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Inhalation therapy – the optimal route of drug administration for the treatment of respiratory diseases

Sirous Sadeghian Chaleshtori[1,2] and Shima Roayaee[1]

[1] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Institute of Biomedical Research, University of Tehran, Tehran, Iran

Summary: The purpose of this review was to introduce inhalation therapy as a suitable method for the consumption of medications in the therapeutic of equine respiratory diseases. Respiratory diseases can reduce the equine's athletic activities and lead to complications and deaths. Therefore, quick identification of respiratory disease and choosing the appropriate treatment method such as inhalation therapy should be prioritized. Inhalation therapy is a route for delivery drugs directly to the airways. The amount of drug deposition in the lung determines the effectiveness of inhalation therapy and pulmonary drug deposition in turn, depends on the size of produced particles. Inhalation therapy has some advantages like the direct delivery of the drug to the intended site of action, the use of a low dose of drug, lower side effects compared to parenteral administration and some disadvantages such as expense, frequency of drug administration and difficulty in determining the precise dose of a medication. Aerosol deployment within the pulmonary system is done under the influence of three main mechanisms of gravitational sedimentation, inertial impaction, Brownian diffusion. Three different types of commercially available inhalation drug delivery which is used in equine include dry powder inhalation, metered-dose inhalers, and nebulizers. Pharmacological agents used in equine inhalation therapy includes antimicrobials, glucocorticoids, mucolytic, bronchodilators and etc. In general, according to the points mentioned above, especially the existence of positive benefits, inhalation therapy in equine should be given more attention and prioritized in the treatment of respiratory diseases.

Keywords: inhalation therapy, drug, respiratory diseases, equine, horse

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Correspondence: Sirous Sadeghian Chaleshtori and Shima Roayaei, Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; s.sadeghian@ut.ac.ir; shima.roayaee@ut.ac.ir

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Introduction

Respiratory diseases are a serious problem in equine and lead to complications and deaths and can reduce the animal's daily activities, especially athletic activities. Therefore, the health of the respiratory tract is important for the performance of athletic horses. Rapid diagnosis of equine respiratory disease and choosing the appropriate treatment method such as inhalation therapy are very important to prevent further problems.

Treatment with inhaled drugs is considered one of the best methods for treating some respiratory diseases (such as recurrent airway obstruction (RAO)) in equine[1]. This method causes the drugs to reach the airways directly. The drugs are changed from powder or liquid forms to aerosol forms, and then the aerosol forms of the drugs are used for inhalation therapy[2]. The effectiveness of a drug in inhalation therapy depends on the amount of the drug that reaches the airways, and the amount of the drug that reaches the airways, in turn, depends on the size of the produced particles. In other word, particle size determines where the particles end up in the respiratory system: Particles with a size smaller than 5μm reach the distal airways and particles with a size smaller than 0.5μm are removed during expiration[2,3].

The objective of this paper was to introduce inhalation therapy as an optimal method for the use of drugs in the management of equine respiratory diseases by review the most relevant papers regarding the inhalation therapy in equine, focusing on the advantage and disadvantage of inhalation therapy, mechanisms of aerosol deposition in airways, factors influencing aerosol deposition in the lung, techniques of inhalation therapy in equine and particularly pharmacological agents used by inhalational route in equine.

Material and methods

This paper was conducted by searching in PubMed, Google scholar and Science direct, Web of sciences, and CAB abstract until August 10, 2023 using some related keywords: (horse – equine), (inhalation – aerosolization – nebulization – inhaled drug – aerosol deposition), (dry powder inhaler – nebulizer – pressurized metered dose inhalers), (antimicrobial – Gentamicin – Amikacin – Ceftiofur – Cefquinome – Marbofloxacin – glucocorticoid – Beclomethasone dipropionate – Fluticasone – Ciclesonide – Budesonide – Dexamethasone – mucolytic – bronchodilators – Albuterol – Levalbuterol – Salmeterol Ipratropium – Nitric oxide – Mast cell stabilizers), (respiratory diseases – recurrent airway obstruction – asthma

– heaves – COPD – chronic obstructive pulmonary disease – inflammatory airway disease – IAD – small airway disease). By searching in this way, some articles were available (230 resources) and the titles and abstracts of them were checked and those related to the topic of our paper (the articles related to inhalation, inhalation method, inhalation mechanism, inhaled drug and the equine species) were selected to be reviewed (106 papers). Since the inhalation therapy in equine is taken from human medicine, in some cases articles related to humans have been used.

The importance of respiratory diseases in equine

The function of the respiratory system is to exchange respiratory gases between cells and the environment. This tract is equipped with the immune and defense systems against pathogens, but it is prone to various diseases due to contact with air and exposure to risk factors. Equine respiratory diseases are separated into circumstances that impress the upper and lower respiratory system. These diseases are a serious problem in equine and depending on the agent can lead to complications and death. Respiratory diseases can negatively affect a horse's athletic potential by interrupting training and causing chronic disabling disorders. After musculoskeletal diseases, respiratory diseases are the most common disorders that limit the performance of horses. Equine have the potential capability to alter gas exchange during exercise because pulmonary functional demands increase considerably during exercise. Therefore, the health of the respiratory tract has a great influence on the performance of athletic horses. Early detection of respiratory disorders in equine is very important because early or subclinical respiratory disease reduces their performance potential. Therefore, it is appropriate to quickly diagnose the equine respiratory disease in the early stages so that the veterinarian can provide special and effective treatments and prevent a respiratory crisis.

All efforts should be made to shorten the duration of the disease because a long period of respiratory disease negatively affects the equine's well-being and can lead to chronic disease consequences if left untreated. Therefore, the use of a treatment method that causes the drug to reach the desired place in the respiratory tract quickly and directly, with a higher concentration and the least side effects, should be prioritized in the management of the treatment of respiratory diseases in equine. One of the suitable treatment methods for this purpose is the inhalation therapy.

Table 1 Advantage and disadvantage of inhalational route for drug administration in respiratory diseases of equines. | *Vor- und Nachteile der inhalativen Verabreichung von Arzneimitteln bei Atemwegserkrankungen von Pferden.*

	Advantage	Disadvantage
Inhalation therapy	Direct drug delivery	The challenge of delivering a precise dose
	Delivering higher concentrations of the drug	Shorter effect duration and more frequency administration
	Avoiding complications of systemic use of drugs	An expensive
	Limiting multidrug-resistant bacteria	The unpredictable drug deposition
	Lower occurrence of complications	Inaccessibility to obstructed airways
	Use in the long-term management	Airway irritation

Inhalation therapy advantage and disadvantage

Some important advantages and disadvantages of inhalation therapy in equine were summarized in Table 1. By inhalation therapy, drug is directly delivered to target sites (airways). However, drug delivery to airways is easily achieved by parenteral administration (in some drugs), but parenteral administration causes other organs also to be exposed to the administered drug, and this will increase the occurrence of unwanted side effects[2]. In other words, inhaled use of a drug permits the drug to be delivered to the target site of action with a high concentration without causing unwanted side effects[4]. For example, inhalation of antibiotics which is associated with toxicities when administered by systemic route (for example, in case of aminoglycosides, nephrotoxicity) allows high doses of antibiotics to reach the lung with low systemic exposure[5] or the parenteral administration of glucocorticoids causes many side effects, including laminitis[6] while their inhalational use will have fewer side effects.

Thus, by inhalation therapy, complications caused by systemic administration of glucocorticoids and antimicrobials are avoided and the emergence of multidrug-resistant bacteria is limited[5]. Also, some drugs such as budesonide or fluticasone propionate after oral administration have low bioavailability and don't reach to lung[6,7]. For delivering these drugs (and drugs with the same problem) to the lung, inhalational route provides a solution.

Although inhalation therapy is not free of side effects and some side effects such as anaphylaxis, skin irritation, overdose, hypersensitivity, kidney damage, cumulative effects, behavioral changes, idiosyncratic reactions, and toxicity may occur with this method, their occurrence is less likely with inhalation therapy than parenteral administration[3]. Inhalation therapy is a more acceptable means in the long-term management of some diseases in equine such as RAO, because in this case, the owners can administrate bronchodilators and corticosteroids as inhalation, which is a more convenient procedure than injections or oral medications[3].

Inhalation therapy, although it has many advantages, it is not without any disadvantages. It is difficult to deliver an accurate dose of a drug to the lungs through inhalation method[3]. Duration of effect in drugs administrated by inhalational route is shorter than that of in drugs administrated by systemic route. Thus, in inhalation therapy, it may be necessary to administer the drug with a higher frequency to be effective[3]. The cost of the equipment needed to administer drugs by inhalation has limited the use of this treatment method[8]. Since there is a different amount of mucus in the airways of equine and also bronchospasm is seen in some patients (these two prevent the drug from reaching the small airways), predicting how much drug reaches the small airways in inhalation therapy is challenging [8,9].

In some respiratory diseases, some airways are obstructed, and by inhalation therapy the drug cannot be delivered to these obstructed airways[2,3]. Some aerosolized drugs irritated airways when administrated by inhalation method and this is another disadvantage of inhalation therapy [2,3].

Aerosol deployment mechanisms

Some aerosol deployment mechanisms, including gravitational sedimentation, inertial impaction, Brownian diffusion, and to lesser extent electrostatic precipitation, turbulence and interception determine the location of the aerosol deposition in the respiratory system[10–12]. Aerosol deployment mechanisms in the respiratory system of equine are summarized in Figure 1.

When the respiratory airflow is diverted (at nasopharynx or at first bifurcation of the airways), particles may not be diverted if the airflow is fast or particles are large. Thus, the particles may impact on the airway wall. This mechanism is called inertial impaction[12]. In addition, a turbulent and fluctuating airflow increases deposition in the airways, including in the trachea[11,12].

Gravitational sedimentation describe deposition of particles under the action of gravity[11,12]. Particles which have not deposited under impaction mechanisms and particles which have penetrated to more peripheral parts of the respiratory tract can be deposited under gravitational sedimentation in these parts of the respiratory tract in which air stream moves relatively slowly[11,12].

The randomic movement of aerosol particles due to collision with gas molecules is known as Brownian diffusion mechanism[12]. Brownian diffusion mechanism resulted in aerosol deposition in bronchioles and alveoli[12].

Fluctuations in respiratory air flow (high breathing rate, local turbulences caused by significant changes in cross-sectional areas for example before reaching the trachea) cause the directions and magnitude of the aerosol particles to change constantly and eventually they settle. This mechanism of deposition is called turbulent mixing[12].

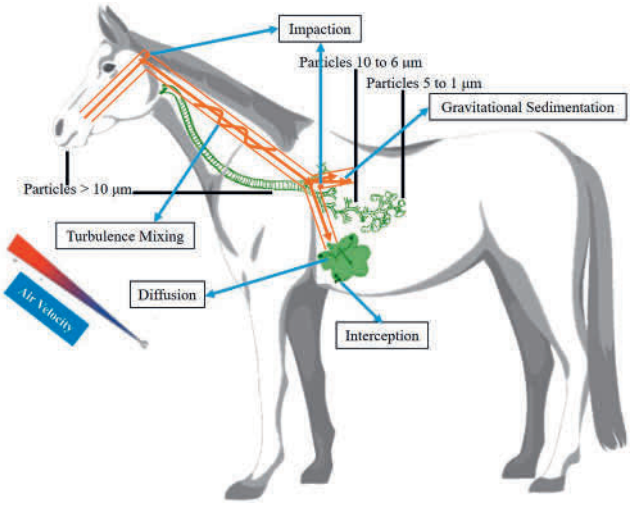

Fig. 1 Schematic of aerosol deployment mechanisms in the respiratory system of equine. | *Schematische Darstellung der Aerosol-Entfaltungsmechanismen im Atmungssystem von Pferden.*

There is a repulsive force between the inhaled charged particles and also an attractive force is established between inhaled charged particles and airway walls. These forces cause the deposition of particles in this way, which is called Electrostatic deposition[12].

When the particles are very close to the airway walls, a small impact causes them to deposit. This is called interception mechanism[12].

Factors influencing aerosol deployment in the respiratory system

Aerosol Properties

Inhalation therapy is delivery of aerosolized drug into the respiratory tract[3]. The shape, size, weight, density and hydrophilicity of particles are factors that affect the deposition of particles in airways. Most of the large particles (more than 10μm) are deposited in nasal cavity and trachea and do not efficiently transmit to lower airways[3,13] (Figure 1). Midsized particles (6 to 10μm) are deposited in trachea, bronchi and large bronchioles, and small particles (1–5μm) are deposited in bronchioles and alveoli[3] (Figure 1).

The hydrophilicity of the particles causes them to absorb water and this leads to them to deposit in the distal airways[3]. The drag force when the particles are non-spherical and have a rough surface will be higher than when the particles are spherical, and this force prevents the particles from settling in the distal parts of the airways[14]. Also, the drag force will be greater when the particles are porous than when the particles are dense and smaller[14,15].

Method of aerosol generation

Method of aerosol generation has a major influence on aerosol deposition in the respiratory tract, as the aerosols generated by each method have a different particle size. For example, a study stated that particle size dispersal in aerosols produced through ultrasonic nebulizers and jet nebulizers is more different than those of generated by metered dose inhalers[2].

It has been shown that the type of device used to produce aerosol determines the efficiency of drug delivery in inhalation therapy[13]. Also, it has been shown that the droplet size distribution is not only dependent on the nebulizer used but also dependent on the duration of the nebulization[16].

6–3-Properties of the inhaled drug

It is obvious that some properties of the inhaled drug will affect the inhalation therapy. For example, particle size distribution is directly influenced by drug viscosity[2]. Also, some properties of the drug (for example tonicity) may induce the protective mechanism of bronchoconstriction in the lung, and thus the distribution of the inhaled drug in the lung indirectly is affected[2].

Most of the drugs considered for inhalation administration are isotonic and this property is important when considering other drug formulations for inhalation administration[2,16]. Also, pharmaceutical formulations intended for intramuscular or intravenous administration when administered by inhalation may contain other compounds that cause bronchoconstriction in the lung and disrupt the deposition of the drug[2]. This issue must be considered when other drug formulations (for example intravenous) are administrated via the inhalation route.

Patient factors

According to the explanations mentioned above about the mechanisms of drug deposition in the lung, the factors related to patient that affect the drug deposition in the lung include depth and rate of breathing, the openness of the airways, the presence or absence of bronchoconstriction, the presence or absence of cough[3]. In patients with asthma, it is more likely that the presence of bronchospasm, excessive secretion of mucus and edema causes the inhaled drug to be deposited in the proximal airways by impaction mechanism[11]. The horse has a low breathing rate and a high breathing depth, and thus inspirate a large amount of air into the lungs, and this air stays in the lungs for a longer period of time. Therefore, the horse is considered a suitable option for inhalation therapy[2]. Variation in the breathing pattern (for example, some horses breathe faster when they are excited and others hold their breath) cause different amounts of the drug to be deposited in the lungs of different patients[2].

Effective pulmonary ventilation is an important factor in inhalation therapy because in this method, the drug reaches different parts of the airways through the air flow. Many pathological and physiological conditions (for example, bronchospasm, lung consolidation, lung atelectasis, mucus accumulation) affect pulmonary ventilation[2]. Any changes in the breathing pattern (for example respiration with high frequency) will affect the delivery of the drug to the lungs through the inhalation route[2].

Techniques of inhalation therapy in equine

There are different techniques for administration drugs by inhalational route in equine (especially the horses) and most of these techniques adapted from equivalent systems used in people. Three different type of commercially available inhalation drug delivery which is used in equine include dry powder inhaler, metered dose inhalers, and nebulizers[17–19].

Nebulization is used for the administration of the drugs with liquid formulation. Ultrasonic nebulizers, jet nebulizers, and mesh nebulizers are some different types of nebulizers[18]. In a jet nebulizer, liquid drug is turned into vapor by air or oxygen, and in this process the liquid is converted to small particles. The droplets of a greater volume are directed back towards the reservoir in order to commence the subsequent cycle, while diminutive particles are conveyed via inhaled air. In ultrasonic nebulizers, high frequency waves pulsate and cause the fluid to become a good fog[17]. In mesh nebulizers, the determination of particle size distribution is primarily influenced by the dimensions of apertures present in the vibrating mesh. In this system, the liquid drug is passed through a plate with microscopic holes by an alternating force and turns into tiny droplets. The droplet's dimensions are estimated to be roughly two times larger than those of the mesh hole[17,20].

Metered dose inhaler is a specially designed device that delivers a specific and exact amount of a drug to the respiratory system in each puff[17]. A typical metered-dose inhaler has three components: (1) a metal reservoir comprising the drug (2) a valve which allows accurate doses of drug to be dispensed and (3) a plastic nozzle[17]. These types of inhalers can be used to deliver single or combined drugs[17].

A dry powder inhaler contains drugs in the form of powder and when the patient inhales through this device, the drug is dispersed into the airways. The drug powder in a dry powder inhaler device usually consists of micronized drug particles that are loosely packed together or of drug particles and lactose (as the carrier). In order to achieve an ideal deposition of the pharmaceutical agent within the pulmonary system, it is imperative that the drug-carrier blend is disaggregated through rigorous inhalation against the internal resistance of the inhalation device[17].

Pharmacological agents used by inhalational route in equine

The pharmacological agents used in equine inhalation therapy include glucocorticoids, antimicrobials, bronchodilators, mucolytic, mast cell stabilizers and etc. A summary of the most important of these agents can be seen in figure 2, and in the following, each one is described separately.

Glucocorticoids

High efficiency of glucocorticoids in relieving airway obstruction has caused to the widespread use of these drugs in the treating some prevalent lung diseases in equine such as RAO, but the parenteral administration of these drugs causes many

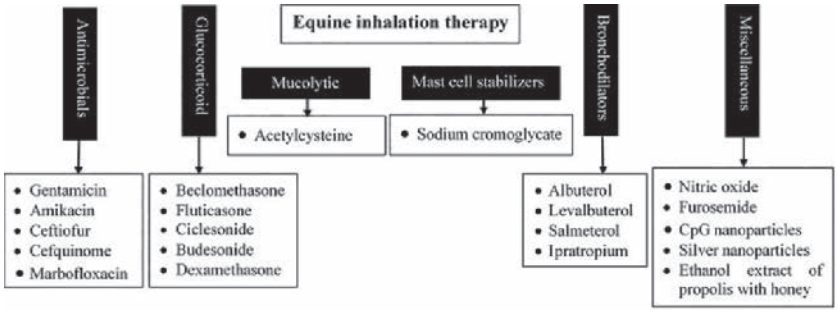

Fig. 2 Major pharmacologic agents used by inhalation therapy in equine. These agents include glucocorticoids, antimicrobials, bronchodilators, mucolytic, mast cell stabilizers and etc. (CpG: cytosine–phosphate–guanosine oligonucleotides). | *Die wichtigsten pharmakologischen Wirkstoffe, die in der Inhalationstherapie bei Pferden eingesetzt werden. Dazu gehören Glukokortikoide, antimikrobielle Mittel, Bronchodilatatoren, Mukolytika, Mastzellenstabilisatoren usw. (CpG: Cytosin-Phosphat-Guanosin-Oligonukleotide).*

side effects, including laminitis[6]. Therefore, inhalational use of these drugs has become popular because by this treatment method, glucocorticoids don't reach to systemic circulation (or reach with low concentration) and as a result, they will have fewer side effects[6]. Some important inhaled glucocorticoids drugs which can be used in equine respiratory diseases are mentioned in Table 2.

Beclomethasone

Beclomethasone dipropionate has low systemic bioavailability because it is a prodrug that is activated in the lung by esterase activity[21]. Inhaled administration of beclomethasone dipropionate has been investigated for treatment of equine asthma and it is reported to be effective[6]. In addition, some studies have been conducted to investigate the beclomethasone dose in the treatment of equine asthma. Improvement in pulmonary function has been achieved after administration beclomethasone in three doses 500, 1000 and 1500 µg by inhalation route in horses affected by recurrent airway disease and beclomethasone at a dose of 500 µg leads to less suppression of endogenous cortisol production than other doses[22]. However, this low dose (500 µg q 12 h for 10 days) in horses which maintain in an antigenic environment during the study did not improve pulmonary functions[23]. Beclomethasone dipropionate administrated as aerosol (1320 µg q 12 h, 7 days) has led to improve lung function and clinical symptom of airway obstacle. However, this improvement was less than that achieved by dexamethasone administration (0.1 mg/kg BW IV q 24 h) (24). Increasing dose of beclomethasone administrated by inhalation route (3750 µg q 12 h, 15 days) was associated with improvement of pulmonary function in horses suffering from chronic obstructive pulmonary disease (COPD)[25]. Nevertheless, in these studies there weren't control groups and their results must be interpreted with cautioun. Recommended dosage for inhaled beclomethasone is 2–8 µg/kg q 12 h[3]. Beclomethasone administrated by inhalation route (1320 µg q 12 h, 7 days) represses endogenous cortisol production, and serum cortisol returns to a normal level 2 days after discontinuation of treatment[24]. This suppression occurs even at lower doses of inhaled beclomethasone ([500 to 1500 µg q 12 h, 1 week[22]] and [528 µg q 12 h, 5 days][26]). It has been reported that beclomethasone (500 µg q 12 h, 10 days) does not change cytology of bronchoalveolar lavage fluid (BALF) in horses kept in an antigenic environment[23]. However, reduction of neutrophils in cytology of BALF at the time of antigenic exposure by treatment with inhaled beclomethasone has been reported[24,27].

Fluticasone

The oral bioavailability of fluticasone is low[21] and it is locally absorbed from the lungs and exerts its effect[28]. Therefore, it is a suitable option for inhalation administration. However, it is identified in plasma when administered by inhalation to equine[29]. Administering fluticasone by inhalation for one month did not lead to changes in the function of adrenocortical[30], but administration of this drug for a long period of 11 months by inhalation has repressed the adrenocortical function[31]. Inhaled fluticasone is a suitable treatment for horses with equine asthma[30,32]. However, some residual bronchospasm can persist, when fluticasone (2000 µg q 12 h) is administered alone to horses with equine asthema[32]. In contrast, a study showed that inhalation of fluticasone (2500 µg q 12 h) combined with salmeterol (250 µg q 12 h) improved pulmonary function and residual bronchospasm was not existent after twelve weeks[33]. Thus, inhalation of a combination of a corticosteroid and a beta2 agonist is more effective than inhalation of each of them alone. When compering inhaled fluticasone and parenteral dexamethasone, fluticasone (3000 µg q 12 h, 15 days) has a similar effect to dexamethasone (0.05 mg/kg IM q 24 h) on reducing airway overreactivity[34] and high-dose fluticasone (6 mg, twice a day, 7 days) has the same effect as dexamethasone (0.1 mg/kg IV q 24 h, 7 days) in prevention of exacerbations of acute RAO[35]. Fluticasone propionate administrated by inhalation is effective in treating RAO in equine[36]. In general, recommended dosage for inhaled fluticasone is 2–4 µg/kg q 12 h[3]. Influence of fluticasone on airway neutrophilia does not completely clear. In some studies, fluticasone does not modify airway neutrophilia[32,34,35], but in others fluticasone decreases airway neutrophilia[37].

Ciclesonide

Ciclesonide, like beclomethasone, is a prodrug that is activated in the lung[21]. This drug has high protein binding capacity and this feature probably restricts the effectiveness of the drug when administrated by parenteral route[21]. Ciclesonide

Table 2 Some important inhaled anti-inflammatory drugs used in the treatment of equine respiratory diseases. | *Some important inhaled anti-inflammatory drugs used in the treatment of equine respiratory diseases.*

Anti-inflammatory	Class	Dosage	Comment
Beclomethasone	Corticosteroid	2–8 µg/kg twice daily	Inhaled beclomethasone even in lower doses causes adrenal suppression
Fluticasone	Corticosteroid	2–4 µg/kg twice daily	Administration for a long period of 11 months by inhalation regresses the adrenocortical function It is recommended that inhaled fluticasone be administrated with inhaled long-acting β2-agonist
Ciclesonide	Corticosteroid	2700 µg twice daily	Ciclesonide administrated by inhalation therapy does not repress endogenous cortisol production
Budesonide	Corticosteroid	1800 µg twice daily	Inhaled budesonide represses the HPA axis in horses as well as dexamethasone
Dexamethasone	Corticosteroid	5 mg daily	Aerosolisation of an injectable form of dexamethasone does not improve lung function and it remarkable represses adrenocortical function

HPA: hypothalamic-pituitary-adrenal

administrated by inhalation (1687.5 µg twice a day) in horses affected with severe airway obstruction was an effective treatment[38]. It seems ciclesonide prescribed by inhalation therapy (up to 2700 µg q 12 h, 14 days) did not repress endogenous cortisol manufacture[38].

Budesonide

It has been shown that budesonide administrated by inhalation method in a dose-dependent manner leads to the improvement of pulmonary functions in horses affected by equine asthma, and the effect of inhaled budesonide at a dose of 1800 µg every 12 hours for two weeks is comparable to that of dexamethasone (0.04 mg/kg, Intravenous, every 24 hours)[6,39]. However, inhaled budesonide at the mentioned dose (1800 µg q 12 h, two weeks) similar to dexamethasone (0.04 mg/kg, Intravenous, every 24 hours) repress hypothalamic-pituitary-adrenal axis in horses affected by severe equine asthma[6,38,39]. Nebulized budesonide at a dose of 1500 µg every 12 h for ten days improves clinical scores and pulmonary functions in horses with severe equine asthma during exacerbation[40]. Budesonide administrated by inhalation method at the dose mentioned above does not seem to change the cytology of BALF of horses with equine asthma maintained in antigenic environment[39].

Dexamethasone

Dexamethasone, which was administered by nebulization (0.04 mg/kg, one time) in horses, could not be detected in blood and urine after 48 hours and 72 hours, respectively[41]. Dexamethasone sodium phosphate (an injectable formulation of dexamethasone) administered by nebulization to horses with severe equine asthma (five mg daily for seven days) did not improve pulmonary functions and remarkably repressed adrenocortical function[42]. However, in a study conducted on healthy horses, inhaled dexamethasone at the same dose does not suppresses the endogenous cortisol production[43]. This difference may be because the drug absorption is more in diseased lungs and also may be because swallowing of the inhaled drug in the diseased horse is higher due to presence of coughing, increased breathing rate, mucus accumulation, and airways obstruction[6].

Antimicrobials

Inhalation of antibiotic drugs improves the delivery of the drug to the site of infection in the respiratory system and also reduces the occurrence of side effects associated with parenteral antibiotic administration[44]. Also, creating high concentrations of drugs locally may be a better therapeutic approach to treat infections with multidrug-resistant pathogens[44]. Some important inhaled antimicrobial drugs for using in equine respiratory diseases are mentioned in Table 3.

Gentamicin

Gentamicin, an antibiotic from the aminoglycoside class, has a good spectrum of action against gram-negative bacteria. Its bactericidal effect is concentration dependent[45]. The parenteral administration of this drug causes many side effects, including nephrotoxicity, and these side effects have limited its parenteral administration[3]. It is better that the concentration of gentamicin administrated by inhalation be 50 mg/ml[46] and it seems diluting gentamicin in 0.45% NaCl solution is better than diluting gentamicin in sterile water because diluting gentamicin in 0.45% NaCl solution will not lead to thedevelopment of mild inflammation of the intrapulmonary conducting airway[47]. The recommended dosage for inhalation of gentamicin is to administer 20 ml of a solution with a concentration of 50 mg/ml every day (2 mg/kg q 24 h)[3]. Inhalation administration of gentamicin at the dose mentioned above in healthy horses leads to higher concentrations in BALF than intravenous injection (6.6 mg/kg)[46,47].

In a study that administered nebulized liposomal gentamicin (6.6 mg/kg, a single dose) and free gentamicin (6.6 mg/kg, a single dose) to foals, it was shown that the concentration of lysosomal gentamicin in BALF was more than the concentration of free gentamicin in BALF[48]. Adding liposome to an antibiotic drug increases the penetration of the drug into the cell and thus efficiency of the drug against intracellular bacterium such as *Rhodococcus equi* will increase[48].

Regular, long-term nebulized gentamicin has been used for treatment of non-cystic fibrosis bronchiectasis in human[49]. Also, aerosolized delivery of gentamicin seems effective for inducing clinical cure in *Bordetella bronchiseptica* infection in dogs[50].

Amikacin

Amikacin, which is a semisynthetic antibiotic belonging to the aminoglycoside class, is used to treat infections produced with gram-negative bacteria and when administrated by parenteral route exhibits a variety of side effects including nephrotoxic-

Table 3 Some important inhaled antimicrobial drugs used in the treatment of equine respiratory diseases. | *Einige wichtige antimikrobielle Inhalationspräparate zur Behandlung von Atemwegserkrankungen bei Pferden.*

Antimicrobial	Class	Concentration	Dosage
Gentamicin	Aminoglycoside	50 mg/mL	2 mg/kg daily
Amikacin	Aminoglycoside	50 mg/ml	3.3 mg/kg daily for ten days
Ceftiofur sodium	Third-generation cephalosporin	25 g/l	2.2 mg/kg daily, for five days
Cefquinome	Fourth-generation cephalosporin	30 mg/ml	0.5 mg/kg daily
Marbofloxacin	Fluoroquinolone	25 mg/ml	300 mg

ity[51]. Nebulized amikacin has been used for the management of infections caused by *Pseudomonas aeruginosa* in human[52–54]. Recommended concentration for inhaled amikacin in horses is 50 mg/ml[55].

Inhaled amikacin (3.3 mg/kg daily for ten days, at a concentration of 50 mg/ml) is effective in the improvement of respiratory clinical sings, reduction of tracheal wash neutrophils count and reduction of bacterial colony forming units (CFU)[55]. It seems that inhaled amikacin is effective as parenteral amikacin and in spite of a lesser inhaled dose, amikacin may be equally efficient for both ways of using. Nevertheless, inhaled amikacin, appears to be capable to persuade a lesser chemotactic act on neutrophils, fewer impairment to the mucosa of lower respiratory system, and as a result of decreasing clinical symptoms[55].

Nebulization of amikacin at a dose of 1750 mg has been conducted on continuously colonized horses with methicillin-resistant *Staphylococcus aureus* with limited success[56,57].

Ceftiofur

Ceftiofur sodium is an antibiotic from the third generation cephalosporins class, which is used to treat infections of the respiratory system of equines, including infections with *Streptococcus zooepidemicus*[58].

Recommended concentration for inhaled ceftiofur in horses is 25 g/l. However, in a study nebulization of ceftiofur at a concentration of 50 mg/ml did not result in any side effects[58]. It has been shown that nebulization of ceftiofur sodium to foals (2.2 mg/kg q 24 h for five days) resulted in higher concentration in the BALF compared with parenteral ceftiofur sodium administration and this concentration remains above the minimum inhibitory concentration (MIC) that inhibits the growth of 90% of S. zooepidemicus for 24 h[58,59].

Cefquinome

Cefquinome is a broad-spectrum antibiotic belonging to fourth-generation cephalosporins class and is active against many pathogens that cause respiratory disease in equines[60,61].

An injectable formulation of cefquinome administered by inhalation to healthy horses (0.5 mg/kg cefquinome at a concentration of 30 mg/ml) did not cause any complications, and within 30 minutes after administration, the concentration of cefquinome in the BALF reached a level that was above the MIC for major respiratory pathogens, including *Streptococcus zooepidemicus*[62]. In another study, inhalation cefquinome in the same dose provided high concentration in BALF, but this concentration was detectable only for 4 hours[63]. These studies showed the effectiveness of inhaled cefquinome in the treatment of lower airway infections in equines, but it must be further studied.

Marbofloxacin

Mabrofloxacin is an antibiotic belonging to the fluoroquinolone class, which is expected to be effective against some infections in the respiratory tract of equine[64]. A study conducted to investigate the effectiveness of inhalation administration of marbofloxacin in healthy horses showed that aerosol administration of marbofloxacin (300 mg of 25 mg/ml marbofloxacin sulotion, a single dose) does not induce any side effects and its concentration in BALF is several times higher than the concentration of this drug in BALF after parenteral administration (2 mg/kg, IV)[65].

Bronchodilators

Anticholinergics and beta-2 agonists are two major classes of bronchodilators. Anticholinergic bronchodilators, which are also called muscarinic receptor antagonists, inhibit the parasympathetic reflexes causing bronchoconstriction via the vagus nerve and thus cause a bronchodilating effect[66]. β-2 agonist drugs bind to β-2 receptors on bronchiole smooth muscles and relax these smooth muscles of the airway and consequently lead to generate a bronchodilatory effect[66]. Anticholinergics include ipratropium, oxitropium, and tiotropium[67].

Short-acting β-2 agonist drugs such as albuterol (Salbutamol), levalbuterol and clenbuterol have a quick beginning of action (in a 5–15 min) and a short period of action (2–4 h). On the other hand, long-acting β-2 agonist drugs such as salmeterol and formoterol have a slow beginning of action (in a 30 min) and a long period of action (8 h)[3]. Combined inhalation therapy of corticosteroids and beta-2 agonist drugs is more effective in the management of disease exacerbation than treatment with each of these drugs alone[33]. Some important inhaled bronchodilators drugs for using in equine veterinary medicine are mentioned in Table 4.

Albuterol

Aerosolized albuterol has been suggested as an effective therapeutic option for the treatment of RAO in equines [9,68–71]. However, aerosolized albuterol is effective only for 30–60 minutes, and thus the use of this drug for long-term control of RAO is limited [9,68,72].

A study that examined the effect of albuterol administered by inhalation (2μg/kg) in anesthetized, non-hypoxemic healthy hors-

Table 4 Some important inhaled bronchodilator drugs used in the treatment of equine respiratory diseases. | *Einige wichtige inhalative bronchodilatierende Arzneimittel zur Behandlung von Atemwegserkrankungen bei Pferden.*

Bronchodilator	Class	Dosage
Albuterol (Salbutamol)	Short acting beta-2 agonists	1–2μg/kg, every 1–4 h
Levalbuterol	Short acting beta-2 agonists	0.5μg/kg, every 4 h
Salmeterol	Long acting beta-2 agonists	0.25–1.0μg/kg, every 6–8 h
Ipratropium bromide	Anticholinergics	1–3 mg/kg, every 6–8 h

es showed that albuterol led to an increase in arterial oxygen tension (PaO_2) in these horses for at least 40 minutes[73]. Also, an increase in PaO_2 after intratracheal administration of albuterol (2 µg/kg, through endotracheal tube) has been observed in anesthetized hypoxemic horses[74]. Therefore, inhalation administration of albuterol at a dose of 2 µg/kg can be useful in anesthetized nonhypoxemic and hypoxaemic horses to increase PaO_2.

Inhaled albuterol (900 µg per a horse) has been shown not to increase performance in healthy racing horses[75]. Inhaled administration of albuterol (720 µg every 4 to 6 h) appears to be beneficial in horses with septic pleuropneumonia[76], although this finding needs further study.

Levalbuterol

Levalbuterol or Levosalbutamol is a short-acting β-2 receptor agonist that has a bronchodilator effect. This drug also inhibits mast cells in the airways, reduces the movement of eosinophils into the airways, and therefore reduces wall edema in the airways[72]. Inhaled levalbuterol (63 µg) has been described to be as effective as albuterol (63 µg) in improving respiratory functions in horses with RAO, although it is a short-acting bronchodilator, the duration of action of levalbuterol is twice that of albuterol[72]. The recommended dose for levalbuterol via inhalation is 0.5 µg/kg, q 4 h[3].

Salmeterol

This drug serves as a β-2 receptor agonist, utilized for the purpose of alleviating bronchospasm that is commonly linked with inflammatory airway disease in equine species. Salmeterol is used to treat equine asthma because it has a bronchodilator effect and clears excess mucus fluid. The inhaled formulation of this drug, allows low doses to be transported directly into the airways for maximum effectiveness while reducing potential side effects [77]. The recommended dose for inhaled salmeterol in horses to achieve a constant effectiveness is 0.5–1 µg twice daily for up to nine treatments[3,77].

Table 5 A number of other inhaled drugs used in treating equine respiratory diseases. | *Eine Reihe anderer inhalativer Arzneimittel zur Behandlung von Atemwegserkrankungen bei Pferden.*

Drug	Class	Dosage
Acetylcysteine	Mucolytic	1.5–2.5 gr/animal
Nedocromil	Mast cell stabilizer	8–14 mg per 500 kg BW every 4–8 h
Cromoglycate	Mast cell stabilizer	10–15 mg per 500 kg BW every 4–8 h
Nitric oxide	Radical	80 ppm
Furosemide	Diuretic	1.0 mg/kg
CpG Nanoparticles	Immunostimulatory	0.075 mg/ml
Silver nanoparticles	–	100 ppm
Propolis + honey	–	1.4 ml + 3.5ml of honey /500 kg

Ipratropium bromide

Administration of ipratropium bromide by dry powder inhalation (2000 µg/horse) or aerosol inhalation (4 ml/100 kg BW of ipratropium bromide with concentration of 75 µg/ml) in horses reveres signs of acute airway obstructive seen in RAO and has no systemic side effects and is currently recommended for the treatment of RAO in equines[78–82]. However, a dose of 1200 µg/horse of dry powder inhalation of ipratropium bromide for the treatment of RAO has been reported[81].

Mucolytic drugs

A large amount of mucus in the airways can be a result of excessive mucus production which occurs with inflammation of the airways or a result of poor mucus clearance and it causes ventilation not to be done well, bacteria and harmful substances remain in the airways and are not eliminated. Thus the presence of excessive mucus in the airways is a problematic situation[83]. In some diseases of the equine's respiratory system, such as RAO, where mucus secretion increases in the airways, the use of mucolytic drugs can be useful. Mucolytics refer to medicinal agents, which modify the biophysical characteristics of respiratory system secretions and subsequently reduce their viscosity. N-acetyl L-cysteine is a well-known mucolytic drug[106]. Acetylcysteine inhalation uses for respiratory disease in humans[84,85] and acetylcysteine inhalation (1.5–2.5 g/animal) uses for dissolving bronchial mucus in equines are listed in table 5[86].

Mast cell stabilizers

Mast cell stabilizers are medications that inhibit the degranulation of mast cells and thus they inhibit the release of inflammatory mediators. Therefore, by reducing inflammation, mast cell stabilizer drugs can be used in inflammatory diseases of the equine's respiratory system, such as RAO, and reduce bronchoconstriction[3,87]. Inhalation administration of cromoglycate sodium, which is a mast cell stabilizer, causes clinical improvement in equines with RAO and this drug administrated by inhalation (80 mg, daily) can also be used as a prophylactic treatment for RAO in equines[3,88]. There are two formulations of cromones, mast cell stabilizers, for inhalation therapy: nedocromil and cromoglycate. Recommended dose for nedocromil and cromoglycate for inhalation therapy is 8–14 mg for a horse weighing 500 kg every 4–8 hour and 10–15 mg for a horse weighing 500 kg every 4–8 hour, respectively[3] (Table 5).

Miscellaneous

Nitric oxide

Nitric oxide which relaxes smooth muscle of the bronchi has a bronchodilator and also pulmonary vasodilation effect[89]. Nitric oxide has some other effects such as antiinflammatory, surfactant production, mucous secretion, ciliary motility stimulation, bronchial hyperreactivity and etc[90]. Nitric oxide administrated by inhalation route (80 ppm) in horses during exercise reduces the pulmonary artery pressure. Since nitric oxide binds to hemoglobin after entering the blood and is immediately de-

activated, the effect of nitric oxide administrated by inhalation route on blood vessels is limited to the pulmonary vessels[91].

It has been shown that there is nitric oxide in the exhaled air of horses during competition, and this suggests that the airways of horses produce nitric oxide during exercise, and this nitric oxide can be useful in the prevention of EIPH in horses and it is possible that the amount of nitric oxide produced in horses during exercise suffering from EIPH is low[91]. However, it has been suggested that inhaled nitric oxide therapy in horses during exercise is not only ineffective, but also worsens EIPH, because the reduction of pulmonary arterial pressure resulting from inhaled nitric oxide probably causes the activation of mechanisms which results in vasoconstriction and this vasoconstriction leads to an increase in the intraluminal pressure of the pulmonary vessels and worsens the stress on the vessels[92–94]. However, the results of these studies must be interpreted with caution because they were performed in horses exercising on a treadmill, the number of studied horses was small and the EIPH severity was not taken into account.

Nitric oxide administered by inhalation route (2.5 µmol/breath for 5 minute) to anesthetized horses resulted in an increase in PaO_2[95]. Therefore, administration of nitric oxide by inhalation route in anesthetized horses can be considered to prevent hypoxemia[95–97].

Furosemide

A study conducted on ponies on the effectiveness of inhaled (1.0 mg/kg at concentration 50 mg/ml) and intravenous (1.0 mg/kg at concentration 50 mg/ml) furosemide treatment on chronic obstructive pulmonary disease showed that the administration of furosemide by both methods leads to the improvement of pulmonary functions and clinical signs of the disease[98]. This study stated that furosemide leads to the relaxation of the smooth muscles of the airways and this effect is a mechanism by which furosemide has a positive effect on chronic obstructive pulmonary disease in ponies[98]. Furosemide administrated by inhalation route is quickly absorbed from the airways and reaches its peak concentration within approximately ten minutes[99].

Cytosine-phosphate-guanosine oligonucleotides (CpG)

One of the therapeutic approaches for the treatment of allergic diseases in equines and humans is based on nanocarrier mediated immunotherapy. CpG can be used for this immunotherapy and nanoparticle seem to be a good delivery system for immunotherapeutic agents[100]. It has been shown that inhaled nanoparticles of CpG have a positive effect in the treatment of RAO in horses, because this therapeutic protocol leads to a decreased percentage of neutrophils in the tracheal wash fluid, and also leads to relieve the clinical symptoms of the disease and to improve pulmonary function[100–102].

In a study, a complex of gelatine nanoparticle and CpG (0.075 mg/ml) was nebulized for 5 to 10 minutes in healthy and RAO-affected horses (100). Three treatments were performed, and there was a two day pause among each treatment. This study showed that this therapeutic protocol leads to the production of antiallergic IL-10 producing cells in the BALF of horses affected with RAO and this increasing in IL-10 is associated with reducing the neutrophile percentage in tracheobronchial fluid. This study concluded, which CpG is a safe and efficient therapy for RAO in horses[100]. However, this therapeutic approach needs to be studied further.

Silver

It is well documented that silver has antibacterial properties and it has been shown that nebulized silver nanoparticle even in low concentrations (100 ppm) can be a good treatment option for the bacterial diseases of the respiratory system of equines[102,103]. A study showed silver nanoparticle have an in vitro bactericidal action against *Streptococcus zooepidemicus* and *Actinobacillus Equuli* and its action is preserved even after nebulization with low concentrations (100 ppm)[104].

Propolis with honey

A study investigating the inhalation of ethanol extract of propolis in combination with honey 3 times per day for 7 days (1.4 ml of ethanol extract of propolis with 3.5 ml of honey for 500 kg/BW) in horses with chronic bronchitis showed that ethanol extract of propolis with honey has an antifungal, antibacterial, and mucolytic effect on the horse's respiratory system, and it also causes a decrease in respiratory and heart rates[105]. However, the use of this material for inhalation therapy needs further investigations.

Future directions for inhalation use of drugs

Inhalation therapy is an ideal method for delivering drugs directly into the airways of equines with respiratory diseases. However, compared to humans, this way of treatment needs more studies to determine the safety and efficacy of various drugs on different respiratory diseases more precisely. Therefore, in the following, some directions are suggested for the inhalation use of drugs in equines:

- The influences of physiological conditions, for example gender, age, and etc, environmental agents, and concomitant diseases on the treatment response to drug inhalation therapy (especially antimicrobial and glucocorticoid) require investigation.
- Some antimicrobial drugs (such as tylosin, tilmicosin, etc.) are not used parenterally in horses because of their side effects. Whether they can be used by inhalation or not has not been determined.
- The impacts of inhalation glucocorticoids and antimicrobial on the inflammatory indicators in airways tissue, BALF, and blood circulation are not well realized.
- Few studies exist on the combination of inhaled glucocorticoids with inhaled bronchodilators in the treatment of respiratory diseases in equines including RAO.
- Encapsulation of drugs in liposomes shows itself as a suitable approach for increasing intracellular drug delivery. Combined liposomes with different antimicrobials must be further investigated. Also, the effectiveness of liposomal

antimicrobials for the treatment of intracellular pathogens such as *Rhodococcus equi* must be further investigated.
- The effectiveness of some drugs used by the inhalation route in respiratory diseases in humans is not yet fully understood in horses and needs to be checked.

Conclusion

This review highlights inhalation therapy in equines and particularly the inhaled pharmacological drugs used in equines. Inhalation therapy has particular advantages and disadvantages and in some situations it can be used for the direct delivery of drugs to airways. The most important benefits of inhalation use of drugs are less side effects compared to systemic use. Due to the existence of such positive benefits, inhalation therapy should be given more attention and prioritized in the management of the treatment of respiratory diseases in equines. Of course, more studies on various aspects of inhalation therapy in equines should be done in future.

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