dosages up to "1600 micrograms over 24 hours in divided doses not to exceed 800 micrograms over 12 hours starting from any dose", which is considered the maximum therapeutic dose for athletes with a so-called Therapeutic Use Exemption (TUE). Froome was in possession of such a TUE, but "the presence in urine of salbutamol in excess of 1000 ng ml^{-1} [...] is presumed not to be an intended therapeutic use of the substance and will be considered as an Adverse Analytical Finding unless the athlete proves, through a controlled pharmacokinetic study, that the abnormal result was the consequence of the use of the therapeutic dose (by inhalation) up to the maximum dose indicated above." Currently, over six months since the urine sample concerned, there is still no news of such a controlled study, which is perhaps not surprising: the burden is with the accused and setting up a robust study requires expertise. Additionally, inter-occasion variability will influence the results of each attempt.

Salbutamol acts on the beta-2-adrenoceptor as a sympathomimetic, commonly prescribed to counteract bronchoconstriction due to allergic and exercise-induced asthma, as well as chronic obstructive pulmonary disease (COPD) [2]. This mechanism of action has led athletes to believe that there might be a performance-enhancing effect of beta-2 agonists both through relaxing smooth muscle cell in the lung, and anabolic effects on skeletal muscles. Two reviews extensively investigated the evidence for these effects and concluded that inhaled beta-2 agonists have no positive effects on muscle strength, sprint or endurance performance, and that only high, systemic dosages can improve muscle strength and sprint performance, but not endurance performance [3, 4]. In summary, beta-2 agonists might give an advantage in sports, but only at very high concentrations and for very short (power) disciplines. It is therefore doubtful that multi-stage (endurance) cyclists like Chris Froome would benefit even from high doses of beta-2 agonists apart from when treating asthmatic symptoms. Nevertheless, WADA is currently of the opinion that all beta-2 agonists are banned substances, necessitating doping control measures. For this purpose, urine analysis is performed, which leads to a problem for the substances that are allowed with a TUE up to a maximum dose: salbutamol, formoterol and salmeterol. For these substances, urine concentration is used to determine whether the maximum dose was exceeded. It is, however, impossible to determine dose from a single urine concentration due to several pharmacological factors that are at play. Salbutamol plasma pharmacokinetics, in particular, are highly variable, mainly due to variability in lung and gut absorption, volume(s) of distribution, metabolism (including first-pass effect) and renal clearance [5–7]. Subsequently, urine pharmacokinetics are even more variable due to the additional factor of urine concentrating abilities of the kidney (urine osmolality between 50 and 1200 mOsm kg^{-1}) [8], volumetric production of urine [9] and micturition. On top of all this, the time since last dose in a doping control setting is unknown. Despite these facts, the WADA rules seem to assume that a urine concentration above the set threshold indicates a high chance of having detected use of more than the allowed dose. This chance is apparently considered sufficiently high to warrant sanctioning the athlete, but supporting evidence in literature is lacking. The aim of this study is therefore to evaluate the current WADA approach to this problem and determine whether this approach is able to differentiate approved use from violations. As a part of that aim, we evaluate whether WADA-approved doses of salbutamol may lead to unacceptable urine concentrations, considering the multiple sources of variability, using a physiologically based pharmacokinetic (PBPK) approach.

Methods

Several literature data sources were used to synthesize a semi-physiological pharmacokinetic model of plasma and urine salbutamol concentrations [5–7]. In short, a PK model of salbutamol in dogs was used as the basis and extrapolated to humans using allometric scaling [7]. Literature data on clearance of salbutamol and its main metabolite, sulphated salbutamol, was added to the base model [5]. A separate compartment was constructed to take into account the production of urine based on factors such as cardiac output and concentrating efforts of the kidneys [9, 10]. Further adjustment of parameters was performed to align a visual predictive check against literature data of salbutamol plasma and urine concentration from Haase *et al*. [6]. All modelling was performed in an environment consisting of Piraña v2.8.1 [11], PsN v4.2.0 [12] and NONMEM v7.3 [13]. The statistical software R® v3.3.0 [14] was used for pre and post-processing of data, and statistical and graphical analysis.

Literature model synthesis

Several pharmacokinetic models exist for salbutamol in the literature; however, none of these include the urinary pharmacokinetics of salbutamol. A number of articles describe the concentrations of salbutamol in both plasma and urine without describing the parameters involved in the transfer of salbutamol between the two fluids. The only available model that approximates the absorption of salbutamol through both lungs and gut was built on data from dogs [7]. The model was implemented in NONMEM and the parameter values extrapolated to humans using allometric theory [15]. Variability on the fraction of inhalation absorbed through the gut and through the lungs was incorporated to account for inter-patient variability in inhalation efficiency, and differences in inhaler types and formulations.

The allometrically extrapolated model was further expanded by introducing a first-pass effect of gut absorbed salbutamol to its main metabolite, sulphated salbutamol (S-SAL). Furthermore, the renal clearances of salbutamol and S-SAL were incorporated from pharmacokinetic parameters reported by Morgan *et al*. [5]. Additional compartments for both salbutamol and S-SAL in urine were constructed, assuming that when the compounds are eliminated from plasma at the clearance rates provided by Morgan *et al*. [5], they are directly introduced to urine without delay.

A separate compartment was built to describe urine volume production, to allow voiding this compartment, similar to micturition, thereby better approximating physiology and allowing for the investigation of several micturition scenarios. Urine formation was assumed to occur at a constant rate, calculated using several physiological parameters, such as cardiac output (CO) scaled allometrically to weight ($CO = 0.166 \cdot weight^{0.79}$) [10]. The CO for a typical person of 70 kg then is 4.76 l, with a coefficient of variance (CV) of 20%. Of this CO, 21% flows through the kidneys. Typical haematocrit is 0.409 (CV: 7%) in trained cyclists [16], thus 59.1% of blood is plasma, with 19% of plasma entering the renal capsule. Of the resulting glomerular filtrate, 99.2% is reabsorbed, leaving 0.8% to leave the

kidneys as urine. Combining these numbers, we calculate the urine production in litres per hour for a typical adult weighing 70 kg as follows [9]:

$$4.76 \text{ L min}^{-1} * 60 \text{ min} * 21\% * 59.1\% * 19\% * 0.8\% = 0.054 \text{ L h}^{-1}$$
(1)

This amounts to 1.2 litres of urine per day, with a 95% prediction interval (PI) of 0.66–1.92 l day^{-1}, corresponding well with the typical volumes of 1–2 l day^{-1}. This also includes variability due to concentrating by the kidney. Cardiac stroke volume is increased in elite athletes, but due to reduced heart rate at rest, the cardiac output is not significantly different from normal healthy controls [17]. Although cardiac output is significantly increased (roughly threefold) during exercise, renal blood flow is restricted, leading to only a mild to moderate reduction in the urine production rate [18, 19].

Model validation and calibration

We extracted data points of plasma and urine salbutamol concentrations from Haase *et al*. [6] using ImageJ v1.50 [20] and the Figure_Calibration plugin [21]. Haase *et al*. administered 13 subjects with a single salbutamol inhalation of 1600 mcg, followed by regular plasma and urine sample collections. Validation of the synthesized literature model was performed through simulation of salbutamol plasma and urine concentrations in 1000 subjects (weight mean: 84 kg, SD: 17 kg), after a single 1600 mcg dose, and bladder voiding at the indicated time points where a urine sample was collected. The resulting simulated concentrations were compared to the plasma and urine concentrations over time in exercised, dehydrated subject data extracted from Haase *et al*. and graphically presented in a visual predictive check plot [22]. Calibration of the parameters derived from the dog model was required, to better correspond to the Haase *et al*. data (for further elaboration, see Supporting Information Data S1).

Model simulation

Subsequently, we simulated a twice-daily 800 mcg inhaled salbutamol administration at steady state (maximal allowed dose) in 1000 virtual subjects and determined the percentage of subjects attaining urine salbutamol concentrations above the 1000 ng ml^{-1} threshold to determine whether approved doses of salbutamol may lead to unacceptable urine concentrations. Finally, we also investigated a two-week regimen of 8 mg oral salbutamol tablets (a dose shown to increase sprint power in elite athletes [23]) in 1000 simulated subjects, to determine the length of washout period associated with non-adverse findings in doping control. Both simulations (inhaled and oral dosing regimens) were used to evaluate whether the current WADA approach is able to differentiate approved use from violations.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from

the IUPHAR/BPS Guide to PHARMACOLOGY [24], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [25].

Results

Model synthesis

The synthesized model consisted of eight compartments; one each for absorption through the gut and lung, one central and one peripheral distribution compartment for parent salbutamol, one central metabolite compartment, one salbutamol and one metabolite urine compartment, and a single urine volume compartment (Figure 1). When administered through inhalation, 20% of the dose reaches central circulation through the lung, with 80% of the dose ingested and absorbed through the gut. Half of the gut-absorbed amount experiences a first-pass effect and is absorbed as sulphate metabolite, with the other 50% reaching the circulation as unchanged salbutamol. Total apparent clearance of salbutamol parent drug is 13.1 l h^{-1} (of which 18.6%, or 2.4 l h^{-1} is the rate of conversion to S-SAL), and clearance of S-SAL is 5.9 l h^{-1}. The higher apparent S-SAL clearance compared to conversion of salbutamol to metabolite in circulation explains why S-SAL only accumulates after oral administration of salbutamol, and not in the case of IV administration [5].

Model validation

After several adjustments of pharmacokinetic parameters (Table 1), plasma and urine salbutamol concentrations were adequately predicted, with only a minor bias, and reasonably similar variation bandwidth (Figure 2). The dual absorption peaks, distribution and elimination phases were well described.
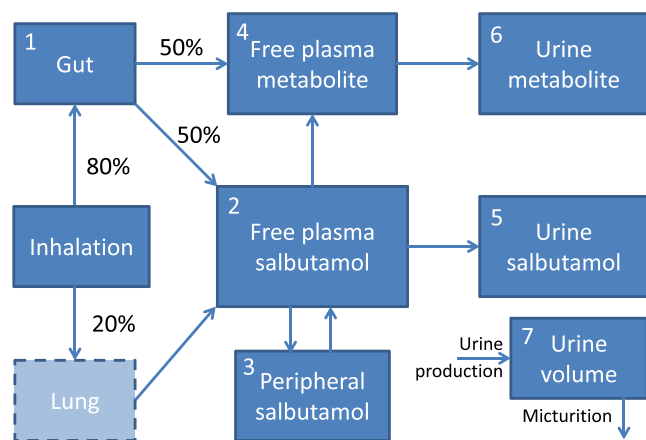

Figure 1

Diagram representing final model structure. Arrows represent the flow of drug amounts. Bioavailability for lung and gut absorption after inhalation is represented as percentages. Lung-absorbed amount is directly introduced into the central compartment and consists of 100% parent salbutamol. Of the amount absorbed from the gut, 50% is parent salbutamol and 50% is directly converted to sulphated salbutamol metabolite (first-pass effect)

Urine concentrations after appropriate salbutamol use

Simulations of steady-state urine concentrations over time resulting from a bi-daily administration of the approved 800 mcg show a large spread, with a significant portion (15.4%) of the simulated population achieving urine concentrations above the threshold of 1000 ng ml^{-1} at the peak concentration at 1 h post-dose (Figure 3). At 12 h after the dose administration, or right before the next inhalation, 0.7% of the population still showed urine concentrations above the threshold. It should be pointed out that these numbers do not take into account voiding the bladder before urine testing. In other words, Figure 3 shows the concentrations that would be measured at a certain time after administration when the bladder is voided for the first time since dose administration.

Urine concentrations after ergogenic salbutamol use

Simulations of urine concentrations over time resulting from a daily 8 mg oral dose (unacceptable usage), show that concentrations decline rapidly below the threshold after ceasing the regimen (Figure 4). Within the first 24 h, the vast majority of subjects are already below the threshold, and after 2–3 days, none of the athletes will produce urine concentrations above the threshold.

Discussion

We synthesized a model based on literature data alone that was able to adequately describe and predict the complex pharmacology of salbutamol in plasma and urine. The developed model was used to simulate possible outcomes of the maximum allowed dose of salbutamol in elite athletes, to show how current (WADA) standards for urine collection and analysis do not adequately take into account the large number of factors contributing to variability (dose amount and timing and physiological variability). This large variability leads to large uncertainty in determining the dose that was used, showing the implemented approach is not fit for purpose. It will lead to incorrect accusations of violation, whereas many violations will go unnoticed.

Applied correction factors by WADA

The general WADA rules for salbutamol use and control depicted in the introduction apply in all cases, but WADA describes two additional relevant specifics for the procedure. First, when collecting the urine sample from the athlete, the doping control officer will check the specific gravity of the urine produced by the athlete. If this is smaller than 1.005 when measured with a refractometer or smaller than 1.010 when measured with lab sticks, the athlete is required to provide further samples, until a suitable sample is collected [26]. The testing authority together with the laboratory decide which samples shall be analysed, although it is not clear on which criteria this decision is based. This procedure is presumably designed to prevent false negative findings due to diluted urine. However, it should be noted that due

Table 1

Model parameters used in the simulations, with their source

Parameter	Typical value	CV%	Source
Cardiac output ($\times$weight$^{0.79}$) (l min^{-1})	0.166	23%[b]	Holt [10]
Haematocrit	0.409	7%	Mørkeberg [16]
Bio-availability Gut (%)	80	23%[b]	Auclair [7]
Absorption constant gut (h^{-1})	0.5[a]	57%	Auclair [7]
Absorption lag gut (h)	1.5	83%	Auclair [7]
Renal clearance salbutamol (l h^{-1})	17.5	25%	Morgan [5]
Renal clearance S-SAL (l h^{-1})	5.91	25%	Morgan [5]
Central volume salbutamol (L/kg)	1.12[a]	63%	Auclair [7]
Peripheral volume salbutamol (l kg^{-1})	1.92[a]	50%	Auclair [7]
Intercompartmental clearance (l h^{-1})	0.56[a]	37%	Auclair [7]
Proportional error salbutamol (%)	23		
Proportional error S-SAL (%)	23		

[a]Adjusted to better correspond to data from Haase et al. [6].
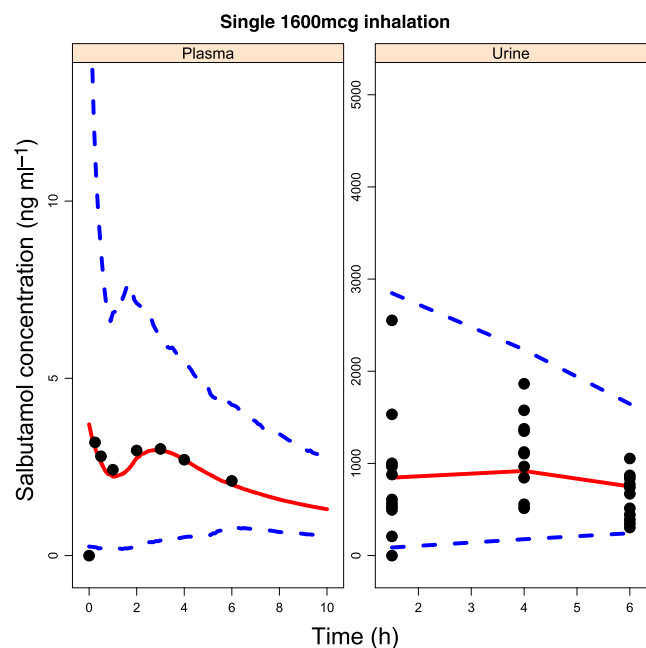[b]Fixed to 23% (omega = 0.05 in NONMEM) due to limited data.

Figure 2

Visual predictive check of simulated concentrations after an inhalation of 1600 mcg salbutamol. Red solid line: median predicted concentrations. Blue dotted lines: 95% concentration prediction interval. Black points: observations extracted from Haase et al. [6]. Left panel: plasma concentrations. Right panel: urine concentrations

to bladder voiding for the first sample, the urine concentration of any subsequent sample will be driven by the plasma concentration at that moment and therefore potentially be a substantial underestimation, as plasma concentrations
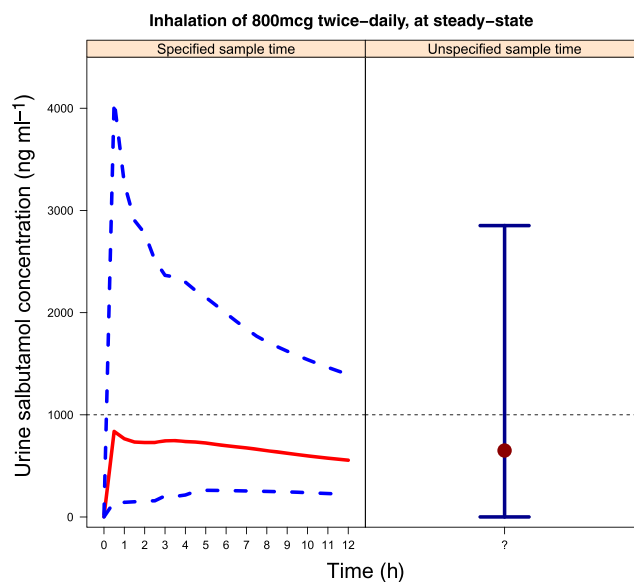

Figure 3

Simulated urine concentrations over time, after administration of the accepted inhalation of 800 mcg salbutamol bi-daily at steady-state (left panel) and the resulting spread in measured concentrations when time is not taken into account in doping control (right panel). Note: the left panel shows the concentrations that would be measured at a certain time after administration when the bladder is voided for the first time since dose administration, with a constant urine production rate. Red solid line: median predicted concentrations. Blue dotted lines: 99.9% concentration prediction interval. Bar plot (right panel): median (red point) and 99.9% prediction interval (bar). Note that the upper limit of this bar is lower than the upper limit peak concentrations in the left panel due to depicting a 99.9% prediction interval of an untimed sample in the right panel

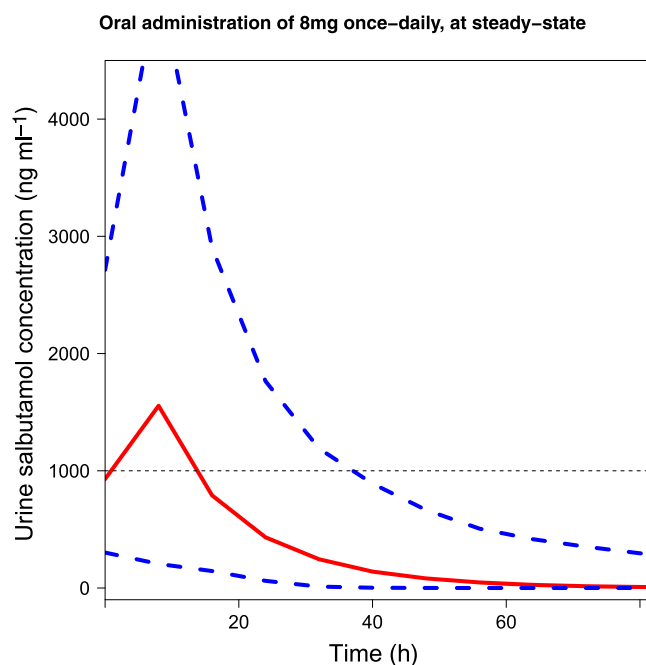**Oral administration of 8mg once–daily, at steady–state**

Figure 4

Simulated urine salbutamol concentrations after the last dose of a two-week treatment with 8 mg oral salbutamol tablets, at regular micturition intervals of 8 h with a constant urine production rate. The last dose of steady-state dosing is shown at 0 h, illustrating that levels decline to below WADA levels of 1000 ng ml^{-1} (dotted black line) well within 48 h of the last dose taken for 99.9% of the simulated study subjects. Red solid line: median predicted concentrations. Blue dotted lines: 99.9% concentration prediction interval

have fallen since dose administration. Correcting urine concentrations for urine osmolality (more accurate than specific gravity) of a sample would therefore be a much more rational way to normalize urine concentrations, and, in addition, avoid the need for multiple samples and their drawbacks.

Secondly, WADA corrects a measured urine concentration for assay variability (measurement uncertainty) by adding to the threshold concentration a guard band. The guard band corresponds to the expanded measurement uncertainty of the assay giving >95% coverage interval for a result at the threshold concentration based on a 1-tailed normal distribution. It is calculated as 1.645 * ucMax (the maximum acceptable combined standard uncertainty of the assay, being 100 ng ml^{-1} for salbutamol), rounded up to two significant figures. A sample is determined to contain an adverse analytical finding only if the concentration is above the threshold plus the guard band, which is called the decision limit (i.e. 1200 ng ml^{-1} for salbutamol) [27]. When this decision limit is applied, 9.95% of subjects were above the limit at 1 h post-dose in our simulated scenario. Throughout the rest of this article, the limit of 1000 ng ml^{-1} is used for clarity, as this is termed the threshold level by WADA, also in the Prohibited List.

WADA deems it unnecessary to account for instability of salbutamol or its metabolite in urine, which potentially could

impact measured salbutamol urine concentrations, as previous research shows that both salbutamol and its conjugate metabolites seem to be stable in urine [28].

Involving pharmacology

So, the procedure involving specific gravity is rather dubious, and although the correction for assay variability seems appropriate, variability due to pharmacological processes are not discussed by WADA. Our developed pharmacokinetic model visualizes the underlying pharmacokinetic theory linking urine concentration and dose, namely, the administered dose being absorbed into the circulation and appearing in urine as a fixed proportion of the amount in blood (renal clearance). This concentration in urine varies with dose amount and time after dose, bioavailability and absorption rate from the lung and gut (for inhalation), distribution over the body, renal clearance, urine volume and voiding. From a clinical perspective, this is very similar to creatinine clearance, and as clinicians might know, it is impossible to calculate the 'dose of creatinine' using only a single urine concentration. Therefore, to be able to make an informed estimate of the dose, one would need to know factors such as the time of dose (which in the doping control setting they do not), the physiological variability of the athlete (not known), volume of urine over a timed period (not known) and the plasma concentration (not known). And because these factors are unknown in the doping control setting, dose cannot be determined from the urine concentration. This is exemplified by simulations from the model in Figure 3, right panel, showing that even from a single dosing scenario, an extremely wide range of urine concentrations can be found. This would perhaps not be as problematic if a certain dose (the maximum allowed dose) would never lead to urine concentrations above the threshold for any subject, but our simulations show that this is not the case either. When collecting urine at 1 h (close to t_{max}), 15.4% of our simulated subjects are above the threshold if they did not void since dosing. Conversely, due to the large variability and the unknown frequency of voiding and time since dosing, there is also a high chance of finding urine concentrations below the threshold with doses above the maximum allowed dose. Given the relatively short half-life of salbutamol, anabolic use (i.e. large oral doses) could even be halted shortly before a race, with no urine concentrations above threshold to be found by urine doping tests. Using our model, simulations of daily oral administration of 8 mg salbutamol over two weeks, with a washout period of 2 days resulted in 0% urine concentrations above the WADA threshold (Figure 4) when applying a normal micturition pattern (three times a day). Moreover, within 24 h, the majority of subjects would already produce a urine concentration below the threshold. This indicates that even high doses that have been shown to improve peak sprint power in elite endurance athletes [23], will not lead to adverse analytical findings in the majority of cases if the athlete ceases dosing at least the day before an event.

Burden of proof

In the current situation, WADA does seem to acknowledge the problem of variability to some extent as an athlete that

produced a urine sample with an unacceptably high salbutamol concentration, is given the possibility to prove this was a result of a dosing scheme within WADA limits by means of a controlled pharmacokinetic study. Hereby WADA transfers the responsibility of resolving the flaws in the rules designed by WADA itself to the athlete. Setting up such a study and getting the desired result will take months at least. And even if an athlete does prove their innocence, this could already do major damage to their reputation (see the Froome case); that is, if showing innocence is successful at all, as this might not prove simple. Although intra-subject variability will be smaller than the previously described inter-subject variability, substantial variability will still be present within a subject. It is therefore not unlikely that many trials will be needed to produce another urine sample that exceeds the threshold with allowed dosages, even more so because it will be difficult to reproduce the circumstances leading to the original finding. Aside from this being a very expensive and time-consuming venture for the athlete, the fact that the foundation on which these WADA rules are based in the first place are flawed as we have shown, makes placing the burden of proof with the athlete completely unacceptable.

Alternative solutions

All these arguments make one wonder why the current procedure is being used by WADA. Speculation about a used dose of salbutamol from a urinary concentration (even when it would be adjusted for osmolality) is open to serious criticism and cannot be used to affect the career of an athlete. A better approach would be to collect timed urine samples over a specified period and possibly take a midpoint blood sample. In addition, S-SAL concentrations in urine could be incorporated, as this metabolite would accumulate more, especially after oral dosing, due to slower clearance and the first-pass effect. One may in fact use an approach using Bayesian hierarchical modelling, as proposed by Mu and Ludden, to determine the most probable dose and dose-administration time [29]. Such an approach would require the development of an accurate population pharmacokinetic model of salbutamol (and S-SAL) in cyclists, including salbutamol in urine, with samples corrected for osmolality. This approach could help approximate the dose, but would require large amounts of samples to be taken to achieve sufficient accuracy and precision. Without such dense data, uncertainty in estimating the dose would remain due to individual pharmacokinetic variability and the unknown factor of time since dosing. Given that the performance enhancing activity of beta-2 agonists is dubious, especially in endurance sports, the question arises whether it is worth the effort of screening for these compounds.

Limitations

In this paper we reviewed the procedure implemented by WADA in the control for salbutamol doping and indicated that these are fundamentally flawed. Many of these problems are no different from those encountered in clinical practice and clinical pharmacology, and so the theory and knowledge from these disciplines were applied in this study

to the issue of doping control. We developed a population pharmacokinetic model based on literature data to substantiate and quantify that theory, but there are some limitations to the model. No individual data on both plasma and urine concentrations were available for model development, such as that of Haase *et al*. [6]. Such data would allow proper estimation of inter-individual variability, leading to better prediction of concentration bandwidths. However, given the good performance of the visual predictive check in Figure 2, there does not seem to be overprediction of the variability in urine concentration. The results of this visual predictive check also make it unlikely that inter-laboratory or inter-assay differences would impact extrapolation of our model to the WADA doping control laboratory setting: the pharmacokinetic analysis in the study used for the plot was performed by the WADA-accredited doping control laboratory in Norway [6]. Furthermore, variability on physiological parameters could only partially be based on actual data. For example, data on additional variability due to the extreme circumstances during professional cycling is not available and thus could not be accounted for. Two variabilities were fixed to 23% (omega = 0.05), which is typically referred to when discussing physiology, and deemed reasonable. In addition, several parameters originally derived from dogs required manual adjustment to properly align with the Haase *et al*. [6] data. Furthermore, the salbutamol concentration in the doping control assay used by WADA is based on the sum of the glucuronide conjugate (expressed as the free drug) and free salbutamol concentrations. For the purpose of this study, we did not explicitly take into account the glucuronide conjugate of salbutamol, as its contribution to the concentration measured in urine is only very limited, with concentrations below the lower limit of detection of 2 ng ml^{-1} in 100% of subjects after inhalation of 800 mcg of salbutamol and 70% of subjects after oral administration of 8 mg (and <3% compared to the unconjugated salbutamol for the remaining subjects) [30]. However, if there is an impact, this would only add to the variability in observed urine concentrations. Similarly, this variability might increase if we incorporated variable urine production (due to increased fluid intake or dehydration) rather than a constant urine production rate that was applied in the model.

Finally, our model is supported by data from a clinical study [30], reporting that out of 28 subjects (including eight asthmatic elite athletes) inhaling a single dose of 800 mcg of salbutamol, there was one subject with a urine concentration above the 1000 ng ml^{-1} threshold when analysing the urine sample taken 4 h post-dose (urine collection between 0 and 4 h). This means in this study, 3.6% of subjects exceeded the urine concentration threshold with allowed use, which is similar to the observed 3.0% when simulating 1000 subjects with our model in this scenario, supporting the validity of our model.

Our approach therefore shows the feasibility of modelling exercises, including integration of pre-clinical and clinical data, with the possibility of clinical trial simulations/not-in-trial simulations and optimal design. Above all, we show that this approach, originating from traditional clinical pharmacology, can also be applied in this setting of doping control.

Conclusion

Pharmacokinetic theory dictates that it is not possible to derive the administered dose with any certainty from a single random urine concentration when there is no information about timing of the dose, urine volume or osmolality and individual physiological variability. Using a pharmacokinetic model based on literature data, we substantiated this notion. We demonstrate that the current approach to detect excessive salbutamol use is fundamentally flawed and cannot differentiate between illegal and allowed use and inadvertently leads to incorrect assumptions of violation. If the community is determined to control for excessive salbutamol use, these procedures should be changed. The expertise present in the field of clinical pharmacology is clearly relevant in doping control, and we therefore advocate a closer collaboration between the two disciplines to work towards a sport that is as clean and fair as possible.

Competing Interests

There are no competing interests to declare. J.H. is a recreational cyclist and is a fan of professional road cycling, but, other than as a spectator and from its clinical pharmacology aspects, is not involved in the sport. S.v.D. and A.C. watch professional cycling but are mainly excited by its clinical pharmacology aspects.

References

1 Union Cycliste Internationale. UCI statement on Christopher Froome [online]. 2017. Available at http://www.uci.ch/pressreleases/uci-statement-christopher-froome/ (last accessed 17 May 2018).

2 Johnson M. Molecular mechanisms of β2-adrenergic receptor function, response, and regulation. J Allergy Clin Immunol 2006; 117: 18–24.

3 Cairns SP, Borrani F. β-Adrenergic modulation of skeletal muscle contraction: key role of excitation–contraction coupling. J Physiol 2015; 593: 4713–27.

4 Pluim BM, de Hon O, Staal JB, Limpens J, Kuipers H, Overbeek SE, et al. β2-agonists and physical performance. Sports Med 2011; 41: 39–57.

5 Morgan DJ, Paull JD, Richmond BH, Wilson-Evered E, Ziccone SP. Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. Br J Clin Pharmacol 1986; 22: 587–93.

6 Haase CB, Backer V, Kalsen A, Rzeppa S, Hemmersbach P, Hostrup M. The influence of exercise and dehydration on the urine concentrations of salbutamol after inhaled administration of 1600 μg salbutamol as a single dose in relation to doping analysis. Drug Test Anal 2016; 8: 613–20.

7 Auclair B, Wainer IW, Fried K, Koch P, Jerussi TP, Ducharme MP. A population analysis of nebulized (R)-albuterol in dogs using a novel mixed gut-lung absorption PK-PD model. Pharm Res 2000; 17: 1228–35.

8 Sands JM, Layton HE. The physiology of urinary concentration: an update. Semin Nephrol 2009; 29: 178–95.

9 OpenStax College. Anatomy & Physiology. Rice University Press, 2013. Available at http://cnx.org/content/col11496/latest/ (last accessed 10 April 2018).

10 Holt JP, Rhode EA, Kines H. Ventricular volumes and body weight in mammals. Am J Physiol 1968; 215: 704–15.

11 Keizer RJ, Karlsson MO, Hooker A. Modeling and simulation workbench for NONMEM: tutorial on Pirana, PsN, and Xpose. CPT: Pharmacometrics Syst Pharmacol 2013; e50: 2.

12 Lindbom L, Pihlgren P, Jonsson N. PsN-Toolkit – a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed 2005; 79: 241–57.

13 Bauer RJ. NONMEM users guide introduction to NONMEM 7.3.0, 2015. Available at https://nonmem.iconplc.com/nonmem730/nm730.pdf (last accessed 20 March 2018).

14 R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2014.

15 Anderson BJ, Holford NHG. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol 2008; 48: 303–32.

16 Mørkeberg JS, Belhage B, Damsgaard R. Changes in blood values in elite cyclist. Int J Sports Med 2009; 30: 130–8.

17 Pluim BM, Chin JC, De Roos A, Doornbos J, Siebelink HMJ, Van der Laarse A, et al. Cardiac anatomy, function and metabolism in elite cyclists assessed by magnetic resonance imaging and spectroscopy. Eur Heart J 1996; 17: 1271–8.

18 Ho CW, Beard JL, Farrell PA, Minson CT, Kenney WL. Age, fitness, and regional blood flow during exercise in the heat. J Appl Physiol 1997; 82: 1126–35.

19 Chapman CB, Henschel A, Minckler J, Forsgren A, Keys A. The effect of exercise on renal plasma flow in normal male subjects. J Clin Invest 1948; 27: 639–44.

20 Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012; 9: 671–5.

21 Hessman FV. Figure Calibration ImageJ plugin. 2009. Available at http://www.astro.physik.uni-goettingen.de/~hessman/ImageJ/ (last accessed 20 March 2018).

22 Post TM, Freijer JI, Ploeger BA, Danhof M. Extensions to the visual predictive check to facilitate model performance evaluation. J Pharmacokinet Pharmacodyn 2008; 35: 185–202.

23 Hostrup M, Kalsen A, Auchenberg M, Bangsbo J, Backer V. Effects of acute and 2-week administration of oral salbutamol on exercise performance and muscle strength in athletes. Scand J Med Sci Sports 2016; 26: 8–16.

24 Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 2018; 46: D1091–106.

25 Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA, et al. The concise guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. Br J Pharmacol 2017; 174 (Suppl 1): S17–29.

26 World Anti-Doping Agency. Urine sample collection guidelines, 2014 [online]. Available at https://www.wada-ama.org/sites/default/files/resources/files/wada_guidelines_urine_sample_collection_2014_v1.0_en.pdf (last accessed 17 May 2018).

27 World Anti-Doping Agency Laboratory Expert Group. Decision limits for the confirmatory quantification of threshold substances, 2017 [online]. Available at https://www.wada-ama.org/sites/default/files/resources/files/2016-12-13_td2017dl.pdf (last accessed 17 May 2018).

28 Berges R, Segura J, Ventura R, Fitch KD, Morton AR, Farre M, *et al*. Discrimination of prohibited oral use of salbutamol from authorized inhaled asthma treatment. Clin Chem 2000; 46: 1365–75.

29 Mu S, Ludden TM. Estimation of population pharmacokinetic parameters in the presence of non-compliance. J Pharmacokinet Pharmacodyn 2003; 30: 53–81.

30 Mareck U, Guddat S, Schwenke A, Beuck S, Geyer H, Flenker U, *et al*. Determination of salbutamol and salbutamol glucuronide in human urine by means of liquid chromatography–tandem mass spectrometry. Drug Test Anal 2011; 3: 820–7.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13619/suppinfo

Data S1 The supplement provides additional information on the model building steps involved in creating the semi-physiological pharmacokinetic model from literature values, as well as the final NONMEM model code