Effect of Aerosol Ascaris suum Challenge in The Horse

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Abstract: Four normal horses were actively sensitized to Ascaris suum extract by repeated intratracheal injections. Three weeks after the last injection, conscious standing animals were challenged with a $10^2$ dilution of A. suum extract administered as an aerosol for 3 min. Pulmonary mechanic and ventilation volumes were measured at 15, 30 and 60 min. after antigenic challenge. Horses responded to A. suum challenge with respiratory distress. Comparisons between baseline (obtained after saline inhalation) and post A. suum challenge values revealed significant increases in the nonelastic work of breathing, maximum change in transpulmonary pressure and pulmonary resistance. Dynamic compliance decreased (p<0.05). While tidal volume did not change, increases in respiration rate and minute volume were highly significant. Arterial blood samples were obtained 15 min. after the inhalation of A. suum and were compared with those obtained after saline inhalation. A significant decrease occurred in PaO2 while PaCO2, pH and HCO3 were not affected. These findings are consistent with those frequently observed in horses suffering from Chronic Obstructive Pulmonary Disease (COPD) as well as those seen in bronchial asthma in man. It is suggested that induced bronchospasm may be used as an experimental model of COPD.

Key words: Chronic obstructive pulmonary disease (COPD), Ascaris suum, bronchial asthma, bronchospasm.

Introduction
Experimental bronchospasm in response to inhaled antigens has been widely used as a valid model of bronchial asthma (reviewed by Wanner & Abraham, 1982). Such studies have significantly contributed to a better understanding of the pathophysiological mechanisms associated with bronchial asthma. Chronic obstructive pulmonary disease (COPD) in the horse appears to be the only naturally occurring non-human disease which can be compared to bronchial asthma (Cook & Rossdale, 1983; Lowal, 1984; Littlejohn, 1979; Mirbahar & Eyre, 1985). In allergic COPD, inhaled antigens are considered to induce and acute asthma-like attack by interacting with antibody, causing the release of pharmacological mediators such as histamine, leukotrienes and kinins. The released mediators are responsible, at least in part, for the clinical manifestations of such cases of COPD (Hanna et al., 1982). The antibodies involved have not been characterized specifically, although reaginic, homocytotropic antibodies have been described in the horse (Eyre 1972; Halliwell et al., 1979; Schatzmann et al., 1973).

Experimentally-induced anaphylaxis in the horse and pony is characterized by respiratory embarrasment and pulmonary edema suggesting lung as a target organ (Eyre & Lewis, 1973; McGavin et al., 1972). In these earlier studies, no attempt was made to quantify altered lung function. Methods to the measurement of the lung function in conscious and unsedated animals have been described which allow the quantification of gas exchange and pulmonary mechanics in normal and diseased horses and calves (Willoughby & McDonell 1979; Collie 1992a; 1992b).

In an attempt to develop a suitable model of allergic bronchospasm associated with equine COPD, we have used A. suum allergen for the active sensitization of horses. This paper describes the effects of A. suum challenge on actively sensitized horses.

Materials and Methods

Animals: Eleven Standard-bred horses were subjected to a detailed clinical examination at rest and after 10 min of trotting exercise, bronchoscopy, bronchoalveolar lavage (BAL) with quantitative cytology and bacteriological examination of the lavage fluid, and selected pulmonary function tests (PFT). On the basis of above tests, four horses were selected as normal and free of pulmonary disease and were used in this study. Three were females (OVC:1; OVC:34 and OVC:66) between 3 and 4 years of age; the fourth was a nine year old gelding (OVC:6). All horses weighed between 345 and 440kg (mean 382kg).

Skin testing and sensitization procedure: An area of the neck of the horse was clipped closely and Intradermal injections of 2ml of serial dilutions of A. suum ($10^{-6}$ to $10^{-2}$ dilution) and control saline were injected approximately 10 cm apart in a zigzag fashion. The horses were examined for skin reaction at 30 min and 5h. Sensitization was accomplished by intratracheal injections of 2ml of A.suum extract (1/10 w/v; Greer laboratories, Inc. Lenoir, N.C; U.S.A.) mixed with 8ml of sterile saline. Three such injections were given at weekly intervals. A latent period of 3 weeks was allowed before horses were challenged with aerosolized A. suum. Four weeks after the last injection of the sensitization procedure, the skin testing was repeated on these horses.

Pulmonary Function Testing (PFT): All PFT was carried out without sedation or anaesthesia on standing animals positioned in a 1 x 2m stock. The restraint was limited to holding the horse via a halter.

A mask made of fiberglass and neoprene was fitted over the muzzle and care was taken to avoid leaks and to minimize dead space. Airflow rates, inspiratory and expiratory volumes and changes in total (mouth to esophageal balloon) and lower (trachea to esophageal balloon) transpulmonary pressure were recorded simultaneously on a photographic recorder (VR-6, Electronics for Medicine, White Plains, New York) as described previously (Willoughby and McDonell, 1979). The primary measurements included the quantifications of tidal volume (VT), and the changes in the total and lower Ppl during
the respiratory cycle. In addition, the inspiratory and expiratory flow rates and changes in total and lower Ppl were calculated at 25, 50 and 75 percent of inspiratory and expiratory tidal volumes.

In the horse, Rl varies through the respiratory cycle and is dependent upon breathing pattern (Gillespie et al., 1986). The Rl was, therefore, calculated at 25, 50 and 75 percent of the inspiratory and expiratory tidal volumes and then averaged to represent the average inspiratory and expiratory Rl, expressed as cmH2O/1/1. Pw was related to the change in volume at the points of zero airflow (in volume, in Ppl) to calculate dynamic compliance (Cdyn) expressed as 1/cmH2O. Inspiratory and expiratory Ppl versus volume were plotted using an X-Y recorder to obtain pressure-volume loops. The area within the loop was determined by planimetry to obtain non-linear work of breathing (Wb), expressed as Kg-cm/1. Upper airway values (upper 2/3 of the trachea and the nasopharynx) were determined by deducting the tracheal to esophageal (lower airways) values.

Arterial blood samples were collected from the carotid artery 15 to 20 min after the animals had been challenged with antigen or saline aerosol, using a 20 gauge 4.5 cm needle and percutaneous puncture. Approximately 5 ml anaerobic blood samples were collected into heparinized glass syringes (10 ml) and immediately analyzed on a blood micro system (BMS-3, MK-2, Radiometer, Copenhagen) to determine PaO2, PaCO2, pH and HCO3 levels.

Nebulization technique: An ultrasonic nebulizer was used to aerosolize saline and antigen. At the beginning of each experiment, the horse was challenged with normal saline to establish baseline values. The animals were then challenged with a 10-2 dilution of A. suum aerosolized for 3 min. Rebreathing of the expired air was prevented by the use of three-way breathing valve. During antigen challenge, the expired air was collected in a meteorological balloon and vented in a safe place. Adequate inspiratory volume was ensured by dividing the inspiratory port using a Y-connector (Mirbahr et al., 1985). One opening was connected to the nebulizer while the other accommodated a flexible tube (5 cm x 3m) opening to room air.

Experimental design: Three weeks after sensitization, each horse was challenged 3 to 5 times with A. suum aerosol at weekly intervals. After each challenge, PFT was performed. Three normal breaths were selected from scalar tracings by visual inspection for analysis and were averaged to represent a single treatment e.g., saline or a give time period after antigenic challenge. Repeated experiments on the same horse were averaged to represent that particular horse. Lung function was measured at 15, 30 and 60 min post antigenic challenge. The pressure-volume loops were constructed at 15, 30, 45 and 60 min. The timing of PFT after antigen challenge was based on pilot studies. Later it was observed that the effect of antigen peaked between 7 and 16 min post-challenge. However, we chose to continue recording lung function at 15 min since we had already completed several experiments.

Statistical analysis: A two-way analysis of variance (ANOVA) was used to compare pre-antigen responses (baseline) with those at 15, 30 and 60 min post-antigen challenge. The Duncan Multiple-Range Test was applied to specify differences between means. A paired t-test was used to compare blood-gas values obtained before and after A. suum challenge.

Results
The results of skin testing performed before and after sensitization were inconsistent. While all 4 horses appeared negative at 30 min compared with saline controls, they responded positively after 5h to the higher dilutions of A. suum. After sensitization, two horses (OVC:1 and OVC:6) were positive at 30 min to the lower doses of A. suum while no change was observed in the remaining two horses. After sensitization, all animals were positive to all dilutions at 5h. Although PFT was not performed after intratracheal injections of A. suum for sensitization, no clinical response was evident after the first and second injections. After the third injection, however, all animals exhibited signs of restlessness manifested as licking of muzzle, sneezing and increased respiratory rate. These signs disappeared within 10 to 15 min.

Response to Ascaris suum challenge: In general, horses were cooperative and tolerated the experimental procedures. The horses responded to antigenic challenge with varying degrees of respiratory distress; 2 horses responded severely, one moderately and one weakly (Fig. 1).

The response to antigenic challenge was immediate, starting during the exposure, peaking at 7 to 14 min post-challenge and lasting approximately 60 min. Other signs noted include: tachycardia, moderate to severe sweating, occasional coughing, severe dyspnea, frequent defecation and urination, and moderate to severe salivation in severely responding animals.

Comparisons of baseline (saline) PFT values before and after sensitization revealed a nonsignificant increase in total and lower Ppl, total inspiratory and expiratory Rl and lower inspiratory Rl with a significant increase in lower expiratory Rl (p < 0.05). The saline baseline values appeared to increase with repeated antigenic challenge throughout the period of study, particularly in the two most severely responding horses. Inhalation challenge with A. suum produced significant increases in respiration rate (f), minute volume (Vv), inspiratory and expiratory flow (Table 1), lower expiratory Rl and total and lower max. Ppl and Wb (Fig. 2, 3 and 4). Cdyn and Pao decreased (p < 0.05) (Table 1) while PaCO2, pH and HCO3 did not change.

As shown in Fig. 1, 2, 3 and 4 the relative dysfunction in lung mechanics after antigenic challenge was most evident in the lower airways, representing essentially intrapulmonary airways. When calculated separately for upper (total minus lower) airways, the relative changes in the pulmonary mechanics were minor and not significant. For example the mean increase in max. Ppl for lower airways was 168, 91 and 74 % at 15, 30 and 60 min respectively after antigen challenge. In the upper airways, however, this change was only 39, 29 and 41 % at the same time intervals. The increases in lower Wb were 320, 152, 161 and 142 percent at 15, 30, 45 and 60 min respectively after the inhalation of A. suum. On the other hand, at these time intervals, the Wb for the upper airways increased only by 79, 74, 12 and 61 percent.

Discussion
Subcutaneous and intravenous injections of foreign proteins constitute the traditional method for the active sensitization of experimental animals. These sensitization procedures appear to be un-natural when investigating allergic lung diseases. Some workers have used the aerosols to achieve or to boost an existing sensitivity to a particular antigen (Wanner et al. 1979; Kenji et al., 1991). Studies in sheep suggested that active
sensitization could be achieved by injecting the antigen directly into the airways (Autenried, 1982). In our horses, the respiratory distress and the changes in lung mechanics observed in response to aerosolized antigenic challenge indicate the success of the present sensitization method. Unfortunately, the horses were not challenged with A. suum to obtain control values before sensitization. This appears to be important since we later discovered that as with dog, monkey and sheep (Wanner & Abraham 1982), a significant proportion of horses also possessed natural allergy to A. suum (Mirbahar 1985). However, horses used in this study did not respond to initial intracutaneous injections of A. suum. A mild clinical response was seen after the third injection. This suggested a gradual development of hypersensitivity to this antigen. In addition, these horses responded more severely to histamine after sensitization when compared to the responses of the same horses to the same dose of histamine before sensitization (Mirbahar et al., 1992). It is of more than passing interest that naturally sensitive horses when discovered are already hyperreactive to histamine and that repeated antigen challenge does not increase airway responses to histamine (Mirbahar et al., 1988).

The responses of each horse to repeated antigen challenge were reproducible. In contrast, the responses of different horses to the same dose of antigen were variable (Fig. 1). Similar variability has been observed in response to inhaled and intravenously administered antigens and histamine in the guinea pig (Douglas et al., 1973), dog (Snapper et al., 1980), sheep (Wanner et al., 1979), monkey (Pera et al., 1976), pony (Dersken et al., 1982) and horse (Mirbahar et al., 1985). The reasons for this variability are not known. Snapper et al. (1980) considered the quantity of the mediators released in response to antigen challenge and the airway sensitivity of individual animals to released mediators to be responsible for individual variability. Wanner et al. (1979) suggested that the differences in the immunological status may account for the observed variability in the airway responsiveness of sheep to inhaled antigen.

It has been reported that the same dose of antigen used for sensitization may not equally be effective in different animals of the same species. While sensitizing sheep, Wanner et al. (1979) used 2 to 11 sensitizing doses of A. suum over a period of 3 to 20 weeks, and achieved only a 50% success with their sensitization method. Out of 10 actively sensitized sheep, all with positive skin reactions to A. suum, only 5 sheep responded with altered lung function on subsequent challenge with aerosolized A. suum.

Comparisons with naturally-occurring equine disease: Equine COPD probably has a multifactorial etiology and could broadly be classified as allergic and nonallergic. In allergic COPD, the development of clinical signs may be acute (Type-I) or subacute. The disease is manifested clinically as severe dyspnea characterized by a double expiratory effort (Cook & Rossdale 1963; Littlejohn, 1979). An increase in values for max dPpl and Wb with parallel decrease in Cmax and PaO2 have been consistently reported (Sasse, 1971; McPherson et al., 1978; Thomson & McPherson, 1984). Similar changes in these parameters were observed in this study (Fig. 2 and 3; Table 1). In the horse, max. dPpl and Wb are considered important in the detection of lower airway abnormalities (McPherson et al., 1978; Sasse, 1971; Willoughby & McDonell, 1979). The increased Wb is thought to be a significant factor in the reduced exercise tolerance seen consistently in these horses (Sasse, 1971). As shown in Fig. 2 and 3, a significant increase in total and lower Wb and max. dPpl accords with these findings and indicate important functional similarities between our horses and those with COPD.

The failure of total inspiratory and expiratory RL to increase significantly may be a reflection of the number of animals studied and variability observed in this study. In addition, it is known that lower RL normally constitutes less than 20 percent of total RL (Thurbeck, 1975). After obstructing half of the peripheral airways, Thurbeck (1975) failed to observe an increase in total RL and thus concluded that considerable disease may be present in lower airway while total RL may be normal. Our system enabled us to isolate lower airway changes and the lower RL increased both on inspiration and expiration, being more prominent during the later (Fig. 4). A greater expiratory RL has also been reported previously by others in horses with COPD (Gillespie et al., 1966; Gillespie & Tyler, 1989; Willoughby & McDonell, 1979). Interestingly, total inspiratory RL decreased at the peak of antigenic effect (Fig. 4). This decrease was in the upper airways since lower inspiratory RL increased (Fig. 4) and may have been due to the flaring of nostrils noted on inspiration. The latter is also a common finding in COPD horses.

In the present study, the decrease in Cmax is comparable to that reported by others in horses with advanced COPD lesions (Sasse, 1971; Thomson & McPherson, 1984; Willoughby & McDonnell, 1979). The measurements of Cmax, particularly at a higher rate of breathing probably do not represent changes in the elastic recoil of the lung. Cmax is influenced by alterations in small airway resistance which may be the result of smooth muscle contraction or partial occlusion of small airways with
Fig. 1: Time-response curves showing absolute values for maximum change in pleural pressure for total and lower airways. Responses are the means ± S.E.M. of 3–5 repeated experiments (R) on each of the four horse to aerosolised saline (C) and A. suum. Animals were challenged with A. suum (AS) at time 0 and the lung function was measured at 15, 30 and 60 minutes after antigenic challenge. * (p<0.05).

Fig. 2: Time response curves of four horses to inhalation challenge with saline (C) and A. suum showing maximum change in pleural pressure for total (○) and lower (○) airways. Animals were challenged with A. suum at time 0 (AS) and the lung function was measured at 15, 30 and 60 minutes after antigenic challenge. Values are expressed as mean ± SEM. * (p<0.05)

Fig. 3: Time-response curves of four horses to inhalation challenge with saline (C) and A. suum showing changes in work of breathing for total (○) and lower (○) airways. The animals were challenged with A. suum at time 0 (AS) and the lung function was measured at 15, 30, 45, 50 minutes after antigenic challenge. Values are expressed as mean ± SEM. * (p<0.05)

Fig. 4: Time-response curves of four horses to inhalation challenge with saline (C) and A. suum showing changes in pulmonary resistance on inspiration and expiration for total (○, □) and lower (○, ■) airways. The values shown are the averages of measurements made at 25, 60 and 75 percent of the tidal volume. The animals were challenged with A. suum at time 0 (AS) and the lung function was measured at 15, 30 and 60 minutes after antigenic challenge. Values are expressed as mean ± SEM. * (p<0.05)

Discrepancies exist in the literature regarding the changes in mucus (Thurlbeck, 1975). The measurements of lung compliance require static conditions and patient cooperation or general anaesthesia. Thus it is impractical to measure static compliance in conscious standing horses.
ventilatory volumes in COPD horses. This may be due to methodological differences or variations in the severity of the airway lesions. Giolespie and Tyler (1969) reported a decrease in inspiratory $V_{I}$, while Sasse (1971) observed a greater $V_{I}$ in horses with COPD. Others have reported no differences in the $V_{I}$ of normal and diseased horses (Willoughby & McDonell, 1979). Similarly, Thomson and McPherson (1984) observed a significantly greater respiratory rate (f) in diseased horses whereas higher f and Vi reported by others did not reach a level of statistical significance (Giolespie & Tyler, 1969; Sasse, 1971). Recently, Nyman et al. (1991) has reported a significant increase of about 100 percent in VI of horses with COPD. In the present study, f and Vi increased significantly and remained high for at least 30 minutes after antigen challenge. $V_{E}$ however, did not change. The increases in f and Vi may have been due to severe hypoxemia, which is a potent stimulator of peripheral chemoreceptors (West, 1979).

The results of arterial blood gases are in general agreement with others. A decrease in $P_{aO_{2}}$ is a prominent feature of chronic pulmonary disorders in the horse. Hypercapnia, on the other hand, is not a common finding in COPD (Sasse, 1971; McPherson et al., 1978; Nyman et al., 1991).

Comparisons with animal models of human asthma: The ethical considerations generally prevent the use of man in laboratory investigations involving antigenic challenge. Experimental bronchospasm in laboratory animals has been widely used as a model of bronchial asthma (Wanner & Abraham, 1982). Increased $R_{L}$ and decreased $C_{v}$ resulting from antigenic challenge have been consistently observed in all animals used and account for their validity as experimental models of bronchial asthma. The present study demonstrates that, in sensitized horses, inhalation of specific antigen causes changes in pulmonary mechanics which closely resemble those observed in asthmatic man. The increase in $R_{L}$ and a decrease in $P_{aO_{2}}$ coupled with unchanged $P_{aCO_{2}}$ indicate ventilation-perfusion inequalities which are highly characteristic of asthma (Thurbeek 1975; West 1978). These findings also accord with frequently reported similar changes in monkey, sheep and dog (Wanner & Abraham 1982). The advantages ad disadvantages of various animal models have been reviewed and the differences in the pulmonary function of bronchial asthmatic man and experimental bronchospasm in animals other than horse, have been pointed-out (Wanner & Abraham, 1982). In addition, all animal models other than the horse appear to share two major disadvantages in common: 1) they possess lungs which are different from those of man at gross and sub-gross anatomical levels (McLaughlin et al., 1961), and 2) they all lack a naturally occurring pulmonary disease which could be compared to asthma on clinical, immuno-pharmacological and patho-physiological basis (Wanner & Abraham, 1982). The horse seem to possess these advantages. Equine and human lungs are considered anathomically similar (McLaughlin et al., 1961). Similarly, naturally occurring equine COPD shares many patho-physiological features and clinical manifestations with asthma in man (Cook & Rossdale 1963; Lowel 1964; Littlejohn 1979).

In view of the known similarities between horse and man, it is surprising that very little attention has been paid to the horse as a possible model of bronchial asthma. This study clearly indicates that experimental bronchospasm can be induced in horses. General anesthesia or sedation is not required in the horse. Unlike dog and monkey, it is very easy in the horse to obtain mechanical measurements of lower airway as distinct from total airway measurements. It is reasonable to suppose that lower airway measurements will be more useful since that represent the site of structural and functional alterations in naturally occurring disease (Thurbeck, 1975). There are some disadvantages of the equine model, the most important being the cost of purchase, the maintenance of animals, the animal size which requires custom designed laboratory equipment, and the temperament of the species.

References


