Original article

Effects of a mandibular advancement device on genioglossus in obstructive sleep apnoea hypopnea syndrome

Chun-yan Liu*, Hai-yan Lu*, Fu-sheng Dong**, Wen-sheng Ma*, Jie Wang***, Xiao-ying Hu* and Wen Wang*

*Department of Orthodontics, ** Department of Oral & Maxillofacial Surgery, ***Department of Oral Pathology, College of Stomatology, Hebei Medical University, The Key Laboratory of Stomatology, Hebei Province, Shijiazhuang, Hebei, China

Correspondence to: Jie Wang, No. 383, East Zhongshan Road, Shijiazhuang, Hebei 050017, People’s Republic of China.
E-mail: wangjiephd@163.com

Summary

Objective: To investigate effects of mandibular advancement device (MAD) therapy for obstructive sleep apnoea hypopnea syndrome (OSAHS) on the genioglossus contractile properties and fibre-type distribution.

Materials and methods: Thirty 6-month old male New Zealand white rabbits were randomised into three groups: OSAHS, MAD, and controls. Rabbits in Group OSAHS and Group MAD were established as OSAHS models by injection, at a dose of 2 ml hydrophilic polyacrylamide gel, via the submucous muscular layer of soft palate. Spiral computed tomography (CT) showed a significant reduced retropalatal upper airway, and apnoeas happened with an increase of Apnea Hypopnea Index (AHI) and a decrease of blood oxygen saturation during polysomnography (PSG), which indicated the OSAHS model developed successfully. OSAHS rabbits in Group MAD were fitted with a MAD made from self-curing composite resin, at 30 degrees to the upper incisors, and the mandible was guided forward 3 to 4 mm. Further, spiral CT and PSG suggested MAD was effective. Rabbits in 3 groups were induced to sleep for 4–6 hours per day for 8 weeks, after which the genioglossus was removed, mounted in a tissue bath, and stimulated through platinum electrodes; maximal twitch tension, contraction time, half-relaxation time, force-frequency relationship, and fatigability were recorded. The percentage of Type I and Type II fibres was quantified.

Results: The fatigability and percentage of Type II fibres of genioglossus increased in Group OSAHS compared with controls; this abnormality was corrected by MAD.

Conclusion: MAD therapy for OSAHS could prevent genioglossus fatigue and abnormal fibre-type distribution of genioglossus in OSAHS.

Introduction

Obstructive sleep apnoea hypopnea syndrome (OSAHS) is a relatively common sleep disorder, characterised by recurrent episodes of partial or complete collapse of upper airway (UA) during sleep. Studies have shown that oral pharyngeal region is usually where closure mostly occurs. Normally, UA muscles, especially the genioglossus, play a crucial role in maintaining the patency of the UA (1, 2), and there is an evidence that injury or reduced activity of these muscles causes the UA collapse during sleep, resulting in OSAHS (3). However, there is also evidence that UA muscles themselves may be abnormal. UA muscle structure is abnormal in the English bulldog, an animal model of OSA (4) and in humans with OSA (5, 6). Since chronic episodic hypoxia causes increased
fatigability in rat geniohyoid and sternohyoid muscle (7) and chronic hypobaric hypoxia caused changes in contractile tension in both sternohyoid and diaphragm muscle of rats (8), this prompted us to consider whether the chronic intermittent hypoxia (CIH) associated with OSA could also affect genioglossus structure and function.

MAD has emerged as an alternative therapy for mild to moderate OSAHS patients and for those who are neither able nor willing to tolerate the standard continuous positive airway pressure (CPAP) therapy (9, 10). There was preliminary evidence that enhanced fatigability of the genioglossus and the abnormal fibre-type distribution observed in OSAHS patients were normalised after CPAP therapy (11). But there was no report on the functional and structural changes of genioglossus in the process of MAD therapy for OSAHS.

We developed an OSAHS and MAD therapy animal model to investigate effects of MAD therapy for OSAHS on the genioglossus contractile properties and fibre-type distribution.

Material and methods
Experimental animals
Experiments were performed on thirty 6-month-old male New Zealand white rabbits (initial weight, 3–3.5 kg). All care and experimental procedures were approved by the Animal Care and Use Committees at Hebei Medical University, Shijiazhuang, China. The animals were housed under normal laboratory conditions. Access to food and water was ad lib. The rabbits were randomised into 3 groups of 10 animals each: OSAHS, MAD, and controls.

Experimental design
Establishment of an animal model
The OSAHS model and treatment by MAD was developed in our laboratory (H. Y. Lu, J. Wang, F. S. Dong, C. Y. Liu, Y. Liu and W. Xiao, manuscript in preparation). After general anaesthesia with sodium pentobarbitone (20 mg/kg, intravenously), rabbits in Group OSAHS and Group MAD were injected with 2 ml hydrophilic polyacrylamide gel, via the submucous muscular layer at the centre of the soft palate, about 1.5 cm away from the junction of the hard and soft palates. The animals in the control group received no gel. All the animals were trained to sleep in a supine position by an animal expert with petting skills. Spiral computed tomography (CT) scanning and polysonomography (PSG) recordings were conducted as described above to evaluate the effectiveness of MAD. The average SaO₂ and AHI were 72.03 ± 8.82 (%) and 33.29 ± 3.62 in Group OSAHS, 90.16 ± 9.07 (%) and 3.55 ± 0.48 in Group MAD, and 96.99 ± 3.70 (%) and 2.55 ± 0.54 in the control group, which suggested MAD was effective. Then all the rabbits were induced to sleep in a supine position for 4–6 hours per day and were studied for 8 consecutive weeks. Access to food and water was ad lib at the rest time.

Genioglossus biopsy
At the end of 8 weeks and after a 12 hours fast, the rabbits were anaesthetised with sodium pentobarbitone (20 mg/kg, intravenously). With the rabbit supine, a midline incision was made in the neck, and genioglossus was identified and exposed. The muscles were removed rapidly and prepared for adenosine triphosphatase (ATPase) staining or for contractile studies. Genioglossus close to CT scanning showed significant reduced retropalatal UA, and PSG recordings suggested the appearance of apnoeas, with an increase of Apneic Hypopnea Index (AHI) and a decrease of SaO₂ suggested OSAHS animal model was established successfully.

OSAHS rabbits in Group MAD had the MAD inserted, made from self-curing composite resin. It was positioned at 30 degrees to the upper incisors and was glued to the 2 upper incisors with glass ionomer. When the rabbit mouth closed, the mandible was guided forward 3–4 mm (Figure 1). After 3–5 days of adaptation, the jaw had flexible movement and no difficulty in taking food. CT and PSG were conducted as described above to evaluate the effectiveness of MAD. The average SaO₂ and AHI were 72.03 ± 8.82 (%) and 33.29 ± 3.62 in Group OSAHS, 90.16 ± 9.07 (%) and 3.55 ± 0.48 in Group MAD, and 96.99 ± 3.70 (%) and 2.55 ± 0.54 in the control group, which suggested MAD was effective. Then all the rabbits were induced to sleep in a supine position for 4–6 hours per day and were studied for 8 consecutive weeks. Access to food and water was ad lib at the rest time.
the inner aspect of the mandible was quickly removed, frozen immediately in isopentane, cooled with liquid nitrogen, and then stored at –80°C for ATPase staining.

Contractile properties of genioglossus

Genioglossus was immediately excised in a longitudinal strip in 2 mm diameter. The strip was mounted vertically in a tissue bath (Radnoti Glass Technology, Monrovia, CA, USA) containing (mM): 135 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, 15 NaHCO₃, and 11 glucose. (pH adjusted to 7.40) (11). One end of the strip was tied to an immobile hook at the bottom of bath and the other end was tied to a calibrated high-sensitivity force transducer. The solution was continuously gassed with 95 per cent O₂ and 5 per cent CO₂ and maintained at 37°C through the water chamber. Muscles were allowed to equilibrate for 30 minutes (14). Fibre length was adjusted by micropositioner until maximal isometric twitch force was obtained (optimal fibre length (Lo)). The strips were stimulated with pulses of 1.0 ms delivered through platinum electrodes. Maximal twitch tension, contraction time, half-relaxation time, and force-frequency relationship in response to stimulation frequencies of 10, 20, 40, 60, 80, and 100 Hz were monitored. All studies were performed at Lo. A 10-minute rest period was allowed and the fatigueability of each muscle was tested. The fatigue protocol was performed at 0.5 Hz for 5 minutes. The loss of force over time was quantified every 1 minute for 5 minutes of the repetitive stimulation protocol. Muscle length was measured in the chamber, and the muscle strip was weighed after being dried at the end of the experiment. Muscle force production was recorded and stored by in a BL-420E four-channel computer recorder for later analysis. Force and time were measured manually offline. The maximum force was normalised to the muscle cross-sectional area. During the fatigue protocol, force was normalised to that produced during the first stimulation train (11).

ATPase staining of genioglossus

Genioglossus stored at –80°C was cut into pieces of 5 × 5 mm². Serial transverse sections 5 μm thick were cut perpendicular to the orientation of muscle fibres in a cryostat (CM1900; Leica Cryo-cut Instruments, Germany) at −23°C. Two adjacent sections were stained for acid and alkali-labile ATPase at room temperature. The sections were subjected to a modified procedure: one section was incubated in ATPase substrate solution for 30 minutes (pH 4.35), the adjacent section was incubated for 15 minutes incubated for 5 minutes in 0.1 M (mol/L) sodium acetate acid solution (pH 7.40) (11). One end of the strip was tied to an immobile hook at the bottom of bath and the other end was tied to a calibrated high-sensitivity force transducer. The solution was continuously gassed with 95 per cent O₂ and 5 per cent CO₂ and maintained at 37°C through the water chamber. Muscles were allowed to equilibrate for 30 minutes (14). Fibre length was adjusted by micropositioner until maximal isometric twitch force was obtained (optimal fibre length (Lo)). The strips were stimulated with pulses of 1.0 ms delivered through platinum electrodes. Maximal twitch tension, contraction time, half-relaxation time, and force-frequency relationship in response to stimulation frequencies of 10, 20, 40, 60, 80, and 100 Hz were monitored. All studies were performed at Lo. A 10-minute rest period was allowed and the fatigueability of each muscle was tested. The fatigue protocol was performed at 0.5 Hz for 5 minutes. The loss of force over time was quantified every 1 minute for 5 minutes of the repetitive stimulation protocol. Muscle length was measured in the chamber, and the muscle strip was weighed after being dried at the end of the experiment. Muscle force production was recorded and stored by in a BL-420E four-channel computer recorder for later analysis. Force and time were measured manually offline. The maximum force was normalised to the muscle cross-sectional area. During the fatigue protocol, force was normalised to that produced during the first stimulation train (11).

Statistical analysis

Results were analysed with SPSS13.0 analysis system (SPSS, Chicago, IL, USA). All data were expressed as means ± SD. The normality test of Shapiro–Wilks and Levene’s variance homogeneity test were applied. The data were found to be normally distributed, and there was homogeneity of variance between the groups. The statistical significance of differences was assessed by analysis of variance (ANOVA), followed by post-hoc analysis when appropriate. The Chi-square test was used to compare the percentage of fibre types. A P value <0.05 was considered significant.

Results

The contractile properties of genioglossus

The contractile properties of genioglossus were summarised in Table 1 and Figure 2. No significant difference (P > 0.05) was observed in maximal twitch tension, contraction time, or half-relaxation time. Figures 3 and 4 show the force-frequency relationship. Differences between groups were not statistically significant (P > 0.05). By contrast, Figure 5 and Table 2 show increased genioglossus fatigueability in Group OSAHS that was not observed in Group MAD or the control group (P < 0.05).

Fibre-type distribution of genioglossus

The fibre-type distribution values showed a significantly higher percentage of Type II fibres in Group OSAHS than in the control group (P < 0.05), but these differences were not observed between Group MAD and the control group (P > 0.05; Figure 6 and Table 3). Figure 7 shows ATPase staining of genioglossus.

The correlation analysis of SaO₂ and genioglossus fatigue is shown in Figure 8.

Discussion

Animal selection and establishment of OSAHS

OSAHS has been studied in many different species, including rats and mice (12–14), cats (15), monkeys (16), and pigs (17). The UA of large animals was similar to humans. However, using large animals is difficult due to their relatively large size and cost. For small animals, it is difficult to induce airway obstruction. In contrast, the overlap of the epiglottis and soft palate is a normal characteristic of rabbit UA anatomy. Any obstruction within the nasal cavity will produce a respiratory wheeze with increased effort. The long nasal cavity is separated from the oral cavity by the hard palate cranially and the soft palate caudally (18). In addition, rabbits are naturally retrognathic, and in humans retrognathia and soft palate hypotrophy are recognised causes of OSAHS (19–20). With the specific oropharyngeal anatomy features of rabbits, the rabbit is regarded as a good model for OSAHS. OSAHS has been successfully developed in rabbits by injecting liquid silicone into the base of the tongue (21) or paralysing the genioglossus (22). This could mimic OSAHS, however,
liquid silicone was not stable or suitable for long-term study. OSAHS model by paralysing the genioglossus could not be used for evaluating the structure and function of genioglossus. Therefore, in this study, we designed to mimic OSAHS by injection of the soft palate to induce stenosis of retropalatal oropharyngeal cavity.

There are multiple sites of obstruction identified and retropalatal airway space was obstructed in this study. The site of injection was decided upon following a preliminary study. Gel easily diffused to the surrounding tissue when it was injected into the loose tissue and could not induce obvious collapse of UA. Therefore, after several trials, soft palate was selected as the safe and effective injection site to induce sleep disordered breathing for long-term studies. The hydrophilic polyacrylamide gel used, which is stable and biocompatible, was originally applied in cell culture and as a filling material for tissue augmentation (23). While sleeping 2 ml hydrophilic polyacrylamide gel was enough to induce apnoea, and it was difficult for injection of large dose because of high tension in the injection site. Histology of the soft palate showed that the injected gel was entirely surrounded with connective tissues, with no spread or shift, similar to soft palate hypertrophy. Persistent and stable obstruction in UA caused apnoea or hypopnea when animals slept in supine position and allowed a long-term observation and evaluation of the OSAHS model. Further, snoring and apnoeas were observed during sleeping in supine position, with increased respiratory efforts and decreased oxygen saturation, suggesting the procedure of developing OSAHS was feasible.
Evaluation of the OSAHS model with MAD treatment

Based on clinical studies (24, 25) and the animals’ tolerance, we chose mandibular advancement of 3–4 mm and jaw opening of 2–3 mm. After OSAHS rabbits were inserted with MAD, it was well tolerated. No rabbits had difficulty in taking food or had signs of distress after acclimatizing for 3–5 days. The device advanced the mandible, increased retropalatal airway space and reduced tendency of collapse during sleep. Therefore, apneic events were reduced and oxygen saturation increased, which were very similar to clinical MAD therapy for OSAHS patients (26, 27), indicating the novel method enabled airway obstruction and MAD in rabbits.

The effects of MAD upon genioglossus of OSAHS

This is the first report of functional and structural changes of genioglossus during MAD therapy for OSAHS. The genioglossus in Group OSAHS was more susceptible to fatigue than in the control group, but was normalised by MAD. In addition, the genioglossus of OSAHS patients has been shown to be structurally and functionally abnormal, with elevated levels of activation while awake (28). We postulated an alternative mechanism of genioglossus fatigue in Group OSAHS that was due to CIH, and MAD therapy increased the SaO₂, further correcting genioglossus fatigue. OSAHS resulting in a long period of CIH, hypoxemia in patients could lead to ‘decreased’ UA dilator EMG activity (29). There was evidence that CIH (30) led to increased genioglossus fatigue, and chronic hypobaric hypoxia caused an increase in contractile tension in rat sternohyoid and diaphragm muscle and an increase in fatigue in the sternohyoid (8). Correlation analysis in present study showed that there was a positive correlation between the SaO₂ and genioglossus fatigue, indicating that genioglossus fatigue was associated with hypoxia.

Another factor contributing to the increased genioglossus fatigue could be muscle decompensation. In this study, polyacrylamide hydrogel was injected into the soft palate of rabbits, which became integral with the soft palate, with no spread or shift. Each episode of pharyngeal obstruction induced by polyacrylamide hydrogel finished with a vigorous contraction of the genioglossus, and so a secondary process of muscle decompensation may ensue. In turn, this may contribute to enhanced genioglossus fatigue and render the pharynx more collapsible, and therefore form a vicious cycle exacerbating the condition. The genioglossus was especially vulnerable to fatigue, which was related to the initial fibre distribution of the muscles since there were more Type II fibres and less Type I fibres (31). The effects were consistent with the changes of fibre-type distribution. Significantly reduced Type I fibres and increased Type II fibres were found in Group OSAHS. This reduction in fatigue-resistant

![Figure 5. Genioglossus fatigue in three groups. Tension was normalised to that produced during the first stimulus train (%). Statistically significant differences compared with control group are indicated by asterisks; *P < 0.05.](image)

![Figure 6. Fibre types of genioglossus in three groups. Statistically significant differences compared with control group are indicated by asterisks; *P < 0.05.](image)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Group OSAHS (n = 10)</th>
<th>Group MAD (n = 10)</th>
<th>Control group (n = 10)</th>
<th>1-2</th>
<th>1-3</th>
<th>2-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88.20 ± 4.80</td>
<td>94.30 ± 3.33</td>
<td>98.80 ± 7.33</td>
<td>0.0180*</td>
<td>0.0002*</td>
<td>0.074</td>
</tr>
<tr>
<td>2</td>
<td>64.80 ± 6.58</td>
<td>83.10 ± 3.51</td>
<td>87.70 ± 4.50</td>
<td>0.0009*</td>
<td>0.0009*</td>
<td>0.051</td>
</tr>
<tr>
<td>3</td>
<td>57.30 ± 4.24</td>
<td>71.60 ± 4.01</td>
<td>74.20 ± 4.99</td>
<td>0.0006*</td>
<td>0.0001*</td>
<td>0.197</td>
</tr>
<tr>
<td>4</td>
<td>37.10 ± 3.38</td>
<td>55.40 ± 2.95</td>
<td>52.00 ± 5.10</td>
<td>0.0005*</td>
<td>0.0004*</td>
<td>0.063</td>
</tr>
<tr>
<td>5</td>
<td>28.30 ± 3.50</td>
<td>45.70 ± 6.27</td>
<td>48.20 ± 4.10</td>
<td>0.0005*</td>
<td>0.0005*</td>
<td>0.387</td>
</tr>
</tbody>
</table>

*P < 0.05.
Type I fibres and increase in fatigable Type II fibres were likely to be related to the increase in genioglossus fatigue. Studies have found that fibre-type transition of genioglossus occurred when exposure to intermittent hypoxia (32). The ratios of Type I and Type II fibres in skeletal muscle are influenced, in part, by passive stretch (33), over/unloading (34), and stimulation (35).

Muscle fibres are dynamic structures capable of changing their phenotype under different conditions. In this study, as discussed above, the genioglossus of rabbits with OSAHS was exposed to repetitive maximal forces during wakefulness, which could explain the increase in Type II fibres. Therefore, MAD therapy may prevent the transition from fast-to-slow fibre types and genioglossus fatigue through increasing SaO\textsubscript{2}, preventing decompensation.

MAD therapy can guide mandible forward, increase UA space, improve SaO\textsubscript{2}, which prevents airway collapse (36) and rests the genioglossus. It was shown that mandibular advancement altered the positions of mandible, tongue, and soft palate to enlarge and stabilise the airway, as well as altered the proportion of sagittal diameter to transverse the diameter of the UA (37), which could lead to increased oropharyngeal diameter and decreased oropharyngeal collapsibility (38). MAD therapy prevented increased genioglossus fatigue and transition from fast-to-slow fibre, similar to what occurred in CPAP therapy for OSAHS.

In conclusion, the present study demonstrated that MAD could prevent the functional and structural damage of the genioglossus in rabbits with OSAHS, which may be attributed to MAD enlargement of the UA.

**Conclusion**

MAD therapy for OSAHS could prevent fatigue and abnormal fibre-type distribution of genioglossus in OSAHS.

**Acknowledgements**

The authors greatly appreciate Dr Mei Qing Yu for providing expert assistance with the CT imaging and Prof. Xin Wang for biotechnical assistance.

**References**


**Table 3.** Fibre-type distribution of three groups (means ± SD). MAD, mandible advanced device; OSAHS, obstructive sleep apnoea hypopnea syndrome; SD, standard deviation

<table>
<thead>
<tr>
<th></th>
<th>Group OSAHS (n = 10)</th>
<th>Group MAD (n = 10)</th>
<th>Control group (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–2</td>
<td>1–3</td>
<td>2–3</td>
<td></td>
</tr>
<tr>
<td>Type I (%)</td>
<td>14.36 ± 3.03</td>
<td>19.79 ± 2.07</td>
<td>21.30 ± 3.34</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Type II (%)</td>
<td>85.64 ± 3.03</td>
<td>80.21 ± 2.07</td>
<td>78.70 ± 3.34</td>
<td>0.0002*</td>
</tr>
</tbody>
</table>

*P < 0.05.

**Figure 7.** Representative adenosine triphosphatase (ATPase) staining tissue sections of genioglossus in one rabbit in Group OSAHS (a and b), one rabbit in Group MAD (c), and one rabbit in control group (d), ×100. Type I (dark staining) and Type II (light staining) fibres in acidic media (pH, 4.35) in a, c, and d. Type II (dark staining) and Type I (light staining) fibres in alkaline media (pH, 10.65) in b. a and b were that two adjacent sections of one genioglossus sample were stained for acid and alkali-labile ATPase activity, revealing mirror structure (as arrows showed).

**Figure 8.** Positive correlation between blood oxygen saturation and genioglossus fatigue.


