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Original Article

Electrophysiological and Histopathological Evaluation of Effects of Sodium–2 Mercaptoethanesulfonate Used for Middle Ear Surgery on Facial Nerve Functions

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OBJECTIVE: Sodium-2-mercaptoethanesulfonate (MESNA) is widely used in medicine because of its antioxidant and mucolytic effects. In recent years, it has been used in otologic surgery. Because it cleaves disulfide bonds, it is used to easily dissect the epithelial matrix in cholesteatoma and atelectasis. In this study, we hypothesized that MESNA does not have any toxic effect on the facial nerve, and the effects of MESNA on the facial nerve were examined histologically and electrophysiologically.

MATERIALS and METHODS: Twenty Wistar albino rats were used. Groups A and B were designated as the control and sham groups, respectively. The animals in groups C and D were administered 20% and 50% of MESNA solution, respectively, after the facial nerve was exposed in the parotid region. Electromyography (EMG) measurements were performed preoperatively and postoperatively at 4 weeks. The animals were subsequently euthanized; facial nerve samples were taken for histopathological examination.

RESULTS: When EMG parameters were compared within and between each group, preoperative and postoperative results were not statistically significantly different. Histopathological examination showed that MESNA did not cause any inflammation, granulation tissue, or foreign body reaction.

CONCLUSION: To the best of our knowledge, the effects of MESNA on facial nerve functions have not been investigated. In this study, the effects of MESNA after direct application to the facial nerve were examined electrophysiologically and histologically, and it was determined that MESNA did not cause any toxic effects. It was concluded that MESNA can, therefore, be safely used during middle ear surgery.

KEYWORDS: MESNA, rat, electromyography, histopathology

INTRODUCTION

Cholesteatoma is a common pathology in chronic otitis media (COM). Residual cholesteatoma and late-term disease recurrence are major problems related to COM surgery [1-3]. Different applications may be used to reduce the incidence of residual cholesteatoma. Oto-endoscopy systems have been introduced for providing better visibility and eradicating "blind spots" [4]. The use of a potassium titanyl phosphate laser provides more effective matrix dissection [5]. In addition, the application of galectin-7 using an immunofluorescence technique enabled visualization of the microscopic epidermal remains [6].

Although new techniques and surgical procedures have been introduced, the incidence of residual cholesteatoma was reported to vary from 5% to 50% [7]. The cholesteatoma matrix is mainly covered with keratin, and it contains numerous disulfide bonds. Chemical separation of these bonds allows dissection to be performed more easily and effectively during surgery [8]. Sodium-2-mercap-

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toethanesulphonate (MESNA), a sodium salt of 2-thiosulphonate anion, is now used for this purpose during otologic surgery; it provides more effective dissection because it cleaves the disulfide bonds [9]. In addition, because MESNA is a synthetic sulfur compound, it enables separation of mucus polypeptide chains by mucolysis of the disulfate bonds [9]. Moreover, owing to its antioxidant, protective, and mucolytic features, it is commonly used in medicine [10]. As a thiol-based cytoprotective agent, it is used for prophylactic treatment in cyclophosphamide-induced hemorrhagic cystitis. Because of its mucolytic effects, it is also used in respiratory medicine (in the treatment of asthma, chronic bronchitis, pharyngolaryngitis, etc.) as well as in some surgical treatments, for example, ear, nose, and throat, orthopedic, and gynecological fields [11].

Previous studies have reported that the use of MESNA in atelectatic and adhesive middle ear and cholesteatoma surgery is not associated with ototoxic effects and had neuroprotective effects; however, to the best of our knowledge, its direct effect on the facial nerve has not been demonstrated [7-10, 12]. The possibility of dehiscence development in the fallopian canal of the facial nerve is very high in the presence of pathologies progressing with dissolving bone structures, such as cholesteatoma; the use of MESNA for separating the cholesteatoma matrix in such surgeries inevitably results in contact with the facial nerve, directly or by passing through bony dehiscence. In this study, we investigated the effects of MESNA, which is commonly used during middle ear surgery, on the facial nerve using electrophysiological and histopathological methods.

MATERIALS and METHODS

This study was approved by the local ethics committee on animal experiments (No.01.11.2016-504). Twenty male Wistar rats raised in the animal experiments laboratory, aged 12-14 weeks and weighing 250-300 g were used in this study. Animals were placed in cages with free access to food and water. The cages had 50%±10% relative humidity at 23°C±3°C and artificial lighting was made available from 8:00 a.m. to 8:00 p.m. The rats were equally divided into four groups. Group A was the control group and group B constituted the sham group. After identifying the facial nerve, the animals in group C were given 20% MESNA solution and those in group D were given 50% MESNA solution. In this study, MESNA (Ureomitexan, MESNA, Eczacıbaşı-Baxter, Turkey) was diluted with physiological saline to obtain 20% and 50% solutions; a single dose was administered to the animals.

The experiments were performed only on the left facial nerve of the animals; electromyography (EMG) recordings were obtained. The rats were given general anesthesia preoperatively and postoperatively at 4 weeks using intramuscular ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg), and EMG was recorded to obtain combined muscle action potentials from the mandibular branch of the facial nerve using PowerLab 26T multiple recording devices and LabTutor (ADInstrumentsPty Ltd.). Latencies, amplitude, wave duration, and supramaximal alert thresholds values were recorded from the test results and compared between the study and control groups.

Electromyography recordings were obtained according to the model described by Salomone et al. [13]. Specially designed bipolar subdermal needle electrodes were used for stimulation and records during EMG. These monopolar electrodes had a thickness of 0.35 mm and length of 12 mm, and they were placed 5 mm apart from each other with a 3-mm gap at the tip for subcutaneous entry and the remaining 9 mm was taped for insulation. Similarly, a monopolar needle electrode wrapped with 9-mm tape was used for grounding. The recording electrode was placed subdermally, 3 mm away from the corner of the mouth, parallel to the lower lip. The stimulation electrode was placed subdermally, 25 mm away from the corners of the mouth, with the anode (+) located proximally and the cathode (-) located distally to stimulate the main body of the facial nerve. The ground electrode was placed subdermally midway between these two electrodes. For electrical stimulation, the stimuli given started at 0.5 mA and were increased to 5 mA in 0.5-mA increments. The duration of each stimulus was 50 ms. After the latency stimulus was given, time until the first wave was observed over the isoelectric line (in ms), distance between the positive and negative wave amplitude peaks (in millivolts), and time between the start and end of the wave–wave duration (in ms) were calculated. Supramaximal stimulation thresholds were determined to be the threshold required for stimulation of all fibers. For comparison at a constant value, the records obtained by giving 2.5 mA which was the appropriate stimulus based on average supramaximal stimulus threshold, were evaluated.

Following preoperative EMG measurements, five rats were separated as the control group without any intervention (group A). In all other animals, the left parotid region was entered subcutaneously using an approximately 2-cm skin incision, and the facial nerve trunk was located on the parotid gland with blunt dissection (Figure 1). Group B was designated as the sham group, and the operation was terminated in five animals, closing the wound with 4/0 Vicryl without any additional intervention. Animals in group C were administered 20% MESNA solution after the facial nerve was located, and the wound was closed after waiting for approximately 5 min. Five animals in group D were administered 50% MESNA solution onto the facial nerve for 5 min, and the operation was subsequently terminated, closing the wound.

After EMG measurements were performed under anesthesia postoperatively at 4 weeks, all rats included in the study were euthanized by guillotine decapitation, and sufficient amounts of facial nerve and surrounding tissue samples were obtained from the region of intervention. Each tissue sample was fixed in formaldehyde solution for 24 h (10%) and embedded in paraffin blocks for histopathological examination. Sections of 4-6 micron thickness were obtained in pathology laboratories using a microtome. The sections were stained using hematoxylin and eosin and S-100 immunohistochemistry stains (S-100, BiocareMedical, USA) and evaluated by a single pathologist under a light microscope (Olympus BX53) to be photographed at 40×, 100×, and 200× magnification fields. All histopathological micromeasurements were quantitatively made using an Olympus DP73 camera and digital microscopy software; each parameter was individually classified as follows:

-Foreign body reaction (Yes/No)
-Inflammation (None: 0, Mild: +, Moderate: ++, Extensive: +++, and Widespread inflammation and necrosis: ++++)
-Granulation tissue (Yes/No)
-Status of the perineural sheath and myelin structure (myelin structure preserved/irregular myelin structure)
-Axonal degeneration (Yes/No)

All samples were assessed by a single pathologist using a protocol blinded to the type of treatment provided to each group.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 22 software (IBM Corp.; Armonk, NY, USA). For the evaluation of test results, preoperative and postoperative mean and standard deviations were calculated for latencies, amplitude, wave duration, and supramaximal stimulus thresholds in the study and control groups. Histopathological data were evaluated together with the statistical results. For comparison of quantitative data between the groups, the Kruskal-Wallis test was used for data not normally distributed, and the Wilcoxon sign test was used for in-group comparisons. A p value of <0.05 was considered to be statistically significant.

RESULTS

One animal in group C (MESNA 20%) was excluded from the study because it died because of anesthesia provided before preoperative EMG measurements.

When the wave amplitude, as measured by EMG, was evaluated between the groups, there was no statistically significant difference between preoperative and postoperative amplitude levels of any of the groups (p>0.05). Compared with the preoperative amplitude level, there was no statistically significant change in the postoperative amplitude level of groups B, C, or D (p=0.893, p=0.068, or p=0.500, respectively, Table 1).

In terms of wave latency levels, as measured by EMG, no statistically significant difference was noted in preoperative and postopera-

Table 1. Evaluation of wave amplitudes, as measured by EMG, within and between groups

Amplitude	Group A	Group B	Group C	Group D	[1]p
Preop	2.49±1.38 (2.2)	2.23±1.01 (2.5)	0.96±0.7 (0.7)	1.99±0.85 (1.7)	0.133
Postop	2.49±1.38 (2.2)	2.36±1.42 (1.8)	1.35±0.79 (1.3)	1.93±0.78 (1.7)	0.514
Preop-postop [2]p	-	0.893	0.068	0.500	

EMG: electromyography

[1]Kruskal-Wallis test

[2]Wilcoxon sign test

Units of the values are millivolts (mV). Values in the parenthesis are mean values

Table 2. Intra- and inter-group evaluation of wave latency periods in EMG

Latency	Group A	Group B	Group C	Group D	[1]p
Preop	1.66±0.31 (1.8)	1.46±0.23 (1.3)	1.75±0.5 (2)	1.62±0.38 (1.8)	0.312
Postop	1.66±0.31 (1.8)	1.78±0.37 (1.8)	1.9±0.62 (1.7)	1.7±0.29 (1.8)	0.964
Preop-postop [2]p	-	0.068	0.713	0.496	

EMG: electromyography

[1]Kruskal-Wallis test

[2]Wilcoxon sign test

Units of the values are milliseconds (ms). Values in the parenthesis are mean values

Table 3. Evaluation of wave durations

Latency	Group A	Group B	Group C	Group D	[1]p
Preop	3.56±0.86 (3.7)	3.28±0.75 (2.8)	3.98±0.34 (4.1)	3.32±0.61 (3.4)	0.438
Postop	3.56±0.86 (3.7)	3.90±0.59 (4)	2.9±0.66 (3)	3.76±0.34 (3.8)	0.156
Preop-postop [2]p	-	0.051	0.068	0.345	

[1]Kruskal-Wallis test

[2]Wilcoxon sign test

Units of the values are milliseconds (ms). Values in the parenthesis are mean values

Table 4. Evaluation of supramaximal stimulation thresholds obtained using EMG

Latency	Group A	Group B	Group C	Group D	[1]p
Preop	2.5±0.5 (2.5)	1.8±0.76 (1.5)	2.75±0.5 (3)	2.7±0.76 (2.5)	0.167
Postop	2.5±0.5 (2.5)	2.6±0.82 (3)	2.5±0.71 (2.8)	2.8±0.91 (2.5)	0.947
Preop-postop [2]p	-	0.066	0.157	1.000	

EMG: electromyography

[1]Kruskal-Wallis test

[2]Wilcoxon sign test

Units of the values are milliampere (mA). Values in the parenthesis are mean values

Table 5. Evaluation of histopathological results

Latency		Group A	Group B	Group C	Group D
Inflammation	0	5 (100%)	5 (100%)	4 (100%)	5 (100%)
	+	-	-	-	-
	++	-	-	-	-
	+++	-	-	-	-
Foreign body reaction	No	5 (100%)	5 (100%)	4 (100%)	5 (100%)
	Yes	-	-	-	-
Granulation tissue	No	5 (100%)	5 (100%)	4 (100%)	5 (100%)
	Yes	-	-	-	-
Perineural sheath and myelin structure	Regular	5 (100%)	5 (100%)	4 (100%)	5 (100%)
	Irregular	-	-	-	-
Axonal degeneration	No	5 (100%)	5 (100%)	4 (100%)	5 (100%)
	Yes	-	-	-	-

Figure 1. Facial nerve trunk on the parotid gland in a rat

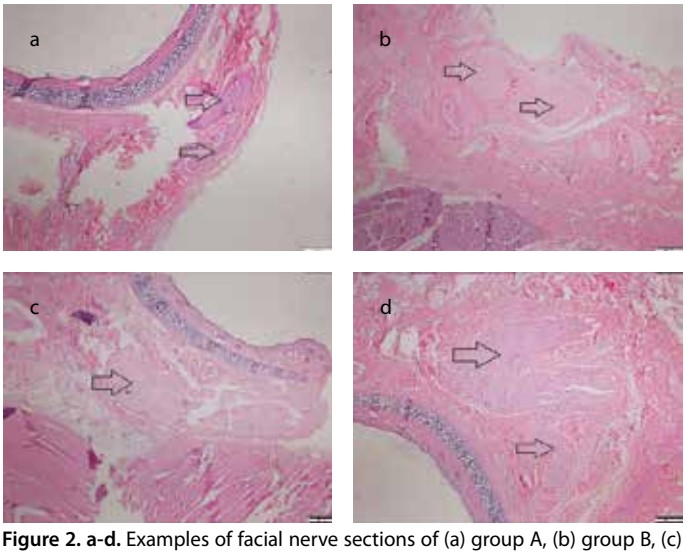

Figure 2. a-d. Examples of facial nerve sections of (a) group A, (b) group B, (c) group C (MESNA 20%), and (d) group D (MESNA 50%) stained with hematoxylin and eosin. No nerve damage is found in any of the 40× magnification images. Nerve samples are indicated using arrows

tive measurements between the groups (p>0.05). Compared with the preoperative latency level, there was no statistically significant change in the postoperative latency level of groups B, C, or D (p=0.068, p=0.713, or p=0.496, respectively, Table 2).

Table 3 denotes the evaluation of wavelength within each group and between all groups. Accordingly, there was no statistically significant difference in the preoperative and postoperative wavelengths between the groups (p>0.05). The increase noted in the wavelength in the postoperative period compared with that in the preoperative period did not show statistically significant difference in groups B, C, or D (p=0.051, p=0.068, or p=0.345, respectively).

When the preoperative and postoperative supramaximal stimulus threshold levels, as measured by EMG, were evaluated between study groups, no statistically significant difference was detected (p>0.05). Compared with the preoperative stimulus threshold level, there was no statistically significant change in the postoperative level in groups B, C, or D (p=0.068, p=0.157, p=1.000, respectively, Table 4).

Table 5 shows the results of histopathological examination. The degree of inflammation in all groups was 0. Foreign body reaction and granulation tissue were not detected in any of the groups. Perineural sheath and myelin structure were normal in all groups, and axonal degeneration was not observed (Figure 2-4).

DISCUSSION

MESNA is the sodium salt of 2-thiosulphonate anion. It is widely used in medicine because of its preservative, antioxidant, and mucolytic properties [12]. It is used as a cytoprotective agent to prevent hemorrhagic cystitis and as a mucolytic agent to improve pulmonary functions. In addition, the sulfhydryl group has the potential to clean the reactive oxygen metabolites and is used as a systemic protective agent against chemotherapy toxicity [9, 11].

In the field of otology, MESNA is used to easily dissect the tissue layers during ear surgeries, such as atelectatic ear and cholesteatoma, and its use has increased in recent years [7]. Some animal studies have shown that MESNA has no harmful effects on the cochlea and does not cause ototoxicity [8, 12]. However, the possibility of dehiscence development

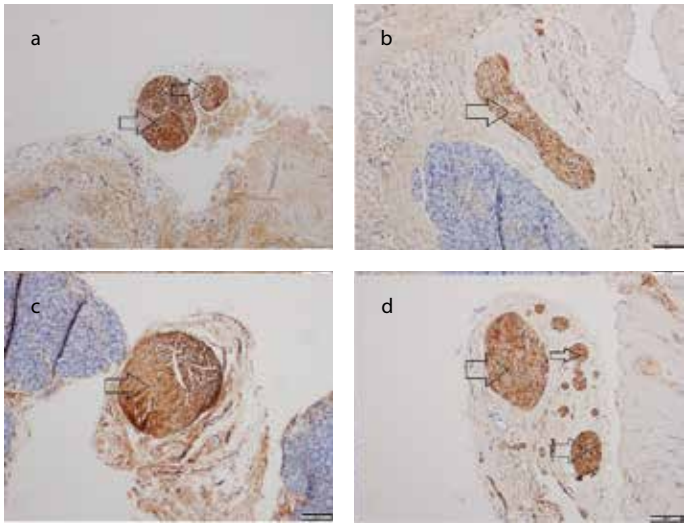

Figure 3. a-d. At 100× magnification, samples of nerve sections with S-100 staining; (a) group A, (b) group B, (c) group C (MESNA 20%), and (d) group D (MESNA 50%). There is no inflammation, foreign body reaction, or formation of granulation tissue in any sections. Myelin sheaths are found to be intact without axonal degeneration. Nerve samples are indicated using arrows

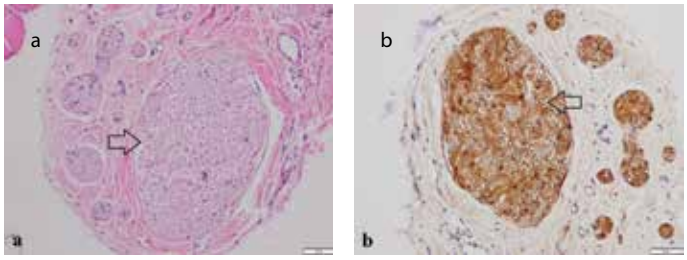

Figure 4. a, b. At 200× magnification, examples of a nerve stained with (a) H&E, (b) S-100. Nerve samples are indicated using arrows

in the fallopian canal of the facial nerve is very high in the presence of pathologies progressing with dissolving bone structures, such as cholesteatoma, and MESNA used to separate the cholesteatoma matrix in such surgeries inevitably comes in contact with the facial nerve, either directly or by passing through bony dehiscence. In a study performed by Ant et al. [10], histopathological results showed that MESNA has no local neurotoxic effects; however, the effects of MESNA on neuron functions have not been evaluated physiologically. Hence, the present study was the first to evaluate this phenomenon.

In this study, the effects of MESNA on facial nerve functions were evaluated using an animal model and based on electrophysiological and histopathological findings. The facial nerve in the parotid region of the rats was preferred because it is easy to reach and has the same characteristics as the facial nerve in the middle ear. As observed in previous studies on MESNA, a single dose of solutions diluted using physiological saline at concentrations of 20% and 50% was tested on separate groups [7, 8, 10, 12]. The wavelength, latency, amplitude, and supramaximal stimuli thresholds, as measured by EMG, were recorded and statistically evaluated within each group as well as between all groups. When the preoperative and postoperative wavelengths, latencies, amplitudes, and supramaximal stimulus threshold levels were evaluated between study groups, no statistically significant difference was detected ($p>0.05$). Compared with all preoperative findings, there was no statistically significant change in the postoperative findings of groups B, C, or D. Histopathological examination did not indicate any difference in terms of inflammation, foreign body reaction, or granulation tissue formation between the groups, and it was observed that the perineural sheath and myelin structure were preserved in all nerves without any axonal degeneration.

Our study has some limitations. The extratemporal portion of rats' facial nerves was evaluated in this study. MESNA was applied subcutaneously by dropping onto the facial nerve, and after waiting 5 min, the skin and subcutaneous tissue were closed. Through the middle ear, the facial nerve was located in a bone canal. During middle ear surgeries, leakage of MESNA into the dehiscent facial canal can increase the pressure on the nerve, and facial paralysis may occur following edema and inflammation. Because this study was unable to evaluate the part of the facial nerve positioned in the middle ear bone canal and because there was lack of knowledge regarding the potential for functional problems after prolonged exposure, studies are needed to investigate the neurotoxic effect of MESNA during middle ear surgery. To the best of our knowledge, this is the first study to investigate the effects of MESNA during otologic surgery, which enables cleavage of disulfate bonds and therefore, allows easy and safe dissection, particularly in cholesteatoma and adhesive otitis media, on the facial nerve in the parotid region by physiological and histopathological methods. The findings suggest that a single application of MESNA does not cause any histopathological and electrophysiological changes that indicate toxicity on the facial nerve.

CONCLUSION

To the best of our knowledge, this is the first study to investigate the effects of MESNA during otologic surgery, which enables cleavage of disulfate bonds and therefore, allows easy and safe dissection, particularly in cholesteatoma and adhesive otitis media, on the facial nerve in the parotid region by physiological and histopathological methods. The findings suggest that a single application of MESNA does not cause any histopathological and electrophysiological changes that indicate toxicity on the facial nerve.

Ethics Committee Approval: Ethics committee approval was received for this study from Yeditepe University Local Ethics Committee on Animal Experiments (Approval No: 01.11.2016-504).

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REFERENCES

1. Vincenti V, Magnan J, Saccardi MS, Zini C. Chemically assisted dissection by means of mesna in cholesteatoma surgery. Otol Neurotol 2014; 35: 1819-24. [CrossRef]

2. Badr-el-Dine M. Value of ear endoscopy in cholesteatoma surgery. Otol Neurotol 2002; 23: 631-5. [CrossRef]
3. Robinson JM. Cholesteatoma: skin in the wrong place. J R Soc Med 1997; 90: 93-6. [CrossRef]
4. Marchioni D, Villari D, Mattioli F, Alicandri-Ciufelli M, Piccinini A, Presutti L. Endoscopic management of attic cholesteatoma: a single-institution experience. Otolaryngol Clin North Am 2013; 46: 201-9. [CrossRef]
5. Hamilton JW. Efficacy of the KTP laser in the treatment of middle ear cholesteatoma. Otol Neurotol 2005; 26: 135-9. [CrossRef]
6. Takagi D, Hato N, Okada M, Hakuba N, Gyo K, Shigemoto K, et al. Galectin-7 as a marker of cholesteatoma residue and its detection during surgery by an immunofluorescent method Y a preliminary study. Otol Neurotol 2012; 33: 396-9. [CrossRef]
7. Kalcioglu MT, Cicek MT, Bayindir T, Ozdamar OI. Effectiveness of MESNA on the success of cholesteatoma surgery. Am J Otolaryngol 2014; 35: 357-61. [CrossRef]
8. Vincenti V, Mondain M, Pasanisi E, Piazza F, Puel JL, Bacciu S, et al. Cochlear effects of mesna application into the middle ear. Ann N Y Acad Sci 1999; 884: 425-32. [CrossRef]
9. Yilmaz M, Goksu N, Bayramoglu I, Bayazit YA. Practical use of MESNA in atelectatic ears and adhesive otitis media. ORL J Otorhinolaryngol Relat Spec 2006; 68: 195-8. [CrossRef]
10. Ant A, Karamert R, Kulduk G, Ekinci Ö, Tutar H, Göksu N. The effects of sodium-2-mercaptoethanesulfonate application on the neural and neurovascular tissues: An experiment alanimal study. Surg Neurol Int 2015; 6: 150. [CrossRef]
11. Casale M, Di Martino A, Salvinelli F, Trombetta M, Denaro V. MESNA for chemically assisted tissue dissection. Expert Opin Investig Drugs 2010; 19: 699-707. [CrossRef]
12. Van Spaendonck MP, Timmermans JP, Claes J, Scheuermann DW, Wuyts FL, Van de Heyning PH. Single ototopical application of mesna has no ototoxic effects on guinea pig cochlear hair cells: a morphological study. Acta Otolaryngol 1999; 119: 685-9. [CrossRef]
13. Salomone R, Costa HJ, Rodrigues JR, Reis e Silva SM, Ovando PC, Bento RF. Assessment of a neurophysiological model of the mandibular branch of the facial nerve in rats by electromyography. Ann Otol Rhinol Laryngol 2012; 121: 179-84.[CrossRef]