의학박사 학위논문

보툴리늄 독소를 이용한 폐쇄성 수면무호흡증 토끼 모델의 확립과 상기도 폐쇄의 방사선학적 확인

Establishment of rabbit model of obstructive sleep apnea using botulinum toxin and radiological identification of upper airway obstruction

2012년 8월

서울대학교 대학원
의학과 이비인후과학 전공
이 명 철

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이 논문을 의학과 박사 학위논문으로 제출함

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Establishment of rabbit model of obstructive sleep apnea using botulinum toxin and radiological identification of upper airway obstruction

by

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논문제목: 보툴리늄 독소를 이용한 폐쇄성 수면무호흡증 토끼 모델의 확립과 상기도 폐쇄의 방사선학적 확인 (Establishment of rabbit model of obstructive sleep apnea using botulinum toxin and radiological identification of upper airway obstruction)

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Abstract

Establishment of rabbit model of obstructive sleep apnea using botulinum toxin and radiological identification of upper airway obstruction

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Background: Obstructive sleep apnea (OSA) syndrome is a common, complex pathology. OSA Animal models have been developed during the last 2 decades. Yet current sleep apnea models are less than ideal in many ways. Here, we present our new botulinum toxin-based rabbit model of OSA and our analysis of upper airway obstruction by dynamic CT using this model.

Methods:

1. *Establishment of the OSA rabbit model*

Twenty-one rabbits were divided into a 2.5 units of botulinum toxin group (Group A, n = 8), a 5 unit group (Group B, n = 10), a 7.5 unit (n = 1), and a normal saline group (control group, n = 2). Botulinum toxin

or normal saline were injected into genioglossus muscle of rabbit transorally after Zoletil/Rompum induced anesthesia. Respiratory parameters (apnea, hypopnea) were measured using ApneaLink^{TM} at 0 day (pre-injection), 1, 2, 3, 4, 6, 8 weeks post-injection.

2. *Identification of upper airway obstruction by dynamic CT*

Another fourteen animals were divided into a 2.5 unit (Group C, n = 7, no overlap with Group A) and a control group (Group D, n = 7). All rabbits in Group C (2.5 unit) and D (Control) underwent respiratory parameters measurement using ApneaLink^{TM} at 0 day (pre-injection), 1, 2, 3, 4, 6, 8 weeks post-injection and dynamic CT (modification of perfusion scan) at pre-injection, 1 week and 2 week post-injection. Airway dimensions including transverse and anterior to posterior (AP) airway lengths at palate and tongue base level were measured.

Results:

1. *Establishment of the OSA rabbit model*

Of the 2.5 unit group (Group A), OSA was successfully induced in 5 of 8 rabbits. AHI scores ranged from 2 to 114. In the 5 unit group (Group B), OSA was successfully induced in 7 of the 10 rabbits. AHI scores ranged from 4 to 262. No difference in OSA induction rate was observed (5/8 vs. 7/10, respectively; $P = 1.0$).

2. *Identification of upper airway obstruction by dynamic CT*

Of seven rabbits in the 2.5 unit group (Group C), 5 rabbits showed

OSA. On the other hand, of seven rabbits in the control group (Group D), OSA was not induced in any rabbit. The success rate of OSA induction was statistically higher in the 2.5 unit group (5/7 vs. 0/7; P = 0.02). In the 2.5 unit group (Group C), all three variables (transverse and AP diameters at the palate level, AP diameters at the tongue base level) except transverse diameters at the tongue base level showed a significant decrease at 2 weeks post-injection. In the control group (Group D), all four variables significantly increased at 2 weeks post-injection.

Conclusion: A new animal model of OSA based on neuromuscular mechanism by injecting botulinum toxin into the genioglossus muscle of the tongue of rabbits was developed in the present study. This model was shown to be able to simulate the obstruction of the upper airway by using the dynamic CT scan.

Keywords: obstructive sleep apnea, botulinum toxin, dynamic CT, animal model, rabbit, sleep

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Introduction

Obstructive sleep apnea (OSA) syndrome is prevalent and known to be an important risk factor for cardiovascular, metabolic, cognitive, and neurological diseases.[1-4] However, mechanisms underlying the developments of these complications are still not completely understood. Furthermore, the pathogenesis of OSA also needs to be studied more. Because investigations in humans have limitations in showing direct causal relationship, animal disease models are useful.

Several kinds of animal models for OSA have been established during the last 2 decades for experiments in controlled conditions without influences of confounders such as disease duration, chronologies of events, contributions of the different OSA components, and genetic and environmental factors that may affect the outcome of clinical investigations. However, current sleep apnea models have some limitations.[5-8]

OSA animal models can be categorized as spontaneous or induced models.[9] The historic natural spontaneous model of OSA is the English bulldog, which has an abnormal upper airway anatomy with a narrow oropharynx and an enlarged soft palate.[10] This model reproduces human features of OSA during rapid eye movement (REM) sleep.

Of the induced models, the non-surgical, non-invasive intermittent hypoxia models have been widely used to assess various consequences arising from sleep disordered breathing.[11] However these models do not replicate upper airway obstruction in OSA.

Surgical invasive models have been designed to induce upper airway obstruction.[12,13] However, most of the models utilized tracheotomy and therefore they were more similar to intermittent hypoxic model rather than upper airway obstruction model.

In the present study, we developed a new non-invasive, physiologic animal model to mimic real pharyngeal conditions during sleep. We speculated that OSA could be induced if the genioglossus muscle, which is a main dilator muscle of the upper airway, is paralyzed. Botulinum toxin was introduced for this purpose. It produces its paralytic effects by preventing vesicles containing acetylcholine from fusing with the cell membrane inside motor nerve terminals, and thus, inhibits acetylcholine release at neuromuscular junctions. The effect of botulinum toxin is generally temporary, and neuronal activity and associated muscle contractions resume over the course of several months through nerve re-sprouting.[14] The present study will demonstrate establishment of a non-invasive neuromuscular model of upper airway obstruction and analyses of changes in the upper airway by dynamic computed tomography (CT) scan[15,16] in rabbits.

Materials and Methods

1. Establishment of the OSA rabbit model

1.1. Induction of OSA with botulinum toxin

Twenty-one 3-month-old New Zealand White male rabbits weighing 2.1 to 2.6 kg were used in this study. All procedures using these animals were approved by the International Animal Care and Use Committee at Seoul National University. All the rabbits were acclimated in the new environment for the first one week and used for further experiments. Sedation was induced by intramuscular injection of 0.3 ml/kg of Zoletil and 0.2 ml/kg of Rompun at the same time. After sleep was induced, botulinum toxin type A (BOTOX®, Allergan, USA) solved in normal saline was injected transorally into the genioglossus muscle to paralyze the tongue in the tongue base of rabbits (Fig. 1).

One rabbit was injected with 7.5 units of botulinum toxin, eight rabbits (Group A) with 2.5 units of botulinum toxin, 10 rabbits with 5.0 units of botulinum toxin (Group B), and two rabbits with normal saline (control group)

1.2. Evaluation of polysomnographic findings with Embletta® X100 during drug-induced sleep

Drug-induced sleep was evaluated using Embletta® X100 (Embla, Denver, USA) in three rabbits (1 rabbit in group B and 2 rabbits in the control group). After induction of sleep as described above, we applied Embletta® X100 to 3 rabbits. Electroencephalography (EEG), electrooculography (EOG), and chin electromyography (EMG), breathing effort, nasal airflow, blood oxygen saturation, and pulse rate were evaluated as shown in Fig. 2. EEG, EOG and EMG records were scored visually with an epoch length of 5 seconds, discriminating between wakefulness (W), REMS and NREMS, according to the following criteria with reference to previous reports[17,18] : W = mixed fast activity in the cortex accompanied by high amplitude EMG records with frequent motion artifacts; NREMS = mixed slow activity with spindles in the cortex and medium-sized EMG records without artifacts; REMS = mixed fast activity in the cortex, and low amplitude EMG recordings with frequent eye movement and occasional twitching artifacts. Remlogic v2.0 software (Embla, Denver, USA) was used for analysis.

1.3. Evaluation of OSA induction with ApneaLink™ (RESMED, San Diego, USA)

After induction of sleep as described above, ApneaLink™ was

applied in all rabbits for about 40 minutes to 1 hour in the supine position. Body position during sleep did not change in any rabbit (Fig. 3). Body weight was measured before each test. Respiratory events were manually analyzed using ApneaLink v7.0 software (RESMED, San Diego, USA) (Fig. 4). Observed parameters were nasal airflow and oxygen saturation.

Apnea was scored when a drop in peak nasal pressure of ≥ 90% versus pre-event baseline was observed. Hypopnea was scored when a drop in the nasal pressure of ≥ 30% versus pre-event baseline occurred with a desaturation of ≥ 3% versus pre-event baseline or when a drop in the nasal pressure of ≥ 50% versus pre-event baseline occurred. We set event duration as two breaths based on baseline breathing patterns in each rabbit according to the guideline for children in American Academy of Sleep Medicine manual.[19] OSA was determined when AHI values (apnea index + hypopnea index) exceeded 5 per hour. OSA induction was considered to be successful when more than half of the ApneaLink™ tests conducted revealed OSA (AHI ≥ 5) after botulinum toxin injection.

1.4 Experimental schedule

On day 0, botulinum toxin or 0.5 ml of normal saline was injected

after baseline respiratory parameters were acquired with ApneaLink^{TM}. At 1, 2, 3, 4, 6, and 8 weeks after injection, respiratory parameters were measured using ApneaLink^{TM} (Fig. 5).

2. Identification of upper airway obstruction by dynamic CT

2.1. Induction of OSA with botulinum toxin

Another fourteen 3-month-old conventional New Zealand White rabbits weighing 2.1 to 2.5 kg were used for evaluation of the upper airway. Animals were divided into OSA (Group C, n = 7) and a control group (Group D, n = 7). In the OSA group, 2.5 units of botulinum toxin was injected into the genioglossus muscle transorally after induction of sleep as described above, on the basis of results from the first part of the study. The control group was also injected with normal saline in the same manner.

2.2. Identification of upper airway obstruction by dynamic CT

All rabbits in OSA group (Group C) and control group (Group D) underwent dynamic CT. After sleep was induced, rabbits were placed supine with the neck in the neutral position while images were taken. A plastic cage was used to fix position during scanning (Fig. 6).

A CT system (SOMATOM definition, 32 channel; Siemens,

Germany) was used to obtain images. A new protocol modified from perfusion scan was developed for this study with help of a radiologist. Only axial views were reconstructed due to limitations of the CT system, with the exception of a few sagittal images. CT parameters were; 1.5 mm slice thickness, examination time 10 seconds, and 10 consecutive scans per each exam. Scans were performed from the ethmoid sinus to cricoid cartilage. Using this protocol, 5 examinations were performed with 2 minute intervals in each rabbit. Briefly, 50 scans were acquired over 8 minutes per a rabbit to include the inspiratory and expiratory breath cycles (Fig. 7).

Images obtained were reviewed in stack mode on a picture archiving and communications system (Maroview; Marotech, Seoul), and patterns of upper airway obstruction were analyzed by the authors. From each set of 50 scans, airway dimensions including transverse and anterior to posterior (AP) airway lengths at palate and tongue base level were measured. The palate level was determined as where the hard palate is best shown, and the tongue base level as where the hyoid bone is best shown (Fig. 8). Mean dimensions were used in the analysis.

2.3. Histologic examination

At 8 weeks after injection, whole tongues, including genioglossus

muscle, were harvested and paraffin blocked. Coronal sections were cut at the foramen cecum level (tongue base). H&E staining was used to compare effects of botulinum toxin injection in the OSA and control groups (Fig. 9).

2.4. Schedule of experiment

On day 0, after induction of sleep, baseline respiratory parameters were measured using ApneaLink^{TM} and baseline dynamic CT scanning was performed. Thereafter, 2.5 units of botulinum toxin or normal saline was injected in the OSA group and control group, respectively. At 1 and 2 weeks after injection, respiratory parameters were measured and each rabbit was scanned by dynamic CT. At 3, 4, 6, and 8 weeks after injection, respiratory parameters were measured (Fig. 10). All rabbits were sacrificed and paraffin block processed for histologic examination after measurements of respiratory parameters at 8 weeks.

3. Statistical analysis

Fisher's exact test was used to compare the rates of OSA induction between Group A and B, and between Group C and D. Mann-Whitney U test was performed to compare upper airway dimensional differences between Group C and D. Wilcoxon's signed-rank sum test was used to

compare the change of upper airway dimensions from baseline to 1 and 2 weeks in Groups C and D. SPSS version 18.0 was used and *p*-values of <.05 was considered to be statistically significant.

Results

1. Establishment of the OSA rabbit model

1.1. Comparison of initial weights

Initial weights prior to injection were not statistically different between Groups A (2.5 unit botulinum toxin) and B (5 unit botulinum toxin) (2.24 ± 0.26 kg vs. 2.32 ± 0.34 kg; $P = 0.77$).

1.2. Evaluation of polysomnographic findings with Embletta® X100 during drug-induced sleep

In all 3 rabbits (1 rabbit in Group B and 2 rabbits in the control group), Embletta® X100 recordings showed mixed slow EEG activities with some sleep spindles, medium-sized EMG records, and rapid eye movements were not observed during about 50 minute to 1 hour (Fig. 11). After about 50 minute to 1 hour, EEG showed faster activity with high amplitude EMG records with frequent motion artifacts gradually.

1.3. Evaluation of OSA induction using ApneaLink™

Out of 21 rabbits in the first part of the study, 2 rabbits in the control group showed no AHI during study period after injection of normal saline. The one rabbit administered with 7.5 units of botulinum toxin

died 1 day after injection due to severe apneic events during drug-induced sleep. When the rabbits in Group A were injected with 2.5 units of botulinum toxin, OSA was successfully induced in 5 of 8 rabbits (R1, 3, 4, 6, 7). AHI scores ranged from 2 to 114 (Table 1). Of 5 rabbits in which OSA was induced, 3 rabbits died of apneic events during drug-induced sleep and 2 rabbits survived for more than 8 weeks (R6:15 weeks, R7:17 weeks). When the rabbits in Group B were injected with 5 units of botulinum toxin, OSA was successfully induced in 7 of the 10 rabbits (R2, 4, 6, 7, 8, 9, 10). AHI scores ranged from 4 to 262 (Table 2). Of the 7 rabbits, 5 rabbits died of an apneic event (R2, 6, 7, 8, 9) and 2 survived more than 8 weeks (R4: 16 months, R10: 1 year). The rate of OSA induction was not statistically different between Groups A and B ($P = 1.0$).

2. Identification of upper airway obstruction by dynamic CT

2.1. Comparison of initial weights and weight gains

Initial weights prior to injecting botulinum toxin or normal saline were not different in the OSA group (Group C) and the control group (Group D) (2.34 ± 0.16 kg vs. 2.34 ± 0.06 kg; $P = 0.79$), and weight gains over 8 weeks were not also different in these groups (0.89 ± 0.23 kg vs. 0.62 ± 0.29 kg; $P = 0.106$).

2.2. Evaluation of OSA induction using ApneaLink™

AHI scores were measured 7 times (baseline and at 1, 2, 3, 4, 6, and 8 weeks post-injection) in all rabbits after inducing sleep with Zoletil/Rompun. Out of 7 rabbits in the OSA group (Group C), 5 rabbits (R2, 4, 5, 6, 7) showed OSA at least 3 times during 6 measurements. AHI scores ranged from 2 to 195 (Table 3). On the other hand, 2 rabbits (R3, 5) in control group (Group D) showed OSA only once (Table 4). The success rate of OSA induction was statistically higher in the OSA group (5/7 vs. 0/7; $P = 0.02$).

2.3. Upper airway dimensions

To identify the changes of the upper airway dimensions, we performed dynamic CT scans three times (baseline and at 1 and 2 weeks after injection of botulinum toxin). In each scan, we measured mean values and the standard deviations of transverse and AP diameters at both the palate and tongue base levels. Baseline dynamic CT scan showed that transverse diameters at the palate level in the OSA and control groups were 5.62 ± 0.80 and 4.99 ± 0.60 mm, respectively. AP diameters at the palate level in the OSA and control groups were 6.41 ± 1.02 and 5.70 ± 0.58 mm, respectively. Transverse diameters at the

tongue base level in OSA and control groups were 5.01 ± 0.55 and 4.90 ± 0.64 mm, respectively. AP diameters at the tongue base level in OSA and control groups were 4.23 ± 0.59 and 3.82 ± 0.34 mm, respectively. All the four variables were not significantly different between OSA and control groups (Table 5).

Transverse and AP diameters at the palate and tongue base levels in baseline dynamic CT scans were compared with those obtained at 1 and 2 weeks to determine the changes of upper airway dimensions during sleep. In the OSA group, the transverse diameters at the palate level significantly decreased from 5.62 ± 0.80 to 4.86 ± 0.45 mm (P = 0.01), and AP diameters at the palate level also significantly decreased from 6.41 ± 1.02 to 5.44 ± 0.53 mm (P = 0.01) at 2 weeks. Transverse diameters at the tongue base level decreased from 5.01 ± 0.55 to 4.95 ± 0.90 mm (P = 0.93), and AP diameters at the tongue base level decreased from 4.23 ± 0.59 to 3.62 ± 0.48 mm (P = 0.01) at 2 weeks. All variables except transverse diameters at the tongue base level showed a significant decrease at 2 weeks after injection (Table 6).

However, in control group, transverse diameters at the palate level increased from 4.99 ± 0.60 to 5.40 ± 0.43 mm (P = 0.03), and AP diameters at the palate level increased from 5.70 ± 0.58 to 6.52 ± 0.56 mm (P = 0.01) at 2 weeks. Transverse diameters at the tongue base level increased from 4.90 ± 0.64 to 5.60 ± 0.52 mm (P = 0.03), and AP

diameters at the tongue base level increased from 3.82 ± 0.34 to 4.27 ± 0.49 mm (P = 0.03) at 2 weeks. All four variables significantly increased at 2 weeks after injection (Table 7).

2.4. Histologic examination

H&E stained specimens were reviewed for general appearance, muscle fiber appearance, and fatty infiltration by authors and a pathologist. No specific differences were found between the two groups.

Discussion

In the present study, we described a new rabbit model of OSA. This model simulates upper airway obstruction through paralysis of the genioglossus muscle using botulinum toxin in rabbits. Therefore, the underlying mechanism of OSA in our novel model can be considered to be neuromuscular. Both apneic and hypopneic events were observed. The obstruction of the upper airway was observed both in the retrolingual and retropalatal airway levels.

Our results indicate that botulinum toxin administration to genioglossus muscle can reduce upper airway dimensions during drug-induced sleep and induce OSA in rabbits. This is the first study to describe a neuromuscular OSA model based on the use of botulinum toxin and measurement of upper airway dimensions based on a dynamic CT.

Recently rabbits have been used for OSA studies because they are easier to handle than larger animals.[20,21] Furthermore, their tongues can be easily injected with botulinum toxin to paralyze the genioglossus muscle, which is the main dilator muscle of the upper airway. In addition, their nostrils and legs were suitable for application of the nasal pressure sensor and pulse oximetry of ApneaLink™, and the

dimensions of the upper airway were enough for evaluation by dynamic CT scan.

In the present study, the sleep stages during drug-induced sleep were identified using Embletta® X100 in the rabbits.[22] There have been a few studies showing the EEG findings of natural sleep or the effects of benzodiazepine on EEG findings in rabbits.[17,18] In contrast to previous studies using electrodes implanted on the surface of brain cortex, we used surface electrode for recording EEG, EOG, and EMG. Because electrodes were attached after Zoletil/Rompun was administered and rabbits fell into sleep, we could not identify sleep onset. During Zoletil/Rompun-induced sleep (about 1 hour), sleep spindles were found over a background of low amplitude mixed frequency activity on EEG and no rapid eye movement was observed on EOG. About one hour after induction of sleep, EEG showed significant shift to faster frequencies and EMG activity increased with frequent motion artifacts. We assumed that these gradual EEG and EMG changes represented transition from NREM stage of sleep to wakefulness. .

In the present study, we first administered 7.5 units of botulinum toxin. Since the rabbit died on one day after injection due to apneic events, we reduced the dosage of botulinum toxin to 2.5 or 5 units. We determined that 2.5 unit was safe, minimal and enough dosage for OSA induction because there was no significant difference in the success rate

of OSA induction between the two dosages. Therefore our experiment measuring the dimension of the upper airway was conducted after injection of 2.5 units of botulinum toxin. We found a few rabbits non-responsive to botulinum toxin administration. We compared characteristics of rabbits responsive and non-responsive to botulinum toxin, but there were no significant differences in ages and body weights. Thus, we speculated that these findings were from technical errors such as botulinum toxin injection methods, injected sites, titers of diluted botulinum toxin, and instability of botulinum toxin.

Many investigators have attempted to identify upper airway obstruction patterns using techniques such as; Friedman staging, computed tomography, magnetic resonance imaging, esophageal pressure manometry, sleep endoscopy, and sleep videofluoroscopy.[23-28] Recently some have reported that dynamic CT images of the pharyngeal airway may be useful for human OSA studies.[15, 16] Inspired by the study conducted by Yucel et al.,[16] we developed a new dynamic CT protocol for a rabbit model of OSA. To our knowledge, this is the first dynamic CT study to be conducted on the evaluation of dynamic upper airway obstruction in a rabbit OSA model. We measured transverse and AP diameters at the palate and tongue base levels to identify obstruction patterns in rabbits during drug-induced sleep. We found that both the transverse and AP diameters at the palate level and

AP diameters at the tongue base level decreased significantly in the OSA rabbits. In contrast the transverse and AP diameters both at the palate and tongue base levels increased in the control rabbits. Although we hypothesized that a decrease in airway dimension would be found only at the tongue base level because botulinum toxin was injected into the genioglossus muscle, the airway dimension also decreased at the palate level. These findings concur with those of an earlier study which reported a multiplicity of obstruction levels.[28] These results indicate that airway collapse at the tongue base level could affect airway dimension even at the palate level via the generation of negative pressure and/or a physical push back of the soft palate and uvula to the posterior pharyngeal wall by the tongue. Conversely, this suggests that correction of airway collapse at the tongue base level might be used to correct the obstruction of the upper airway at the palate level.

Histologic findings showed no differences between OSA group and the control group at 8 weeks, which agrees with the findings of a previous study, in which no change was observed in the contractile materials of rectus femoris muscle injected with botulinum toxin in rabbits.[29]

A variety of methods have been used to make animal models of OSA.[5-9] However, the previously developed techniques have some innate problems; 1) invasiveness in models such as tracheotomy,[12,13]

intraventricular and brain cortical implantation of an electrode for EEG recording,[17,18] and restrained neck position during sleep[30], 2) no simulation of the upper airway during sleep in models such as hypoxia models[11], 3) difficult handling, high cost of large animals such as pig, dog, monkey[8], or 4) limited reproducibility in spontaneous OSA models such as pig, dog[10]. In comparison with other models, our OSA rabbit model has several advantages. Firstly, this model is simple and relatively inexpensive. Furthermore, botulinum toxin injection to genioglossus muscle, ApneaLink™ application, and dynamic CT scanning are not difficult to perform. Secondly, the use of a botulinum toxin injection to collapse the upper airway during drug-induced sleep represents the first non-invasive, functional, neuromuscular attempt to devise an OSA model. We showed that this model can simulate the upper airway obstruction during sleep in the analysis using dynamic CT. Thirdly, this model can be used to develop site-specific devices, such as nerve or muscle stimulators or traction devices for the treatment of pharyngeal airway collapse around the tongue base level.[20,21] In addition, the dynamic CT protocol proposed allows the effects of treatment modalities to be easily visualized.

However, our model has some drawbacks. Firstly, Zoletil (tiletamine-zolazepam) and Rompun (xylazine) were used to induce sleep. Zolazepam is a benzodiazepine drug and is used as a sedative, a

hypnotic (sleep-inducing), an anxiolytic, and as a muscle relaxant, and these effects can induce and aggravate OSA.[31] However, OSA was found minimally in the control group (Group D) in the present study. Thus, Zolazepam is less likely to induce sleep respiratory events, such as apnea or hypopnea in rabbits. Secondly, events during drug-induced sleep may differ from what occurs during normal sleep and may only reflect a small portion of sleep events. Furthermore, hypnotics including benzodiazepine affect sleep architecture and OSA patterns.[31-33] However, it is not possible to measure sleep events in animals during natural sleep using a portable sleep monitoring device without restraint, and night-long restraint is too stressful for animals and can bias results. Accordingly, it might be more practical to devise OSA model in a drug-induced setting. Thirdly, we applied a level 4 portable sleep monitoring device (ApneaLink^{TM}) rather than a more sophisticated device with more channels to measure both sleep and respiratory parameters. ApneaLink^{TM} has been found in several studies to be a simple, easy-to-use, highly sensitive, and specific device for calculating AHI comparable to full polysomnography when it is performed in an attended setting.[23] In the present study, we focused on developing respiratory events and calculating AHI, and thus, considered ApneaLink^{TM} adequate. However, in the future study, both sleep and respiratory events would be better to be checked at the same time to

identify in which stage of sleep the respiratory events are more severe.

Conclusions

A new animal model of OSA based on neuromuscular mechanism by injecting botulinum toxin into the genioglossus muscle of the tongue of rabbits was developed in the present study. This model was shown to be able to simulate the obstruction of the upper airway by using the dynamic CT scan. This new model may contribute to identifying the pathogenesis of upper airway obstruction in OSA and to developing new diagnostic or treatment devices targeting specific obstruction sites.

References

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Table 1. Apnea-hypopnea indexes in the group of 2.5 unit botulinum toxin for establishment of rabbit OSA model (Group A)

	Weeks after injection of botulinum toxin						
	0	1	2	3	4	6	8
R1	0	91	29	17	0	2	
R2	0	0	0	0	0		
R3	0	88	26	14	5	0	6
R4	0	4	32				
R5	0	0	0	0	0		
R6	0	114	0	0	6	16	0
R7	0	9	14	6	0	0	0
R8	0	0	0	0	0		

R, rabbit.

Table 2. Apnea-hypopnea indexes in the group of 5 unit botulinum toxin for establishment of rabbit OSA model (Group B)

	Weeks after injection of botulinum toxin						
	0	1	2	3	4	6	8
R1	0	0	0	0	0		
R2	0	86	6				
R3	0	0	0	0	0		
R4	0	18	98	110	0	65	0
R5	0	0	0	0	0		
R6	0	89	52				
R7	0	27	50	13			
R8	0	59	213	202	70	190	242
R9	0	9	0	6	0		
R10	0	8	4	45	57	262	0

R, rabbit.

Table 3. Apnea-hypopnea indexes in the OSA group for upper airway evaluation by dynamic CT (Group C)

	Weeks after injection of botulinum toxin						
	0	1	2	3	4	6	8
R1	0	0	0	0	0	0	0
R2	0	13	0	0	0	195	101
R3	0	0	0	0	0	0	0
R4	0	26	0	0	37	88	8
R5	0	0	16	144	35	76	36
R6	0	0	0	14	6	0	12
R7	0	5	5	2	132	0	0

R, rabbit.

Table 4. Apnea-hypopnea indexes in the control group of normal saline (Group D)

	Weeks after injection of normal saline						
	0	1	2	3	4	6	8
R1	0	0	0	0	0	0	0
R2	0	0	0	0	0	2	0
R3	0	0	0	0	0	0	8
R4	0	0	0	0	0	0	0
R5	0	0	6	0	0	0	0
R6	0	0	0	0	0	0	0
R7	0	0	0	0	0	0	0

R, rabbit.

Table 5. Comparison of baseline upper airway dimensions between the OSA (Group C) and control group (Group D) in dynamic CT

Upper airway dimension	OSA (Group C)	Control (Group D)	
Palate – Transverse	5.62 ± 0.80	4.99 ± 0.60	$P = 0.08$
Palate – Ant to Post	6.41 ± 1.02	5.70 ± 0.58	$P = 0.11$
Tongue base – Transverse	5.01 ± 0.55	4.90 ± 0.64	$P = 0.70$
Tongue base – Ant to Post	4.23 ± 0.59	3.82 ± 0.34	$P = 0.09$

Ant, anterior; Post, posterior

Values are means ± standard deviation in millimeters.

Table 6. Changes of upper airway dimension in the OSA group (Group C)

Upper airway dimension	Weeks			*p*-value	
	0 wk	1 wk	2 wk	0 wk vs. 1 wk	0 wk vs. 2 wk
Palate – Transverse	5.62 ± 0.80	5.22 ± 0.69	4.86 ± 0.45	0.03	0.01
Palate – Ant to Post	6.41 ± 1.02	5.81 ± 0.64	5.44 ± 0.53	0.03	0.01
Tongue base – Transverse	5.01 ± 0.55	4.86 ± 0.42	4.95 ± 0.90	0.43	0.93
Tongue base – Ant to Post	4.23 ± 0.59	3.77 ± 0.54	3.62 ± 0.48	0.01	0.01

Ant, anterior; Post, posterior; wk, week

Values are means ± standard deviation in millimeters.

Table 7. Changes of upper airway dimension in control group (Group D)

Upper airway dimension	Week			*p*-value	
	0 wk	1 wk	2 wk	0 wk vs. 1 wk	0 wk vs. 2 wk
Palate – Transverse	4.99 ± 0.60	5.30 ± 0.49	5.40 ± 0.43	0.15	0.03
Palate – Ant to Post	5.70 ± 0.58	5.91 ± 0.68	6.52 ± 0.56	0.11	0.01
Tongue base – Transverse	4.90 ± 0.64	5.51 ± 0.64	5.60 ± 0.52	0.01	0.03
Tongue base – Ant to Post	3.82 ± 0.34	3.97 ± 0.41	4.27 ± 0.49	0.07	0.03

Ant, anterior; Post, posterior; wk, week

Values are means ± standard deviation in millimeters.

Figure Legends

Fig. 1. Injection of botulinum toxin. Tongue is pulled out from rabbit's oral cavity (A). Schematic diagram depicting injection site and direction. Arrow shows injection site and direction. Star marks genioglossus muscle (B).

Fig. 2. Application of Embletta® X100. Observed parameters are electroencephalography, electrooculography, electromyography, breathing effort, nasal airflow, oxygen saturation, and arterial pulse.

Fig. 3. Application of ApneaLink™. Observed parameters are nasal airflow and oxygen saturation. Supine body position has not changed throughout the experiment.

Fig. 4. Data of ApneaLink™ on desktop computer. Events of apnea, hypopnea, and desaturation are shown (red box).

Fig. 5. Protocol of experiments for establishment of obstructive sleep apnea rabbit model. ▲ means respiratory parameters measurement using ApneaLink™. ★ means injection of botulinum toxin 2.5 units

(Group A), 5 units (Group B), 7.5 units, or 0.5ml of normal saline to genioglossus muscle. ‘d’ means day and ‘w’ means week.

Fig. 6. Scanning of dynamic CT. A rabbit is in the supine position with the neck in the neutral position. Plastic cage was used to fix position during scanning.

Fig. 7. Protocol for dynamic CT. 5 exams at 2 minute intervals were done in each rabbit. ▬ means 1 exam including 10 scans during 10 seconds. ‘min’ means minute.

Fig. 8. Scanned images demonstrating upper airway dimensions using dynamic CT. Axial views show airway at palate level (A) and tongue base level (B). Mid-sagittal view shows upper airway of rabbit and anatomic landmarks (hard palate and hyoid bone) (C). Star marks hard palate, and arrow marks hyoid bone.

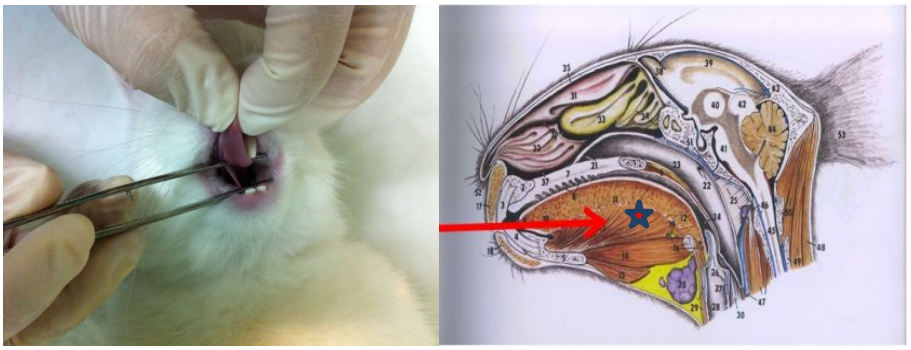
Fig. 9. Microscopic findings of H&E stain of coronal tongue section. Black arrow shows genioglossus muscle (A). Microscopic finding (x100 magnification) shows muscle fibers and intervening connective tissues (B).

Fig. 10. Protocol of experiments for identification of upper airway dimensions in OSA rabbit model with dynamic CT. ▲ means respiratory parameters measurement using ApneaLink™. ★ means injection of botulinum toxin 2.5 units (Group C) and 0.5ml of normal saline (Group D) to genioglossus muscle. ◆ means scanning of dynamic CT. ↓ means sacrifice and tongue harvest. 'd' means day and 'w' means week.

Fig. 11. Data of Embletta® X100 on desktop computer. Electrooculography, electroencephalography, electromyography, nasal air flow, breathing efforts are shown. Sleep spindles are identified on electroencephalography (black arrows).

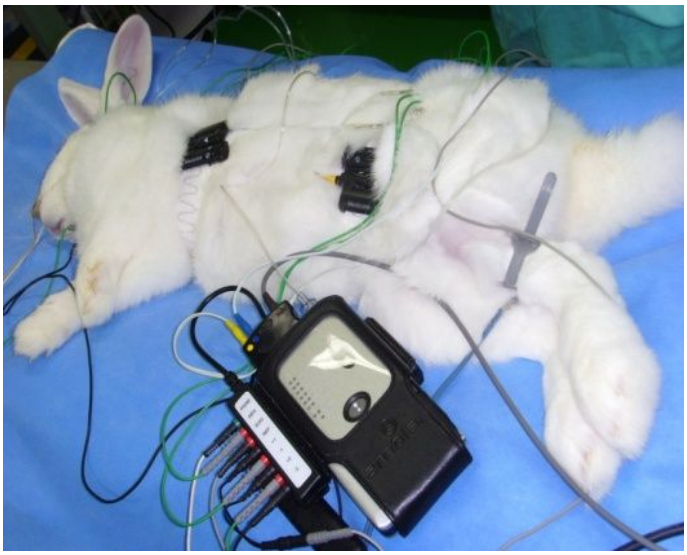
Figures

Figure 1.



A B

Figure 2.



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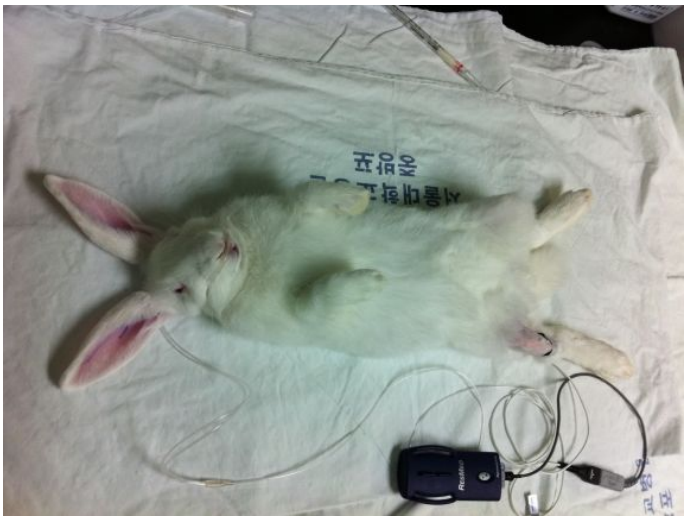

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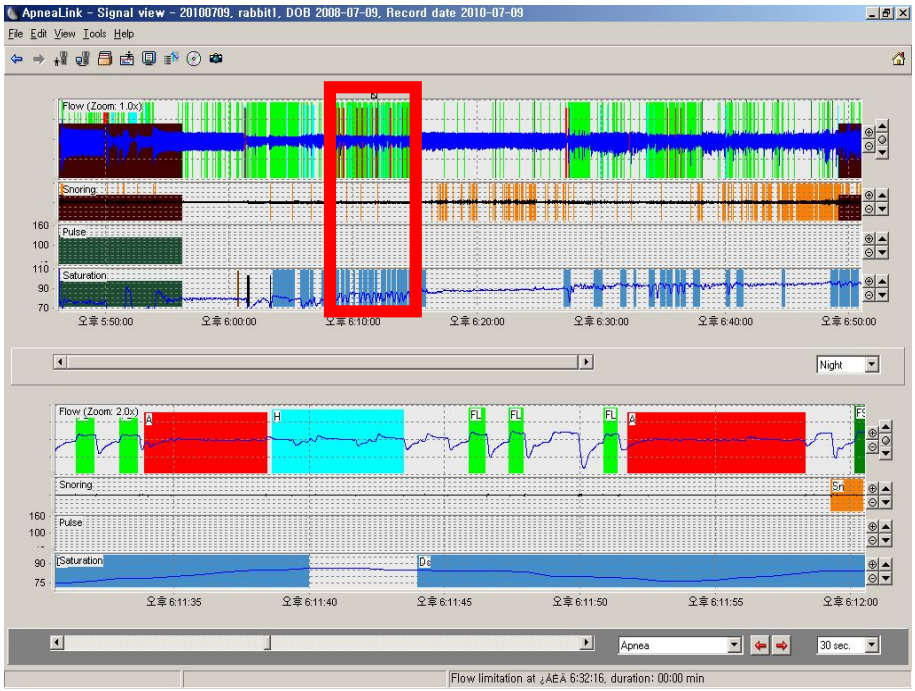
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Snoring
Pulse
Saturation
160
100
110
90
70
오후 5:50:00
오후 6:00:00
오후 6:10:00
오후 6:20:00
오후 6:30:00
오후 6:40:00
오후 6:50:00
Night
Flow (Zoom: 2.0x)
A
H
FL
FL
FL
A
Snoring
Pulse
Saturation
160
100
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75
오후 6:11:35
오후 6:11:40
오후 6:11:45
오후 6:11:50
오후 6:11:55
오후 6:12:00
Apnea
30 sec.
Flow limitation at ¿AEA 6:32:16, duration: 00:00 min

Figure 5.

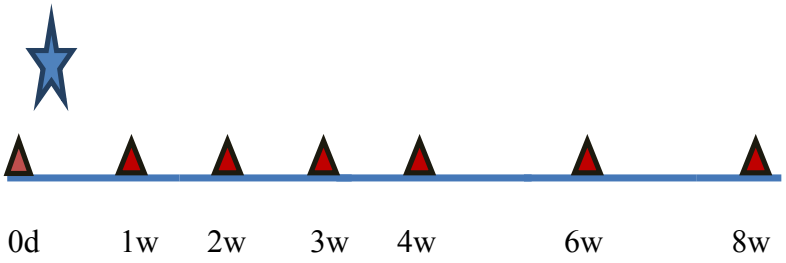


Figure 6.

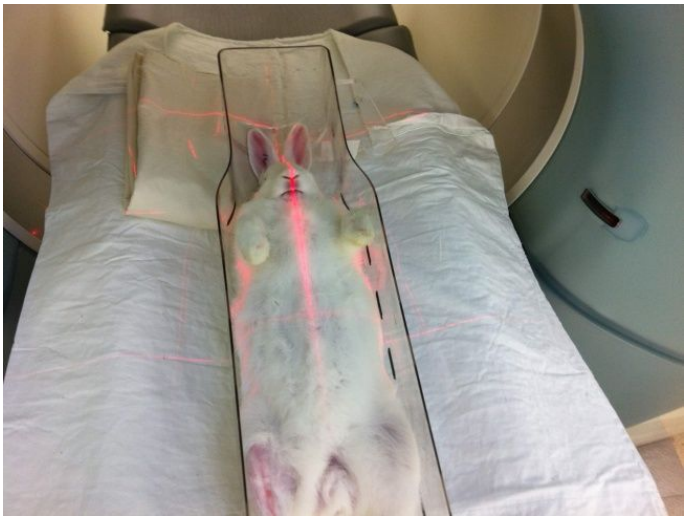
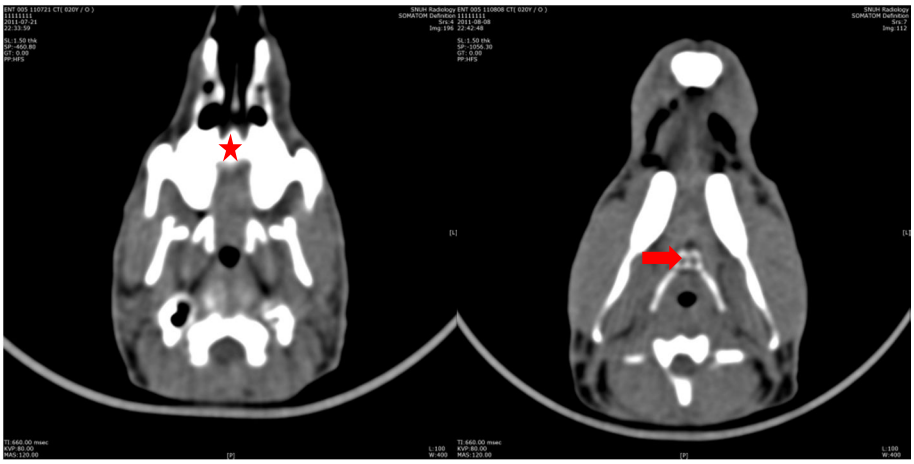
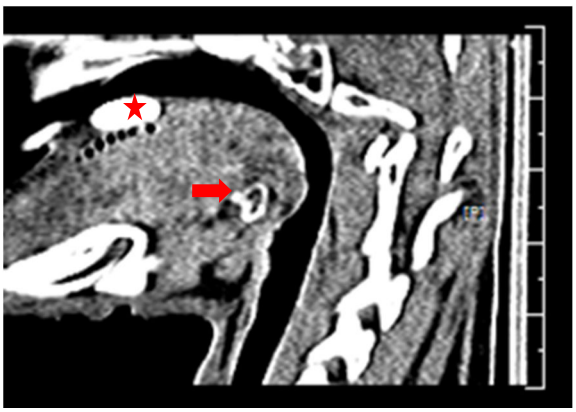


Figure 7.

Figure 8.

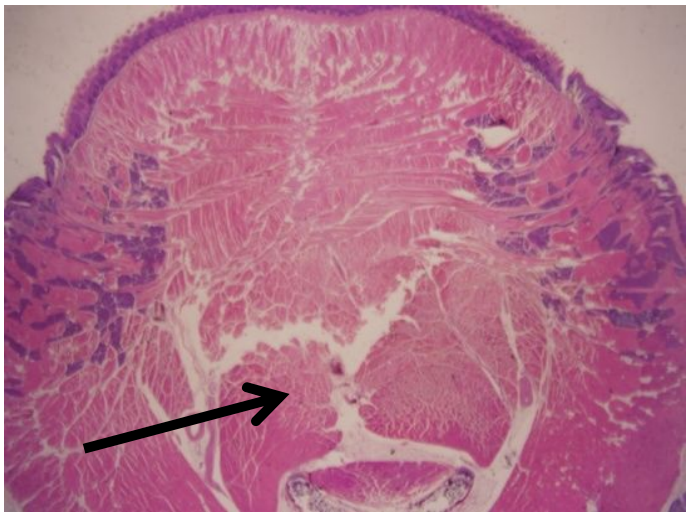


A B

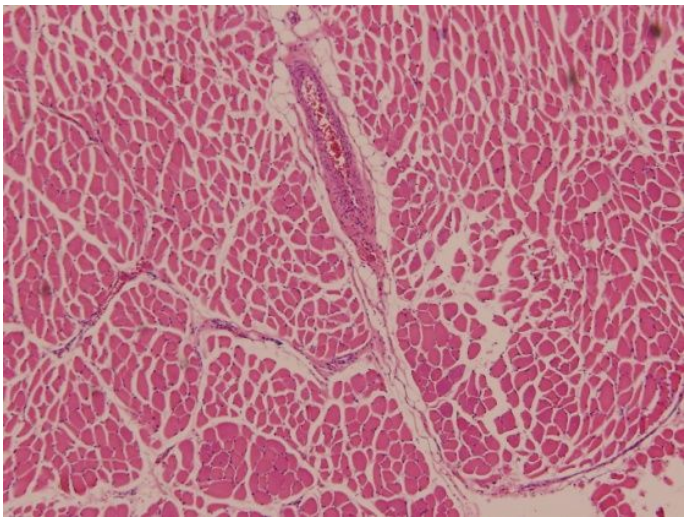


C

Figure 9.



A



B

Figure 10.

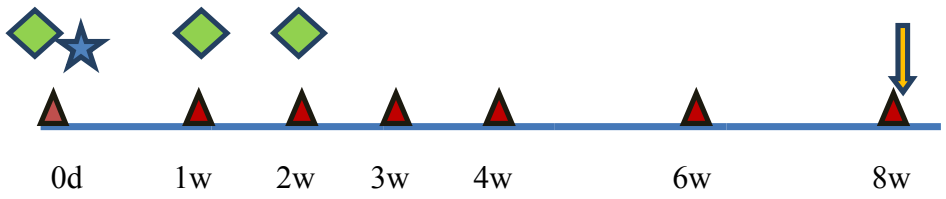
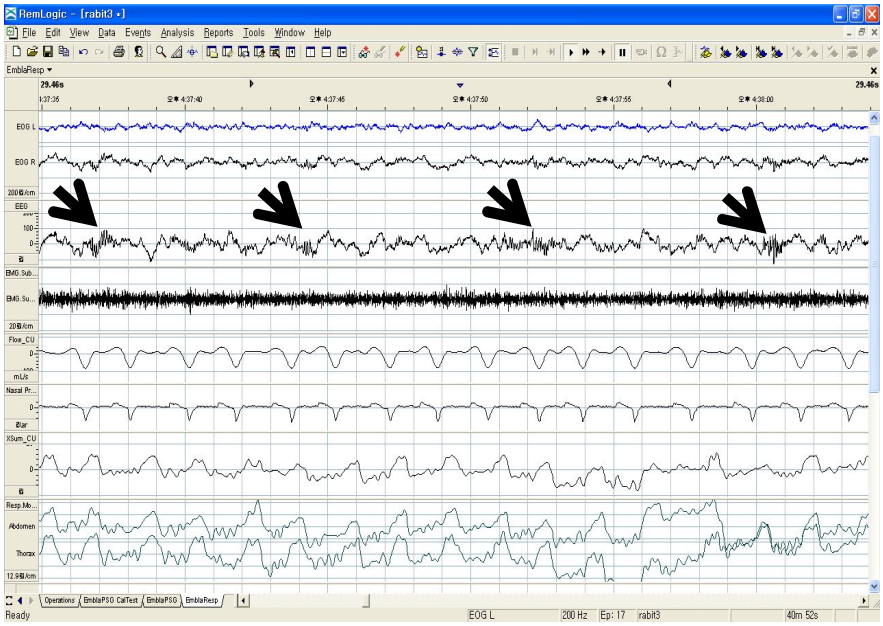


Figure 11.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국문초록

배경: 폐쇄성 수면 무호흡 증후군은 흔하지만 아주 복잡한 질환군이다. 폐쇄성 수면 무호흡증후군 동물 모델은 최근 20년 동안 개발되어 왔다. 하지만 이러한 동물 모델은 여러 가지 면에서 부족한 점이 많다. 본 연구에서는 보툴리늄 독소를 이용한 토끼의 새로운 수면 무호흡증 모델의 확립과 이 모델을 활용하여 역동적 컴퓨터 단층화 촬영(dynamic CT)을 이용한 상기도 폐쇄의 분석을 제시하고자 한다.

방법:

1. 폐쇄성 수면 무호흡 토끼 모델의 확립

21 마리의 토끼를 보툴리늄 독소 2.5 유닛(그룹 A, 8 마리), 5 유닛 (그룹 B, 10마리), 7.5 유닛(1마리), 생리식염수(대조군, 2마리) 총 4군으로 나누었다. 보툴리늄 독소나 생리식염수를 Zoletil/Rompun 마취 후에 경구강으로 토끼의 이설근(genioglossus muscle) 에 주사하였다. 주사하기 전과 주사 후 1,2,3,4,6,8 주에 ApneaLink™ 을 이용하여 수면 시 호흡지표 (무호흡, 저호흡)를 측정하였다.

2. Dynamic CT 를 활용한 상기도 폐쇄의 확인

14 마리의 토끼를 보툴리늄 독소 2.5 유닛(그룹 C, 7마리), 생리식염수(그룹 D, 7마리) 두 군으로 나누었다. 모든 토끼를 보툴리늄 독소나 생리식염수를 주사하기 전과 주사 후 1,2,3,4,6,8 주에 호흡지표를 ApneaLink™ 을 이용하여 측정하였다. 동시에 dynamic CT 를 주사전과 주사 후 1,2 주에 찍었다. Dynamic CT 를 통해 구개 부분과 설기저 부분에서 기도의 횡경(transverse diameter)과 종경(anterior to posterior diameter)를 측정하였다.

결과:

1. 폐쇄성 수면 무호흡 토끼 모델의 확립

그룹 A 의 8마리 토끼 중 5마리에서 수면무호흡증이 유발되었다. 무호흡-저호흡 지표(AHI)는 2에서 114까지 측정되었다. 그룹 B 의 10마리 중 7마리에서 수면무호흡증이 유발되었고, 무호흡-저호흡 지표는 4에서 262까지 측정되었다. 두 군간의 수면무호흡증이 유발된 비율은 통계적으로 차이는 없었다 (5/8 vs. 7/10 ; P = 1.0).

2. Dynamic CT 를 활용한 상기도 폐쇄의 확인

그룹 C 의 7마리 토끼 중 5 마리에서 수면무호흡이 유발되었으나 대조군 7 마리 토끼에서는 유발되지 않았다. 보툴리늄 독소를 주사한 그룹에서 통계적으로 유의하게 수면무호흡 유도율이 높았다 (5/7 vs. 0/7; P = 0.02). Dynamic CT 를 활용해 확인해 본 결과 그룹 C 에서 구개 부분 기도의 횡경과 종경, 설기저 부분 기도의

종경이 주사 전에 비해서 주사 2주 후에 통계적으로 유의하게 줄어들었다. 반면에 대조군에서는 구개와 설기저 부분의 횡경과 종경 모두 주사 전에 비해서 주사 2주 후에 늘어났다.

결론: 본 연구에서는 토끼 혀의 이설근에 보툴리늄 독소를 주사하여 토끼의 새로운 수면 무호흡증 모델을 확립하였고, dynamic CT를 이용하여 상기도 폐쇄의 양상을 확인하였다.

주요어: 폐쇄성 수면 무호흡, 보툴리늄 독소, 역동적 전산화 단층촬영, 동물모델, 토끼, 수면

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감사의 글

우선 이비인후과 입문에서부터 이비인후과 박사학위수여까지 부족한 저를 무한한 애정과 정성으로 이끌어 주신 은사 이철희 교수님께 깊은 존경과 감사를 드립니다. 심사위원장을 흔쾌히 맡아주신 윤인영 교수님과 바쁘신 중에도 심사를 위해 귀중한 시간을 내어주신 이승훈 교수님, 윤필영 교수님, 김정훈 교수님께 감사를 드립니다.

심사위원장을 맡아주신 윤인영 교수님께서는 자칫 이비인후과적인 (외과적 또는 해부학적) 관점에만 빠질 수 있었던 논문에 대해서 수면에 대한 소중한 정보를 추가할 수 있게 도움을 주셨습니다. 이승훈 교수님께서는 용어의 오류, 연구 디자인의 미흡했던 부분에 대한 지적과 친절한 조언으로 체계적인 논문이 되는데 많은 지도편달을 해주셨습니다. 윤필영 교수님께서는 논문에 대한 세세한 지적을 통해서 영어논문으로서 자리를 잡는데 도움을 주셨습니다. 김정훈 교수님께서는 실질적으로 본 연구를 저자와 함께 설계하고 수행하신 분으로 실험 디자인부터 논문완성까지 어느 한 곳 손길이 미치지 않은 곳이 없을 정도로 많은 도움을 주셔서 뭐라 감사의 말씀을 드려야 할지 모르겠습니다. 특히 지도교수님이신 이철희 교수님은 수면 동물모델에 대한 주제를 주시고 연구의 시작부터 종심에 이르기까지 학문적 관심과 조언으로 제게 많은 가르침을 주셨습니다. 뿐만 아니라, 늘 모든 일에 성실하고 원칙을 따르라는 가르침을 주시고, 제자에게 자상한 선생님으로서 인생의 본보기가 되어 주고 계십니다. 다시 한번 선생님의 지도와 사랑에 감사 드립니다.

또한 다른 여러 선생님들의 도움과 격려는 오늘의 논문이 완성되는 밑거름이 되었습니다. 석사 지도 교수님이신 이재서 교수님은 항상 저에게 관심을 가져 주시며, 학위를 또 학업을 계속하도록 격려와 충고를 아끼지 않으시고 계십니다. 항상 감사 드리며 선생님의 은혜에 보답하면서 의사로서, 또 학자로서의 길을 묵묵히 걷도록 하겠습니다. 원자력 병원 이국행 과장님, 이병철 과장님께서는 당신들이 바쁘신 가운데에서도 후배가 박사학위 과정을 밟는데 물심양면으로 많은 도움을 주셔서 힘든 박사 과정을 지탱할 수 있는 보루 역할을 해주셨습니다. 감사 드립니다. 또한 기쁜 마음으로 궂은 일도 마다하지 않았던 서울대학교 전임상 실험실 최준재 연구원에게 깊은 감사의 말씀 올리고 싶습니다. 진심으로 최준재 연구원이 도와주지 않았다면 제 학위 획득은 힘들었을 것입니다. 감사 드리고 하는 일 다 잘 되시기를 기원합니다.

마지막으로 저를 낳아주시고 길러주시고 교육시키느라 평생을 보내시고, 지금은 지병으로 고생하고 계신 우리 어머니, 그 옆에서 그런 어머니를 보살피고 계신 아버지께 부족하지만 사랑하고 감사한다는 말씀을 전하고 싶습니다. 바쁘다는 핑계로 자주 찾아 뵙지는 못하지만 항상 제 마음속에 두 분이 있다는 것은 잊지 말아주십시오. 늘 지켜봐 주시고 격려해 주신 장인, 장모님께도 감사의 말씀을 올립니다. 언제나 옆에서 힘이 되어주는 사랑하는 아내 이지민과 눈에 넣어도 아프지 않을 예쁘고 총명하고 착한 우리 나흔이, 유담이가 이 학위를 만들어 준 것임을 진심으로 알고 있습니다. 가족 모두의 깊은 관심과 사랑에 앞으로 더욱 정진할 힘을 얻습니다. 이 논문을 사랑하는 가족에게 바칩니다.