Study of the effect of Bronchipret on the lung function of five Austrian saddle horses suffering recurrent airway obstruction (heaves)

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The effects of an oral preparation containing an extract of thyme and primula (Bronchipret; Bionorica) on the lung function of five horses suffering heaves were determined in a longitudinal study. The horses accepted the product well. The plasma concentrations of the marker substance, thymol, indicated that at least one of the substances in the extract had been absorbed from the gastrointestinal tract. The compliance, pulmonary pressure and airway resistance of the horses' lungs were all significantly improved after one month of treatment. However, the severity of their clinical signs and their arterial oxygen partial pressure had not improved significantly.

RECURRENT airway obstruction or heaves is a common pulmonary disorder of middle-aged stabled horses (Robinson and others 2001). Dust, moulds and spores from poor quality hay and straw bedding are the main environmental factors that trigger the clinical signs of the syndrome. Most horses in Austria are kept in livery stables and it is difficult to provide conditions that suit every horse and owner. As a result, other strategies for treating the condition must be found. At present, despite its potential side effects, corticosteroid therapy appears to be the best choice for the long-term treatment of severe cases (Robinson and others 2001); it would therefore be helpful to discover new active substances. Edelman and others (2000) reported the clinical efficacy of an orally administered leukotriene inhibitor in children with asthma, but Stark and others (2003) have shown that it has little activity in horses, probably owing to the poor oral bioavailability of the tablets. Nevertheless, some of the results of that study and some findings by Marr and others (1998) suggest that leukotriene inhibitors could be of potential therapeutic value in the treatment of heaves.

Bronchipret (Bionorica), a herbal product containing extracts of *Thymus vulgaris* and *Primula veris*, is a popular medicine for the treatment of human bronchitis. Thymol is the main monoterpene component of thyme oil, others being carvacrol and 1,8-cineol; the last is also known to be the major component of eucalyptus oil. Thymol is quickly absorbed from the human gastrointestinal tract and is excreted unchanged via the lungs by exhalation and in the urine. The primula root extract contains large quantities of saponins, which are probably not absorbed.

Strong evidence for the clinical efficacy of Bronchipret in controlling bronchitis was obtained in a matched-pair comparison of 7783 patients (Ernst and others 1997). Pharmacological studies have shown that thymol, one of the major ingredients of Bronchipret, has bronchospasmolytic and anti-inflammatory activities (Morgenstern 1998, Meister and others 1999), which possibly include the inhibition of leukotrienes. Moreover, the thyme and primula extracts have some mucolytic, antioxidant, antiviral and antibacterial activity (Leslie 1978, May and Willuhn 1978, Haen 1996). The expectorant and secretolytic activities of thymol and the primula saponins are probably the results of gastromucosal vagal reflex activity and the direct effect of thymol on the bronchial mucosa (Leslie 1978).

The purpose of this pilot trial was to investigate the pulmonary respiratory parameters of horses with recurrent airway obstruction after treatment with Bronchipret and to assess its oral tolerance.

MATERIALS AND METHODS

Bronchipret film-coated tablets containing 160 mg dried extract of *T vulgaris* and 60 mg dried *P veris* root extract per tablet were used. Five warmblood saddle horses, aged between 12 and 24 years, with a confirmed history of recurrent airway obstruction were given 15 tablets twice daily in their feed. The horses were individually stabled, but shared a common airspace. They were fed 8 kg poor quality pasture hay once a day and 2 kg oats/muesli mix twice a day, and they were bedded on average quality straw. They had self-drinkers delivering tap water. The stables were lit by daylight or artificial light, depending on the time of the day, and at night the lights were out. No special management changes were made to improve the stable environment before or during the trial. The study was a longitudinal self-controlled study. The horses' clinical signs and pulmonary function were measured before and after treatment with Bronchipret tablets for a month. Their pulmonary function was tested basically according to the methods described by Sasse (1971), but modified so that intrapleural pressure was measured by using an oesophageal balloon sealed over the distal end of a polypropylene catheter which was passed into the thoracic part of the oesophagus. The catheter was attached to a pressure transducer. Respiratory flow was measured with a Fleisch No 5 transducer attached to a face mask. Airflow and pleural pressure during breathing were processed by a lung function computer program (Buxco-Biosystems XA for Windows) to calculate tidal volume, pulmonary resistance, dynamic compliance and maximal intrapleural pressure. Blood samples taken from the carotid artery were analysed for blood gases immediately after collection. Bronchoalveolar lavage fluid (BALF) was collected according to the methods of Viel (1980), and analysed cytologically on four slides per sample. The percentages of granulocytes and mononuclear cells were counted, and the total impression of visual microscopic abnormalities in the samples was graded on a scale from 0 to 3. The lung auscultation results, the degree of dyspnoea, coughing, the size of the lung percussion field, and nasal discharge were similarly graded on a scale from 0 to 3. A composite clinical score was calculated as the sum of the scores for dyspnoea, coughing, nasal discharge and auscultation. Red and white cell parameters were determined with an automatic cell analyser (ADVIA 120; Bayer). Plasma biochemical constituents were measured with a Hitachi 911 (Roche Diagnostics).

Determination of the plasma thymol concentrations

The plasma thymol concentrations were determined by means of a newly developed method using solid-phase micro-

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extraction (SPME) and gas chromatography/mass spectrometry (GC/MS). On the basis of human data it was considered that sampling over eight hours should provide adequate data for the pharmakokinetic analysis. Blood samples were taken immediately before and one, two, three, four, five, six, seven and eight hours after the first oral dose, and centrifuged at 11,000 *g* for 10 minutes; the plasma was decanted and samples of 1000 µl were each stabilised with 40 µl 0·58M acetic acid (Riedel-de Haen) and stored at –20°C until assayed.

For analysis, the plasma samples were thawed and 40 µl 0·58M acetic acid (Riedel-de Haen) and 100 µl glucuronidase (Sigma) were added. The mixture was incubated at 37°C for 30 minutes in a waterbath and 50 µl of internal standard solution (40 mg o-cresol [Sigma] + 2 ml methanol made to 100 ml with water and diluted 1:10 with water) was added. This mixture was added to a 10 ml vial containing 1·0 g sodium chloride (Suprapur; Merck) and 100 µl 85 per cent phosphoric acid (Merck), and a cylindrical magnetic stirring bar was added. For the SPME the vial was sealed with a PTFE/butyl septum for headspace analysis and a crimp cap (Agilent), and the septum was perforated with a needle in order to insert a crimped 65 µm polydimethylsiloxane-divinylbenzene (PDMS-DVB) fibre (Supelco). While the fibre was in the headspace of the sample, the vial was incubated on a magnetic mixer/heater at 80°C for 35 minutes at 1500 *g*. The PDMS-DVB fibre was then put into the injector of the GC/MS and desorbed for 5 minutes.

The GC/MS analyses were made on an HP 6890 coupled with an HP 5972 MSD (Hewlett-Packard) and fitted with an HP 30 m x 0·25 mm capillary column coated with HP-5MS (0·25 µm film thickness; Hewlett-Packard). The analytical conditions were as follows: carrier gas, helium; liner, 0·75 mm internal diameter (Supelco), injector temperature 250°C; split ratio: 1:1; temperature programme, 60°C for 5 minutes; 60°C to 120°C at a rate of 4°C per minute and 120°C to 240°C at a rate of 20°C per minute. The components were identified by comparing their retention indices and mass spectra. Each sample was analysed in duplicate.

The analyses were calibrated by examining blank plasma samples from blood samples taken before the administration of Bronchipret to which thymol had been added to give concentrations of 25·0, 50·0, 100·0, 200·0, 250·0 and 400·0 ng/ml.

Clinical parameters

Before and after the treatment seven measurements on each horse were selected over a 10-minute recording period. Only suitable data recorded at respiratory frequencies between 10 and 36 per minute were used. The data were analysed by a two-factorial analysis of variance for repeated measurements, and differences between the treatments were analysed by a *t* test. The semi-quantitative data from the analysis of BALF and the clinical scoring were analysed by Wilcoxon's rank test.

RESULTS

The horses all accepted the tablets well, and consumed them within minutes of their being put on top of the oats/muesli mix. Thymol was considered as an indicator of the pharmaceutical activity of the mixture of volatile terpenes, saponins and flavonoids. The plasma thymol concentrations differed considerably among the horses, and most of them still had measurable plasma thymol concentrations after eight hours (Fig 1). The concentration-time curves were unsuitable for reliable pharmacokinetic calculations owing to the unsuitable choice of sampling times. In two of the horses it was estimated that the mean residence time of a thymol molecule was about four hours.

The effects of the treatment on the horses' pulmonary functions are summarised in Table 1. All the lung function parameters, except arterial oxygen partial pressure (pO_2), had

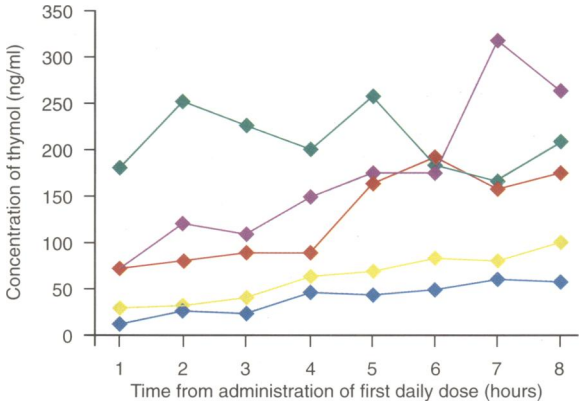

FIG 1: Plasma concentrations of thymol in five horses given 15 Bronchipret tablets (equivalent to 2·4 g of thyme extract) orally, at intervals after the dose

improved signficantly after the month of treatment. There was a significant interaction between horses and treatment ($P<0·01$), some horses having improved more than others.

The effect of the treatment on the cytology of BALF was not significant (Tables 1 and 2). The clinical signs such as cough, nasal discharge, nasal flaring, size of the lung percussion field and character of lung auscultation were nearly all scored between 2 (moderate) and 3 (severe), and were not clearly improved by the treatment, although the composite clinical score based on dyspnoea, nasal discharge, cough and auscultation tended to have improved ($P=0·06$). Neither the endoscopic appearance of the trachea nor the arterial PO_2 were significantly changed by the treatment (Table 2).

None of the haematological or blood biochemical measurements were outside the normal ranges for horses.

DISCUSSION

Although medicinal herbs and plant-based remedies are popular among horse owners and horse healers, most of the veterinary community have a sceptical attitude towards phytotherapy because most label claims of herbal preparations are not supported by sound scientific studies. One problem with herbal remedies is that many are extracts of medicinal herbs which contain a variety of chemically related and unrelated, potentially pharmacologically active, substances in

TABLE 1: Mean (sd) effect of Bronchipret on the pulmonary function parameters and semi-quantitative cytology of bronchoalveolar lavage fluid of five horses with heaves

Parameter	Before treatment	After treatment	P
ΔPpl_{max} (cmH_2O)	17·1 (7·8)	11·7 (5·2)	<0·01
C_{dyn} (cmH_2O/litre)	1·02 (0·36)	1·53 (0·85)	<0·01
R_L (cmH_2O/litre/second)	1·17 (0·57)	0·72 (0·30)	<0·01
pO_2 (mmHg)	87·2 (14·2)	86·6 (7·0)	NS
Granulocytes (median % [range])	30 (10-90)	80 (70-90)	NS
Mononuclear cells (median % [range])	50 (10-98)	20 (2-50)	NS

ΔPpl_{max} Maximal intrapleural pressure, C_{dyn} Dynamic compliance, R_L Pulmonary resistance, pO_2 Arterial oxygen partial pressure, NS Not significant

TABLE 2: Median (range) of the clinical scores of five horses with heaves before and after treatment with Bronchipret

	Before treatment	After treatment
Composite clinical score	8 (3-10)	7 (3-10)
Cytology score	3 –	3 (2-3)
Endoscopy score	3 (2-3)	3 (2-3)

varying proportions and quantities. Often these are essential volatile oils and flavonoids, for example, the apigenin, cirsilineol, thymonin and primulagenin A in Bronchipret. Unless they can be standardised the pharmacological effect of these preparations cannot be assessed reliably.

However, new botanical culture techniques for medicinal herbs and the standardisation of extraction techniques have resulted in pharmaceutically homogeneous products, which can be better characterised in terms of their pharmacological effects. One such product is Bronchipret, which contains an extract from carefully cultured thyme and primula root. Because thymol is the major component of the thyme extract, it was considered to be a marker of the product's overall pharmaceutical activity. Meister and others (1999) showed the pharmacological effects of an extract of thyme on the guinea-pig trachea. After pretreatment with prostaglandin $F_{2\alpha}$, histamine, barium chloride or carbachol, the extract inhibited the contraction of the tracheal muscle by 89, 73, 49 and 42 per cent, respectively, Morgenstern (1998) demonstrated the anti-inflammatory properties of Bronchipret in a rat paw oedema model. Ernst and others (1997) showed that it had clinical effects on human bronchitis comparable to those of synthetic drugs. Unfortunately, that study could not be blinded, so the patients could have been biased towards the herbal remedy. Nevertheless, these observations provide sufficient evidence to suggest that Bronchipret might be useful for treating horses suffering recurrent airway obstruction.

The horses described here had had the condition for a long time and were stabled under poor environmental conditions before and during the trial. The dose administered was based on the dose used for human beings, and the duration of treatment was based on the treatment of the condition with other drugs. After the treatment, there was some improvement in the pulmonary function of all five horses, suggesting a reduction of airway spasm. However, the clinical signs, such as the results of lung auscultation and the size of the lung percussion field, coughing, nasal discharge and the endoscopic appearance of the trachea, were not significantly improved, possibly owing to the inevitably subjective interpretation of the severity of these signs. The cytological analysis of BALF did not demonstrate a clear anti-inflammatory effect of Bronchipret on the influx of granulocytes into the airways, but it is possible that the method is not sensitive enough to pick up subtle changes. On the other hand, the poor environmental conditions for the horses were deliberately left unimproved, and it is thefore likely that the continuous inhalation of allergens kept irritating their airways. Under these conditions, it is still possible that the release by the infiltrating inflammatory cells of inflammatory mediators with bronchospasmic properties, such as leukotrienes, were inhibited by Bronchipret.

The concentration-time curves of plasma thymol were not adequate for reliable pharmacokinetic calculations owing to the poor choice of the sampling times, which was based on human data. The maximum concentrations of thymol were reached between five and seven hours after the administration of Bronchipret and the concentration had not decreased by much eight hours after the first oral dose, when the last blood sample was taken. The maximum plasma thymol concentration ranged from 62·4 to 315·9 ng/ml in the five horses. However, the curves suggested that a twice daily treatment regimen probably results in measurable plasma levels for 24 hours.

Despite the small number of horses, and the fact that the study design did not include blinding and cross-over procedures, the authors consider that the statistically significant improvement in lung function parameters was caused primarily by the mixed pharmacological activities of the active substances in Bronchipret on the horses' airways. These initial results support further clinical investigations of the product.

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Notes