Clenbuterol

**Background**

Clenbuterol is a $\beta_2$-adrenoceptor agonist. It is assigned a 3/B in the ARCI’s Uniform Classification of Foreign Substances. For Quarter Horse racing it is designated as a prohibited substance and assigned a Class A penalty. Clenbuterol is commonly administered orally as a bronchodilator and mucolytic agent in the management of various respiratory disorders, including recurrent airway obstruction (RAO).\(^i\)

Clenbuterol is a prescription medication and can only be dispensed by or upon the request of a veterinarian. It is commercially available as an oral syrup, Ventipulmin™.\(^ii\) The lowest recommended dose of Clenbuterol is 0.8 mcg/kg however studies have indicated that only 25% of horses respond at this level of administration.\(^iii\) Doses of up to 3.2 mcg/kg have been used, to improve clinical efficacy, with dosing increased incrementally by 0.8 mcg/kg to avoid adverse effects. Clenbuterol’s $\beta_1$ sympathomimetic activity can cause sweating, excitement, urticaria, trembling, and tachycardia when treatment is initiated or when a high dose is administered non-incrementally. Clenbuterol overdose can lead to complications including laminitis, acute renal failure, rhabdomyolysis, and cardiomyopathy, and death.\(^iv\)

Clenbuterol causes relaxation of airway smooth muscle by activating adenylyl cyclase and stimulating the production of cAMP. This increase in cAMP leads to activation of protein kinase A(PKA) that down regulates myosin light chain kinase activity and opens $K^+$ channels thus inhibiting smooth muscle contraction in the airways.\(^v\) Clenbuterol has also been determined to cause lipolysis and increase muscle mass in numerous species, including horses. Reports have shown a decrease in the percent body fat and increase in fat-free mass following the administration of high-dose clenbuterol.\(^vi\)

There is documented evidence that clenbuterol has been routinely administered absent any medical indication to racehorses, likely due to the increase in muscle mass associated with leptin- and adiponectin-mediated repartitioning effects. When examined closely, however the potential muscle building benefits are offset by the negative effects of long-term administration, including a decrease in aerobic capacity, time to fatigue, cardiac function, and maximal oxygen consumption.\(^vii\) Studies have shown that even minimal concentrations of clenbuterol adversely
affect aerobic performance, high-intensity exercise capacity, and the horse’s ability to recover from exercise. viii Clenbuterol has also been demonstrated to induce changes in the structural dimensions of the equine heart, including both cardiac remodeling and an enlarged aorta immediately after exercise. This increases the risk of aortic rupture and sudden death in the equine athlete. ix Finally, studies have shown that clenbuterol becomes less effective as a bronchodilator when the treatment period extends beyond 14 consecutive days. x

Administration Study

Clenbuterol was administered orally, at a dose of 0.8 mcg/kg, twice a day for a total of thirty days to 22 exercise-conditioned Thoroughbred horses (geldings and mares). A second group of six horses received clenbuterol according to the incremental dosing protocol outlined on the manufacturer’s label: 0.8 mcg/kg twice daily for 3 days, 1.6 mcg/kg twice daily for 3 days, 2.4 mcg/kg twice daily for 3 days and 3.2 mcg/kg twice daily for the remainder of the 30-day dosing protocol. This study was performed at the University of California, School of Veterinary Medicine (Davis, CA) by personnel in the Maddy Equine Analytical Pharmacology Laboratory under the direction of Dr. H. K. Knych. xi

Blood samples were obtained immediately before dose administration and at the following times after dosing: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 hours after the administration of the first and last dose and at 18, 24, 36, 48, 60, 72, 84, 98, 108, and 120 hours after the administration of the last dose. Additionally, blood samples were collected every 12 hours immediately prior to dosing throughout the entire course of the administrations.

Urine samples were collected at 12, 36, 60, 84, 108, 136, and 160 hours after the final administration and subsequently at 7-day intervals until the drug was below the limit of detection (LOD).

Extraction and Analysis Procedures

Quantification of clenbuterol in plasma and urine samples was performed at the Maddy Equine Analytical Pharmacology Laboratory (Davis, CA) using validated methods. Clenbuterol concentrations were determined in plasma and urine by liquid chromatography-mass spectrometry (LC-MS/MS) using a stock solution of clenbuterol-d9 as internal standard to verify quantitative accuracy and precision. The method was characterized by a Limit of Quantification (LOQ) of 10 pg/mL and 25 pg/mL in plasma and urine, respectively. The plasma LOD was 1 pg/mL.

Pharmacokinetic Modelling

Average clenbuterol plasma concentrations vs. time curves following chronic low-dose and escalating protocol administration are shown in Figure 1.1. Pharmacokinetic analysis was
performed on individual plasma concentrations vs. time data using Phoenix® WinNonlin® Version 6.0 pharmacokinetic analysis software (Pharsight Corporation, St. Louis, MO).

**Results and Discussion**

Clenbuterol plasma concentrations were below LOD in all horses in the low dose protocol and in 5 of 6 horses in the escalating protocol by 7 days after the last administration. The terminal elimination half-life ranged from 5.5 to 21.5 hours with a mean of 10.4 (±4) hours following low dose administration.

Urine clenbuterol concentrations were below the LOD between 21 and 28 days in all horses that received the low-dose administration and 5 out of 6 horses receiving the escalating protocol. Mean, median, and range of the urine concentrations at 14 days after low-dose administration in all twenty-two horses are shown in Table 1.1.

*Figure 1.1* Mean ± s.d. plasma clenbuterol concentrations vs. time following oral administration of 0.8 μg/kg bwt Ventipulmin® b.i.d. for 30 days to 22 racing fit Thoroughbred horses. Inset graph depicts plasma concentrations following the final dose. LOD = limit of detection; LOQ = limit of quantification.
Table 1.1 Urine clenbuterol mean, median, and range at 14 days following last oral administration of 0.8 μg/kg BID of clenbuterol to 28 horses for 30 days.

<table>
<thead>
<tr>
<th>Time after last administration (days)</th>
<th>Mean(±SD) pg/mL</th>
<th>Median pg/mL</th>
<th>Range pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>35.28 ±20.61</td>
<td>33.25</td>
<td>12.5-76.1</td>
</tr>
</tbody>
</table>

Scientific Advisory Committee Recommendation

The 95/95 tolerance interval was calculated on the natural logarithmic (i.e., ln) transformed urine concentration data from the 14-day collection time point for all 28 horses. and yielded a value of 136.7 pg/mL that was rounded to a threshold recommendation of 140 pg/mL of urine. Due to the concerns for the performance enhancing effects of race day administration, the RMTC Scientific Advisory Committee (SAC) recommended Limit of Detection for the threshold in serum or plasma (based upon the experimentally observed LOD).

Both thresholds must be met to ensure control of both serial oral administrations and a single race day administration of clenbuterol. To adequately control the use of clenbuterol it is important to submit paired (blood and urine) samples for analysis. The serum threshold is intended to control for race day administration. The urine threshold is intended to support a 14-day withdrawal interval. The submission of blood-only samples should be discouraged as it represents a vulnerability with respect to the control of the use of clenbuterol.

In 2020, based on evidence that clenbuterol was being administered to horses absent a diagnosis of lower airway disease and for extended periods of time, the RMTC recommended increased constraints on its use. These included the mandatory reporting of prescriptions or treatments, with a maximum treatment interval of 30 days. Treated horses would be placed on the Veterinarians’ List (VL) and established as ineligible to race until the horse underwent the standard protocol for release from the VL including an observed published work with clenbuterol not detected in post-work blood and urine samples. Clenbuterol would also be regulated by LOD in blood and urine for post-race samples. The detection of clenbuterol in an out of competition sample from a horse with no corresponding treatment report would result in the horse’s placement on the VL pending the outcome of an investigation. The ARCI adopted this Model Rule for Thoroughbred racing but elected to maintain the 14-day withdrawal interval (and corresponding thresholds) for Standardbred racing.

Practice Tips

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**Important Note:**

Currently there is considerable variation among racing jurisdictions with respect to the regulation of clenbuterol. Trainers and veterinarians are advised to consult the authorities where horses race and train to ensure compliance with clenbuterol regulations. It is further recommended that these communications occur well in advance of a horse’s race to afford sufficient time to meet eligibility criteria.

Clenbuterol was established as a prohibited substance by the American Quarter Horse Association in recognition of its misuse as an anabolic agent. The use of clenbuterol in Quarter Horses is additionally controlled with hair testing both in- and out-of-competition.

There is no withdrawal guidance associated with the revised Model Rule. Eligibility to race is not based on a withdrawal interval or restricted administration time but rather release from the Veterinarians’ List. As a practical matter, the scheduling of a work for a treated horse seeking release from the VL should occur no less than 21 days after the last dose of clenbuterol. It is likely that analysis of a sample collected at less than 21 days after treatment will result in a positive test for clenbuterol, thus preventing the horse’s release from the VL.

To the extent that the 14 day withdrawal guidance and corresponding thresholds continue to apply, note that they are based upon a maximum of 0.8 mcg/kg twice daily, orally administered dose of clenbuterol as Ventipulmin™ for a maximum of thirty days. Different formulations of clenbuterol, administration of higher doses, compounded products, or combinations of clenbuterol with other substances may result in concentrations above the threshold unless an extended withdrawal time is observed. Veterinarians are advised to assess the impact of these potential risk factors on the withdrawal time and to advise their clients accordingly.

Because there is an FDA-approved clenbuterol-containing product (Ventipulmin™) commercially available, the compounding of clenbuterol from any other source is not permitted pursuant to FDA Guidelines. Illegally compounded clenbuterol has been acquired through investigations and in many cases laboratory analysis has determined that the concentration of clenbuterol differs widely from the labelled concentration. Administration of these illegal products has been associated with adverse events, including death.

Additionally, it is important to note that although clenbuterol can decrease fat mass percentage and increase muscle mass percentage, multiple studies have shown that long term administration of clenbuterol causes a decrease in athletic performance from cardiac remodeling and decreased aerobic capacity, time to fatigue, cardiac function, and maximal oxygen consumption.\textsuperscript{xiii}

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References


