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Influence of carbon dioxide laser irradiation on the morphology and function of guinea pig cochlea

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Abstract

Both experimental and clinical studies have demonstrated that carbon dioxide laser is suitable for stapedotomy. The aim of this study was to investigate morphological, electrophysiological and functional changes in the inner ear after irradiation with CO_2 laser set with different energy parameters.

A cochleostomy in the basal cochlear turn of guinea pig cochleae was performed with CO_2 laser of 1, 2 and 3 w, respectively. The cochleae were removed three weeks after laser irradiation. The auditory evoked brainstem response (ABR) was measured before and after laser application and immediately before removal of the cochlea. Immunohistochemical methods were used to examine inducible nitric oxide synthase (iNOS/NOSII) and heat-shock protein 70 (Hsp70) concentrations in the cochlea after laser application. The organ of Corti was studied by scanning electron microscopy.

Worse hearing loss was observed in animals receiving higher-power CO_2 laser. These findings correlated with more intense injury of the cochlear ultrastructure and with positive expression of iNOS and Hsp70 in spiral ganglion cells, nerve fibres, supporting cells of the organ of Corti and cells of the spiral ligament.

The CO_2 laser as a noncontact procedure is shown to be effective and safe if the total amount of energy is kept within the limits applied in this study. Nitric oxide and stress proteins play important roles in the traumatic mechanism of the inner ear, which are related to hearing loss and injury of the ultrastructure of the inner ear.

Key words: Stapes Surgery; Cochlea; Laser Surgery; Scanning Electron Microscopy; Nitric Oxide

Introduction

Surgical techniques opening the inner ear must meet two principal requirements that are apparently difficult to reconcile: the ability to ablate bone, and a high degree of safety in preventing damage to the sensory structures of the inner ear. Using modern laser technology, surgery can be performed without mechanical contact, reducing the risk of direct mechanical trauma. The application of different lasers has been described in experimental and clinical studies. [1–4] *In vitro* and *in vivo* animal studies and clinic application have shown that carbon dioxide laser seems appropriate for use in inner-ear surgery. [3,5]

Laser systems may be classified as continuous-wave lasers, such as argon and CO_2 lasers, or pulsed lasers, such as erbium–yttrium–aluminium–garnet (YAG), erbium:yttrium–scandium–gallium–garnet and holmium:YAG. The application of all of these laser systems for stapedotomy has been reported by different authors.[1,2,4–6] The CO_2 laser has a wavelength of 10.6 μm and is well absorbed in water. It can be applied in either a continuous or pulsed mode. In continuous mode its effect is primarily thermal, giving rise to coagulation and carbonization at the borders of the application area. In the pulsed mode its effect becomes more ablative because of a higher energy density and a shorter application time. As exposure times are very short, heat conduction to adjacent tissues is minimal and tissue is ablated with little or no marginal effects such as coagulation or carbonization.[7]

Nitric oxide is involved in the regulation of various physiological and pathological mechanisms in the inner ear, such as mediating neurotransmission and regulating vascular tone and pathophysiology. Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) in an unusual reaction that converts arginine and oxygen into citrulline and NO.[8] At least three isoforms of NOS have been characterized; the constitutive endothelial (eNOS, ecNOS and NOSIII) and neuronal (nNOS, ncNOS and NOSI) types are calcium-dependent, while the third form of the enzyme is calcium-independent inducible NOS

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(iNOS, NOSII) (usually not expressed constitutively but inducible under pathological conditions, e. g. endolymphatic hydrops, inflammation, adminstration of anticancer drugs or noise exposure).[9–14] These reports suggested that large amounts of NO are associated with various pathological conditions of the inner ear.

Heat-shock proteins (Hsps) are self-protective proteins that maintain cell homeostasis against various forms of stress as an adaptive response.[15] These proteins are induced by a wide variety of stressors and have broad cytoprotective functions. The 70-kDa family of Hsp (Hsp70) plays a particularly vital role in cellular protection and has been detected in various tissues subject to stress.[16] In the inner ear, Hsp70 becomes immunogenic in the host because of overexpression (secondary to inflammation caused by another agent) and causes cochlear dysfunction through an autoimmune reaction in which a cellular immune response plays a central role.[17]

On the one hand, the aim of this animal experiment was to determine whether *in vivo* application of CO_2 laser caused inner-ear damage and, if so, to what extent. Scanning electron microscopy (SEM) enabled precise morphological evaluation of the organ of Corti in the basal turn of the cochlea and thus provided data on the possible type, extent and site of damage. Results obtained by measuring auditory evoked brainstem response (ABR) after laser application enabled a comparison of morphological and electrophysiological data.

On the other hand, this experiment also aimed to explore the possible traumatic mechanisms of the inner ear, by immunohistochemical examination of iNOS/NOSII and Hsp70.

We compared the effects of cochleostomies performed with CO_2 lasers of various power densities. Guinea pigs were chosen as the animal model because the basal turn of the cochlea is easily accessible and the thickness of the cochlea wall (1 mm anterior to a round window of approximately 120–160 μm) is comparable with the dimensions of a slightly thickened otosclerotic footplate (150–200 μm).[5] The results of this study may therefore be related to the anticipated effects of stapedotomy in humans.

Materials and methods

Animal preparation

Experiments were performed on 32 albino guinea pigs (both sexes, 250–400 g). All animals were obtained from the Animal Center of Anhui Medical University and their outer and middle ears were confirmed to be healthy by otomicroscopy. All animals had a positive Preyer reflex. The animals were divided into four groups: 'A group', eight animals receiving cochleostomy with 1-w CO_2 laser (796 w/cm^2); 'B group', eight animals receiving cochleostomy with 2-w CO_2 laser (1592 w/cm^2); 'C group', eight animals receiving cochleostomy with 3-w CO_2 laser (2388 w/cm^2); and 'D group', eight animals receiving a surgical cochleostomy without CO_2 laser (this group served as the control group). For all experimental procedures, the animals were anaesthetized with sodium pentobarbital (30 mg/kg). All the procedures were performed in the left ears of the guinea pigs.

Surgery

Guinea pigs were anaesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). The local anaesthetic lidocaine 0.5 per cent (2 ml) was infiltrated into the postauricular skin before the surgical procedure. Experiments were carried out on a feedback-controlled heated blanket adjusted to 37°C. A postauricular incision was made behind the left ear, and the bulla and the basal turn of the cochlea were exposed by careful fracture and removal of the bony shell of the bulla (to avoid any acoustic damage to the inner ear). Under microscope (Zeizz, Jena, Germany) guidance, a cochleostomy was performed 1 mm anterior to the round window niche using CO_2 laser (Sharplan 30C, Yokneam, Israel) with a continuous pulse, until a circular opening of approximately 0.5 mm in diameter was obtained. The endothelium was opened as verified by an effusion of perilymph. The cochleostomy was closed 10 mins after completion of the opening with a free muscle graft, fixed with fibrin glue. The bulla was closed with dental cement and the skin sutured.

Auditory evaluation

The hearing of each animal was assessed before surgery, immediately after surgery and three weeks post-operatively. Auditory evoked brainstem response thresholds were measured bilaterally in all animals, with surface electrodes, according to the method described by Gong and Wang.[18] Responses that clearly demonstrated a reproducible wave form were interpreted as threshold responses.

Scanning electronic microscopy

Three weeks after the cochleostomy, two guinea pigs from each group were sacrificed (by decapitation). The tympanic bullae were dissected from the surrounding tissues and opened. Under magnification, the bony wall of the cochlea was removed with a pick and forceps to expose the upper aspect of the organ of Corti. Cochleae were fixed in a 2.5 per cent solution of glutaraldehyde in 0.1 mol/L sodium cacodylate buffer. Dissected cochleae were dehydrated in 10 per cent, 30 per cent, 70 per cent, 90 per cent and 100 per cent (three times) concentrations of ethanol and critical-point-direc with an Emitec 850 stem, plated by a Bio-Rad SC-650 and observed under a JSM 6700F scanning microscope.

Cochleae processing

Four guinea pigs from each group were sacrificed immediately after cochleostomy and two guinea pigs from each group were sacrificed three weeks later. The temporal bone was quickly removed and the bone surrounding the cochlea trimmed. To aid

penetration of the fixative (4 per cent paraformaldehyde in 0.01 mol/L phosphate buffered saline (PBS)), the footplate of the stapes was dislocated and exposed the oval window. The cochlea was then fixed for 24 hours and decalcified in 7 per cent HNO_3 for 24 hours. Subsequently, the tissues were embedded in paraffin. Each specimen was sectioned at a thickness of 3 μm along the midmodiolar plane of each cochlea and sections collected on glass slides coated with 3-amino-propyltriethoxysilane (Boster Scientific, Wuhan, China).

Immunohistochemical staining of anti-Hsp70

Specimens were deparaffinized (by immersion in graded series of ethanol), immersed in 3 per cent hydrogen peroxide for 10 mins (after rinsing in distilled water three times) and blocked with 5 per cent bovine serum albumin (BSA) for 20 mins. They were then incubated in mouse monoclonal anti-Hsp70 (1:100, Boster Scientific) at 4°C overnight. Normal mouse serum was substituted for the monoclonal antibody in the sections that served as immunohistochemical controls. The antibody was detected by the StreptAvidin-biotin-peroxidase complex (SABC) method (Boster Scientific) with diaminobenzidine hydrochloride as the chromogen.

Immunohistochemical staining of anti-iNOS

After deparaffinization as above, sections were immersed in 3 per cent H_2O_2 for 20 mins (after rinsing in distilled water three times) and blocked with 5 per cent BSA for 20 mins. They were then incubated in rabbit polyclonal anti-iNOS (1:100, Boster Scientific) at 4°C overnight. Normal rabbit serum was substituted for the polyclonal antibody in the sections that served as immunohistochemical controls. Antibody was detected by the SABC method (Boster Scientific) with diaminobenzidine hydrochloride as the chromogen.

Statistical analysis

Mean (± standard error of the mean) values of ABR were compared using a pair-sample *t*-test. All analyses were performed using the SPSS statistical package; $p < 0.05$ was considered statistically significant.

Results

Auditory brainstem response thresholds

Auditory brainstem responses were recorded before surgery, immediately after surgery and three weeks post-operatively in the four groups (Figure 1). In groups A, B and C, ABR thresholds immediately after irradiation were higher compared with pre-operative results ($p < 0.05$). In groups B and C, ABR thresholds three weeks after irradiation were lower than those immediately after irradiation (but still higher than those before irradiation) ($p < 0.05$); no significant change was noted in group A ($p > 0.05$). The ABR thresholds of the D group showed no significant pre-to post-operative alteration ($p > 0.05$). Immediately after irradiation, ABR thresholds were higher in the A group than in the D group ($p < 0.05$) whereas after three weeks the ABR thresholds of these two groups did not differ significantly ($p > 0.05$).

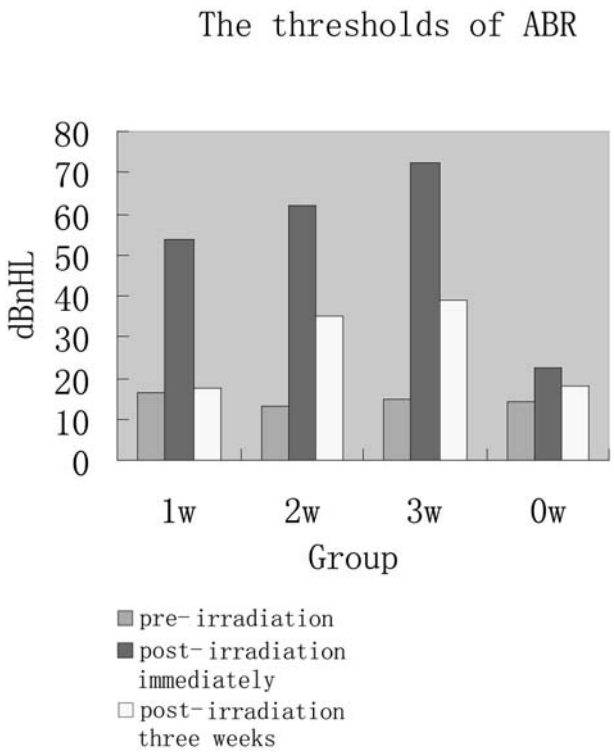

FIG. 1

Mean auditory brainstem response thresholds recorded pre-irradiation, immediately post-irradiation and three weeks post-irradiation, in four groups: A (1 w), B (2 w), C (3 w) and D (0 w).

Morphology

From each group, two cochleae were harvested three weeks post-operatively and viewed by SEM.

The D group cochleae (i.e. controls) yielded normal SEM findings for the stereocilia and cuticular plates of the inner and outer hair cells of the basal turn. The supporting cells had a normal configuration in all examined turns. Electrophysiological recordings were likewise normal for this group throughout the entire examination period (Figure 2).

In two cochleae from group A (irradiated with 1 w (796 w/cm^2)), the basal turn showed no SEM abnormalities. A partially irregular structure of the cilia on the third row of outer hair cells was observed, with normal visualization of the remaining outer hair cells and the inner hair cells (Figure 3).

In two cochleae from B group (irradiated with 2 w (1592 w/cm^2)), irregularly structured outer hair cells of the basal turns were observed on SEM, with collapse of the stereocilia and loss of individual outer hair cells (Figure 4).

In two cochleae from group C (irradiated with 3 w (2388 w/cm^2)), SEM showed fusion of the stereocilia

tips with formation of giant cilia. The stereocilia of the inner hair cells were partially lost throughout the cochlea (Figure 5).

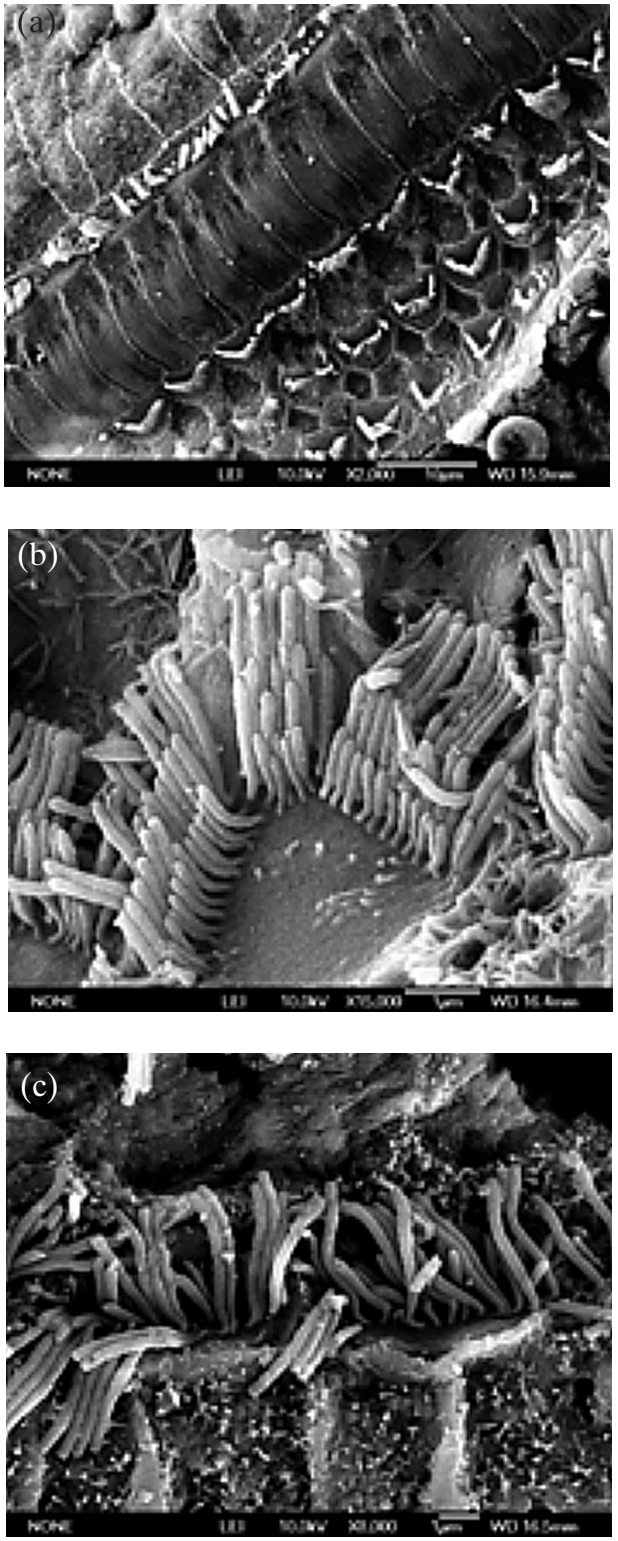

FIG. 2

Scanning electron microscope image of the basal turn of a cochlea three weeks after surgery without CO_2 laser irradiation. The stereocilia and cuticular plates of the inner and outer hair cells and supporting cells show normal configuration. (a) Inner and outer hair cells ×(2000); (b) outer hair cells ×(15000); (c) inner hair cells ×(8000).

Immunohistochemical expression of Hsp70

A faint Hsp70-like immunostaining was seen in the six control cochleae, with staining mainly in the cytoplasm of interdental cells and spiral ganglion cells (Figure 6). Cochleae from those animals without obvious hearing threshold shifts showed an identical immunostaining pattern to controls, with low immunoactivity in the interdental cells and spiral ganglion cells. In animals with a hearing threshold elevation of 10 dB or more, intense immunostaining was detected in 16 of the 18 ears, with staining mainly present in the spiral ganglion cells and spiral ligment (Figure 7), organ of Corti (Figure 8), and stria vasularis. There was no positive staining in immunohistochemical control sections incubated with normal mouse serum only.

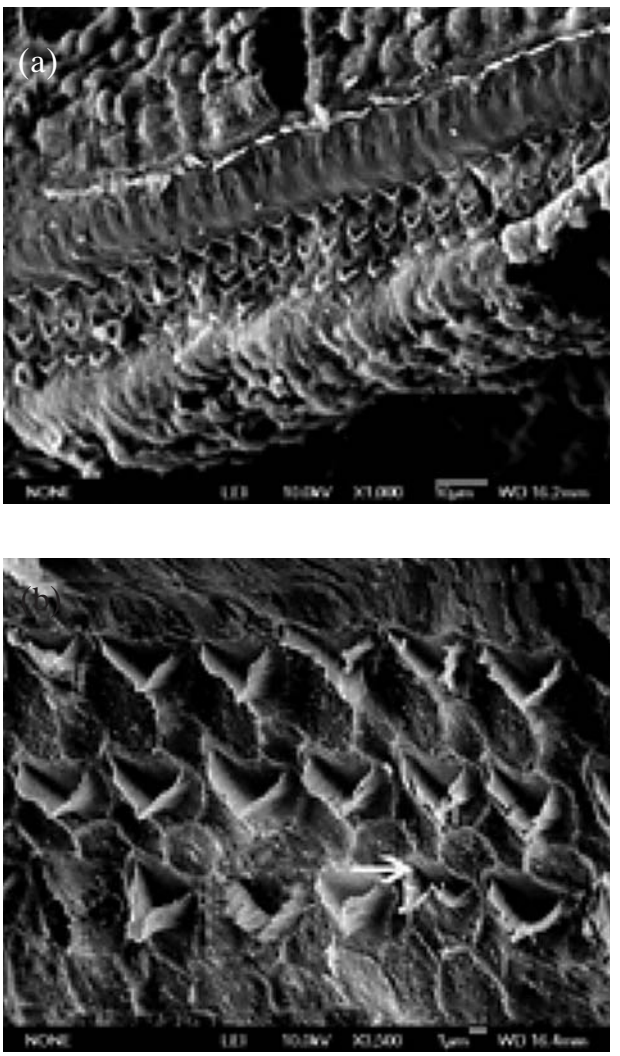

FIG. 3

Scanning electron microscope image of the basal turn of a cochlea three weeks after applying CO_2 laser irradiation of 1 w (796 w/cm^2). The stereocilia and cuticular plates of the inner and outer hair cells and supporting cells show normal configuration. (a) Inner and outer hair cells ×(1000); (b) most of the three outer rows of hair cells show normal configuration (although partially irregular structure of the cilia is seen in the third row of outer hair cells (arrow)) ×(3500).

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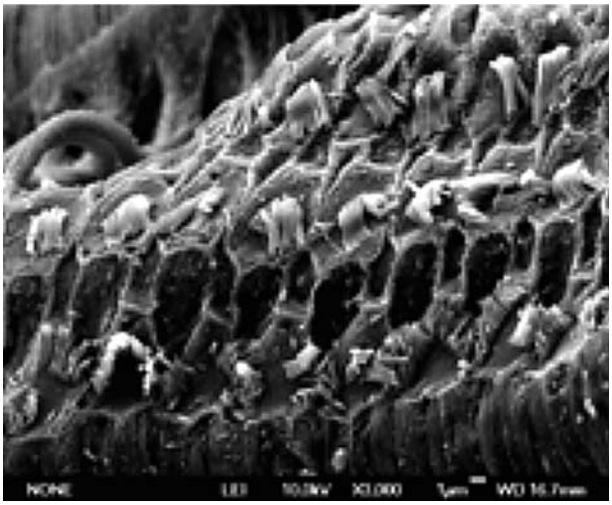

FIG. 4

Scanning electron microscope image of the basal turn of a cochlea three weeks after applying CO_2 laser irradiation of 2 w (1592 w/cm^2). Most of the outer hair cells are collapsed, and derangements and loss of stereocilia are seen.

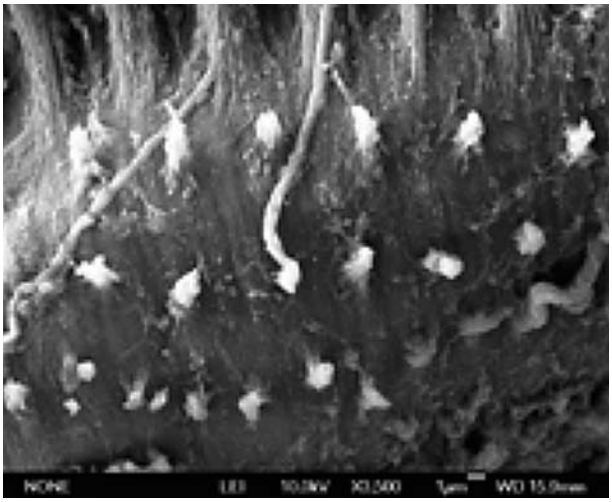

FIG. 5

Scanning electron microscope image of the basal turn of a cochlea three weeks after applying CO_2 laser irradiation of 3 w (2388 w/cm^2). Fusion of stereocilia tips of all the outer hair cells is evident, with loss of the third row of outer hair cells.

Immunohistochemical expression of iNOS

A faint iNOS immunostaining was seen in the cochleae of the six control animals, with staining mainly in the cytoplasm of the spiral ligment and the spiral ganglion cells (Figure 9). Cochleae from animals without obvious hearing threshold shifts showed an identical immunostaining pattern, compared with controls, and only a few immunoreactive stained interdental cells and spiral ganglion cells were seen. In animals with hearing threshold elevations of 10 dB or more, intense immunostaining was detected in 16 of the 18 ears, with staining mainly present in the spiral ganglion cells (Figure 10) and in the spiral ligment, stria vascularis and organ of Corti (Figure 11). There was no positive staining in immunohistochemical control sections incubated with normal rabbit serum only.

Discussion

Laser inner-ear surgery enables greater precision and control than the manual technique. However, the cochlear structures can be directly and indirectly damaged by laser-induced thermal or acoustic transfer of energy. To be safely applied, laser should be assessed for detrimental effects to the inner ear. Our present study shows a strong relationship between ABR testing results and morphological changes (viewed on SEM) in cochlea cells following inner-ear laser surgery. Scanning electron microscopy was particularly effective for investigating changes in the sensory and supporting cells of the organ of Corti. The basal turn of the cochlea was selected for all evaluations because both the controls and laser-treated animals showed various changes in this part of the cochlea; we found identical changes in the apex of the cochlea, including torsions, a collapse and irregular structure of the stereocilia. Jovanovic *et al.* observed similar changes in the cochlear apex when evaluating the organ of Corti after exposure of guinea pigs to laser irradiation.[3]

In our SEM examinations, CO_2 laser with low energy (1 w, 796 w/cm^2) did not cause morphological changes in the organ of Corti in any cochlear areas, compared with controls, and ABR thresholds were higher following irradiation (compared with preceding it) but resolved in three weeks. A faint Hsp70 and iNOS immunostaining was seen in the cytoplasm of spiral ganglion cells, similar to results in controls. These experimental results demonstrate the safety of CO_2 laser irradiation to the basal cochlea when an energy density of 796 w/cm2 and total energy of approximately 1 w are used, parameters effective for human foot-plate perforation.

In contrast, in group B, with a CO_2 laser energy of 1592 w/cm^2 used for cochleostomy, we observed that the organ of Corti showed pathologically altered sensory and supporting cells. There were more marked changes of outer than inner hair cells, particularly in the second and third rows. These alterations included irregular structure of the stereocilia and collapse of the outer hair cells in the second and third rows. Some of the pathological changes were so marked that they resulted in irreversible hearing loss, with higher thresholds detected by ABR. Moreover, in group C, it was demonstrated that an even higher energy density resulted in severe damage to the inner ear. Scanning electron microscope changes included torsion and fusion of the stereocilia and loss of some outer hair cells, and electrophysiological examination revealed severe, irreversible ABR threshold changes.

Various calibrations have been used to assess different lasers. Auditory brainstem responses, compound action potentials and electrocochleographic thresholds have been used to determine electrophysiological inner-ear function in guinea pigs.[3,12,19–21] Morphological investigation of the inner ear has been carried out via cochlea

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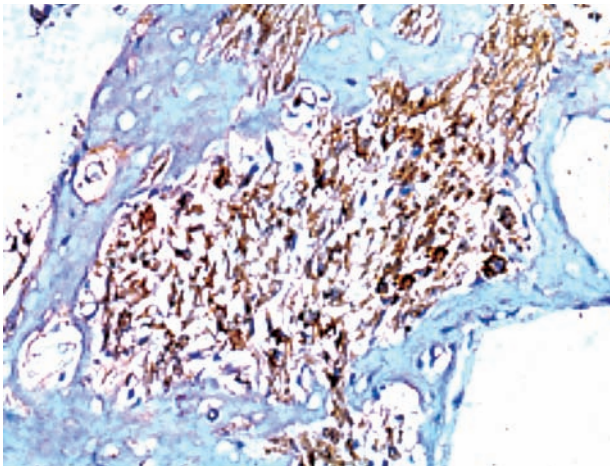

FIG. 6

Faint Hsp70 immunostaining indicates expression of heat-shock protein 70-like proteins in the cytoplasm of spiral ganglion cells in control animals with normal hearing (StreptAvidin-biotin-peroxidase complex method; ×400).

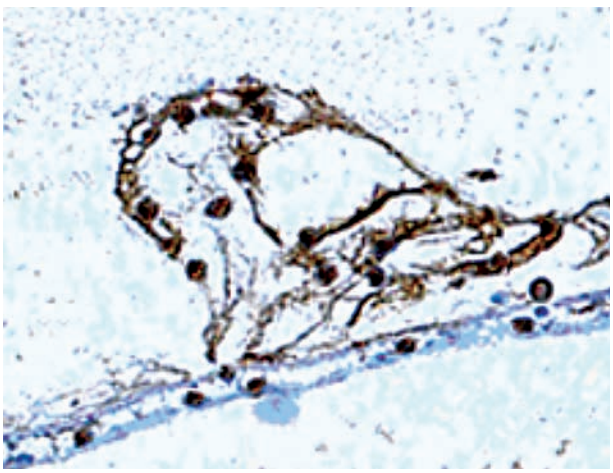

FIG. 8

Staining of hair cells, pillar cells and supporting cells indicates expression of heat-shock protein 70-like proteins in the organ of Corti in experimental animals with significant hearing loss (StreptAvidin-biotin-peroxidase complex method; ×400).

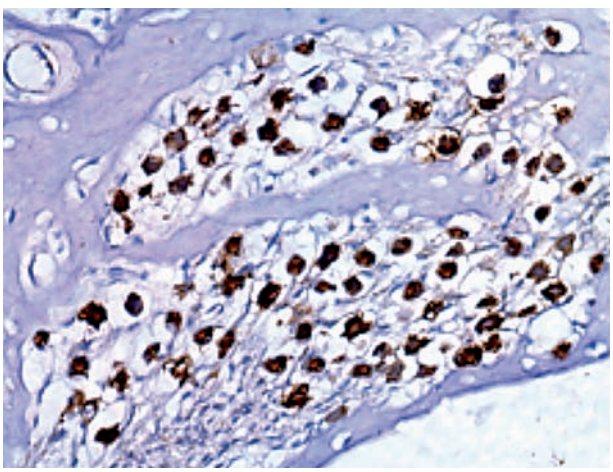

FIG. 10

Intense immunostaining indicates expression of inducible nitric oxide synthase in the cytoplasm of spiral ganglion cells in experimental animals with significant hearing loss (StreptAvidin-biotin-peroxidase complex method; ×400).

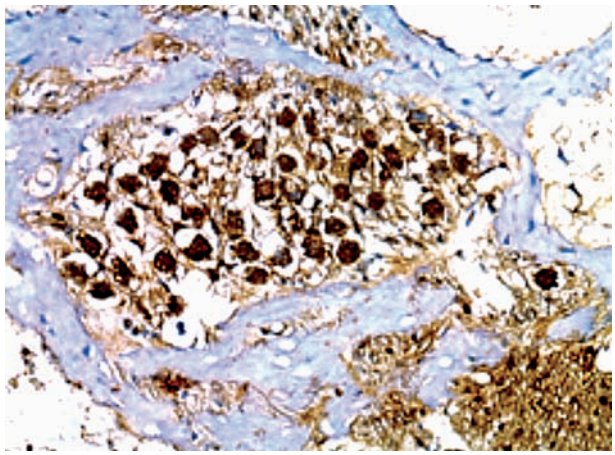

FIG. 7

Intense immunostaining indicates expression of heat-shock protein 70-like proteins in cytoplasm of spiral ganglion cells and spiral ligament in experimental animals with significant hearing loss (StreptAvidin-biotin-peroxidase complex method; ×400).

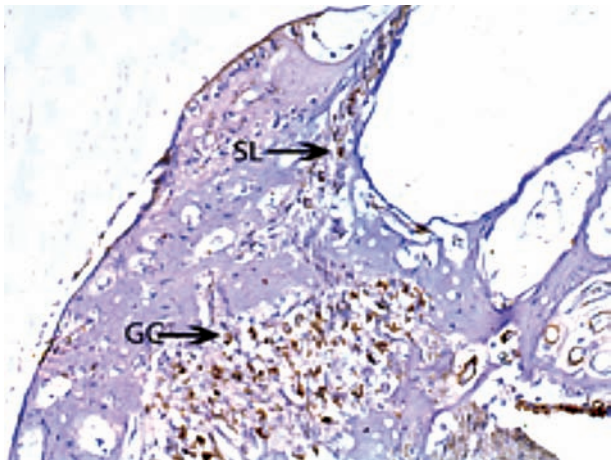

FIG. 9

Faint inducible nitric oxide synthase (iNOS) immunostaining indicates expression of iNOS in the cytoplasm of spiral ganglion cells (GC), spiral ligament (SL) and organ of Corti in control animals with normal hearing (StreptAvidin-biotin peroxidase complex method; ×200).

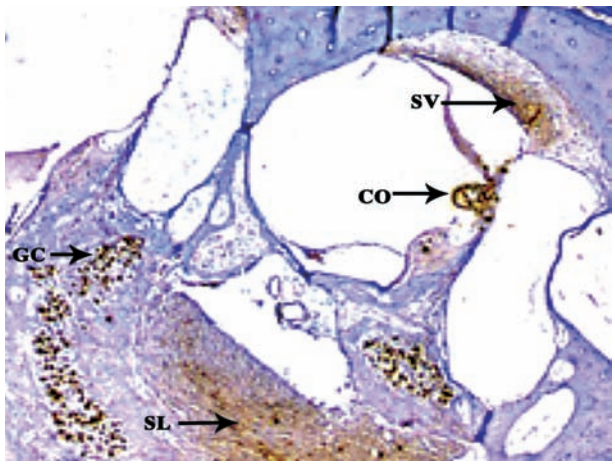

FIG. 11

Expression of inducible nitric oxide synthase in the spiral ganglion cells (GC), organ of Corti (CO), stria vascularis (SV) and spiral ligament (SL) in experimental animals with significant hearing loss (StreptAvidin-biotin-peroxidase complex method; ×200).

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basilar membrane stretched preparation, celloidin-embedded section, paraffin section with haematoxylin and eosin staining, and SEM.[3,20–22] Although SEM examinations can investigate changes in the sensory and supporting cells of the organ of Corti, the mechanical damage to the cochlear basilar membrane sustained when making electron microscopic specimens should be considered when using this method to evaluate CO_2 laser effects. Accordingly, in our experiments, we not only researched electrophysiological changes via ABR testing and morphological changes via SEM examination, but also assessed the immunoactivity of iNOS and Hsp70 via immunohistochemistry (as the latter two substances play important roles in the traumatic mechanism of the inner ear).

Heat-shock proteins are believed to protect cells by dissolving and refolding misfolded or denatured protein. They are induced by various forms of stress including heat, ischaemia, inflammation and toxic agents. It has been reported that acoustic over-stimulation induces the synthesis of Hsp72 in outer hair cells, which suggests that the increase in Hsp72 may confer protection or trigger release of tissue components. Detection of Hsp72 hours after cisplatin injection could indicate that the outer hair cells are one of the major groups of cells affected in the injured rat cochlea.[23] Moreover, the immune response of the inner ear could induce expression of Hsp70 in the guinea pig cochlea.[20]

In our study, a faint Hsp70 expression in the guinea pigs with normal hearing was conformed by SABC, and Hsp70 immunostaining was mainly seen in interdental cells and spiral ganglion cells. The present data support the conclusion that a baseline level of expression of other heat-shock proteins that share common antigenic epitopes with Hsp70 occurs in the normal guinea pig cochlea, and that there is little or no expression of Hsp70 itself in the normal inner ear.[24] However, intense Hsp70 immunostaining was detected in cochleae from guinea pigs with an elevation of hearing thresholds of 10 dB or more. Expression of Hsp70 may be induced by thermal damage and infectious agents following irradiation with high-energy CO_2 laser. While preparing the cochlea specimens from guinea pigs with obvious hearing damage, we observed that most showed inflammatory change, with hyperaemia and some inflammatory exudate. This supports the conclusion that bacterial Hsps are immunologically cross-reactive with their mammalian counterparts; thus, antibacterial Hsp70 antibodies or activated lymphocytes could also react with Hsp70-like proteins expressed in inner-ear cells, and this immune response may damage the inner-ear tissues.[20] This response may lead to an immune-related inner-ear injury and, ultimately, to auditory dysfunction. Increased expression of Hsp70 may serve to protect inner-ear cells and help to limit the aggregation of denatured cellular proteins. In addition, Hsp70 can also facilitate the repair of these proteins.

Nitric oxide is a diffusible, free-radical gas that serves as a multifunctional messenger affecting many diverse aspects of mammalian physiology, such as regulation of vascular tone, macrophage-mediated cytotoxicity and cell-cell interactions in the nervous system.[25] In the auditory system, iNOS is weakly constitutively expressed in some cochlea nerve fibres and has also been reported to be involved in initial neuronal differentiation during inner-ear develoment.[26] In our experiments, we observed faint iNOS immunoactivity in the cochleae of guinea pigs with normal hearing, mainly in the spiral ligment and the spiral ganglion cells. Intense iNOS immunostaining was observed in the cochleae of guinea pigs with hearing loss and injury of the inner-ear ultrastructure. Intense expression of iNOS has some relationship with the constitutive release of NO, an important factor responsible for pathological damage to the cochlea. In the central nervous system, generation of NO by the inducible isoform has been demonstrated in some astrocytes and in microglia after endotoxin and cytokine stimulation. Lowenstein *et al*. reported that endotoxin can trigger rapid induction of iNOS in rat brain *in vivo*,[27] while Galea *et al*. demonstrated that astrocytes and glial cells express iNOS activity *in vitro*.[28] Nitric oxide produced from iNOS may play an important role in tissue damage of the inner ear after high-energy CO_2 laser irradiation. It is well known that, once induced, iNOS can produce large amounts of NO for prolonged periods of time. Marked pathological changes in the inner ear, including damage to the cochlear ultrastructure, may be induced by large amounts of NO generated from iNOS.

To further investigate the level of trauma after CO_2 laser cochleostomy and the possible mechanisms, we considered the change in immunoactivity of Hsp70 and iNOS as complementary criteria. Intense immunoactivity of Hsp70 and iNOS coincided with auditory damage and change of the cochlear ultrastucture. Assessing Hsp70 and iNOS immunoactivity compensated for the possibility that cochlear ultrastructure changes seen with SEM resulted from specimen processing rather than CO_2 laser irradiation. We also performed identical cochleostomy surgery without CO_2 laser on eight control animals, to exclude the possibility that the damage resulted from the surgery itself.

Laser application to the basal turn of the guinea pig cochlea was the model selected for laser stapedotomy, as described by Thoma *et al*.[29] The basal turn of the cochlea, 1 mm from the round window, was chosen as the application site for the laser beam, since its thickness at this point is similar to that of the human stapes footplate and is easily accessible surgically. In our experiments, we found increasing thickness of the cochlea wall in relation to the age of the animal. This variation might imply possible differences in the thickness of the footplate in humans with otosclerosis.

It should be noted, however, that anatomical and pathophysiological conditions in laser stapedotomy in humans differ from those in this animal experiment. In humans, a perforating stapedotomy is most likely to expose the saccule

and the utricle, whereas, in the guinea pig cochleae, lasering the basal turn involves a higher risk to the structures of the organ of Corti (e.g. the basilar membrane, the stria vascularis, and the outer and inner hair cells). In addition, the guinea pig cochlea has been reported to be 10 times more sensitive to acoustic trauma than the human cochlea.[30] Since this probably results in greater vulnerability of these anatomical structures in our animal experiment, inner-ear damage would be more likely following laser application in our animal model than when lasering the human stapes footplate and vestibulum; thus, the detrimental effects we have seen may be less pronounced in humans.

- **The application of laser surgery in the middle ear for otosclerosis surgery allows greater precision and control than manual techniques. The carbon dioxide (CO_2) laser is suitable for stapedotomy**
- **This study concludes that the CO_2 laser with the limited parameters applied in animal experimentation is effective and safe in human stapedotomy**
- **Nitric oxide (NO) and stress proteins play important roles in the mediation of trauma to the inner ear**

Nevertheless, our results indicate that different energy densities of CO_2 laser have different potentials for trauma; this is relevant to the safe use of CO_2 laser stapedotomy in humans. It can be assumed that the laser parameters shown to be safe in our animal model can also be applied safely in the human cochlea.

Our results demonstrate good tolerance and high application safety of CO_2 laser, within the parameters tested, in a guinea pig model. The high ablation rate and low damage potential of CO_2 laser could render it a useful tool in stapedotomy in humans. The mechanisms of trauma to inner-ear structures during use of lasers were considered to be: thermal load to tissue structures; acoustic load; direct irradiation and damage to the sensory cells; and inner-ear toxicity from products resulting from thermal effects.[10] Our research assumed that the higher immunoactivity of Hsp70, induced by thermal damage and inflammation following CO_2 laser irradiation, was a possible mechanism of cochlear trauma. Concurrent production of large amounts of NO, indicated by intense iNOS immunostaining, may also be an important factor in cochlear injury.

These results are only valid for the lasers used in this study. The mechanism and extent of inner-ear damage was observed *in vivo* to relate to the laser parameters and patterns applied.

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