The Journal of Laryngology & Otology
August 2005, Vol. **119,** pp. 600–605

Effect of thermal energy produced by drilling on the facial nerve: histopathologic evaluation in guinea pigs

Asim Aslan, MD, H Seda Vatansever, MD*, Gulay Guclu Aslan, MD, Gorkem Eskiizmir, MD, Gulsen Giray, MD*

Abstract

The effect of thermal energy due to drilling around the facial nerve canal on the facial nerve was histopathologically evaluated in four guinea pigs. The bony canal of the facial nerve was drilled using a 3-mm diamond burr for one minute. The temperature changes on the facial nerve canal were noted before and after dissection. The temporal bones of the animals were histopathologically examined under light microscopy using haematoxylin & eosin (H&E) and solochrome cyanine staining for myelin, and immunohistochemical staining for neuronal nitric oxide synthase (nNOS). Compared to the control group, it was observed with H&E staining that there was oedema among the axonal fibres and with solochrome cyanine staining that the thickness of the myelin fibres was decreased, and that the severity and extent of nNOS activity was decreased in the axonal fibres. It was concluded that a temperature increase on the facial canal may potentially lead to inflammation of the nerve, and may also cause deterioration of nerve conduction to some extent.

Key words: Facial Nerve; Otological Surgical Procedures; Wounds and Injuries; Temperature

Introduction

Facial nerve (FN) paralysis is one of the most dreadful complications of otologic surgery. It has been reported that the intra-operative FN paralysis rate ranges from 0.6 to 3.6 per cent.[1,2] There are two likely causes: direct trauma to the nerve during surgical manipulations; and trauma due to thermal energy produced by drilling around the FN canal.

Although there is an insufficient number of clinical studies, many otologic surgeons frequently emphasize that thermal energy produced by drilling of the mastoid bone might potentially be conducted to the FN, and cause injury to the nerve.[2,3] Lavy and Fagan[3] reported that heat produced during trans-canal procedures caused transient FN paralysis. They suggested continuous water irrigation to decrease the amount of heat produced.

That the diamond and cutting burrs used for bone dissection during mastoidectomies cause heating was first demonstrated experimentally by Call.[4] Abbas and Jones[5] found, using cadavers, that when the bone around the facial canal was drilled with 4-mm burrs there was a heat increase of 33°C inside the canal without irrigation, and an increase of 4°C with irrigation. Mills *et al.*,[6] however, reported an increase of 0.9°C within the facial canal from drilling of the stapes. These two studies were made on cadavers and thus not under *in-vivo* conditions. Blood flow might decrease the heat change to some extent, but such an effect can only be verified by animal studies. To our knowledge, there is no study histologically examining the effect of heat produced by drilling on the FN. We aimed to examine whether the heat produced by drilling causes any histopathologic changes in the FN in guinea pigs.

Material and methods

Obtaining the specimens

Six New Zealand guinea pigs weighing 200–250 mg were used in the study. An approval from the ethical committee of Celal Bayar University School of Medicine was obtained prior to the study. All animals were anaesthetized with intra-peritoneal ketamine and xylazine. After retroauricular skin incision temporal bone was exposed. The bulla was entered using a 3-mm diamond burr. Temperatures over the FN canal were measured by a thermistor (Digitek 3½ digit multimeter, DT-890D) of which the diameter was 1 mm (Figure 1). Then, the bone around the facial nerve canal was drilled with a 3-mm diamond burr for 60 seconds with a drill of speed 15 000 rpm. A thin covering of the bone was

From the Departments of Otorhinolaryngology and *Histology and Embryology, Celal Bayar University, School of Medicine, Manisa, Turkey.
Accepted for publication: 14 April 2005.

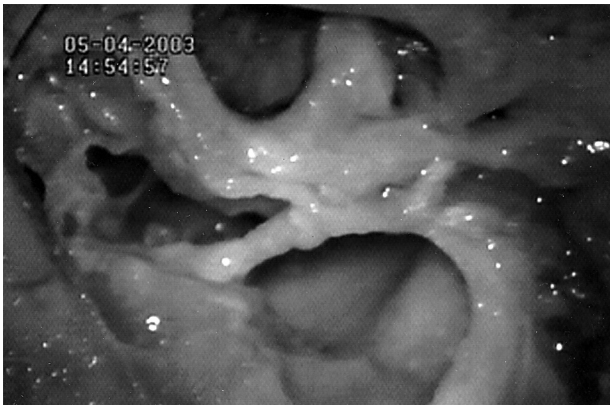

FIG. 1
Mastoid portion of FN canal in a guinea pig.

left. The temperature was measured again. All procedures were performed by the same surgeon (A A). The animals were killed by intra-cardiac ketamine infusion and the temporal bones removed.

Two animals were used in the control group. In one animal (C1-right) dissection around the facial nerve was carried out using a 1-mm diamond burr, but no temperature change occurred (it remained at 33.0°C). However, contraction of the facial muscles was observed at 40 seconds. It was considered that mechanical trauma might have been responsible for the contraction. The left ear of this animal (C1-left) was used as a negative control group without any drilling. In order to decide on the diameter of the burr to be used in the study, bone dissection was performed with a 1-mm diamond burr in the right ear of the second animal (C2-right) and with a 3-mm diamond burr in the left ear (C2-left). The temperature changed from 30.9 to 30.3°C with the 1-mm burr, while it increased from 31.1 to 36.4°C with the 3-mm burr. Thereafter, it was decided to continue the dissections in four animals in the study group using 3-mm diamond burrs.

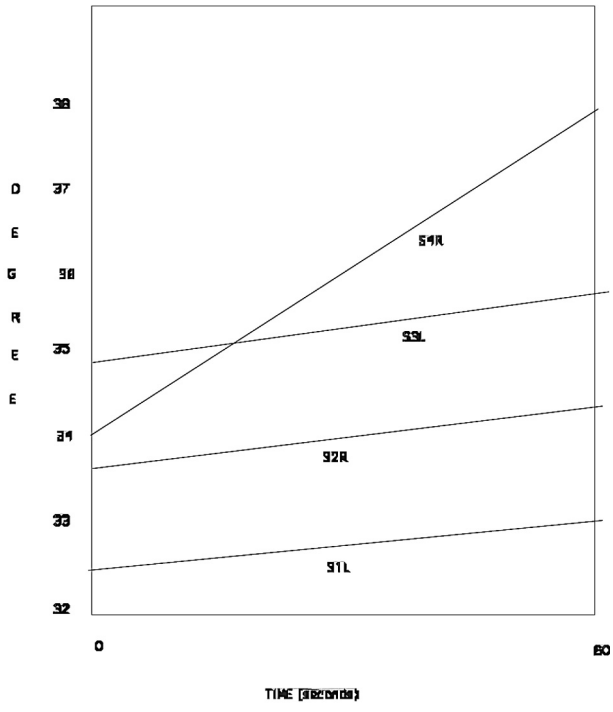

FIG. 2
Graphic demonstration of temperature changes in the study group.

Tissue collection and fixation

All samples were fixed in 10 per cent formalin solution for 48 hours. Samples were then washed with tap water and soaked in a graded series of 50 per cent, 60 per cent, 70 per cent, 80 per cent and 90 per cent ethanol for 30 minutes, and then in 95 per cent and 100 per cent ethanol for one hour. Then they were held in a solution of 100 per cent ethanol and xylene at a 1:1 ratio for 30 minutes, before being embedded in paraffin and held at 60°C for one hour to make paraffin blocks. Transverse sections (5 μm) were taken from the blocks and prepared for histochemical and immunohistochemical staining. Haematoxylin & eosin (H&E) staining for histological observation and solochrome cyanine staining for demonstration of myelin were used.

Sections were kept at 60°C overnight and then incubated in xylene for 30 minutes. After washing with a decreasing series of ethanol, while some of the sections were stained with H&E using a routine procedure, the other sections were stained with solochrome cyanine solution (Eriochrome cyanine RC; Sigma E-2502, St Louis, MO, USA) for 15 minutes. After washing with tap water, they were incubated in iron alum solution (Ammoniumeisen [III] sulfate dodecahydrate; Merck A993675, Darmstadt, Germany) for 10 minutes. They were then washed in tap water again, dehydrated with ethanol and mounted using entellan.

Immunohistochemistry

After deparaffination at 60°C overnight, sections were held in xylene for one hour. After washing with decreasing concentrations of ethanol (95 per cent, 80 per cent, 70 per cent and 60 per cent, two minutes each), the sections were washed with distilled water and phosphate-buffered saline (PBS) for 10 minutes. Then they were treated with in 2 per cent trypsin in Tris buffer at 37°C for 15 minutes, and given three five-minute washes in PBS. The limits of sections were drawn with a Dako pen (Dako S-2002, Glostrup, Denmark) and incubated in 3 per cent hydrogen peroxidase for 15 minutes to inhibit endogenous peroxidase activity. The sections were then washed with PBS and incubated with a polyclonal anti-nNOS (neuronal nitric oxide synthase) antibody in a 1:100 dilution (Zymed 617000, San Francisco, CA, USA) for 18 hours. Afterwards, sections were washed three times for five minutes each in PBS, followed by incubation with biotinylated anti-rabbit IgG and then with streptavidin peroxidase conjugate (Zymed Histostain kit 85-9043). After washing the secondary antibody in PBS three times for five minutes each, the sections were incubated with a substrate solution containing diaminobenzidine (Zymed 00-2020) and

https://doi.org/10.1258/0022215054516250 Published online by Cambridge University Press

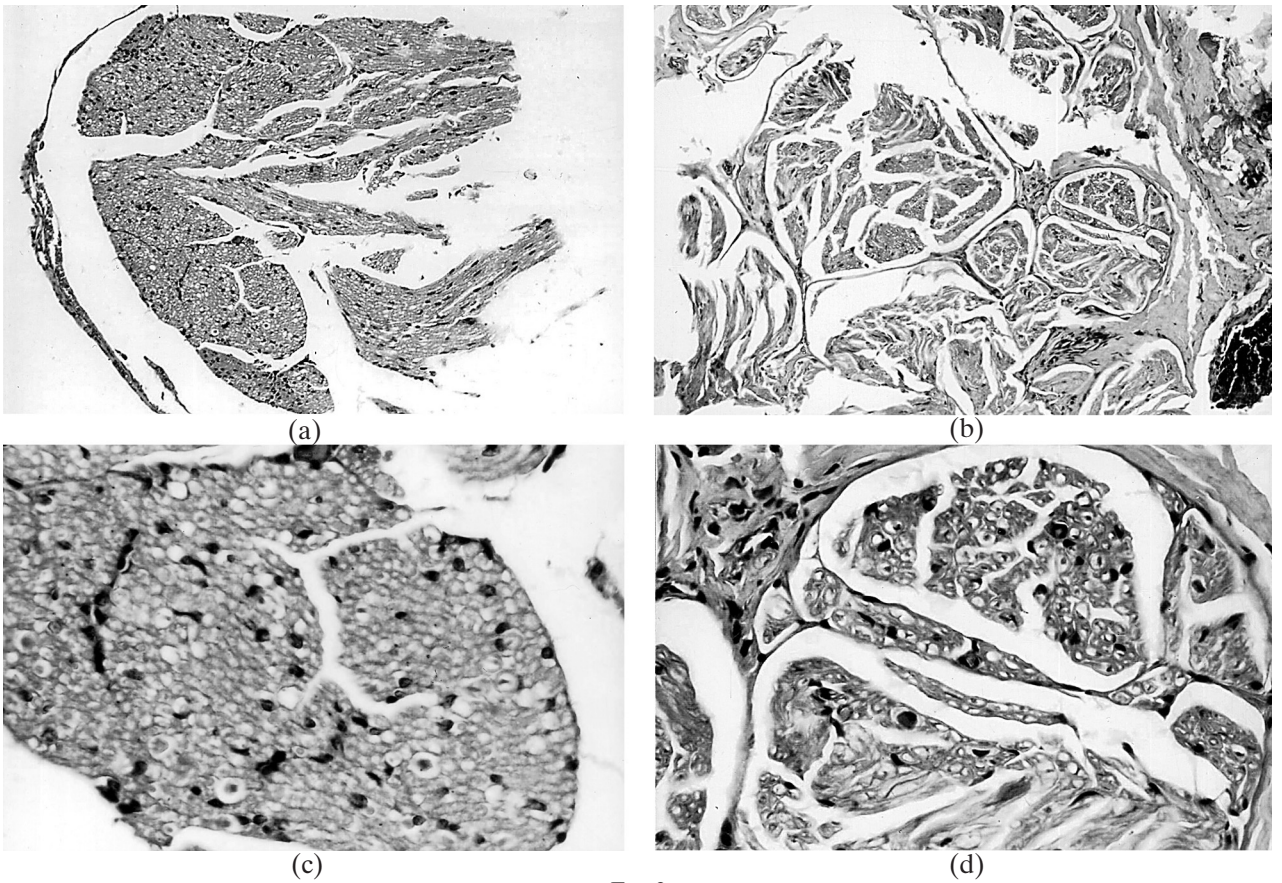

FIG. 3

Photomicrographs showing H&E staining of FN from study (a, c) and control (b, d) groups. While there was no oedema or increase in connective tissue elements in the control group (b, d), localized oedema scattered in the axonal fibres was detected in the study group (a, c). Magnifications: ×100 (a, b) and ×400 (c, d).

H_2O_2 for five minutes to visualize peroxidase activity, and were then counterstained with Mayer's haematoxylin. They were covered with entellan and analysed with a BX 40 light microscope (Olympus, Tokyo, Japan). Pictures were taken using 100 ASA Fuji colour film. Control samples were processed in an identical manner, but the primary antibody was replaced by normal rabbit serum.

Evaluation of sections

Sections were covered with glass cover slips prior to viewing and photographed under the Olympus BX-40 light microscope; two observers, blinded to clinical information, evaluated the staining scores independently. Staining intensity was assigned using a semiquantitative immunohistochemical scoring system as follows: mild (+), moderate (++) and strong (+++).

Results

Temperature findings

Four animals were included in the study group. The temperature changes before and after bone dissection around the FN canal were as follows (Figure 2): 32.6 to 33.1°C (0.5°C) in the first animal (S1); 35.0 to 35.8°C (0.8°C) in the second animal (S2); 33.7 to 34.5°C (0.8°C) in the third animal (S3) and 34.1 to 38.0°C (3.9°C) in the fourth animal (S4). The mastoid portion of the temporal bone in S4 was found to be sclerotic. No facial muscle contractions due to FN stimulation were observed in any animal in the study group during dissections.

Histochemical findings

Control group. H&E staining showed normal histology of the FN with preservation of epineurium, perineurium and endoneurium. There was no oedema or increase in connective tissue elements. Axonal fibres could easily be detected (Figure 3b and d). After solochrome cyanine staining, myelin sheath around the axonal fibres could easily be distinguished and had the same thickness (Figure 4b and d). In addition, nNOS immunostaining was observed in each axon and its intensity was strong (Figure 5b and d).

Study group. Using H&E staining, epineurium, perineurium and endoneurium were found to be preserved (Figure 3a and c). There was no cell infiltration or oedema within the connective tissue. Localized oedema scattered in the axonal fibres was observed. In the specimens of S2 and S4, there was oedema also in the endoneurium and the axonal fibres could not be distinguished very well. Solochrome cyanine showed that the thickness of myelin sheath varied, and degenerative changes were also observed (Figure 4a and c). Mild-to-moderate immunostaining of nNOS was observed in axonal fibres (Figure 5a and c; Table I).

https://doi.org/10.1258/0022215054516250 Published online by Cambridge University Press

TABLE I

DISTRIBUTION INTENSITY OF NNOS IMMUNOREACTIVITY AND NUMBER OF POSITIVE CELLS

Animal No.	Amount of temperature change	nNOS intensity	Percentage of nNOS positive cells
C1-left		+++	78
C1-right	33.0 – 33.0	+	58
C2-left	31.1 – 36.4	++	65
C2-right	30.9 – 30.3	+++	71
S1-left	32.6 – 33.1	++	43
S2-right	35.0 – 35.8	+++	81
S3-left	33.7 – 34.5	++	58
S4-right	34.1 – 38.0	+	60

Discussion

Mastoidectomy is one of the most frequently performed otologic surgical procedures. The metal burrs used for drilling lead to heat production during this procedure. It has been proposed that the thermal energy produced by drilling around the FN canal might be conveyed to the nerve, causing FN paralysis.[1–3] Although an increase in temperature within the facial canal caused by drilling has been reported,[4–6] since these studies were performed in cadavers it is difficult to state that this increase would also cause an injury to the FN. The effect of heat production on the FN can be studied in animal models. However, there is a lack of studies addressing histological changes in the FN in an animal model.

Histopathological evaluation using H&E staining in this study showed that there might be oedema both in axonal fibres of the FN and in the endoneurium (Figure 3a and c). This indicates that temperature elevation may lead to inflammation. Similar findings were observed by Ator *et al.*,[7] who found significantly increased oedema in the FN after CO_2 laser application. Furthermore, solochrome cyanine staining in our study demonstrated that the temperature increase may also cause thinning of the myelin fibres, and even degenerative changes (Figure 4a and c).

We also studied nNOS activity in the FN. NOS is a gaseous neurotransmitter, and nNOS is used as a neuromodulator molecule in nerve fibres. It is known that it plays an important role in neurotransmission.[8–10] We found that temperature elevation may cause a decrease both in intensity of nNOS and in the number of cells having nNOS activity in the axonal fibres of the FN (Figure 5a and c). The most significant decrease in intensity of nNOS was seen in the animal with sclerotic bone and

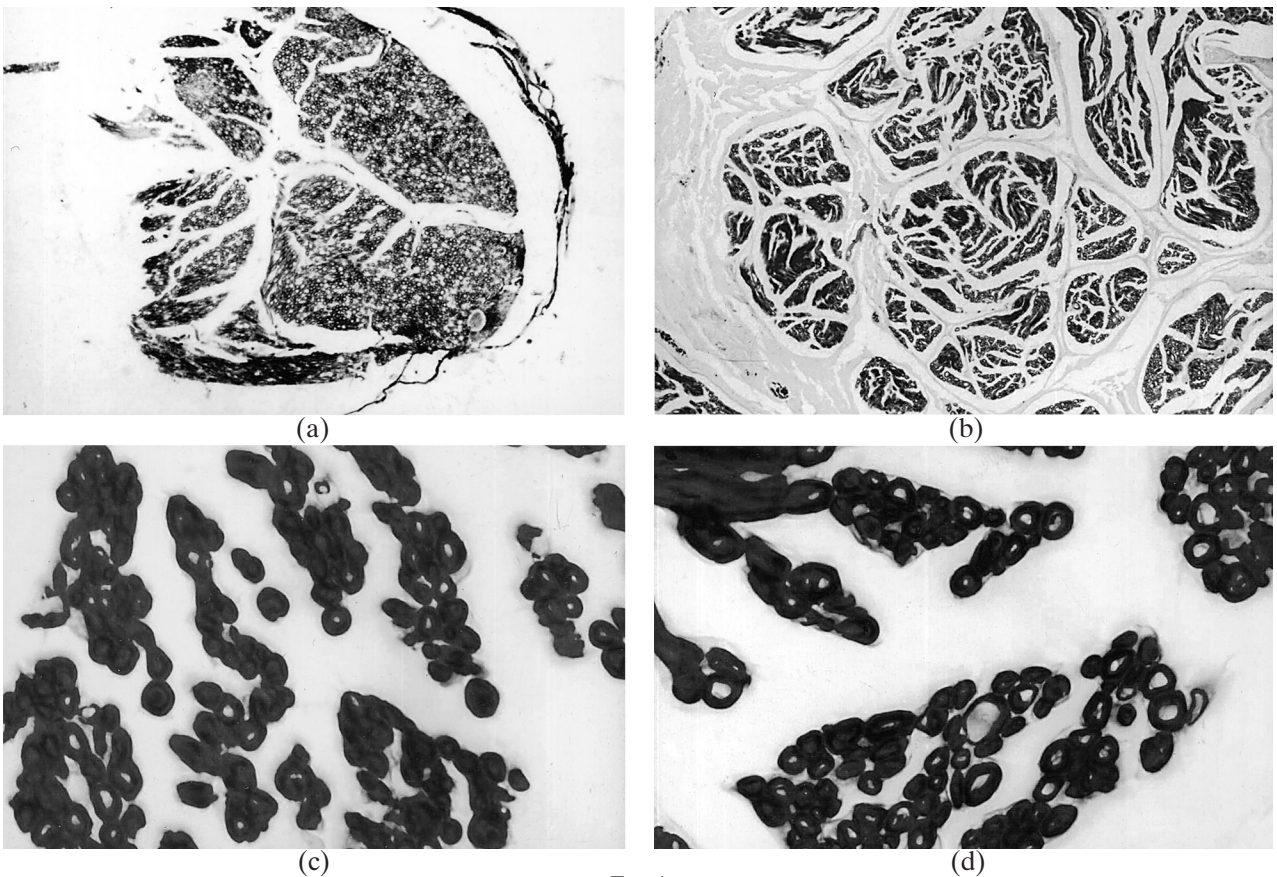

FIG. 4

Photomicrographs showing solochrome cyanine staining of FN from study (a, c) and control (b, d) groups. There are differences in the thickness and degenerative changes in the myelin sheath in the study group (a, c) when compared to the control group (b, d). Magnifications: ×100 (a, b) and ×1000 (c, d).

https://doi.org/10.1258/0022215054516250 Published online by Cambridge University Press

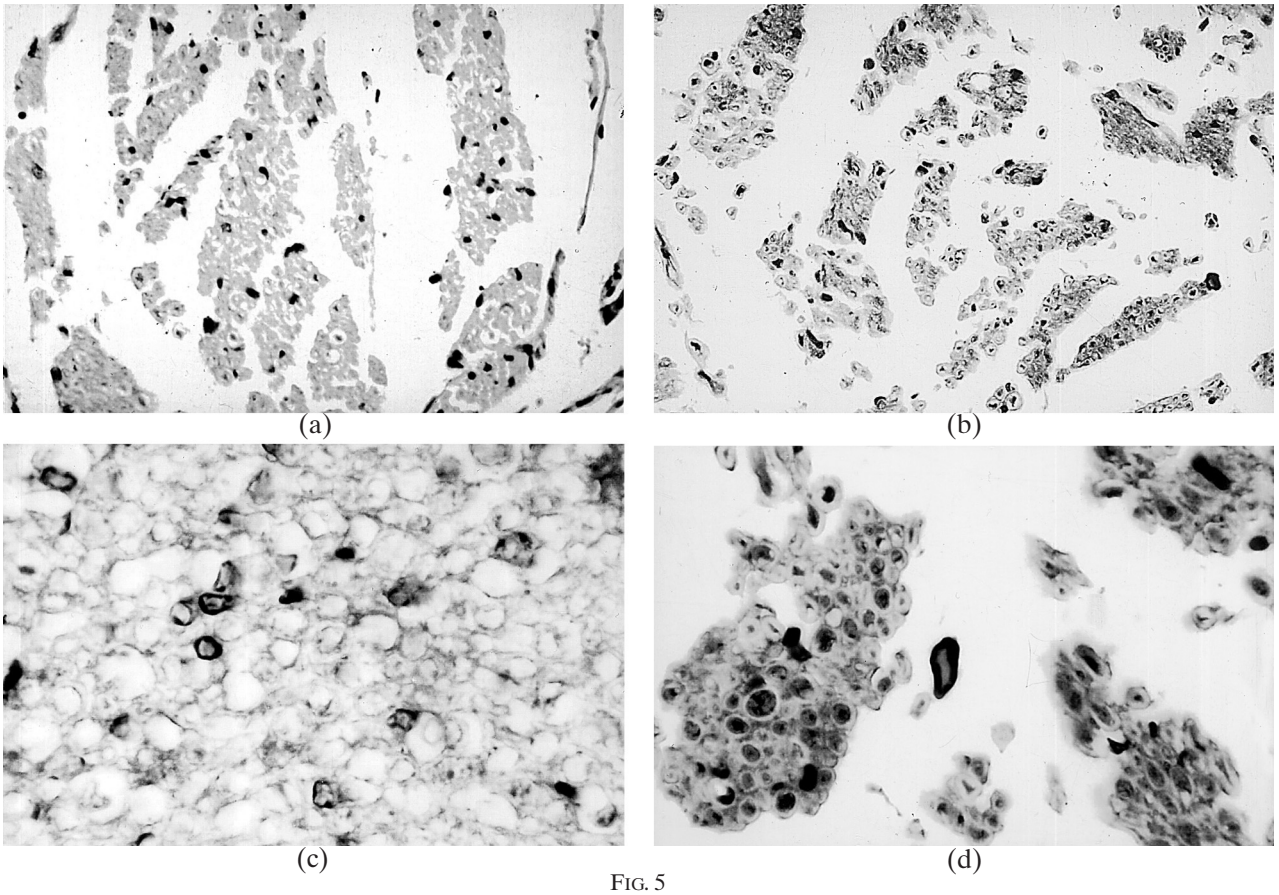

FIG. 5

Photomicrographs of immunolocalization of nNOS in FN from study (a, c) and control (b, d) groups. While mild-to-moderate immunostaining of nNOS was observed in the study group (a, c), immunoreactivity was detected strongly in the control group (b, d). Magnifications: ×100 (a, b) and ×1000 (c, d).

in which the temperature elevation was highest. This shows much similarity to the human temporal bone in which the facial canal is surrounded by compact bone. The finding of decreased nNOS activity together with changes observed in the myelin fibres may indicate that temperature elevation leads to changes in nerve conductibility. However, it is impossible to infer to what extent these histopathological changes reflect the real functional status of the FN, since the design of the study cannot assess the electrophysiological properties of the nerve concomitantly.

A similar decrease in nNOS activity was observed in the animal C1-left, in which facial muscle contraction was observed. Since there was no temperature elevation, it is possible that this contraction was due to touching of the FN. This finding could be of interest. In routine otologic surgery, FN function is followed by observation of any facial muscle contraction. Decreased nNOS activity indicates that facial contraction might lead to a decrease in nerve conductibility. During surgical procedures repeated stimulation of the FN causing muscle contraction leads to unresponsiveness. This might be explained by decreased nNOS activity. However, whether this decrease is permanent remains unclear. The thermal tolerance of the FN has not been well defined and no information exists regarding return of function to the damaged nerve. Further studies are necessary to elucidate this subject.

The amount of increase in temperature measured in our study can be questioned. In the study group the temperatures ranged from 0.5 to 3.9°C. One may speculate that these values are much lower than those reported in some experiments,[4,5] although Mills *et al.* reported that dissection with microdrills caused an increase of 0.9°C within the facial canals of cadavers.[6]

Thermogenesis due to drilling may depend on factors such as the design and size of the burr, the pressure applied, and the speed of turning.[4] Since the diamond and cutting burrs work using different thermodynamic principles, the temperature change they create also differs. An increase in thermogenesis by a cutting burr is proportionate to a higher rotation speed, amount of pressure applied and amount of surface contact area. An increase in thermogenesis by diamond burrs, however, has been reported to be proportionate only to the amount of pressure applied, and not to the speed of rotation and contact area of the burr.[4] Since all the dissections were carried out by the same surgeon (A A), it can be considered that the amount of pressure was standardized as much as possible. In addition, before dissecting the study group animals we evaluated the effect of diamond burr size on heat production. Although, using 2-mm and 4-mm diamond burrs, Call[4] reported that the less the

https://doi.org/10.1258/0022215054516250 Published online by Cambridge University Press

diameter the greater the temperature generated, we observed that 1-mm diamond burrs did not cause any change in temperature while 3-mm burrs caused a significant elevation. This indicates that the conclusion put forward by Call can only be valid for diamond burrs larger than 1 mm in diameter.

- **This study looks at the possible thermal injury caused to the facial nerve by drilling. Guinea pigs were used as an experimental model**
- **Histologic evidence of thermal facial nerve injury was found in the form of inflammatory changes**
- **The possibility of clinical injury to the facial nerve during middle ear surgery caused by thermal injury needs to be borne in mind by otologists. Further studies are needed to correlate the histologic features of nerve injury with impaired nerve conduction**

In addition, there may be significant differences between temperatures measured in cadavers and *in vivo,* as it is highly possible that blood flow decreases the amount of elevation in temperature. Moreover, the facial canal in guinea pigs is isolated within an air-filled cavity (Figure 1); unlike in humans there is no compact bone surrounding it. As the thickness of the bone surrounding the facial canal increases, there would be higher temperature elevations during dissections. In an animal which had intense sclerotic bone around the facial canal (S4), we detected a significantly higher level of temperature elevation (3.9°C).

The degree of temperature change is important since it was reported that temperature elevations of more than 9°C may be the cause of injury to the FN. Angell-James *et al.*[11] reported that an elevation in temperature of more than 9°C resulted in FN damage in patients with Ménière's disease in which the labyrinth was ultrasonically irradiated. However, this criterion cannot be applied to guinea pigs since there are some anatomical differences. It is highly possible that in guinea pigs smaller elevations in temperature cause some histological changes as observed in our study.

In conclusion, the temperature created by drilling around the FN can potentially cause some histological changes. Temperature increase of the facial canal might lead to inflammation of the nerve. In addition, it may cause a deterioration in nerve conduction to some extent, indicated by changes in the thickness of myelin fibres and in nNOS activity. However, there is a need for further studies examining the correlation of the histopathological changes in nerve conduction with the electrophysiological status of the FN.

Acknowledgement

This study was supported by the Scientific Research Projects Committee of Celal Bayar University School of Medicine (No: 2002/24).

References

1 Cawthorne T. The surgery of the temporal bone. Part 2. The non-suppurative surgery of the temporal bone. *J Laryngol Otol* 1953;**67**:437–48
2 Wiet RJ. Iatrogenic facial paralysis. *Otolaryngol Clin North Am* 1982;**15**:773–80
3 Lavy J, Fagan P. Canalplasty: review of 100 cases. *J Laryngol Otol* 2001;**115**:270–3
4 Call WH. Thermal injury from mastoid bone burs. *Ann Otol* 1978;**87**:43–9
5 Abbas GM, Jones RO. Measurements of drill-induced temperature change in the facial nerve during mastoid surgery: A cadaveric model using diamond burrs. *Ann Otol Rhinol Laryngol* 2001;**110**:867–70
6 Mills RP, Szymanski M, Abel EW. Delayed facial palsy following laser stapedotomy: in vitro study of facial nerve temperature. *Clin Otolaryngol* 2003;**28**:211–14
7 Ator GA, Coker NJ, Jenkins HA. Thermal injury to the intratemporal facial nerve following CO2 laser application. *Am J Otolaryngol* 1985;**5**:437–42
8 Kiechle FL, Malinski T. Nitric Oxide: Biochemistry, pathophysiology, and detection. *Am J Clin Pathol* 1993;**100**:567–75
9 Billiar TR. Nitric oxide: Novel biology with clinical relevance. *Ann Surg* 1995;**221**:339–49
10 Michel O, Hess A, Krolzig M, Stennert E, Addick K, Bloch W. Involvement of nitric oxide synthase in the physiology and pathophysiology of facial nerve function and dysfunction. *Eur Arch Otorhinolaryngol* 2000;**257**:188–92
11 Angell-James J, Dalton G, Freundlich H. Histological, thermal, and biochemical effects of ultrasound on the labyrinth and temporal bone. *Acta Otolaryngol (Stockh)* 1964;**57**:306–12

Author for correspondence:
Asim Aslan, MD,
Posta Kutusu, 82,
45010, Manisa, Turkey

Fax: 90-236-2370213
E-mail: asimas98@yahoo.com

Dr A Aslan takes responsibility for the integrity of the content of the paper.
Competing interests: None declared