

**Audiometric Outcomes of Topical IGF1 Treatment for Sudden Deafness
Refractory to Systemic Steroids**

Takayuki Nakagawa, MD, PhD; Eriko Ogino-Nishimura, MD; Harukazu Hiraumi, MD, PhD; Tastunori Sakamoto, MD, PhD; Norio Yamamoto, MD, PhD; Juichi Ito, MD, PhD
Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine,
Kyoto University, Kyoto, Japan

Running head: Topical IGF1 therapy for sudden deafness

Corresponding author: Takayuki Nakagawa

Department of Otolaryngology, Head and Neck Surgery,
Graduate School of Medicine, Kyoto University,
Kawaharacho 54, Shogoin, Sakyo-ku, 606-8507 Kyoto, Japan

Tel: +81-75-751-3346 Fax: +81-75-751-7225

E-mail: tnakagawa@ent.kuhp.kyoto-u.ac.jp

Conflicts of Interest and Source of Funding: This study was supported by grants for Research on Sensory and Communicative Disorders from the Japanese Ministry of Health, Labour and Welfare. The authors declare no conflicts of interest.

Abstract

Objective: To examine the effects of topically applied recombinant human insulin-like growth factor 1 (IGF1) via gelatin hydrogels on alterations in pure-tone audiometry (PTA) in patients with sudden sensorineural hearing loss (SSHL) refractory to systemic steroids.

Study Design: Retrospective chart review. **Setting:** A single University Hospital.

Patients: Twenty-five patients with SSHL refractory to systemic steroids who received topical IGF1 treatment.

Intervention: Single topical application of IGF1 to the round window niche using gelatin hydrogels.

Main Outcome Measures: The primary outcome was alterations in PTA thresholds at frequencies of 0.25, 0.5, 1, 2 and 4 kHz following topical IGF1 application. Secondary outcomes included differences in final improvements in PTA thresholds among frequencies tested and cumulative numbers of patients showing 10- or 20-dB recovery in PTA during the observation period.

Results: Topical IGF1 application via gelatin hydrogels significantly altered PTA thresholds at each frequency tested and at the average frequency. The numbers of patients with a 10-dB recovery in PTA increased until four weeks after treatment and then stabilized, while those patients showing 20-dB recovery gradually increased during the observation period.

Conclusions: Topical IGF1 application via gelatin hydrogels contributes to the recovery of PTA levels in patients with SSHL refractory to systemic steroids. Major recovery of

PTA levels occurs within four weeks after treatment.

INTRODUCTION

Sudden sensorineural hearing loss (SSHL) is the most common cause of acute sensorineural hearing loss and is one of the few types that can be cured, with spontaneous recovery occurring in 30–60% of cases (1, 2). Most recoveries occur within the first two weeks of onset, and, after this time, it can be difficult to detect further improvement in hearing. The most common therapy for SSHL is the systemic application of glucocorticoids (3, 4), but about 20% of patients do not respond to this treatment (5). In addition, no therapy has been demonstrated to be effective according to the evidence criteria (6).

Various experimental studies have been performed to identify alternative therapeutics for acute sensorineural hearing loss including SSHL, and, of several potential candidates, insulin-like growth factor 1 (IGF1) was shown to be crucial for the development and maintenance of the cochlea (7); its clinical use has also been permitted, so it is a reasonable treatment for us to focus on. IGF1 delivery to the cochlear fluid at appropriate concentrations for sufficient durations has proved difficult, however, as most drugs, particularly growth factors, require sustained delivery for their appropriate actions. Topical application can increase drug concentrations in the cochlear fluid, but simple tympanic injection does not achieve sustained delivery (8). To overcome this, we have utilized gelatin hydrogels for the sustained release of proteins or peptides in topical cochlear treatment (9, 10).

Based on our pre-clinical studies (11-13), we performed a prospective clinical trial to test the safety and efficacy of topical IGF1 application via gelatin hydrogels for SSHL refractory to systemic steroids (14). The main outcome measures of this clinical

trial were the proportions of patients showing hearing improvement, as determined by hearing recovery of 10-dB or above in the mean level of five frequencies tested in pure-tone audiometry (PTA) at 12 and 24 weeks after the test treatment. The null hypothesis was that 33% of patients would show hearing improvement, as was reported for a historical control after hyperbaric oxygen therapy (15). At 12 weeks after treatment, 48% of patients showed hearing improvement, and this proportion increased to 56% at 24 weeks, which was statistically significant (14). These findings indicate that topical IGF1 application may be efficacious for hearing recovery in patients with SSHL refractory to systemic steroids. However, details of audiometric alterations following topical IGF1 treatment have not yet been elucidated, and might prove crucial for understanding mechanisms of IGF1 therapeutic effects on SSHL and for the design of a consecutive randomized trial. The present study therefore aimed to understand these details.

MATERIALS AND METHODS

Patients

Medical records were reviewed of 25 patients (13 females and 12 males, of median age 49 years at registration [range, 23–72 years]) with SSHL who received topical IGF1 treatment in our previous clinical trial (14). Briefly, the main inclusion criteria of our previous clinical trial were as follows: diagnosis of SSHL within 29 days of onset at the Department of Otolaryngology, Head and Neck Surgery of Kyoto University Hospital, Kyoto, Japan, and hearing recovery of less than 10-dB for an average of PTA thresholds at five frequencies (0.25, 0.5, 1, 2 and 4 kHz) after systemic steroid treatment. We excluded patients with active chronic otitis media, acute otitis media, otitis media with effusion or dysfunction of the auditory tube, malignant tumors, severe liver dysfunction (aspartate aminotransferase [AST] >100 and alanine aminotransferase [ALT] >100), uncontrolled diabetes (haemoglobin A1C [HbA1c] >10), pituitary or adrenal dysfunction, severe systemic illness that affected life expectancy, a history of severe drug allergy, or a history of alcohol or drug dependence within the past 1 year, and pregnant or lactating women. All patients registered in this study had no previous history of noise exposure. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and approved by the Ethical Committee of the Graduate School of Medicine, Kyoto University (registered number, C165). Each patient gave written, informed consent to participate in this study.

Intervention

Patients received topical IGF1 treatment using gelatin hydrogels at a median of 23 days

(range 15–32 days) after the onset of SSHL, between 2007 and 2009 at Kyoto University Hospital. Gelatin hydrogels were made from porcine skin gelatin (Nitta Gelatin Inc., Osaka, Japan) in a clean room at the Department of Pharmacy, Kyoto University Hospital. Topical IGF1 treatment procedures were performed in the Day-Surgery Unit of Kyoto University Hospital. Mecasermin (recombinant human IGF1, Somazon[®], 10 mg for injection; Astellas Pharma Inc., Tokyo, Japan) was dissolved in physiological saline at a final concentration of 10 mg/ml. A 30- μ l sample of mecasermin solution was mixed with 3 mg gelatin hydrogels 60 min before application. The single treatment involved placing hydrogel containing 300 μ g mecasermin in the round-window niche of the middle ear following tympanostomy under local anesthesia with 1% lidocaine. Patients were hospitalized for four days after the surgical procedure, and their general and local conditions were examined at the outpatient clinic of the Department of Otolaryngology, Head and Neck Surgery, Kyoto University Hospital, for 24 weeks after the test treatment.

Audiometric examination

PTA was performed on the day of registration and at one, two, four, 12 and 24 weeks after the test treatment. Thresholds in air-conductance at frequencies of 0.25, 0.5, 1, 2, and 4 kHz were determined.

Outcome measures

A retrospective chart review was performed with a primary outcome measure of

alterations in PTA thresholds for each frequency tested and for the average of five frequencies during the observation period. Secondary outcome measures included differences in final improvements of PTA thresholds among frequencies tested, and cumulative numbers of patients showing 10- or 20-dB recovery in the average of PTA thresholds at each time-point following treatment.

Statistical analysis

Alterations in PTA thresholds during the observation period and differences in final improvements of PTA thresholds among frequencies tested were examined by the repeated measurement analysis of variance (ANOVA). The final recovery in PTA thresholds following topical IGF1 treatment was compared with that in a historical control of hyperbaric oxygen therapy for 199 patients with glucocorticoids-resistant SSHL in Kyoto University Hospital between October, 2000 and September, 2006 (15) by unpaired t-test. $P < 0.05$ was considered statistically significant.

Results

Alterations in PTA thresholds

Figure 1 shows alterations in mean PTA hearing levels between at registration and 24 weeks after treatment for each patient. The mean PTA hearing level at registration was 81.2 dB (95% confidence interval [CI], 71.2–91.1) and that of 24 weeks after treatment was 69.3 dB (95% CI, 59.8-78.7). The time course of alterations in averages of PTA thresholds is shown in Figure 2A and was statistically significant ($P < 0.001$). Figure 2B-F shows the time courses of alterations in PTA thresholds at frequencies of 0.25, 0.5, 1, 2 and 4 kHz, which were all found to be statistically significant ($P < 0.001$). These findings indicate that topical IGF1 treatment via gelatin hydrogels has significant effects on the recovery of PTA thresholds in patients with SSHL refractory to systemic steroids.

Difference in PTA recovery among frequencies

The mean recovery for the average PTA threshold of five frequencies tested 24 weeks after treatment was 11.9 dB (95% CI, 5.8–17.9), and means of recoveries in PTA thresholds for each frequency are shown in Figure 3. Differences in the recovery of PTA thresholds among frequencies were statistically significant ($P = 0.0003$), and we observed a trend that hearing recovery at 2 or 4 kHz was smaller than at lower frequencies. These findings indicate that PTA hearing recovery following topical IGF1 treatment predominantly occurred at low frequencies.

Time-points for hearing recovery

Cumulative numbers of patients showing 10- or 20-dB recovery in PTA during the observation period are shown in Fig. 4. For the average PTA threshold of five frequencies tested, 14 patients showed 10-dB recovery and eight showed 20-dB recovery at the end of the observation period. Four weeks after treatment, 10-dB recovery was found in 11 patients (79%) and 20-dB recovery in four patients (50%) (Fig. 4). For each frequency, 10-dB recovery at 4 weeks was found in 17/19 patients (89%) at 0.25 kHz, 17/19 (89%) at 0.5 kHz, 13/19 (68%) at 1 kHz, 12/14 (86%) at 2 kHz and 8/12 (67%) at 4 kHz (Fig. 3B-F). At 4 weeks, 20-dB recovery appeared in 7/11 patients (64%) at 0.25 kHz, 11/13 (85%) at 0.5 kHz, 7/10 (70%) at 1 kHz, 3/6 (50%) at 2 kHz and 5/6 (83%) at 4 kHz. These findings demonstrate that PTA hearing recovery after topical IGF1 treatment occurred during the initial four weeks in the majority of patients, but that further recovery was evident in some patients at a later stage.

Comparison with a historical control

The recoveries of PTA thresholds for each frequency tested and for the average of five frequencies 24 weeks after topical IGF1 treatment were compared with those in a historical control of hyperbaric oxygen therapy (Fig. 3). The recovery of the average PTA thresholds after topical IGF1 treatment was larger than that of a historical controls (11.9 ± 2.9 [mean \pm standard error] for IGF1 v.s. 8.0 ± 0.9 for historical control), but the difference was not statistically significant ($P = 0.08$). For each frequency, there is a trend that topical IGF1 treatment shows better PTA threshold recoveries than a historical control (Fig. 3). The difference in PTA threshold recoveries at 1 kHz between the two group was statistically significant at $P = 0.04$, while at other frequencies no

significant differences in PTA threshold recoveries were observed.

Discussion

The present study demonstrates that topical IGF1 treatment has significant effects on the PTA thresholds of patients with SSHL refractory to systemic steroids. Topical IGF1 application using gelatin hydrogels was performed at 23 days after SSHL onset, and led to an average recovery of 11.9 dB in PTA thresholds over five frequencies. In comparison with a historical control of hyperbaric oxygen therapy, there is a trend that topical IGF1 treatment shows better hearing recovery than hyperbaric oxygen therapy, and a significant difference in hearing recovery is found at 1 kHz. After topical IGF1 treatment, recovery at or above 10 dB was found at 0.25, 0.5 and 1 kHz frequencies. Maximum recovery of 16.0 dB occurred at 0.5 kHz, while hearing recovery at 2 or 4 kHz was less than 10 dB, suggesting that irreversible degeneration might occur in the high frequency region of cochleae. On the other hand, significant hearing recovery was also observed at low frequencies, suggesting that damage in the low frequency region may be reversible and respond to IGF1 delivered by gelatin hydrogels from the round window.

Regarding the time course of alterations in PTA thresholds for the average of five frequencies, hearing recovery was initiated two weeks after treatment and had stabilized by four weeks, which corresponds to recovery starting approximately five weeks after the onset of SSHL symptoms and continuing until seven weeks after the onset. In general, spontaneous SSHL recovery has been shown to occur between days 3–14 after its onset (1, 2), so the PTA recovery observed in the present study may not be spontaneous. PTA thresholds at 1 kHz exhibited a trend of linear recovery during the observation period, while those at other frequencies and alterations in cumulative numbers of patients with a 10 dB recovery showed a similar trend to the average of all

five frequencies. An increase in cumulative numbers of patients showing 20 dB recovery was slightly delayed in comparison with that showing 10 dB recovery, which initiated at four weeks after treatment and continued to 12 weeks. In short, hearing recovery following topical IGF1 treatment appeared to take approximately four weeks, and, in cases with good recovery, further delayed recovery occurred.

Previous animal experiments have demonstrated crucial roles of IGF1 in the physiopathology of hearing (16, 17). We have also revealed that IGF1 has otoprotective effects against noise- and ischemia-induced cochlear damage using *in vivo* animal models (11-13). As for mechanisms of hearing recovery by IGF1, two possible explanations are aroused. One possible mechanism is the rescue of cochlear cells from cell death via IGF1 receptor-mediated actions, which has been indicated by animal experiments. IGF1 binding to IGF1 receptors activates several intracellular signaling pathways associated with the promotion of cell survival (18). Another is the regulation of glucose transport in cochlear cells. IGF1 also has insulin-like actions including promoting glucose transport (19), which can be associated with the survival of cochlear hair cells (20, 21). Both mechanisms might be involved in hearing recovery by topical application of IGF1. Further studies are required for elucidation of detailed mechanisms.

Recently, the intratympanic injection of steroids has gained considerable attention as an alternative for systemic steroids that may reduce the risk of developing systemic side effects and enables the delivery of more steroids into the cochlea. Rauch et al. reported that the intratympanic injection of steroid effects as an initial treatment for SSHL are not inferior to those of systemic steroids (22), and there is an increasing number of case series evaluating intratympanic steroids as a salvage therapy for SSHL refractory to

systemic steroids (23-28). Table 1 shows a summary of six studies, of which evidence levels are superior to IV, and of which sample sizes are larger than 20 subjects. The median PTA recovery obtained in these studies is 14.0 dB (range, 5–22 dB), which is in agreement with that obtained in the present study.

Of previous studies on intratympanic steroids as salvage treatment, two describe detailed time courses of PTA thresholds. Ho et al. (29) reported a time course of alterations in PTA thresholds following the intratympanic injection of dexamethasone. Four weeks after the initiation of treatment (approximately six weeks after the onset), an average recovery of 24.3 dB was reported, with little further improvement. Similarly, Kilic et al. (26) examined PTA thresholds for an average of four frequencies following intratympanic injections of methylprednisolone every three days for a total of five applications. At four weeks after the initiation of treatment (approximately eight weeks after the onset), an improvement of 19.8 dB was found, with no further improvements occurring during the observation period. The time course of hearing recovery after topical IGF1 treatment therefore appears to be similar to that which occurs after intratympanic steroid application.

Conclusions

The present results indicate that topical IGF1 application via gelatin hydrogels may be efficacious for SSHL refractory to systemic steroids. Although the level and time course of hearing recovery following topical IGF1 treatment is similar to that following IT steroids, the baselines of PTA thresholds and methods for evaluation vary among studies. In addition, pharmacological actions of IGF1 differ from those of steroids. Therefore,

the difference in therapeutic potential for SSHL refractory to systemic steroids between topical IGF1 and intratympanic steroid treatment is unclear. To clarify this, we are currently performing a randomized trial to compare the efficacy of topical IGF1 treatment with that of intratympanic steroids for SSHL refractory to systemic steroids, which may provide critical information on salvage treatments for refractory SSHL.

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Figure Legends

Fig. 1. Alterations in hearing levels in pure tone audiometry (PTA) between at registration (regist.) and 24 weeks after topical IGF1 treatment in each patients.

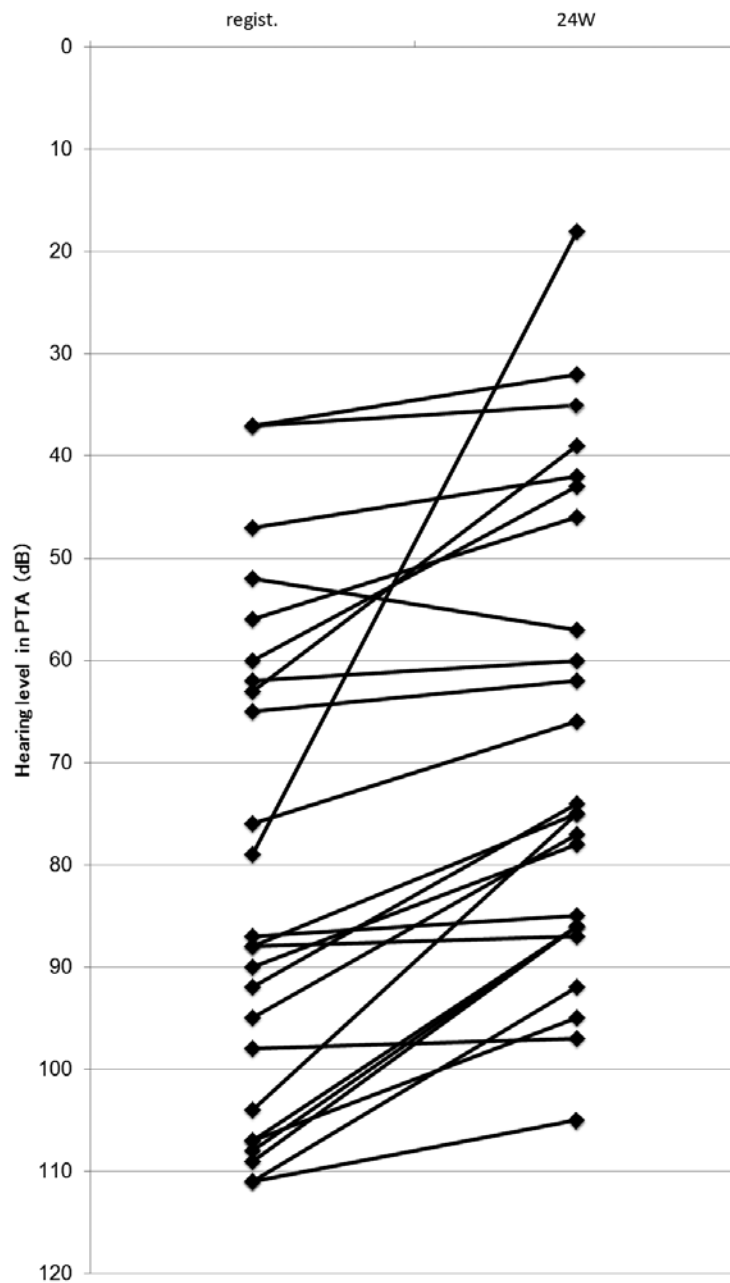


Fig. 2. Hearing levels in pure tone audiometry (PTA) at registration (regist.) and following topical IGF1 treatment, in the average of five frequencies tested (A), at 0.25

kHz (B), 0.5 kHz (C), 1 kHz (D), 2 kHz (E) and 4 kHz (F). Bars represent standard errors.

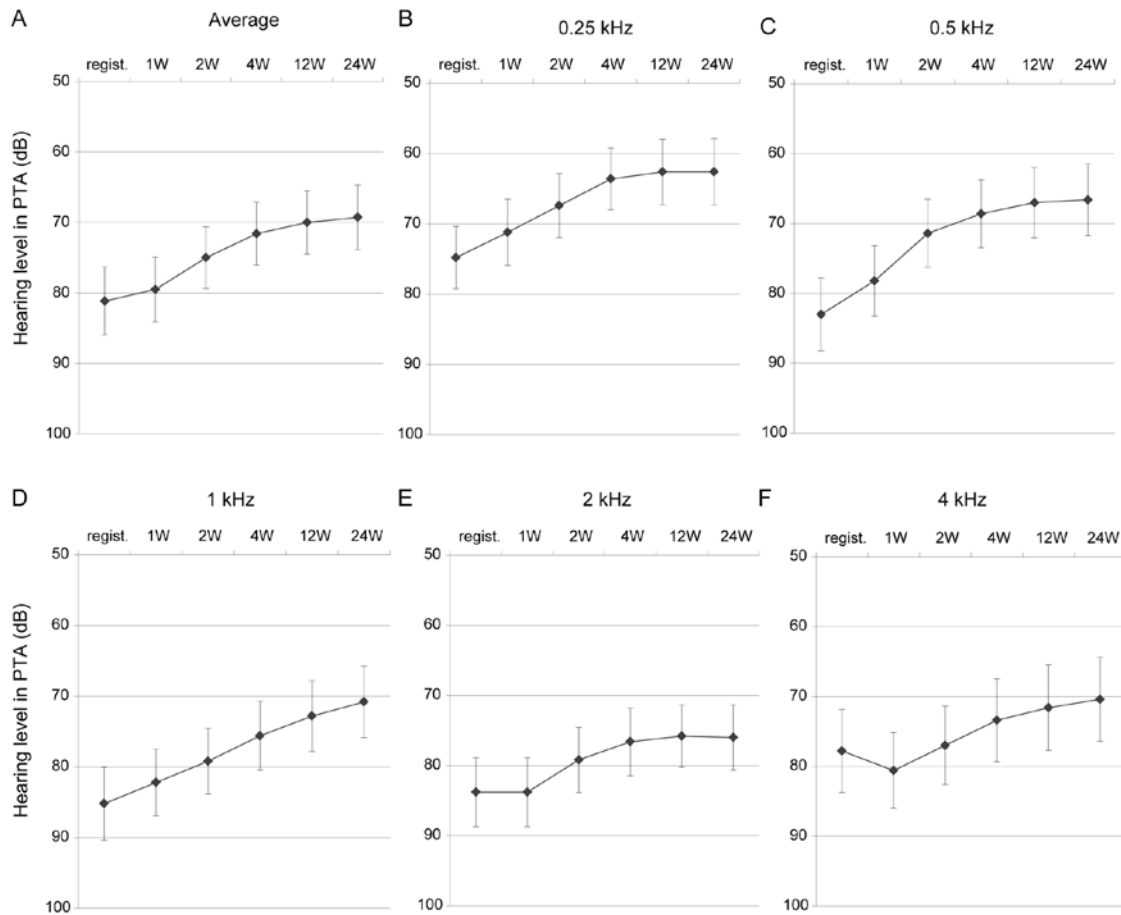


Fig. 3. Final hearing recovery in the average of five frequencies tested and at each frequency tested after topical IGF1 treatment or in a historical control of hyperbaric oxygen therapy (HBO). An asterisk indicates a significant difference in hearing recovery between topical IGF1 treatment and HBO. Bars represent standard errors.

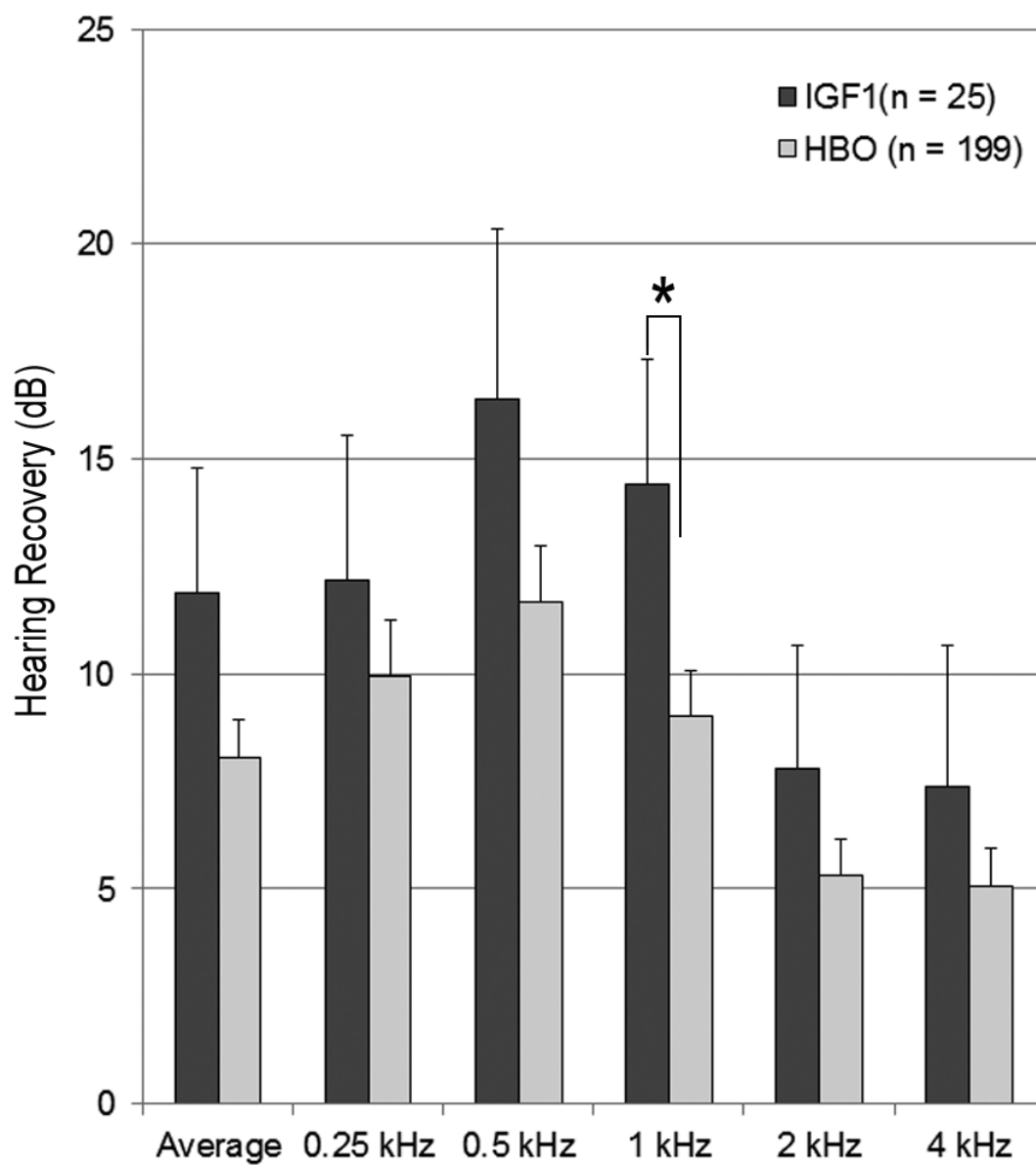


Fig. 4. Cumulative numbers of patients showing 10- or 20-dB recovery in the average of five frequencies during the observation period. Bars represent standard errors.

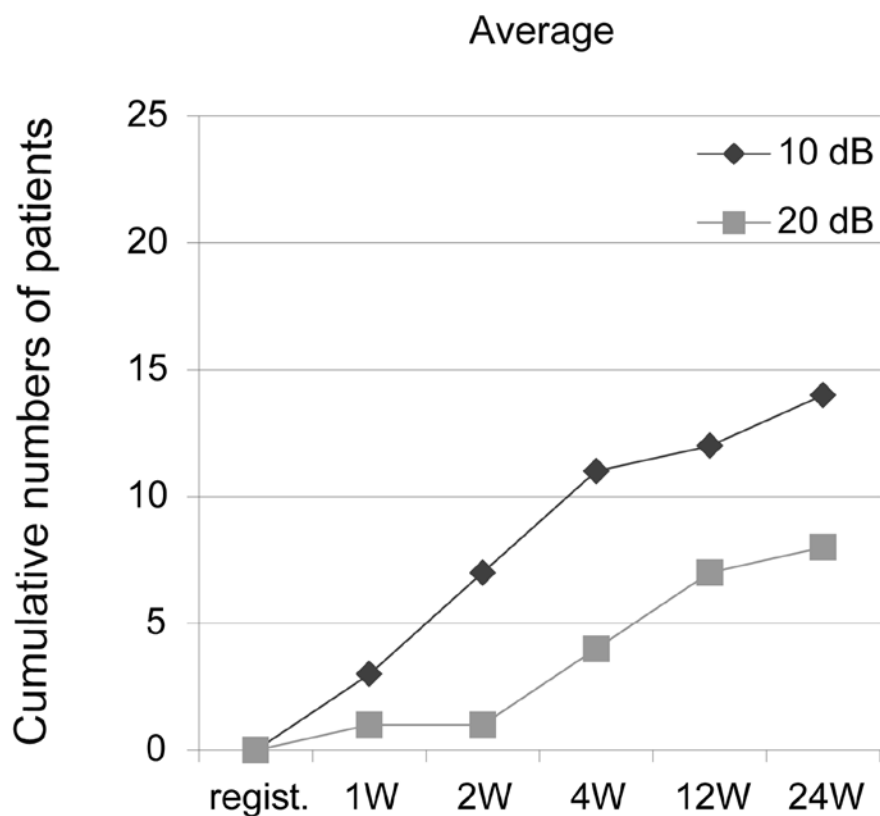


Table 1. Audiometric outcomes in studies of the intratympanic injection of steroids for sudden sensorineural hearing loss refractory to systemic steroids. PTA: pure tone audiometry, RW: round window, Dex: dexamethasone, Mp: methylprednisolone, NA: not available.

First author	Sample size	Steroid, Dose (mg/ml)	Treatment protocol	PTA baseline (dB)	Hearing improvement (dB)
Plontke, 2005 [17]	23	Dex (4) or Mp (40)	RW microcatheter for 4 weeks	103	16
Choung, 2006 [18]	34	Dex (5)	2 injections/week for 2 weeks	72	9
Roebuck, 2006 [19]	31	Dex (24)	1 injection	69	12
Kilic, 2007 [20]	20	Mp (62.5)	5 injections at 3-day intervals	65	22
Haynes, 2007 [21]	40	Dex (24)	1 injection	NA	5
Kakehata, 2011 [22]	24	Dex (4)	1 injection/day for 8 days	75	17