Clenbuterol and the Horse

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Clenbuterol is a β_2-agonist bronchodilator and mucokinetic drug. When administered at the recommended dose of 0.8 μg/kg q 12 h, it reaches plasma levels that should relax airway smooth muscle. However, the measurable degree of bronchodilation achieved may not always be clinically obvious even in horses with heaves. After a ten-day treatment (0.8 μg/kg q 12 h), the half-life of elimination from plasma is about ten hours and plasma levels are below the pharmacological threshold within three days. Urinary elimination is prolonged and irregular so that control of clenbuterol usage in racehorses should be based on blood rather than urine levels. Author's address: Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824-1314. © 2000 AAEP.

Introduction

In May 1998, the U.S. Food and Drug Administration (FDA) approved the use of Ventipulmin® (clenbuterol HCl) syrup for the management of horses affected with airway obstruction, such as occurs in chronic obstructive pulmonary disease (COPD). In other parts of the world, Ventipulmin had been available for many years but, because it was not available in the U.S., it was frequently used illicitly. As with most things forbidden, a mythology developed around Ventipulmin and its active ingredient clenbuterol. The purpose of this paper is to report what is known about clenbuterol so that practicing veterinarians and racing regulators can make informed decisions.

The active ingredient in Ventipulmin syrup is clenbuterol hydrochloride. The Association of Racing Commissioners International (ARCI) classifies clenbuterol as a Class 3 agent. Identification of clenbuterol in post-race samples can therefore lead to sanctions.

What is Clenbuterol?

During the "flight and fright" reaction, catecholamines (epinephrine and norepinephrine) are released from sympathetic nerves and the adrenal gland. The catecholamines activate several types of adrenoceptors (α_1, α_2, β_1, β_2, and β_3) so that heart rate and contractility increase, blood flow increases to the muscles and is reduced to splanchnic organs, glycogenolysis and lipolysis increase, the pupils dilate, gastrointestinal motility is decreased, and the uterus and bronchi relax. Over the years, the physiological actions evoked by activation of specific adrenoceptors has been determined. Pharmaceutical companies have been able to develop specific drugs that activate (agonists) or block (antagonists) specific adrenoceptors. Such drugs are widely used in human and veterinary practice.

Clenbuterol is one of a large group of compounds that are selective β_2-adrenergic agonists. Other drugs in this class include albuterol (salbutamol), pirbuterol, and fenoterol. Selectivity of drugs for β_2-adrenoceptor activity is expressed by their β_2/β_1 ratio. The β_2/β_1 ratio for clenbuterol is 4.0, the same as that for albuterol but less than pirbuterol, for which the ratio is 200. Because of its moderate selectivity, clenbuterol at low doses preferentially

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activates β_2-adrenoceptors but at higher doses it begins to activate β_1-adrenoceptors.

Clenbuterol is more lipid soluble than other β_2-agonists such as albuterol and this endows it with the following properties. It is well absorbed from the intestinal tract. For example, in humans, the oral bioavailability of clenbuterol is 80–90% and albuterol is 40–50%. Clenbuterol crosses the blood brain barrier better than less lipid soluble β_2-agonists such as albuterol. The higher lipid solubility also results in accumulation of clenbuterol in tissues with a high lipid content such as fat depots and liver.

Pharmacological Activity

The most important action of clenbuterol and other β_2-agonists in the lung is relaxation of airway smooth muscle. For this reason, such drugs are widely used for relief of bronchospasm in human asthma and similar diseases in animals. When these drugs bind to β_2-adrenoceptors, they activate adenylyl cyclase, which leads to an increase in the intracellular concentration of the second messenger cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA). In the tracheobronchial tree and in the uterus, β_2-agonists, cAMP, and PKA inhibit smooth muscle contraction by opening K^+ channels and by down-regulation of myosin light chain kinase activity.

The pharmacological activity of clenbuterol on equine airway smooth muscle has been defined in vitro.[1,2] The drug concentration that exerts half the maximal effect is known as the EC_{50}. The EC_{50} for clenbuterol has been reported as 2.1 nM (95% confidence interval, 0.9–6.8 nM)[2] and 5.7 nM.[1] These concentrations correspond well with the plasma concentrations (1.6–2.7 nM) obtained during twice-daily oral treatment with 0.8 μg/kg of clenbuterol.[3] In vitro, 0.1 nM is the lowest concentration at which clenbuterol has a measurable effect on airway smooth muscle and maximal effect is achieved at 100 nM.[1,2]

In vivo, the bronchodilator effect of clenbuterol has been demonstrated by measurements of lung function and by clinical observations of the effort of breathing. The most commonly reported measurements of lung function are pulmonary resistance, dynamic compliance, and change in pleural pressure. Pulmonary resistance is a measure of the degree of obstruction of the air passages and ranges in value from as low as 0.25 cm H_2O/l/s in a normal horse up to more than 3.0 cm H_2O/l/s in a horse with severe heaves. Dynamic compliance indicates the function of peripheral airways and ranges in value from as high as 2.0 l/cm H_2O in normal horses down to 0.1 l/cm H_2O in severe heaves. The change in pleural pressure indicates how much effort the horse is using to breathe. Resting normal horses develop a change in pleural pressure of around 10 cm H_2O during each breath while heavy horses may develop a pressure of more than 75 cm H_2O.

In normal horses, a single intravenous bolus of clenbuterol (0.8 μg/kg) decreased the pulmonary resistance from 0.38 to 0.25 cm H_2O/l/s within ten minutes. This very small decrease persisted for at least 3 hours.[4] In horses with airway disease, clenbuterol has been shown to decrease the pulmonary resistance and work of breathing,[5] decrease the change in pleural pressure,[6] and improve clinical signs of airway obstruction.[7] The effect of clenbuterol in the airways is not limited to bronchodilation. Clenbuterol increases ciliary beat frequency and therefore improves airway mucociliary clearance rate in horses with and without COPD.[8]

In the cardiovascular system, administration of a bolus of clenbuterol (0.8 mg/kg, IV) to normal horses is followed almost immediately by a decrease in blood pressure. The blood pressure decrease is followed by a reflex increase in heart rate and pulmonary arterial pressure. All these changes last less than 2 minutes.[4] Presumably the decrease in systemic blood pressure is due to vasodilator effects of the bolus concentration. The vasodilator effect has also been demonstrated in the bronchial circulation, a systemic vascular bed.[9] The β_2-agonists have some anti-inflammatory activity. They can decrease release of mediators from mast cells and inhibit the increase in vascular permeability that occurs in allergic inflammation. These changes have been demonstrated when clenbuterol is administered to rats[10] but the clinical significance of such action is in doubt.

Injectable clenbuterol is used in obstetrical practice to prevent uterine contraction in cattle. By relaxing uterine muscle, clenbuterol can be used to delay the onset of labor or to facilitate uterine repair following Caesarian section.

The β_2-agonists facilitate lipolysis and increase nitrogen retention. These properties have led to their use as repartitioning agents. When repartitioning agents are fed to growing animals, they favor the formation of muscle over deposition of fat. Feeding clenbuterol to cattle, sheep, pigs, and poultry results in a carcass that has less fat and more muscle. The doses used for repartitioning are either much larger than those used for bronchodilation or the treatment periods are long. For example, the dose used for repartitioning in steers was 30 μg/kg for 98 days.[11] In young growing female rats, 10 μg/kg/day for 15 days increased muscle mass, whereas in male rats, 200 μg/kg/day increased the rate of protein accretion within 4 days.[12] There is no published information on the repartitioning effect of clenbuterol in horses.

Therapeutic Uses and Effectiveness

In horses, the primary therapeutic use of clenbuterol is as a bronchodilator. Heaves, the severe form of COPD, is a disease in which bronchospasm is a major cause of airway obstruction. The effectiveness of clenbuterol has therefore been tested most frequently in this syndrome. Denac and Pfister[5]

administered clenbuterol IV to horses with airway disease and obtained a decrease in the work of breathing and resistance. Sasse[6] administered clenbuterol (0.8 mg/kg, IV) to horses affected with COPD and measured the change in pleural pressure three hours later. Clenbuterol significantly decreased pleural pressure change (a measure of the severity of airway obstruction) from 35.5 to 25.2 cm H_2O (SEM = 2.9). In practical terms, these horses would have moderate signs of heaves before treatment. After treatment, their signs would be somewhat less but not gone.

A large clinical trial of clenbuterol syrup was conducted in COPD-affected horses by Erichsen et al.[7] These investigators used a score based on clinical signs of respiratory distress, and administered incremental oral doses of clenbuterol from 0.8 to 3.2 μg/kg q 12 h. Twenty-five percent of horses responded to the lowest dose and, overall, 75% of 239 horses improved with one of the incremental doses. Improvements persisted throughout the 30-day trial. In contrast to these observations of the efficacy of clenbuterol, Traub-Dargatz et al.[13] were unable to show any clinical benefit of the drug (0.4 mg/horse q 12 h) in COPD-affected animals. It is worth noting that in none of the trials of clenbuterol efficacy were the investigators blinded as to treatment and only in the study of Erichsen et al.[7] was there any attempt to determine a dose response to the drug.

The varying observations about the efficacy of clenbuterol in horses with COPD can be understood if one considers a) the relationship between lung function and clinical signs, and b) the pharmacology of clenbuterol. In COPD-affected horses with severe airway obstruction, measurable improvements in lung function can occur without a noticeable clinical improvement.[14] When clenbuterol was administered intravenously and its effect was measured shortly thereafter, i.e., when plasma concentrations were high, the improvement in lung function was significant.[6] However, the change in pleural pressure after treatment (25.2 cm H_2O) would still be sufficient to be associated with signs of airway obstruction.[14] Oral dosing is likely to result in lower peak concentrations of clenbuterol than is intravenous dosing. Following oral dosing, one would therefore expect a lesser improvement in lung function and even more difficulty in detecting this by clinical signs alone. Hence the negative report by Traub-Dargatz et al.[13] and the need for incremental doses to obtain a definitive clinical effect.[7]

There is no doubt that clenbuterol is a bronchodilator and mucokinetic drug, is absorbed from the intestinal tract, and achieves blood concentrations[3] in a range that can relax airway smooth muscle.[1,2] It is therefore logical to use clenbuterol for relief of airway obstruction due to bronchospasm and mucus accumulation. The most obvious example of this is COPD. Bronchospasm and mucus accumulation can occur to varying degrees whenever the airways are irritated and inflamed. For the latter reason, clenbuterol may provide relief in horses that have inhaled smoke, have nonspecific airway inflammation, or are recovering from pneumonia.

It is important to realize that bronchodilator drugs do not cure disease. They provide symptomatic relief. Treatment of COPD requires reduction of the dust load to which the horse is exposed[15] in combination with use of a potent corticosteroid such as dexamethasone.[16]

Ventipulmin® Syrup

Ventipulmin syrup is the only form of clenbuterol available for horses in the United States and is the first β_2-agonist approved for use in the horse in that country. In Europe, Australia, Canada, and South America, Ventipulmin has been available in many forms for up to fifteen years. In these countries, Ventipulmin is administered at a dose of 0.8 μg/kg q 12 h. It is used as an adjunct to other treatments that will reduce airway inflammation, for example, corticosteroid administration or antigen avoidance by environmental modification. In the United States, the FDA required proof of efficacy in the absence of these other treatments. In many horses, this required a dose greater than 0.8 μg/kg.[7] Hence the label for Ventipulmin in the USA recommends a variable dose schedule.

Even though Ventipulmin syrup can be administered at up to 3.2 μg/kg, this dose must be approached with care. Initially giving this high dose will result in side effects of sweating, trembling, tachycardia, and excitement. If the dose is approached gradually as recommended on the label, tolerance to side effects will develop.

The label states that Ventipulmin syrup is indicated for management of horses with airway obstruction *such as COPD*. Thus the indication is not restricted to horses with heaves, but the drug can be used in any animal in which the veterinarian judges there is airway obstruction as a result of inflammation, bronchospasm, and mucus accumulation. Inflammatory airway disease of racehorses in training would fall into this category.

Untoward effects of Ventipulmin are like those of other β_2-agonists. These agents are only selective for β_2-adrenoceptors but, when used in high enough doses, will activate β_1-adrenoceptors. Activation of β_1-adrenoceptors causes tachycardia, excitement, and sweating. If a horse is exhibiting signs of β_2-agonist overdose, it should be left in a quiet stall until signs pass, generally in one to two hours. Subsequent doses should be decreased. Because of the potential tachycardia, Ventipulmin syrup is not recommended for use in horses with cardiac disease. Ventipulmin syrup should not be used in near-term mares because it prevents uterine contraction and could delay the onset of labor.

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Effects of Clenbuterol on Performance

Because it is a bronchodilator and a repartitioning agent, there has been great concern about clenbuterol's ability to enhance performance. The amount of bronchodilation that occurs in normal horses is very small. In healthy human athletes, a measurable dilation of the airways induced by a β_2-agonist is not associated with an improvement in oxygen consumption.[17,18] Likewise, intravenous administration of clenbuterol to thoroughbred horses 30 min before exercising on a treadmill does not improve oxygen consumption or cardiovascular function.[19] Because the plasma concentration would be lower, it is even less likely that oral administration would have any effect on oxygen consumption. This is indeed the case. Kallings et al.[3] found no effect of 10-day oral treatment with clenbuterol on lactate accumulation of oxygen tension in Standardbred horses exercising on a treadmill.

As stated above, there are no data on the repartitioning effect of clenbuterol in horses. Even if some repartitioning occurred at the therapeutic dose, there is no evidence that the increased muscle mass is associated with increased ability to exercise. Indeed, in mice treated with clenbuterol, measures of performance were reduced even though muscle mass was increased by clenbuterol.[20]

Elimination of Clenbuterol from Plasma

During twice-daily oral administration of 0.8 μg/kg clenbuterol, plasma concentrations average 350 pg/ml and may reach 750 pg/ml (mean $\pm$ 2 SD). When administration ceases, clenbuterol is cleared from blood with a half-life of 10.4 h (SD = 2.25).[3] If a horse is two standard deviations above the mean, its plasma concentration during administration will be 750 pg/ml and its half-life of elimination will be 14.9 h. In five half-lives, approximately 75 hours, the plasma concentration in this horse will be 23 pg/ml. This theoretical calculation has been borne out by observations in 14 Warmbloods treated for 10 days.[21] In these animals, plasma concentrations were all below the limit of quantification (50 pg/ml) by the third day of withdrawal. Recent data obtained using a more sensitive assay showed serum concentrations less than 30 pg/ml at 48 h and below 10 pg/ml at 72 h after cessation of administration.[a]

Withdrawal Times

At the time of writing (April 2000), regulations regarding clenbuterol in racehorses vary widely from state to state ranging from three days withdrawal to zero tolerance for the drug in urine based on a very sensitive detection method. The American Horse Shows Association and American Quarter Horse Association allow a 24-hour withdrawal as long as clenbuterol is a declared medication while Federation Equestre Internationale has zero tolerance.

The threshold concentration of clenbuterol to have any bronchodilator effect is 0.1 nM (31.5 pg/ml).[1]

Clearly, the plasma concentration of drug is below this level by 72 h after the end of a 10-day oral administration of the usual dose. One can safely conclude that clenbuterol has no pharmacological effect on β_2-adrenoceptors at this time and therefore the withdrawal time of 72 h used in Canada and several states has a sound scientific basis.

The problem with the regulation of clenbuterol arises solely from the use of very sensitive detection methods in urine samples. Following a 10-day administration, clenbuterol can be detected in urine samples for long periods. Depending on the limit of detection, positive urine samples can occur for 13 days[21] or longer[a] and horses can shift from negative to positive and back again during this period. No such problem occurs with the use of blood samples.

Intratracheal Injection of Clenbuterol

The sensitivity of urine tests for clenbuterol was increased in part because of anecdotal reports that clenbuterol was being injected directly into the trachea. We recently studied the effect of such an administration. In a crossover study, we compared the effect of 90 μg of clenbuterol or an equivalent volume of saline injected into the trachea of heaves-affected horses. Lung function improved slightly after the intratracheal injections but there was no difference between the saline or clenbuterol injections. Two and four hours after injections, lung function tended to be worse rather than better after clenbuterol injection. In the same study, we also were unable to demonstrate any effect of intratracheal injection on either heart rate or excitement.[22] It thus appears that the concern over the effects of intratracheal injection is unfounded especially if it occurs 2 to 4 h before a race. Furthermore, newer sensitive measurements of serum concentrations of clenbuterol make it easy to detect such an injection if it occurs just before a race.

What Do We Need to Know?

The pharmacokinetics of clenbuterol and its therapeutic efficacy have been established at the usual dose of 0.8 μg/kg. It is clear from studies of clenbuterol elimination that it will be much easier to regulate the use of this drug by use of blood rather than urine levels. One of the points of debate may be the in vivo pharmacological threshold I have derived from in vitro data. However, establishing such a threshold will take extensive studies and the result will most likely be higher than my estimate of 31 pg/ml that is achieved with a 72-h withdrawal period.

References and Footnotes

1. Törneke K, Ingvast Larsson C, Appelgren L-E. Relaxation of equine tracheal muscle *in vitro* by different adrenoceptor drugs. *J Vet Pharmacol* 1997;20:216–219.
2. Törneke K, Ingvast Larsson C, Appelgren L-E. A comparison between clenbuterol, salbutamol and terbutaline in relation to receptor binding and *in vitro* relaxation of equine tracheal muscle. *J Vet Pharmacol* 1998;21:388–392.

3. Kallings P, Ingvast-Larsson C, Persson S, et al. Clenbuterol plasma concentrations after repeated oral administration and its effects on cardio-respiratory and blood lactate responses to exercise in healthy Standardbred horses. *J Vet Pharmacol Ther* 1991;14:243–249.

4. Shapland JE, Garner HE, Hatfield DG. Cardiopulmonary effects of clenbuterol in the horse. *J Vet Pharmacol Ther* 1981;4:43–50.

5. Denac M, Pfister R. Der Einfluß des β_2-Rezeptoren-stimulierenden Sympathiko-mimetikums Ventipulmins® (NAB-365) auf die Atmungsmechanik des Pferdes. *Tierärztl Umschau* 1981;36:188–194.

6. Sasse HHL. Ein Vergleich des Effektes nach einmaliger parenteraler Gabe von Ventipulmin oder Euphyllin auf die Lungenfunktionsprüfung bei an C.O.P.D. erkrankten Pferden. *Tierärztl Umschau* 1984;39:656–662.

7. Erichsen DF, Aviad AD, Schultz RH, et al. Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD). *Equine Vet J* 1994;26:331–336.

8. Turgut K, Sasse HH. Influence of clenbuterol on mucociliary transport in healthy horses with chronic obstructive pulmonary disease. *Vet Rec* 1989;12:526–530.

9. Sanders EA, Gleed RD, Hackett RP, Dobson A. Action of sympathomimetic drugs on the bronchial circulation of the horse. *Exper Physiol* 1991;76:301–304.

10. Inagaki N, Kawasaki H, Hiyama H, et al. Inhibitory mechanisms of β-adrenoceptor agonists for immunoglobulin E-- mediated experimental allergic reactions in rats. *Eur J Pharmacol* 1997;336:225–231.

11. Ricks CA, Dalrymple RH, Baker PK, et al. Use of a β_2- agonist to alter fat and muscle deposition in steers. *J Anim Sci* 1984;59:1247–1255.

12. Reeds PR, Hay SM, Dorwood PM, et al. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. *Br J Nutr* 1986;56:249–258.

13. Traub-Dargatz JL, McKinnon AO, Thrall MA, et al. Evaluation of clinical signs of disease, bronchoalveolar and tracheal wash analysis, and arterial blood gas tensions in 13 horses with chronic obstructive pulmonary disease treated with prednisone, methyl sulfonmethane, and clenbuterol hydrochloride. *Am J Vet Res* 1992;53:1908–1916.

14. Robinson NE, Olszewski MA, Boehler D, et al. Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. *Equine Vet J* accepted Oct. 1999.

15. Jackson CA, Robinson NE, Berney C, et al. Prednisone—is it really effective in the treatment of chronic obstructive pulmonary disease? In: *Proceedings.* 45th Annu Conv Am Assoc Equine Practnr 1999;304–305.

16. Rush BR, Raub ES, Rhoads WS, et al. Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. *Am J Vet Res* 1998;59: 1039–1043.

17. Heir T, Stemshaug H. Salbutamol and high-intensity treadmill running in nonasthmatic highly conditioned athletes. *Scand J Med Sci Sports* 1995;5:231–236.

18. Sandsund M, Sue-Chu M, Helgerud J, Reinertsen RE, Bjermer L. Effect of cold exposure (-15 degrees C) and salbutamol treatment on physical performance in eline nonasthmatic cross-country skiers. *Eur J Appl Physiol Occup Ther* 1998;77:297–304.

19. Rose RJ, Evans DL. Cardiorespiratory effects of clenbuterol in fit Thoroughbred horses during a maximal exercise test. In: *Proceedings.* 2nd Int Conf Equine Exer Physiol 1987; 117–131.

20. Ingalls CP, Barnes W, Smith S. Interaction between clenbuterol and run training: effects of exercise performance and MLC isoform content. *J Appl Physiol* 1996;80:795–801.

21. Kleemann R, Goossens L, Reder E, Quirke J-F. Depletion kinetics of clenbuterol hydrochloride in competition horses. *Equine Vet J* 1999;31:339–341.

22. Harkins JD, Robinson NE, Jackson CA, et al. Intratracheal clenbuterol in the horse: its pharmacological efficacy and analytical detection. *J Vet Pharmacol Ther* submitted 1999.

aTobin T, (Lexington, KY) personal communication, June 2000.

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