EFFECTS OF ATROPINE ON ANTIGEN-INDUCED BRONCHOSPASM IN THE HORSE

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ABSTRACT

The efficacy of atropine to attenuate *A. suum* induced bronchospasm was studied in 6 conscious standing horses. Animals were challenged with saline and a 10^{-2} dilution of *A. suum* aerosolized for 3 minutes. Pulmonary function tests (PFT) were performed at 15, 30 and 60 minutes after antigen challenge. Pulmonary mechanics and ventilation values were measured using a differential pressure transducer and a Fleisch Pnemotachograph. One week later, animals were treated with atropine sulfate (6.0 mg administered IM) and rechallenged with saline follwed by same dose of A. suum. Clinical signs noted after the inhalation of *A. suum* alone included hyperpnea, dyspnea, sweating and salivation. The effect of antigen was rapid in onset starting during the inhalation and lasting for over 60 minutes. The PFT revealed significant ($P<0.05$) increases in W_b, max. ΔP_{pl}, R_L, V_I, f, and V_T whereas the C_{dyn} decreased ($P<0.05$). The changes were more severe in lower airways. Atropine abolished the clinical signs. Comparison of post atropine saline and *A. suum* challenge values revealed significant increase in W_b max ΔP_{pl} at 15 minutes post antigen challenge. Changes in R_L, f and C_{dyn} were abolished. Comparison of responses to *A. suum* in the presence and absence of atropine revealed a significant ($P<0.05$) inhibition of changes in max. P_{pl}, W_b, inspiratory and expiratory R_L, V_I, f and flow. The study suggested that the *A. suum* induced bronchospasm in the horses is mediated, at least in part by vagal reflexes.

Key words: Equine COPD; antigenic bronshospasm, atropine.

INTRODUCTION

The term chronic obstructive pulmonary disease (COPD) was introduced in the veterinary literature to describe a variety of respiratory disorders in the horse, originating primarily in the lung (Sasse, 1971; Gerber, 1973; Breeze, 1976). COPD has a multifactorial etiology and despite exhaustive investigations, over the past three decades, the pathophysiological mechanism associated with equine COPD remain obscure (Mirhabar and Eyre, 1986). Analogous to bronchial asthma in men, the COPD horses also suffer from an ill-defined state of hyperreactivity (Mirhabar *et al.*, 2000). The latter in man, is believed to render sujects more susceptible to bronchospasm in response to specific and non specific stimuli such as cold air, dust, exercise and gases like ozone and sulfur di-oxide (Boushey *et al.*, 1980).

Many workers have suggested the role of non-specific nervous reflex in hyperreactive airways and the use of atropine in the treatment of bronchial asthma supports this content. In experimental animals, reports on the ability of atropine to inhibit experimental bronchospasm are in consistent. While Yu *et al.* (1972) reported the inhibition of bronchospasm by atropine in dogs, others have described only a partial attenuation (Krell *et al.*, 1976). Similarly, atropine has no effect on the antigenic response of allergic sheep (Abraham *et al.*, 1981) and monkey (Patterson nd Harris, 1976). In the pony, vagotomy only partially blunts the antigenic effect (Derksen *et al.*, 1982).

Atropine is also one of the common drugs used in the management of equine COPD (Muylle and Oyaert, 1973, Murphy *et al.*, 1980). In an attempt to evaluate the equine model of bronchial obstruction, the efficacy of atropine sulfate to attenuate the antigenic bronchospasm, was tested in *Ascaris (A.) suum* allergic and histamine hyperreactive hosres.

MATERIALS AND METHODS

Six standard bred horses of mixed weight (350 to 460 Kg), age 3 to 7 years and of either sex were used in this study. All these horses were hypesensitive to *A. suum* as confirmed by reported aerosol challenge with *A. suum* to which they responded with severe bronchospasm and highly significant changes in pulmonary mechanics and ventilation volumes (Mirhabar *et al.*, 2000a). Ascaris allergic horses were hyper reactive to histamine (Mirbahar *et al.*, 2000).

Methods

It was established from preliminary experiments that frequent antigernic challenge induced de-sensitization and or tachyphylaxis to antigen effect. Thus each horse was challenged to *A. suum* at weekly interval to avoid tachyphylaxis. To be on safe side, the horses were challenged with antigen alone on experimental day one and three (one week before and after atropine treatment). The data of these two experiments was averaged to represent control. The effect of atropine was studied on experimental day two.

Pulmonary function tests (PFT)

The measurements were made in conscious standing horses with out sedation. The methods have been described previously in detail (Willoughby and McDonell, 1979; Mirbahar *et al.*, 1985) and will only be mentioned briefly here. A fleisch pnemotachgraph and esophageal balloon were used to measure airflow rates ventilation volumes and transpulmonary pressure (P_{pl}) and recorded simultaneously on a photographic recorder (VR-6, Eelctronics for Medicine, U.S.A.). Two differential pressure transducers were used to determine the total (mouth to esophagus) and lower (Trachea to esophagus) airway pressure changes. The dynamic compliance was measured by relating the changes in voluem to changes in P_{pl} at the points of zero airflow while pulmonary resistance was calculated by relating the changes in P_{pi} to instantaneous air flow at 25, 50 and 75 per cent of inspiratory and expiratory tidal volumes (V_T) which were then averaged.

Nebulization method

The concentration of antigen and the time of exposure (a 10^{-2} dilution aerosolized for 3 minutes) was determined from our preliminary experiments while the dose of atropine (6.0 mg bolus administered intramuscularly) was selected from personal clinical experience. *A. suum* was diluted in 0.9% saline to obtain baseline values. After antigenic challenge, the lung function was measured at 15, 30 and 60 minutes. During *A. suum* inhalation, the expired air was collected in 50001 meteorological balloon and disposed off a safe place.

Statistical analysis

A two way analysis of variance was applied to compare pre antigen challenge (Saline) values with responses at 15, 30 and 60 minutes post antigen challenge in the presence ad absence of atropine. A Duncan Multiple Range test was applied to specify differences between means.

RESULTS

As shown in Fig. 1 and 2 and Table 1 and 2, all horses responded with severe respiratory distress to *A. suum* challenge. The effect was rapid in onset, starting during the exposure and lasting for more than one hour. The clinical signs noted include: tachypnea, moderate to severe sweating, moderate salivation, occasional coughing, tachycardia and frequent defecation and urination.

The quantitation of pulmonary mechanics revealed a significant increase ($P<0.05$) in work of breating and transpulmonary pressure which remained high for upto 60 minutes post challenge (Fig. 1 aand 2). The ventilation volume and frequency increased while C_{dyn} decreased significantly (Table 1). Airflow was greater on inspiration as well as on expiration. While the total R_L did not change after antigen challenge, the increases in inspiratory and expiratory lower R_L were highly significant (Table 2).

After the injection of atropine, at least 45 minutes were allowed before the horses were rechallenged with saline to obtain post atropine baseline values. The clinical signs noted after the injection of atropine included restlessness, drying of mouth, frequent licking of mouth and occasional pawing and kicking.

Atropine abolished the antigen induced clinical signs such as respiratory distres, techypnea, salivation and sweating almost completely on 4 to 6 horses tested, while in the remaining two horses, the effect was less pronounced. The measurements of pulmonary mechanics revealed significant inhibition of the antigenic effect. In the presence of atropine, the antigenic challenge failed to produce changes in max ΔP_{pl}, total W_b, R_L C_{dyn} and frequency when compared to baseline values (Fig. 1 ad 2, Table 1 and 2). Lower W_b, however, increased reaching statistical significance only at the peak of antigenic effect (Fig. 1). In the presence of atropine, the changes in V_I and flow rates were small but statistically significant (Table 1 and 2).

The comparison between post antigen challenge values in the presence and absence of atropine indicated a significant inhibition of changes in total and lower max. ΔP_{pl}, W_b, flow rates, V_I, f and lower R_L. Since atropine raised the baseline total R_L ($P<0.05$). The antigenic challenge in atropine treated animals caused a decrease in total R_L. Nevertheless the values were still significantly higher than those obtained with antigen in the absence of atropine.

Table 1: Pulmonary mechanics and ventilation values (mean±SEM) in six horses challenged with aerosolized *Ascaris suum* in the (a) absence (b) presence of atropine.

		Baseline	Minutes after *A. suum* inhalation		
			15	30	60
VT (L)	a)	4.2±0.3	4.0±0.6	4.1±0.5	4.2±0.3
	b)	4.2±0.7	3.5±0.7	4.2±0.8	4.6±0.5
Vl (L/min)	a)	48.0±4.9	115.8±17.8*	92.6±7.7*	70.6±10.2
	b)	33.6±3.7	53.8±3.6*#	51.2±4.0*#	42.9±2.3#
f (B/min)	a)	11.9±1.8	34.8±6.6	27.8±6.3*	17.9±3.3
	b)	8.4±0.7	19.1±4.6	13.5±2.2	9.7±1.0#
Cdyn (L/CmH_2O)	a)	2.3±0.3	0.7±0.2	0.9±0.1*	1.0±0.2
	b)	1.4±0.3	1.0±0.3	1.1±0.3	1.3±0.2

* = significantly (P<0.05) different from base line (ANOVA); # = significant inhibition by atropine (t-test)

Table 2: Average airflow rate and pulmonary resistance values (mean±SEM) in six horses in the absence (a) and presence (b) of atropine.

		Baseline	Minutes after *Ascaris suum* inhalation		
			15	30	60
Air flow (L/sec)					
Inspiration	a)	1.99±0.27	4.48±0.56 *	3.45±0.31 *	2.81±0.39
	b)	1.45±0.16	2.11±0.17 *#	2.04±0.20 *#	1.83±0.08
Expiration	a)	1.63±0.18	4.83±0.17 *	3.28±0.41	2.43±0.40
	b)	0.97±0.12	1.98±0.15 *#	2.00±0.25	1.42±0.18
Pulmonary Resistance (CmH2O/L/Sec) total airways					
Inspiration	a)	2.04±0.75	1.33±0.33	1.74±0.62	1.91±0.63
	b)	3.69±0.94 #	2.18±0.41	2.66±0.97	3.45±0.88
Expiration	a)	1.65±0.56	1.47±0.30	1.58±0.47	1.93±0.53
	b)	3.23±0.66 #	2.24±0.33	2.34±0.48	2.54±0.64
Pulmonary Resistance (CmH2O/L/Sec) lower airways					
Inspiration	a)	0.118±0.033	0.325±0.078 *	0.350±0.049 *	0.234±0.042
	b)	0.027±0.015 #	0.111±0.036 *#	0.055±0.028	0.083±0.052 *
Expiration	a)	0.0250±0.039 *	0.605±0.83 *	0.650±0.103 *	0.571±0.074
	b)	0.419±0.112	0.462±0.130 #	0.506±0.121	0.653±0.277

* = Significantly (P<0.05) different from baseline (ANOVA); # significant (P<0.05) effect of atropine (t-test)

DISCUSSION

A. suum challenge caused severe respiratory distress in horses manifested as dyspnea, hyperpnea, sweating, and moderate urination and defecation. The effect was rapid in onset and started during the aerosolization of *A. suum*. Although the clinical sings apparently subsided within 10-15 minutes post challenge, the changes in pulmonary mechanics remained significantly higher at 60 minutes post challenge (Figure 1 and 2 and Table 1 and 2). The response to *A suum* was in complete agreement with our previous reports (Mirbahar *et al.*, 1988, 2000). Atropine inhibited the tachypneic and hyperpneic response to antigen, and this accords with similar findings in dogs (Cotton *et al.*, 1977), monkeys (Pare *et al.*, 1976) and ponies (Derksen *et al.*, 1982). Almost complete blockade of antigenic effect by atropine at a low dose (0.6 mg) appears to suggest that the *A. suum* induced bronchospasm in horse may have been mediated by mechanism involving cholinergic pathways. Interestingly, atropine did not attenuate *A. suum* induced *in vitro* contractions of isolated bronchial strips of the same horse, which may however, reflect the lack of intact vagal pathways in the isolated strips (Mirbahar *et al.*, 1990).

The data shown in Fig. 1 and 2 and Tables 1 and 2 with regard to pulmonary mechanics are however, difficult to interpret due to atropine induced increases in baseline values. Although the R_L, max. ΔP_{pl}, W_b and C_{dyn} did not change significantly in response to antigen in the presence of atropine, the results may have been a reflection of higher baseline values. The later change cannot be explained properly at the present time. Atropine can stimulate the vagus through its effect on CNS. However, this effect is immediate and transient and probably occurs only at doses higher than those used in this study (Weiner, 1980). In this study, the atropine was used at a dose of 6.0 mg (approximately 0.15 mg/kg b.wt) administered intramuscularly. This is lower that that used in COPD horses by Muylle and Oyaert (1973) and Murphy *et al.* (1980), who used 0.02 mg/kg b.wt and 10 mg bolus respectively. Krell *et al.* (1976) and Gold *et al.* (1972) administered 0.5 mg and 0.2 mg/kg in the dog respectively while Abraham *et al.* (1981)

used 0.2 mg/kg atropine in the sheep. It, therefore, appears unlikely that atropine at this dose will have sufficient central effects to influence vagal activity. Alternatively, the drying effects of atropine may have contributed to the observed increases in baseline values. Antigen challenge increases airway secretions and impairs mucocilliary activity (Phips *et al.*, 1983). The latter may remain impaired for upto seven days (Allerga *et al.*, 1983). In the present study, each horse was repeatedly exposed to antigenic aerosol challenge. It is therefore, possible that frequent exposure to antigen may have caused accumulation of mucus in the airways as a result of either increased secretion or decreased removal or a combination of both. The drying effect of atropine may, therefore, have caused mucus solidification, plugging of the airways, and thus an increase in R_L. Under these circumstances, however, one would have expected mucus plugs in the lower airways and thus an increase in lower RL which was not the case in this study. Lower R_L decreased significantly after atropine treatment and did not rise after inhalation challenge.

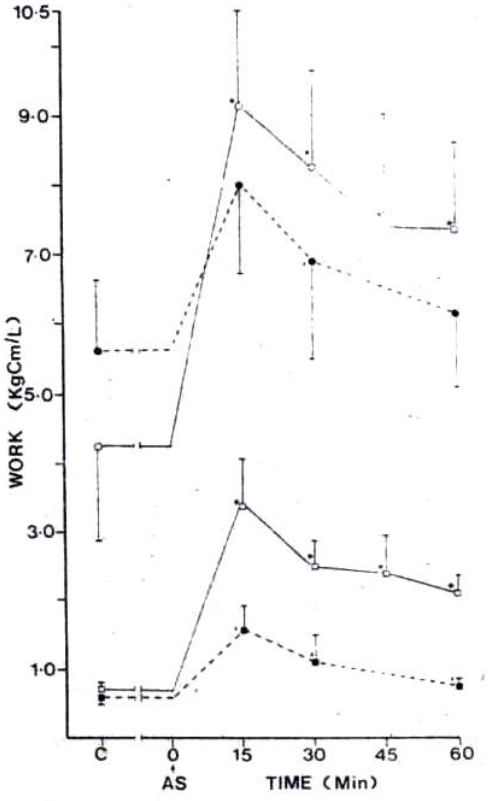

Figure 1: Time response curves showing absolute changes (mean±SEM) in work of breathing for total and lower airways before and after atropine sulphate administered I.M. 45 minutes before antigenic challenge.

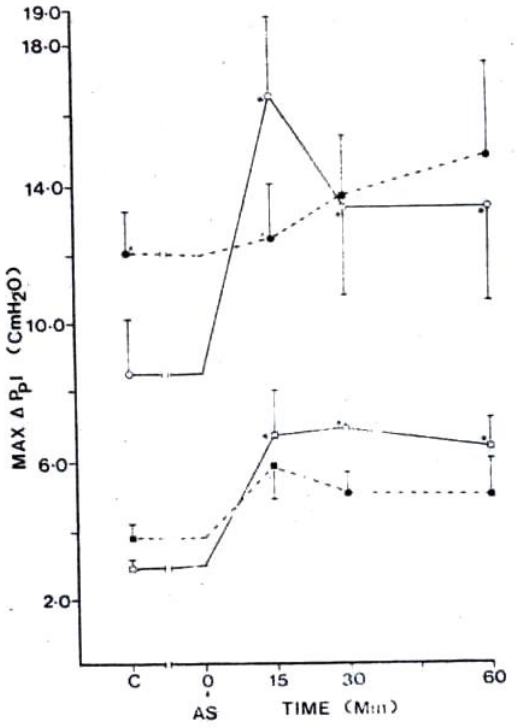

Figure 2: Time response curves showing absolute changes (mean±SEM) in maximum change in traspulmonary pressure for total and lower airways in the absence and presence of atropine sulphate administered I.M. before 45 minutes before antigenic challenge.

It is important to note that the data in the literature are generally normalized and expressed as per cent changes in the baseline. If the same approach was to be adopted instead of absolute values, as expressed in this study, then one would only note the post challenge results and this might conclude that pulmonary mechanics did not alter after atropine treatment.

Atropine has frequently been employed in the management of equine COPD. Administered either parentally or as an aerosol, atropine decreases W_b and max Δ P_{pl} in horses (Muylle and Oyaert , 1973; Murphy *et al.*, 1980). The contradiction between the findings of the resent study and those of above workers may be due to the fact that in affected horses, pre-atropine base line values were very high. For example, Murphy *et al.* (1980) used horses with max. v.P_{pl} of approximately 18 to 20 mmKg while the values for W_b in horsed used by Muylle and Oyaert (1973) were 10.99±4.93 KgCm/L as opposed to a mean max. ΔP_{pl} and W_b of 8.5 CmH_2O and 4.2 KgCM/L respectively in this study. With higher resting tone, atropine may act as functional antagonist to pharamcological mediators (Hana, 1979). It is, therefore, possible that the beneficial effect of atropine in COPD horses may be due to this property.

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