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Influence of Laser-Assisted Cochleostomy on Acoustically Evoked Compound Action Potentials in the Guinea Pig

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Hypothesis: Making a cochleostomy with a laser can affect the inner ear function.
Background: Different types of lasers can be used to create a fenestration in the footplate of the stapes during stapedotomy. Because of variations in absorption spectra of the laser light in various tissues or fluids, each laser has its own characteristics and possible side effects.
Materials and Methods: The basal turns of the cochleae of 20 guinea pigs were fenestrated using 4 types of lasers (thulium, KTP, CO_2, diode; all groups n = 4). A control group (n = 4) was included to correct for the effects of the surgery alone. At 3 different time points, acoustically evoked compound action potentials (CAPs) were recorded at 5 frequencies and at different sound pressure levels. N_1-P_2 amplitudes were measured, and subsequently, thresholds were calculated. A repeated measures analysis of variance was used to investigate differences between groups.
Results: There was a decrease in CAP amplitudes and an increase in CAP thresholds after cochleostomy with each laser. The increase in thresholds was significantly larger for higher frequencies. The thulium laser evoked the largest threshold shifts, the KTP laser the smallest with the CO_2, and diode lasers in intermediate positions. Overall, there was an increase in latencies after treatment.
Conclusion: Laser treatment on or near the cochlea can cause damage to the sensitivity of the cochlea for sound. The thulium laser seems to be the worst choice in this respect. **Key Words:** Cochleostomy—Guinea pig—Hearing—Laser—Trauma.

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Using a laser for fenestration of the footplate in stapes surgery can reduce the complication rate because of conventional contact methods of perforation (1). With a MicroPick instrument or microburr, substantial mechanical energy and sounds are generated, which can damage inner ear structures. The use of a noncontact method does not entail this risk. Until now, various laser types have been proposed to be safe for use in stapedotomy. However, each of these lasers has its own specific characteristics, which influence its potential risk of damaging the inner ear structures. The wavelength of the laser light will determine the level of absorption in different tissues and media, for instance, in the bone, perilymph, or basilar membrane, and therefore will determine the associated side effects and their size.

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The traditionally used lasers, such as the argon (wavelength [λ] = 488 nm) and potassium titanyl phosphate (KTP; λ = 532 nm) lasers, bear the risk of damaging the inner ear directly (2,3). Because of their light transmission through watery liquids, such as the perilymph, unwanted energy is absorbed in the area of the neuroendothelium of the vestibule. It has been suggested that this could explain the (temporary) vertigo complaints in patients after KTP laser–assisted stapedotomy (2–4).

The CO_2 laser (λ = 10.6 μm), used in both continuous wave and pulsed wave mode, is well absorbed in both fluid and bone, implying a controlled perforation. Excess energy is highly absorbed by the perilymph and therefore will not reach the neuroendothelium (5,6). However, this fast absorption can cause local heat formation, potentially causing vertigo, tinnitus, and hearing loss (7).

Recent developments include the introduction of the continuous wave 2-μm laser (usually referred to as the thulium laser) and the 980-nm diode laser. The thulium laser has the advantage of a relatively high absorption in water. Excessive energy, however, will be able to penetrate the vestibule much deeper than with a CO_2 laser (5).

The 980-nm diode laser is interesting to use in current economical times. The devices are small, battery driven, and relatively inexpensive. The 980-nm wavelength has certain surgical advantages, its high absorption in hemoglobin makes it an excellent candidate to treat vascularized lesions (8,9). Its effects in stapedotomy are more difficult to predict. The energy at this wavelength is partially absorbed in the perilymph, but most of it will be transmitted through the perilymph. It could theoretically cause both heating near and direct damage to the inner ear cells.

Previous research in artificial models on heating, mechanical, and acoustic effects showed remarkable differences between the different lasers previously mentioned (5,10). The question remains whether these effects actually affect inner ear function or whether they are clinically irrelevant. We chose a guinea pig model to investigate in vivo effects because the basal turn of the cochlea in this species is easily accessible and the thickness of the cochlear wall of approximately 120 to 160 μm is comparable to the 150 to 200 μm of a slightly thickened otosclerotic footplate (11–13). Furthermore, the cochlear function can be measured by recording acoustically evoked compound action potentials (CAPs).

The aim of this study is to compare the effects of different laser types (KTP, diode, CO_2, and thulium) on inner ear functionality in the guinea pig. The results will help in establishing the optimal laser choice for stapedotomy procedures.

MATERIALS AND METHODS

Animals

Twenty albino guinea pigs (weight, 350–550 g; strain: Dunkin Hartley; supplier: Harlan Laboratories, Horst, The Netherlands) were used. The study protocol was approved by the animal ethical committee of the University Medical Center Utrecht (under number DEC2012.I.12.126).

All 4 laser groups consisted of 4 animals. A fifth group (n = 4) was designated as a control group. Animals in this group underwent the same procedures, but no laser fenestration occurred. The group was added to control for influences caused by the surgical procedures or anesthesia.

Anesthesia was initiated with 0.5 ml/kg Hypnorm (0.315 mg/ml fentanyl + 10 mg/ml fluanisone) administered intramuscularly. Further anesthesia was induced with a gas mixture of N_2O (2 L/min), O_2 (1 L/min) and 2% isoflurane using a mouth cap. To prevent bradycardia after anesthesia, 0.1 ml/kg atropine was administered intramuscularly. The animal was subsequently tracheostomized and artificially ventilated with a gas mixture of N_2O and O_2 (2:1) and 1% to 1.5% isoflurane (±35 cycles/min respiration rate at maximum 2–2.3 kPa) throughout the experiment. Heart rate was monitored, and body temperature was maintained with a heating pad. After surgery, the animals were kept under general anesthesia for 4 hours, to be able to detect late effects.

Surgery

After local analgesia with xylocaine (1% lidocaine, 2% epinephrine), a retroauricular incision was made to expose the right bulla. The bulla was opened, and a clear overview of the middle ear was created. The basal turn of the cochlea was identified. Fenestrations were made by one of the lasers. In total, a rosette of 4 fenestrations was made (Fig. 1). In the control group, animals underwent the same surgical procedures, but no fenestration in the cochlea was made.

To measure the functionality of the guinea pig cochlea, CAPs were recorded. A golden ball electrode was positioned in the round window niche (just superior to the round window membrane). A reference electrode was positioned rostrally of the brain in the skin on the forehead, and finally, a ground electrode was placed in the right hind leg. The pinna of the right ear was repositioned to assure normal access of sound to the outer ear canal. Four hours after fenestration, the animals were euthanized with an intracardial injection of 0.5 ml Nembutal (pentobarbital).

Stimulus Generation and Electrophysiologic Methods

To record acoustically evoked CAPs, the following procedures were performed: Stimuli were generated by a pc with custom-designed software in a Delphi 7 (Borland) programming environment and were fed to a 24-bit DA converter (RP2.1, Tucker-Davis Technologies (TDT, Florida, USA) at a sampling rate of 49 kHz. Acoustic stimuli for CAP measurements were presented as 12 ms (0.5 kHz) or 8 ms (1–16 kHz) tone bursts, with $\cos^2$-shaped rise and fall times of 4 ms at 0.5 kHz; 2 ms at 1 kHz; 1.5 ms at 2 kHz; and 1 ms at 4, 8, and 16 kHz. The acoustic signal was fed via a pair of attenuators (PA5, TDT) and

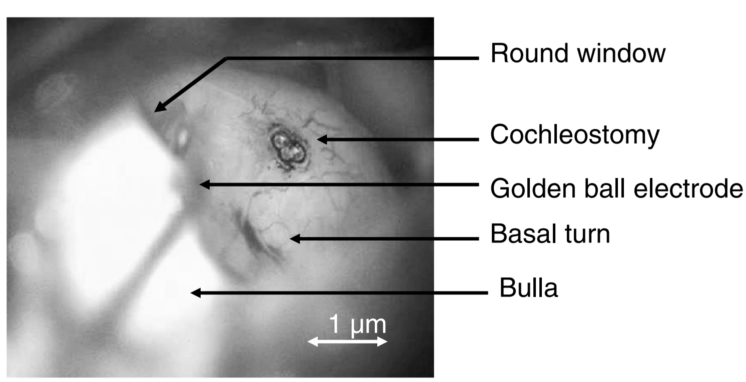

FIG. 1. Intraoperative view. The bulla was opened. A rosette cochleostomy is created in the basal turn (in this example by the CO_2 laser). The golden ball electrode is placed in the round window niche.

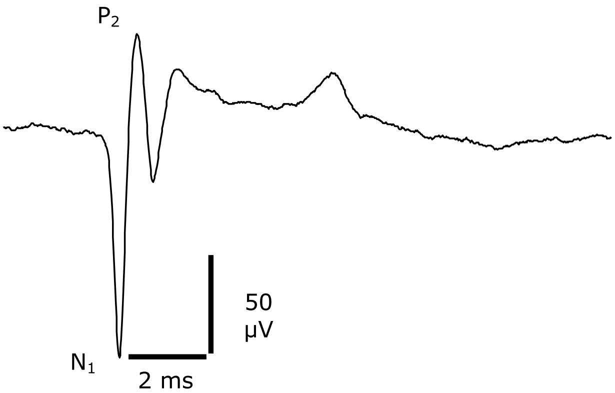

FIG. 2. Example of compound action potential (16 kHz, 93 dB SPL). Amplitude is measured in microvolts between first negative peak (N_1) and subsequent positive peak (P_2).

a headphone amplifier (HB7, TDT) to a speaker (Blaupunkt, Berlin, Germany, PCxb352, 4 Ohm, 30 W) approximately 5 to 10 cm from the right ear of the animal. Sound levels were determined with a sound level meter (2610, Brüel & Kjær, Nærum, Denmark) and a ¼-in. condenser microphone (4136, Brüel & Kjær), calibrated with a 94 dB SPL 1 kHz reference source. Tonebursts were presented in trains with an interstimulus interval of 99 ms. Consecutive tonebursts were of opposite phase (condensation first or rarefaction first). Cochlear potentials were differentially amplified (5,000× or 10,000×), band-pass (1 Hz to 30 kHz) filtered (preamplifier 5113, EG&G Instruments, Princeton, USA) and AD converted at 49 kHz (RP2.1, TDT). Responses to stimuli with opposite phases were separately averaged (to a maximum of 250 sweeps/polarity) and stored for offline analysis. The sum of the responses to the 2 opposite phase acoustic stimuli yielded the CAP waveforms.

CAPs were measured at 3 different times: 1) ''prelaser,'' just before laser fenestration; 2) ''directly postlaser,'' directly after laser fenestration; and 3) ''late postlaser,'' 4 hours after laser fenestration. During the experiment, the bulla was kept clean from blood. Per frequency, we started with a high level of stimulation. In subsequent steps of 10-dB attenuation, CAPs were recorded until reaching the noise threshold value of approximately 3 μV.

Using MATLAB software (version 7.11.0; Mathworks, Natick, MA, USA) the amplitudes of the CAP between the first negative peak (N1) and the subsequent positive peak (P2) were measured (Fig. 2). Threshold levels were defined as an isoresponse level at 3 μV.

Lasers

Four different lasers were investigated: KTP, diode, CO_2, and thulium laser. The 532-nm KTP laser (IDAS, Quantel Derma, Erlangen, Germany) was coupled to a fiber hand piece (Endo-ENT, Biolitec, East Longmeadow, Massachusetts, USA; spot size, 200 micron). A 980-nm diode laser (Atos Medical BV, Zoetermeer, The Netherlands) was used with a 200 micron fiber (Atos Medical BV, Zoetermeer, The Netherlands). The 2-μm continuous wave thulium laser (RevoLix Jr. thulium laser system, Lisa Laser Products, Katlenburg-Lindau, Germany) was used with a 273 micron silica fiber. An articulated arm and micromanipulator was used for the CO_2 experiments (λ = 10.6 μm, Ultrapulse Encore CO_2 laser, Lumenis Ltd., Yokneam, Israel; spot size, 250 micron).

All lasers were used in a single pulse mode, where the pulse lasted 100 ms. KTP, diode, and thulium fiber laser tips were carbonized before starting the experiment, by firing 5 laser pulses on a wooden spatula. This increases speed of the ablation mechanism and is standard surgical procedure (14). The laser settings were the lowest settings needed to ensure bone ablation, known from current clinical practice (15–19). The fluencies (in J/cm^2) are higher when a laser wavelength has less bone ablating capacity; more energy is needed per surface to ensure bone ablation. The laser settings and fluencies are displayed in Table 1.

Four hours after fenestration, the animals were euthanized with an intracardial injection of 0.5 ml Nembutal (pentobarbital).

Statistical Analysis

We used SPSS for Windows (version 20) for the statistical analyses. We used a repeated measures analysis of variance (ANOVA) to compare differences in thresholds between the control and laser groups, with time (prelaser, directly postlaser, and late postlaser) and frequency as within factors. When the assumption of sphericity was violated, the Greenhouse Geisser correction was applied.

We also performed a post hoc Bonferroni and Dunnett analysis to compare laser groups directly with the control group.

RESULTS

Figure 3 shows input-output curves for all 5 groups at 16-kHz stimulation. At this frequency, the effects were largest. In the control group, amplitudes deteriorated slightly over time. However, much larger deteriorations of CAP amplitudes over time were found in all laser treated groups, with the thulium laser at the most extreme end of the damage spectrum.

CAPs were characterized and further analyzed on the basis of isoresponse levels of 3 μV, which is the point at which the input-output curves cross the horizontal axis in Figure 3. These thresholds are depicted as a function of frequency and time in Figure 4. In the control group, thresholds remained stable throughout the experiment. The minimal changes in the higher frequencies were not statistically significant. Also, rmANOVA on possible differences between the thresholds before the use of the laser for all groups did not show a significant difference ($F_4 = 0.9$, $p > 0.05$) for all frequencies.

An rmANOVA on the entire dataset with laser as between-groups factor and time and frequency as within groups factors revealed a significant interaction between group and time (Greenhouse Geisser: $F_{6.8} = 3.4$, $p = 0.011$),

TABLE 1. *Laser specifications*

Group	Wavelength	Power	Time	Spot diameter	Fluency
Control	—	—	—	—	—
KTP	532 nm	1 W	100 ms	200 μm	318 J/cm^2
Diode	980 nm	2.5 W	100 ms	200 μm	796 J/cm^2
CO_2	10.6 μm	2 W	100 ms	250 μm	407 J/cm^2
Thulium	2 μm	5 W	100 ms	273 μm	857 J/cm^2

Laser specifications are shown of each laser, wavelength, and laser settings. Laser settings consist of power per laser pulse (in W), time of laser pulse (in ms), and laser spot diameter (in μm). The total energy used per surface (fluency, J/cm^2) is shown in the rightmost column.

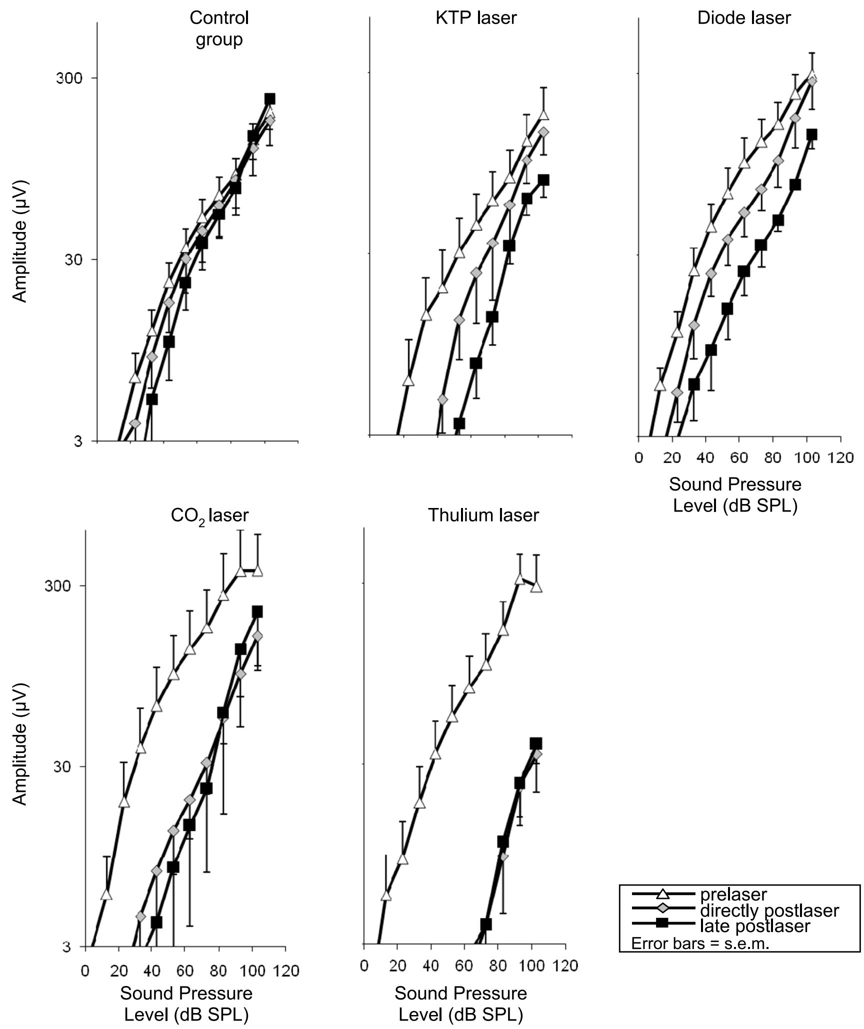

FIG. 3. CAP amplitudes are shown for all laser groups and the control group. For each group, measurements are shown before creating the cochleostomy ("prelaser"), directly after ("directly postlaser"), and 4 hours later ("late postlaser"). Error bars represent standard error of means (SEM). Amplitudes were measured at different stimulation levels (in dB SPL), until they reached the 3 μV threshold.

between group and frequency (Greenhouse Geisser: $F_{8.6}$ = 2.9, p = 0.015), and finally, between time and frequency (Greenhouse Geisser: $F_{4.3}$ = 19.6, p < 0.001), indicating that the effect of the laser was different for each group but also dependent on frequency. Across groups, the course of this increase was significantly different (rmANOVA) with laser as between factor and both time and frequency as within factors (Greenhouse Geisser: $F_{17.1}$ = 2.4, p = 0.005). Close inspection of the figure shows that we can interpret this as follows: the effect of laser treatment is larger at higher frequencies and is dependent on the type of laser used.

Differences between prelaser–directly postlaser and prelaser–late postlaser were calculated. RmANOVA with laser as between factor and time as within factor and post hoc analysis for these differences showed that the thulium laser significantly increased thresholds compared with the control group (Bonferroni: p = 0.015). The KTP laser was the only laser significantly less damaging than the thulium laser (Bonferroni: p = 0.041; Dunnett: p = 0.003). For 16 kHz, the threshold differences of the CO_2 and thulium groups were significantly increased compared with the control group (Dunnett: thulium, p = 0.001; CO_2, p = 0.015).

Latencies of the P_1 peak of the CAP at 16 kHz stimulation and 63 dB SPL are shown in Figure 5. This level was chosen because meaningful latencies can only be measured when a CAP actually occurs, and at higher levels, ceiling effects can occur (e.g., in Fig. 3, the KTP and the diode laser). An rmANOVA with laser as between groups factor and time as within factor revealed a significant interaction of laser and time ($F_{1.6}$ = 24.5, p < 0.001).

DISCUSSION

The aim of these animal experiments was to gain more information on the safety of various laser systems used

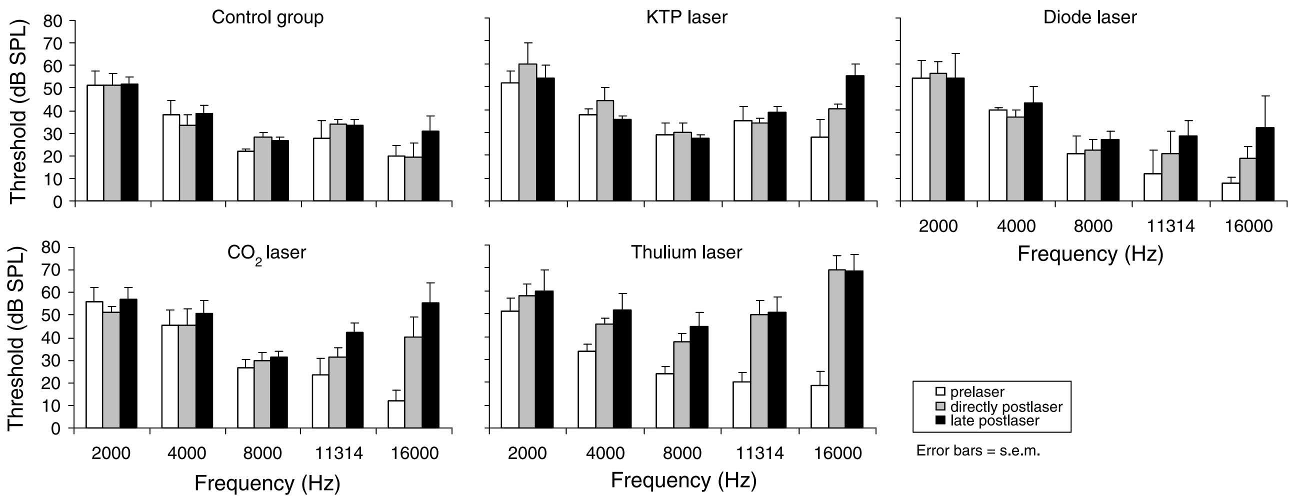

FIG. 4. For each control and laser group, thresholds (CAP isoresponse levels at 3 μV) are presented for the different frequencies. For each frequency, thresholds are shown before creating the cochleostomy ("prelaser"), directly after ("directly postlaser"), and 4 hours later ("late postlaser"). Error bars represent standard error of the means (SEM).

in stapedotomy. As a model, the basal turn of the guinea pig cochlea was chosen because it resembles the human stapes footplate in structure and thickness (13,20). To assess damage, we measured CAPs to evaluate inner ear function.

In summary, we found that the damaging effects of laser treatment are frequency dependent, with the largest effects at the highest frequency we measured (16 kHz). If we assess the effects on threshold at 16 kHz (Fig. 4), we can order the different groups according to their damaging effect as follows: thulium > CO_2 > diode ≈ KTP > control.

There seems to be a relation between the damage we measured and the way laser energy is absorbed in perilymph and more solid structures in the cochlea. For KTP and diode lasers, it was to be expected that damage predominantly occurs locally because their energy will only limitedly be absorbed in the perilymph. Most energy will be absorbed at vascularized structures behind the perforation, like the basilar membrane, causing direct damage to the neuroendothelial cells. The minimal threshold changes we saw in diode and KTP occurred at 16 kHz. When taking into consideration the tonotopic arrangement of the guinea pig cochlea, this is approximately the location of the fenestration; 16 kHz is the specific frequency for the location approximately 4 mm from the oval window and centrally located in the basal turn (21). In contrast, we see a more generalized shift in frequency-dependent thresholds for the thulium and CO_2 laser. These laser wavelengths are strongly absorbed in the perilymph, causing heating (5,6,22). Because the volume of the cochlea is small, heating will spread quickly, potentially causing a shift in thresholds in the surrounding frequencies. Earlier research, visualizing thermal effects in the vestibule, showed CO_2 laser energy is absorbed quickly, giving temperature rise very locally. In contrast, in these experiments, thulium laser energy penetrated the vestibule for 1 to 2 mm, before it was absorbed (5). This deeper heating might be related to the observed damage to a larger area in the present experiment.

When threshold shifts occur because of heating, there is a possibility that these threshold shifts are temporary and might recover over time. Possible recovery can occur in weeks. Previous research by Jovanovic et al. (20) showed a (partial) recovery of CAPs after 7 days, in guinea pigs treated with high power pulses of CO_2 laser. Ren et al. showed the same recovery in auditory brainstem responses (ABRs) after CO_2 laser application in the guinea pig cochlea (23). Both authors found only limited threshold shifts, when lower (corresponding to clinical) settings were used. In long-term follow-up (6 mo), minor degrees of hearing loss, up to 15 dB, were reversible, and higher degrees remained permanent (11).

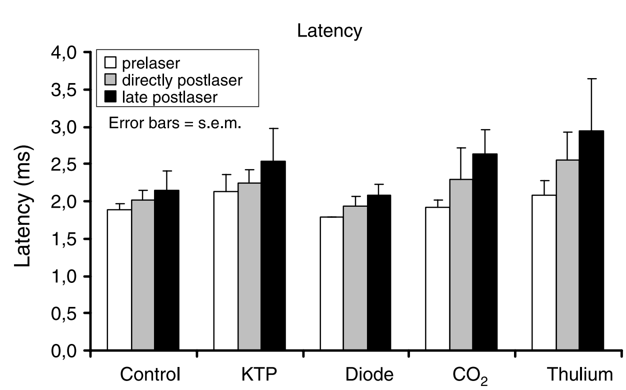

FIG. 5. For each control and laser group, latencies of the P_1 peak of the CAP at 16-kHz stimulation and 63 dB SPL are shown. Latencies are shown before creating the cochleostomy ("prelaser"), directly after ("directly postlaser"), and 4 hours later ("late postlaser"). Error bars represent standard error of the means (SEMs).

It needs to be said that the stimuli used in the experiments described previously were broadband acoustic stimuli. We used small band, frequency-specific stimuli, therefore measuring different parts of the cochlea independently. When the observed effects occur more locally, they might be overlooked when using a broad spectrum stimulus, therefore underestimating true and clinically relevant effects.

In laser-assisted stapedotomy in humans, local effects will not occur in the cochlea but in the vestibule, located just behind the stapes footplate. Several authors have reported (transient) vertigo after KTP or argon laser stapedotomy in patients, ranging from 6.6% to 39% (17,24–26). No shift in bone conduction thresholds has been described. For CO_2 laser, a (mainly temporary) threshold shift in higher frequencies has been described, in early postoperative bone conduction thresholds (27–29). Comparing this with our results, this might be explainable by the heating of the fluids in the vestibule, which communicates with cochlear contents.

Comparing the 4 lasers, we must conclude that KTP, diode, and although slightly less, CO_2 laser systems seem safe to use for stapedotomy. The effects on hearing thresholds are small and may be temporary. With the thulium laser, the threshold shifts are larger and more broadly spread across frequencies. Potential irreversible harm to inner ear function with the thulium laser cannot be excluded.

CONCLUSION

Laser-assisted cochleostomy in the guinea pig shows deterioration in amplitudes and prolonged latencies of CAPs, especially in the high frequencies. For KTP and diode laser, threshold shifts are small and occur locally at the site of perforation, at approximately 16 kHz; CO_2 laser shows a threshold shift over a larger area, but changes are still small. Thulium, however, shows larger thresholds shifts broadly spread over the different frequencies. Therefore, this laser is potentially harmful when used in clinical cases of stapedotomy.

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