

Short Communications

Inhalation with NDS27 attenuates pulmonary neutrophilic inflammation in recurrent airway obstruction

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RECURRENT airway obstruction (RAO) or heaves is a chronic respiratory disease that affects mature horses. It is characterised by neutrophilic inflammation and delayed neutrophilic apoptosis (Turlej and others 2001) leading to lower airway inflammation, bronchoconstriction and mucus accumulation. Curcumin has been demonstrated to have a potent inhibitory effect on neutrophilic migration and myeloperoxidase (MPO) release and to induce neutrophilic apoptosis (Hu and others 2005). However, due to its low bioavailability and solubility, its clinical use is limited (Anand and others 2007). The aim of the study was to test the effects of inhalation with the modified highly soluble curcumin derivative NDS27 (World Intellectual Property Organization 2009), on clinical signs, broncho-alveolar lavage fluids (BALF) cytology, and lung function in RAO-affected horses.

Five RAO-susceptible horses from the University of Liege's research horses' herd were used for this blinded randomised cross-over study. The study was approved by the Committee for the Use of Animals of the University of Liege (approval number 611). Horses were kept on low dust wood shavings and were fed with haylage before the experiments and during the two-month wash-out period. Clinical exacerbation of RAO was induced by exposure to dusty hay in all five horses on two different occasions, two months apart. Environmental conditions were maintained during the whole treatment period. All measurements were performed before (T1) and after a treatment period of seven days (T7) and consisted of clinical examination with RAO score determination (Rush and others 1998), endoscopy with determination of tracheal mucus score (Gerber and others 2004), respiratory mechanics by impulse oscillometry (IOS) (van Erck and others 2004), arterial blood gas analysis, and bronchoalveolar lavages. Total nucleated cell count and relative neutrophil counts (percentage) were immediately determined from BALF. BALF samples were then centrifuged and supernatants were frozen for later analysis. MPO

concentrations in BALF samples were measured by an equine-specific ELISA (Bioptis) according to Franck and others (2005). For the first treatment period, horses were randomly assigned to either the treatment group (n=3), which received a twice-daily inhalation (MedicAid, Pagham, UK) of 100 mg of NDS27 (corresponding to 2 mg of pure curcumin), or control group (n=2), which received a twice-daily inhalation of 100 mg of the excipient hydroxypropyl- β -cyclodextrine alone. After a remission period of two months, horses were re-exposed to dusty hay and administered the opposite treatment. Each variable was analysed separately with a two-way analysis of variance (treatment x time) with repeated measurements ($P < 0.05$). A Tukey post-test was used to compare mean values between the treated group and the control group at each treatment time (OpenStat; Miller). Non-parametric variables (RAO and tracheal scores) were compared by Wilcoxon test. All values are expressed as mean (se).

Successful RAO exacerbation was confirmed in all horses after exposure periods of seven to 52 days by clinical signs and by at least four out of five of the following measurements at T1: R_{5Hz} more than 0.10 kPa; R_{5Hz} more than R_{10Hz} ; negative values of X_{rs} between 5 and 20 Hz of IOS measurements; BALF relative neutrophil count more than 25 per cent; and partial pressure of oxygen (PaO_2) less than 90 mmHg.

Significant interactions (treatment x time) were observed for the variations of the mean total nucleated cells and the MPO. The mean (se) total nucleated cell count in the control group was 134.8 (40.2) $\times 10^5$ /mL at T1 and 139.4 (40.5) $\times 10^5$ /mL at T7. Seven days of NDS27 treatment led to significant reduction of mean total nucleated cell count from 156.6 (29) $\times 10^5$ /mL to 77.6 (13.5) $\times 10^5$ /mL in the treated group. At T7, mean total nucleated cell count was significantly lower in the treated group than in the control group. At T1, the mean relative BALF neutrophil count was 42 (6) per cent in the treated group and 39 (8) per cent in the control group. After six days of NDS27 treatment, mean relative neutrophil count was lower in the treated group (25.6 [8.3] per cent) than in the control group (45.8 [11] per cent). MPO concentrations were significantly lower at T7 in the treated group (94 [13.1] ng/ml) than in the control group (764 [456.8] ng/ml), while the two groups were not significantly different from each other at T1. Results are displayed in Table 1. Treatment with NDS27 induced improvement of clinical RAO scores in treated horses. Mean RAO score was 5.7 (0.75) at T1 and 3.9 (0.75) at T7 in the treated group, and 5.2 (0.75) at T1 and 5.5 (0.75) at T7 in controls. However, this observation was not statistically significant ($P = 0.0625$). Mean values for IOS measurements, partial pressure of oxygen and carbon dioxide in the arterial blood and tracheal secretion scores were not significantly modified by the treatment.

Treatment with NDS27 for one week induced a reduction in BALF neutrophil counts in RAO-affected horses. This is probably mediated by various proapoptotic mechanisms attributed to curcumin, such as the activation of p38 mitogen-activated protein kinase and the increase in caspase-3 activity (Hu and others 2005), or the inhibition of the transcription factor NF- κ B (Ward and others 1999). Furthermore, the MPO concentration was lower in the BALF of treated horses when compared with those treated with the excipient (control) alone. Art and others (2006) showed that the measurement of MPO in BALF is a good marker for the presence and activation of neutrophils in the lower airways of RAO-affected horses. The inhibitory effects of curcumin on neutrophilic MPO release have been described in various animal models of lung inflammation (Punithavathi and others 2003, Suzuki and others 2009, Bansal and Chhibber 2010) as well as on the ex vivo degranulation of equine neutrophils (Franck and others 2008).

Horses were maintained in dusty hay conditions during the whole treatment period, which might have partly obscured the results. Couetil and others (2005) tested various routes of glucocorticoid treatment in RAO-affected horses and underlined the importance of environmental control. They demonstrated that neither a single injection

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TABLE 1: Total nucleated cell count, relative neutrophil count and myeloperoxidase (MPO) concentration in the bronchoalveolar lavage fluid (BALF) of five horses with recurrent airway obstruction exacerbation, before (T1) and after (T7) treatment with NDS27 (treatment) or cyclodextrine (control)*L

Group	Treated horses (n=5)			Control horses (n=5)			P at T7 (treated v controls)
	T1	T7	P (T1 v T7)	T1	T7	P (T1 v T7)	
Total nucleated cell count	156.2 (29)	77.6 (13.5)	0.003	134.8 (40.2)	139.4 (40.5)	0.95	0.008
Relative neutrophil count (%)	39 (8.4)	25.6 (8.3)	0.27	42 (6)	45.8 (11)	0.92	0.097
MPO concentration in BALF (ng/ml)	305 (134)	94 (13.1)	0.21	520.8 (334.2)	764 (456.8)	0.15	0.005

* Statistical analysis by Tukey post-test. Significantly different values (P < 0.05) in bold. All values expressed as mean (se)

of dexamethasone, nor two weeks of beclomethasone inhalation had an effect on BALF cytology or NF- κ B and AP-1 activity when horses were kept continuously in dusty environment.

The development of non-toxic neutrophil apoptosis regulating molecules may provide a novel therapeutic strategy for the treatment of RAO in horses (Cunningham and Dunkel 2008). The results of the present study underline the potential effect of NDS27 to regulate neutrophilic inflammation in the lower airways of RAO-affected horses.

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