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Histological and biomechanical effects of palatal sclerotherapy in the horse using sodium tetradecyl sulfate

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ABSTRACT

Palatal sclerotherapy using sodium tetradecyl sulfate has been suggested as a treatment for dorsal displacement of the soft palate in young Standardbred horses. The present study evaluated histological and biomechanical changes in the equine soft palate following trans-endoscopic treatment with a low dose of this compound. Two horses were euthanased and examined at 2 weeks and at 1, 2, 4 and 6 months post-sclerotherapy, while two further horses served as untreated controls. The technique was easily performed in all cases without major complications. On histological examination there was no evidence of palatal necrosis, inflammation or fibrosis in any of the treated or control animals. There was no variation in the density of palatal connective tissue between individuals, and on biomechanical assessment no significant difference in the stiffness of the palatal tissue was found between treated and control horses at any time. The lower dose of sodium tetradecyl sulfate used in this study relative to previous reports, might explain the absence of tissue alterations. This method of sclerotherapy did not alter the morphology or biomechanical properties of normal equine soft palates.

Introduction

Dorsal displacement of the soft palate (DDSP), a common cause of upper airway obstruction in racehorses, leads to exercise intolerance and poor performance (Franklin et al., 2002; Parente et al., 2002; Franklin et al., 2004). The clinical signs of DDSP normally appear during strenuous exercise and are characterised by a fluttering expiratory noise (Rehder et al., 1995; Holcombe et al., 1998; Parente et al., 2002). While the aetiology and pathophysiology of the disorder are unclear, the pathogenesis may involve neuromuscular dysfunction leading to flaccidity of the soft palate (Blythe et al., 1983; Holcombe et al., 1999).

Retrospective studies of surgical and medical treatments of DDSP report success rates of 60–75% (Anderson et al., 1995; Duncan, 1997; Llewellyn and Petrowitz, 1997; Tulleners et al., 1997; Harrison and Raker, 1998; Bonen Clark et al., 1999; Barakzai and Dixon, 2005). A 100% success rate in six symptomatic horses using systemic corticosteroids and rest has been reported (Parente et al., 2002), however, the number of horses involved in this work was low and the report has not been substantiated by controlled studies. Recently a new surgical technique termed 'laryngeal tie-forward' was reported to improve performance in 80–82% of cases of DDSP (Woodie et al., 2005). The wide variety of methods previously employed to treat DDSP most likely reflects a multifactorial aetiology.

Microscopically, the normal soft palate is lined dorsally by a nasopharyngeal mucosa composed of a pseudostratified columnar ciliated epithelium and ventrally by oral mucosa composed of a non-keratinised stratified squamous epithelium that periodically projects into the submucosa (Poyrazoglu et al., 2006). The nasopharyngeal submucosa consists of elastin-rich connective tissue among which lymphoid follicles, lymphocytes and mucous glands are interspersed, and the oral submucosa is composed of loose connective tissue and a small lymphoid and glandular compartment. The submucosae converge at a central stalk of skeletal (*palatinus*) muscle around which variably dense bundles of elastin fibres are scattered.

In a reproducible canine model developed to assess new techniques for treating palatal flutter snoring, palatal sclerotherapy was found to stiffen the soft palate while diminishing snoring (Lafrentz et al., 2003). In humans, palatal sclerotherapy (also known as 'injection snoreplasty') using 3% sodium tetradecyl sulfate is a simple, safe and effective treatment for primary snoring. Advantages

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over other procedures include simplicity, low cost, decreased post-treatment pain and minimal to no required time for convalescence (Brietzke and Mair, 2001). Poly-L-lactic acid (Cehak et al., 2006) or 3% sodium tetradecyl sulfate, as used in humans and in canine experimental models (Marcoux et al., 2008), have been used in equine palatal sclerotherapy. In the latter study in eight juvenile Standardbred racehorses affected with DDSP, six showed improvement in racing performance while respiratory noise disappeared in seven of the animals (Marcoux et al., 2008).

The aim of the present study was to evaluate any histological and biomechanical changes in the equine soft palate following palatal sclerotherapy using a relatively low dose sodium tetradecyl injection treatment.

Material and methods

Animal selection

Nine French Trotters and three Selle Français (six geldings and six mares), aged 2–12 years and weighing 400–550 kg were used in the study. On clinical and endoscopic examination, performed at rest, the upper respiratory tracts were normal. Horses were maintained in paddocks and fed hay throughout the study. Two Selle Français horses, aged 11 and 12 years of age, which had not been clinically or endoscopically evaluated, and which were being sent for slaughter, served as controls.

The experimental protocol was reviewed and approved by the Animal Care Committee of the Ecole Nationale Vétérinaire de Lyon, following directive 86/609 of the European Community Council and following guidelines published by the Laboratory Animal Resources Commission on Life Sciences National Research Council, Washington DC, USA.

Sclerotherapy procedure

Palatal sclerotherapy was performed as previously described (Marcoux et al., 2008), with the horse standing in stocks and sedated with 80–120 μg/kg of romifidine chlorhydrate (Sedivet, Boehringer Ingelheim) and, when necessary, 0.01 mg/kg of butorphanol (Torbugesic, Fort Dodge) was given IV. A nose twitch was applied throughout the procedure.

The metal portion of a 20 gauge hypodermic needle was attached and glued to a semi-rigid plastic tube which was passed down the instrument channel of a 10 mm video endoscope until the needle tip was positioned 2 mm beyond the end of the channel. The nasal and pharyngeal mucous membranes were then anaesthetised by the topical application of 40 mL of 2% lidocaine chlorhydrate (Xylovet, Ceva) through the accessory canal of the endoscope. Before injection of the sodium tetradecyl sulfate and in order to visualise the distal border of the soft palate, dorsal displacement of the free distal margin was achieved with a blunt-end hook inserted through the contra-lateral nostril. Sodium tetradecyl sulfate injections were directed into the submucosa of the distal two-thirds of the soft palate with each horse receiving 10 injections of 0.5 mL (Trombovar 3%, Innothera Laboratoires). The distribution of the injections was as illustrated in Fig. 1. The time taken to perform the technique was recorded and immediately following the procedure each animal received an 4.4 mg/kg IV injection of phenylbutazone (Butasyl, Fort Dodge), with a further 2.2 mg/kg of phenylbutazone administered twice daily per os (Equipalazone, Intervet) for the following 4 days.

Clinical and endoscopic evaluation

Clinical examinations were performed on each horse daily prior to, and for 15 days following sclerotherapy. Endoscopic examination was carried out on each animal just before the procedure (D0), at 2 (D2), 7 (D7), 14 (D14) days after the treatment, and on the day prior to euthanasia (D > 14). The macroscopic appearance of the soft palate, including any evidence of nodular pharyngitis (graded from 0 to IV), was noted, along with the presence, quantity and quality of pharyngeal and tracheal secretions. If DDSP was induced, the time required for spontaneous correction was measured. The tone of the soft palate, determined by palatal movement during a nasal occlusion test and by resistance to traction of the soft palate with the blunt-end hook, was the consensus assessment of two of the authors (VP and JM). The tone of the soft palate was described as normal or flaccid while the resistance to traction was graded at one of four levels (++, +, +/− and −).

Soft palate sampling

Horses were randomly assigned to five groups of two animals each (A, B, C, D and E) and these groups were sequentially euthanased at 2 weeks and at 1, 2, 4 or 6 months following sclerotherapy. Premedication consisted of 0.04 mg/kg, of acepromazine IV (Vetranquil, Ceva,) while euthanasia was performed via administration of 100 mL of a mix of embutramide, mebezonium and tetracaine IV (T 61, Intervet). After death was confirmed, the head was removed at the atlanto-occipital joint and held in a vertical position for 5 min to facilitate bleeding out.

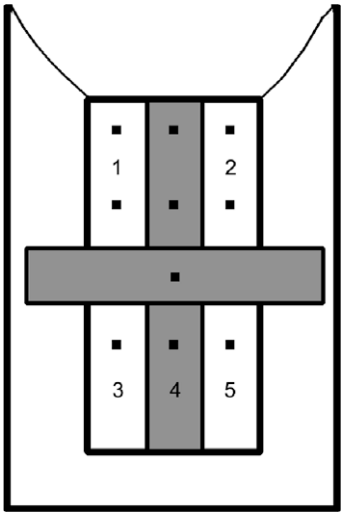

Fig. 1. Diagram representing the caudal (upper portion) and nasal portions of the equine soft palate. The black squares represent the points of injection of 3% sodium tetradecyl sulfate which diffused outwards for approximately 1 cm^2. The strips shaded grey represent the location of samples taken for histological examination. The strips labelled 1, 2, 3, 4 and 5 represent the samples taken for biomechanical evaluation. In the case of strips 1 and 3, the sample consisted of only the nasal mucous membrane.

The cheeks and masseter muscles were longitudinally incised and osteotomy of both mandibular rami was performed. With the head placed upside-down, the mandible was retracted caudally by incising the lateral walls of the oropharynx and the stylohyoid bones. The exposed soft palate was transected at its junction with the hard palate as far back as the palatopharyngeal arch. The soft palates were collected in a similar way from the two control slaughterhouse animals that were assigned to group F.

Each soft palate was evaluated macroscopically and its thickness measured using callipers at the medial free border as well as at the midpoint, 5 cm rostral to the free border. The soft palate was then placed on a polystyrene pad to which it was fixed with 24 gauge needles. Two tissue strips for histological evaluation were dissected from each palate, the first measuring 5 cm by 1 cm and the second 3 cm by 1 cm (Fig. 1). These samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. A further five tissue strips measuring 3 cm by 1 cm were taken for biomechanical evaluation (Fig. 1). These samples were snap-frozen in liquid nitrogen, stored at −80 °C and thawed in water at 37 °C immediately prior to evaluation (Paul et al., 1997; Krag, 1998; Chan, 2003). Tissue strips 1 and 3 as indicated in Fig. 1 consisted of nasal mucous membrane only.

Histological evaluation

Samples of formalin-fixed, paraffin-embedded soft palate were cut 3–5 μm thick and stained by the haematoxylin and eosin method to assess tissue morphology and by the Van Gieson method to assess the abundance of elastin and collagen fibres. The slides were evaluated qualitatively and semi-quantitatively using a scale from 1 to 5 (1, minimal; 2, slight; 3, moderate; 4, marked; and 5, severe) (Shackelford et al., 2002; Poyrazoglu et al., 2006; Richardson et al., 2006). The following criteria in particular were assessed: alterations to the epithelia of the oral and nasopharyngeal mucosae; the number and size of glandular acini, lymphoid follicles or elastin fibre bundles; tissue degeneration or necrosis; tissue inflammation and fibrosis; the connective tissue density.

Biomechanical evaluation

Biomechanical evaluation was performed using a model 5544 single column 2 kN materials testing system (Instron, Canton), equipped with pneumatic side action 2 kN grips (model number 2712-016, Instron). The thickness and width of the samples were measured in mm using electronic callipers. Samples were oriented longitudinally and were securely placed in the grips. The required force on the grips (10 PSI for mucosa samples, 30 PSI for full-thickness samples) had previously been determined by testing three necropsy specimens. The jaws were moved apart at a rate of 10 mm/s (Viola et al., 1992). The data were recorded by Bluehill Materials Testing software (Instron) and scanned into a personal computer (GX270, Dell). The slope of the load deformation curve (i.e., the stiffness), tensile stress, and tensile strain, were normalised to the cross-sectional area of the specimen prior to loading.

Statistical analysis

A mixed linear model, with time (at four levels) as a between-subject factor and patient identification within each time level as a random factor, was used to determine the effects of treatment on the stiffness of the palatal tissue samples. When this statistical model indicated an overall significant difference, a priori contrasts were used to compare the individual means obtained at each post-treatment time to pre-treatment values. All analyses were carried out with a P value < 0.05, using SAS version 9.1.

Results

Sclerotherapy technique

The technique was easily performed in all cases. Chemical restraint in combination with local anaesthesia and the use of a nose twitch resulted in satisfactory horse compliance.

The duration of the procedure ranged from 8 to 32 min with a mean of 17.6 min.

Immediately following injection of the sodium tetradecyl sulfate, visible deformation of the tissue with 'bubble' formation was observed at 54 of the 100 injection sites. Four horses bled slightly at one or two injection sites while four further animals bled at more than two positions. Leakage of injected material was observed during ten injections, though never more than twice for any given animal. Bleeding from the ventral nasal conchae occurred in one horse due to mucosal laceration by the sclerotherapy needle during insertion of the endoscope. Once the haemorrhage stopped, sclerotherapy was performed successfully. In the 14 days following the procedure, palpation of the external throat region in treated animals did not appear painful and no coughing or dysphagia was observed. All horses presented slight enlargement of their submandibular lymph nodes from the 4th to the 14th day post-treatment. A slight serous to seromucinous nasal discharge was present in all animals throughout the 14 day post-treatment period.

The findings of post-sclerotherapy endoscopic examination are summarised in Table 1. Endoscopic examinations performed at D0, D2, D7 and D14 revealed soft palate congestion and oedema in zero, eight, four and three animals, respectively. Degrees of nodular pharyngitis between grade 0 and III were noted. Soft palate tone was considered flaccid in five horses at D0 and in three horses at D2 but normal in all horses for the remainder of the observation period. Resistance to traction varied between horses. The endoscopic appearance of the injection sites is summarised in Table 2. Endoscopic examination was not performed on the control (group F) horses.

Post-mortem macroscopic examination of the soft palates revealed petechiae which appeared to be an agonal change, associated with euthanasia as these were not observed on endoscopy prior to euthanasia. One group E horse had a centrally-positioned 2 mm diameter hard nodule located 3 cm from the free border of the soft palate. At the same location, one group A and one group B horse exhibited submucosal haematomas. All remaining soft palates appeared normal. The mean thickness of the soft palate at its midpoint, 5 cm from the free border, was 4.33 ± 0.55 mm. The mean thickness of the free border at its axial point was 0.72 ± 0.07 mm. The mean thickness of tissue samples 4 and 5 as indicated in Fig. 1 was 12.81 ± 0.57 mm and 12.48 ± 0.61 mm, respectively. The thickness of samples 4 and 5 varied by 4 mm in four horses, one of which was a control animal, while the remainder of the horses showed less or no variation. No significant difference in thickness was detected between the groups.

Histological evaluation

Qualitative examination revealed that the proportion of each tissue type (glandular, muscular, and connective) was consistent with that expected for the most caudal portion of the soft palate (Poyrazoglu et al., 2006). The glandular, muscular and connective tissue portions represented 5–30%, 25–60% and 25–50% of each tissue section, respectively. Significant necrosis, inflammation or fibrosis was not observed in samples from either treated or control animals. There was no variation between animals in the density of the connective tissue present. One group C horse exhibited slight focus of myofibre necrosis with associated regeneration and a mild focal lymphoid cell infiltrate.

The density and thickness of elastin fibres within connective tissue and muscle bundles varied between animals. The size of subepithelial lymphoid follicles also varied between horses, ranging from 50 to 200 μm in diameter, with larger follicles correlating with greater degrees of lymphocytic infiltration of the adjacent connective tissue and epithelia. The thickness of the nasopharyngeal epithelium was similar in all animals, ranging from 2 to 8 cells deep according to location, with the thinnest portion more caudally located. The thickness of the oral epithelium also varied between horses, ranging from 200 to 500 μm, and was observed to form pseudoepitheliomatous projections into the submucosa in all cases.

All samples had some degree of parakeratotic hyperkeratosis of the oral mucosa while one animal exhibited minimal focal cellular crust formation (Table 3). Group A, E and F horses as well as one group D animal had no (Fig. 2), or minimal (grade 1) increases in the size of their lymphoid follicles and in the attendant lymphocytic infiltration (Fig. 3). However, group B and C horses, along with the remaining group D animal, exhibited larger follicles and lymphocytic infiltrates (grade 2) (Fig. 4).

Biomechanical evaluation

Technical errors occurred during the sampling of the soft palates of four horses from groups A and B so that the biomechanical data obtained from these horses were excluded from the analysis.

When testing the samples in tension, most failures 32/40 (80%) occurred near the grip while a smaller number 8/40 (20%) failed in the mid-portion of the tissue strip. The force applied by the pneumatic grips was lower for the mucosal than for full-thickness sam-

Table 1
Results of endoscopic examination prior to (D0) and 2 (D + 2), 7 (D + 7), 14 (D + 14) and >14 (D > 14) days post-palatal sclerotherapy.

	D0	D + 2	D + 7	D + 14	D > 14
Numbers of horses with soft palate congestion	0	8	4	3	0
Degree of nodular pharyngitis[a]	0–III	0–II	0–II	0–II	0–III
Nature of pharyngeal secretion	Seromucinous	Seromucinous to purulent	Seromucinous	Seromucinous	Seromucinous
Soft palate tone	5/10 flaccid 5/10 normal	3/10 flaccid 7/10 normal	All normal	All normal	All normal
Numbers of horses with various degrees of resistance to traction (score)	3 (+), 2 (−), 5 (±)	8 (+), 2 (±)	6 (+), 4 (±)	6 (+), 4 (±)	4 (+), 1 (−), 3 (±)

[a] Degree of nodular pharyngitis varied from 0 to IV.

Table 2
Results of endoscopic examination of the 10 injection sites per horse 2 (D + 2), 7 (D + 7), 14 (D + 14) and >14 (D > 14) days post-palatal sclerotherapy.

Injection sites	D + 2[a]		D + 7[a]		D + 14[a]		D > 14[b]	
	Median	Total	Median	Total	Median	Total	Median	Total
Visible	3.4	10	3.4	10	3	10	0.75	3
With severe swelling	2	8	0.9	6	0.1	1	0	0
With slight swelling	0.8	4	1.8	9	2.8	9	0.75	3
With necrosis	0.5	3	1.4	8	0.6	5	0	0
With haematoma formation	0.5	4	0	0	0	0	0	0

Total, the total number of horses with visible injection sites.
[a] $n = 10$ horses.
[b] $n = 8$ horses.

Table 3
Grading of the histological findings in the various group of animals post-palatal sclerotherapy.

Histological finding	Group F (controls)		Group A		Group B		Group C		Group D		Group E	
Necrosis and regeneration of myofibres	2											
Increase in size of lymphoid follicles	2	1			3	2	2		2	1	1	
Lymphoid cell infiltration	2	1	1	1	2	1	2	1	2	1	1	
Hyperkeratosis of oral mucosa												1

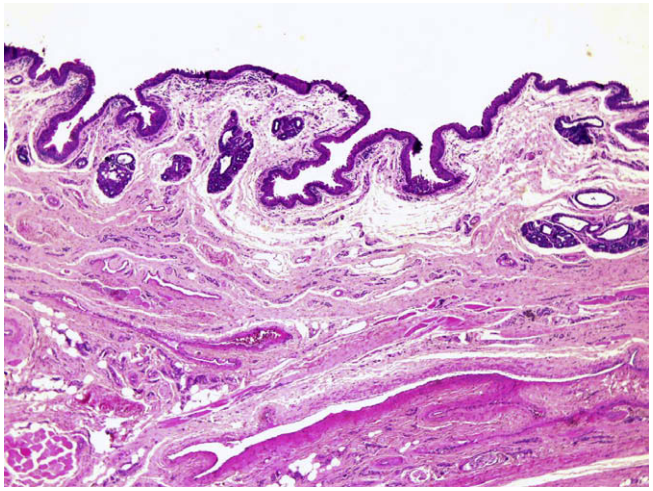

Fig. 2. Photomicrograph illustrating the nasopharyngeal mucosa of the soft palate of one of the horses in group A. Very few lymphocytes and no lymphoid follicles are present in the submucosal loose connective tissue. Haematoxylin and eosin stain. Magnification factor 10×.

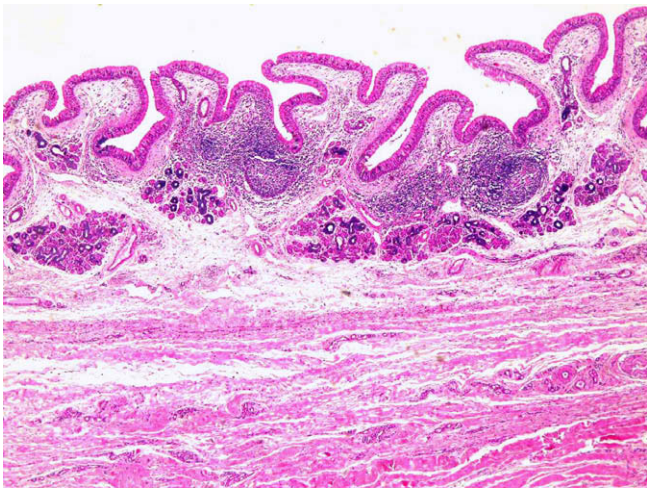

Fig. 3. Photomicrograph illustrating the nasopharyngeal mucosa of the soft palate of one of the horses in group E. There is a minimal (grade 1) number of lymphoid follicles and lymphoid cell infiltration in the submucosal loose connective tissue. Haematoxylin and eosin stain. Magnification factor 10×.

ples in an effort to ensure correct positioning of the specimens yet limiting tissue laceration.

The mean stiffness values for the full-thickness samples are presented in Fig. 5. No significant differences in stiffness, tensile strain or tensile stress were found between the treated and control samples, at any time. Furthermore, no significant change was detected in any of the biomechanical parameters over time in the treated horses. Biomechanical evaluation of the mucosal samples also failed to reveal any significant differences between treated and control samples or between treated samples over time (data not shown).

Discussion

Successful palatal sclerotherapy in humans has been objectively appraised at 75%, a success rate similar to that reported for other treatment methods (Brietzke and Mair, 2004). In a study comparing the effects of cautery and palatal sclerotherapy on palatal stiffness in dogs, sclerotherapy involving injection of sodium tetradecyl sulfate proved 100% efficacious and induced submucosal fibrosis without infection (Lafrentz et al., 2003). Anatomical and pathophysiological differences suggest that the effects of sclerotherapy in humans and dogs should not necessarily be extrapolated to horses. In our study this was illustrated by the fact that the sclerosing agent did not induce submucosal fibrosis in our horses as it did in dogs (Lafrentz et al., 2003). This may have been due to species variation in tissue response or possibly due to differences in the amount of sclerosing agent injected.

Subjective evaluation by trainers or veterinarians indicates a reduction in respiratory noise in 7/8 (87.5%) juvenile Standardbred racehorses treated for DDSP by palatal sclerotherapy (Marcoux et al., 2008). Results of racing performance were available for 7–13 months following this therapy and suggested a success rate of 75%. In these cases, a dose of 1 mL of 3% sodium tetradecyl sulfate was used at each injection site, twice the amount used in the present study. We chose to use 0.5 mL in order to

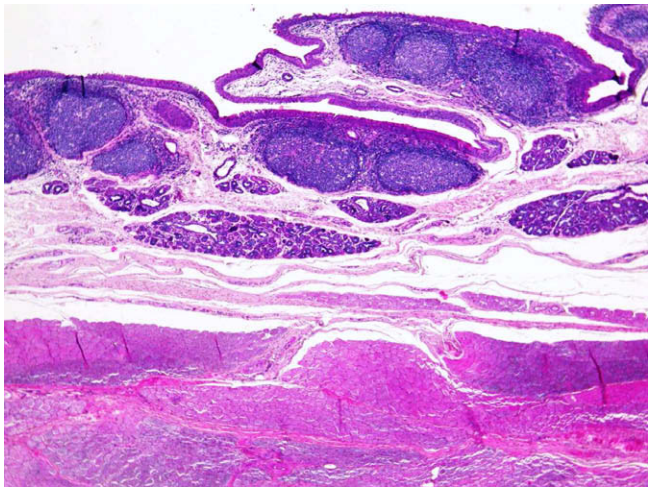

Fig. 4. Photomicrograph illustrating the nasopharyngeal mucosa of the soft palate of one of the horses in group C. There is a slight (grade 2) number of lymphoid follicles and lymphoid cell infiltration in the submucosal loose connective tissue. Haematoxylin and eosin stain. Magnification factor 10×.

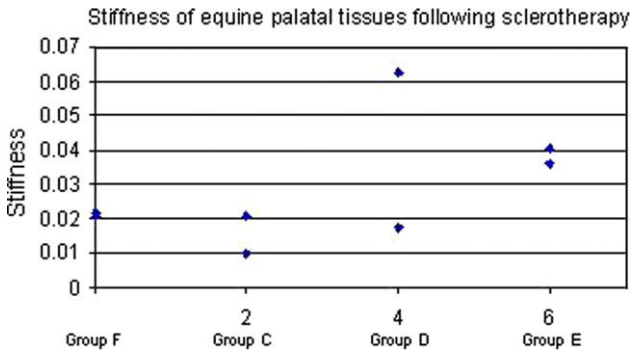

Fig. 5. Mean stiffness values of the full-thickness palatal samples from the various treatment groups post-palatal sclerotherapy. Each point represents the mean value for each horse.

minimise the necrosis and swelling reported by Marcoux et al. (2008). This factor may have been significant in moderating post-injection mucosal swelling and the subsequent lack of fibrosis and increased stiffness. Interestingly, 50% of the dogs in the study by Lafrentz et al. (2003), where a single injection of 2 mL of sclerosing agent was used, required re-injection to attain the desired clinical outcome.

In the current study trans-endoscopic palatal injection was minimally invasive and was easily performed on the standing, sedated horse with few or no side effects. Only one horse developed epistaxis due to laceration with the sclerotherapy needle during insertion of the endoscope. This risk was minimised by adopting the approach described by Marcoux et al. (2008) of ensheathing the needle within the endoscopic canal.

The injection of 3% sodium tetradecyl sulfate induced congestion and oedema observable macroscopically for up to 14 days. Microscopic examination revealed differences between groups A, E and F and groups B and C in terms of lymphoid follicular size and lymphoid cell infiltration. These differences were not age related and may have resulted from previous inflammation unrelated to the sclerotherapy procedure.

A finite element model was recently constructed to predict displacement of the equine palate based on the estimated force of the *tensor veli palatini* and *palatopharyngeus* muscles together with published values of peak inspiratory nasopharyngeal pressure (Lester et al., 2003). This study provided evidence that the coordinated contraction of the palatal musculature is essential in preventing DDSP during exercise. While a biomechanical study has been performed in pigs to compare different surgical techniques used to treat human snoring and mild sleep apnoea (Courey et al., 1999), our study represents the first objective analysis of the effect of sclerotherapy on the biomechanical properties of the equine soft palate.

In the present experiment, the effect of palatal sclerotherapy was evaluated in mucosal as well as in full-thickness samples so as to determine the effects of the intervention on a component of the palate and to maximise the detection of any changes. Full-thickness samples were found to facilitate quantification of palatal stiffness, which may more closely reflect the clinical impact of the procedure. While it is possible that the changes induced by our treatment regime were too subtle to be detected by this methodology, this biomechanical testing system was considered the most appropriate.

No significant differences in stiffness, tensile stress or tensile strain were found between the treated and control palates or over time in treated horses. The biomechanical data from four horses were omitted from the statistical analysis because the palates of these animals were damaged during the sampling procedure at 15 days and 1 month post-treatment. Given that horses treated for DDSP by sclerotherapy return to work between 15 and 30 days post-treatment (Marcoux et al., 2008), the missing data may have revealed some pertinent differences. However the data from later time points should be more relevant clinically since the goal of this therapy is to induce fibrosis and a prolonged effect.

The limited number of horses used in our study (eight following exclusions due to damage during sampling) and the important differences encountered between samples, especially from group D horses, complicate data interpretation. There were variations in stiffness, tensile stress and tensile strain between two samples from the same animal such that the true effect of sclerotherapy was difficult to ascertain. This variation can be attributed to disparity in soft palate size prior to treatment (as in the control group), and to the difficulty in obtaining homogenous samples due to the equine palate's complex anatomy. Variation might also relate to the injection procedure. While it is recommended that the sodium tetradecyl sulfate is injected into the submucosa, it is possible that, under endoscopic guidance alone, some injections were inadvertently delivered into the muscular layer.

It is also important to consider that the tissue response to the sclerotic agent in this study might differ from that of palates with pre-existing inflammation such as those encountered in horses with DDSP (Blythe et al., 1983). The biomechanical properties of the soft palate may be altered by obstructive sleep apnoea syndrome (OSAS) and with age in humans (Veldi et al., 2001). A study using three-dimensional models, found that pharyngeal length increased with age and that increases in soft palate length and thickness and in its contained fat component, were associated with increased body mass index (BMI). Furthermore, as age and BMI increased, airway narrowing appeared more severe (Shigeta et al., 2008). Disorders of lipid metabolism may be present in humans with OSAS and fatty infiltration of the oropharyngeal region might be important in the pathogenesis through altering the size and shape of the airway and the compliance of the pharyngeal wall (Zhou et al., 2003).

In our study, age, gender, BMI and breed were not factored into the statistical analysis since study numbers were small. However age and DDSP in the horse may affect the biomechanical properties of the soft palate. Moreover, it is possible that inflammation resulting from the sclerotherapy injection could reduce the diameter of the intra-pharyngeal ostium which includes the caudal border of the soft palate, and this in turn would allow the latter to remain

lodged under the epiglottis even during intensive exercise. Radiographic assessment of soft palate thickness could be useful in evaluating this parameter (Cehak et al., 2006), and might contribute to a positive clinical response to therapy in the absence of biomechanical measurements. Although treadmill endoscopic examination was not used in this study, this would have been a useful additional technique in evaluating anomalies in the upper respiratory tract.

Conclusions

Palatal sclerotherapy, as carried out in this study, did not alter the morphology or biomechanical properties of normal equine soft palates. As a result, our findings do not support the hypothesis that the sclerosing effect of this therapy increases soft palate rigidity, preventing its displacement over the epiglottis during exercise. However, the reduced dose and volume of sodium tetradecyl sulfate used in the present study may have minimised its sclerotic effect. Furthermore, it is also possible that the tissues of clinically affected animals might react differently to this therapy than do normal soft palates.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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References

Anderson, J., Tulleners, E., Johnston, J., Reeves, M., 1995. Sternothyrohyoideus myectomy or staphylectomy for treatment of intermittent dorsal displacement of the soft palate in racehorses: 209 cases (1986–1991). Journal of the American Veterinary Medical Association 206, 1909–1912.

Barakzai, S., Dixon, P., 2005. Conservative treatment for thoroughbred racehorses with intermittent dorsal displacement of the soft palate. Veterinary Record 157, 337–340.

Blythe, L., Cardinet, G., Meagher, D., Brown, M., Wheat, J., 1983. Palatal myositis in horses with dorsal displacement of the soft palate. Journal of the American Veterinary Medical Association 183, 781–785.

BonenClark, G., Bryan, J., Hernandez, J., Ferrel, E., Colahan, P., 1999. Sternothyroideus tenectomy or sternothyroideus tenectomy with staphylectomy for the treatment of soft palate displacement. In: Proceedings of the 45th Meeting of The American Association of Equine Practitioners, Albuquerque, New Mexico, USA, pp. 58–86.

Brietzke, S., Mair, E., 2001. Injection snoreplasty: how to treat snoring without all the pain and expense. Otolaryngology—Head Neck Surgery 124, 503–510.

Brietzke, S., Mair, E., 2004. Injection snoreplasty: investigation of alternative sclerotherapy agents. Otolaryngology-Head and Neck Surgery 130, 47–57.

Cehak, A., Deegen, E., Drommer, W., Lutkefels, E., Ohnesorge, B., 2006. Transendoscopic injection of poly-L-lactic acid into the soft palate in horses: a new therapy for dorsal displacement of the soft palate? Journal of Equine Veterinary Science 26, 59–66.

Chan, R., 2003. Effect of postmortem changes and freezing on the viscoelastic properties of vocal fold tissues. Annual Review of Biomedical Engineering 31, 482–491.

Courey, M., Fomin, D., Smith, T., Huang, S., Sanders, D., Reinisch, L., 1999. Histologic and physiologic effects of electrocautery, CO_2 laser, and radiofrequency injury in the porcine soft palate. Laryngoscope 109, 1316–1319.

Duncan, D.W., 1997. Retrospective study of 50 thoroughbred racehorses subjected to radical myectomy surgery for treatment of displacement of the soft palate. In: Proceedings of the 43rd Meeting of The American Association of Equine Practitioners, Phoenix, Arizona, USA, pp. 237–238.

Franklin, S., Naylor, J., Lane, J., 2002. Effect of dorsal displacement of the soft palate on ventilation and airflow during high-intensity exercise. Equine Veterinary Journal 34 (Suppl.), 379–383.

Franklin, S., Price, C., Burn, J., 2004. The displacement of equine soft palate as a source of abnormal respiratory noise during expiration. Equine Veterinary Journal 36, 590–594.

Harrison, I., Raker, C., 1998. Sternothyrohyoideus myectomy in horses: 17 cases (1984–1985). Journal of the American Veterinary Medical Association 193, 1299–1302.

Holcombe, S., Derksen, F., Stick, J., Robinson, N., 1998. Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. American Journal of Veterinary Research 59, 504–508.

Holcombe, S., Derksen, F., Stick, J., Robinson, N., 1999. Pathophysiology of dorsal displacement of the soft palate in horses. Equine Veterinary Journal 30 (Suppl.), 45–48.

Krag, S., 1998. Effect of freezing on lens capsule mechanical behavior. Ophthalmic Research 30, 280–285.

Lafrentz, J., Brietzke, S., Mair, E., 2003. Evaluation of palatal snoring surgery in an animal model. Otolaryngology—Head Neck Surgery 129, 343–352.

Lester, J., Franklin, S., Burn, J., 2003. Can Tension in the soft palate reduce dorsal displacement during inspiration? In: Proceedings of the 42nd Meeting of The British Equine Veterinary Association, Birmingham, UK, p. 258.

Llewellyn, H., Petrowitz, A., 1997. Sternothyroideus myotomy for the treatment of dorsal displacement of the soft palate. In: Proceedings of the 43rd Meeting of The American Association of Equine Practitioners, Phoenix, Arizona, USA, pp. 239–243.

Marcoux, M., Picandet, V., Céleste, C., Macieira, S., Morisset, S., Rossier, Y., Schambourg, M., Jean, D., 2008. Palatal sclerotherapy: a potentially useful treatment of intermittent dorsal displacement of the soft palate in juvenile Standardbred racehorses. Canadian Veterinary Journal 49, 587–591.

Parente, E., Martin, B., Tulleners, E., Ross, M., 2002. Dorsal displacement of the soft palate in 92 horses during high-speed treadmill examination (1993–1998). Veterinary Surgery 31, 507–512.

Paul, R., Tarlton, J., Purslow, P., Sims, T., Watkins, P., Marshall, F., Ferguson, M., Bailey, A., 1997. Biomechanical and biochemical study of a standardized wound healing model. The International Journal of Biochemistry and Cell Biology 29, 211–220.

Poyrazoglu, E., Dogru, S., Saat, B., Güngor, A., Cekin, E., Cincik, H., 2006. Histologic effects of injection snoreplasty and radiofrequency in the rat soft palate. Otolarynogology—Head and Neck Surgery 135, 561–564.

Rehder, R., Ducharme, N., Hackett, R., Nielan, G., 1995. Measurement of upper airway pressures in exercising horses with dorsal displacement of the soft palate. American Journal of Veterinary Research 56, 269–274.

Richardson, L., Wakley, G., Franklin, S., 2006. A quantitative study of the equine soft palate using histomorphometry. The Veterinary Journal 172, 78–85.

Shackelford, C., Long, G., Wolf, J., Okerberg, C., Herbert, R., 2002. Qualitative and quantitative analysis of non-neoplastic lesions in toxicology studies. Toxicology and Pathology 30, 93–96.

Shigeta, Y., Enciso, R., Ogawa, T., Clark, G., 2008. Changes in three dimensional simulation models of the airway which are due to increases in age or body mass index. Studies in Health Technology and Informatics 132, 460–462.

Tulleners, E., Stick, J., Leitch, M., Trumble, T., Wilkerson, J., 1997. Epiglottic augmentation for treatment of dorsal displacement of the soft palate in racehorses: 59 cases (1985–1994). Journal of the American Veterinary Medical Association 211, 1022–1028.

Veldi, M., Vasar, V., Hion, T., Kull, M., Vain, A., 2001. Ageing, soft-palate tone and sleep-related breathing disorders. Clinical Physiology 21, 358–364.

Viola, R., Kempski, H., Nakada, S., del Cerro, M., Aquavella, J., 1992. New model for evaluating corneal wound strength in the rabbit. Investigative Ophthalmology and Visual Science. 33, 1727–1733.

Woodie, J., Ducharme, N., Kanter, P., Hackett, R., Erb, H., 2005. Surgical advancement of the larynx (laryngeal tie-forward) as a treatment for dorsal displacement of the soft palate in horses: a prospective study 2001–2004. Equine Veterinary Journal 3, 418–423.

Zhou, B., Ji, C., Zhou, D., 2003. Clinical study on oropharyngeal fatty infiltration on the pathogenesis of obstructive sleep apnea syndrome. Journal of Clinical Otorhinolaryngology 17, 535–538.