厚生労働科学研究費補助金

障害者対策総合研究事業（感覚器障害分野）

新世代人工内耳に対応した内耳薬剤徐放技術の開発

平成22年度～23年度　総合研究報告書

研究代表者　吉　川　弥　生

平成24（2012）年3月

厚生労働科学研究費補助金

障害者対策総合研究事業

（感覚器障害分野）

新世代人工内耳に対応した内耳薬剤徐放技術の開発

平成 22 年度～23 年度　総合研究報告書

研究代表者　吉川　弥生

平成 24（2012）年　3 月

目　　次

厚生労働科学研究補助金（障害者対策総合研究事業（感覚器障害分野））
総合研究報告書

新世代人工内耳に対応した内耳薬剤徐放技術の開発

研究代表者　吉川　弥生　東京大学医学部附属病院　耳鼻咽喉科・頭頸部外科
病院診療医

研究要旨

人工内耳治療には、近年になり適応拡大・両耳装用、さらには海外での人工内耳と補聴器の併用（EAS）といった大きなパラダイム・シフトが起きている。これを実現するには、埋込術後の急性期の蝸牛障害を予防する技術の開発が必須である。

本研究課題では、研究代表者らが有するバイオマテリアル技術および内耳アポトーシス予防技術を統合し、薬剤徐放機能付き人工内耳などの新たな内耳治療手技の開発を行った。

まずはゼラチン・ハイドロゲルを用いてダミー人工内耳電極を作成し、IGF-1 および HGF を含浸させたハイドロゲル製剤が人工内耳挿入時の蝸牛障害を有意に軽減する効果を持つことを明らかにした。さらに新たなコート素材として３つの候補の比較実験を行い、最終的に MPC（2-メタクリロイル オキシエチルホスホリルコリン）ポリマーを選定、目的の薬剤徐放電極を作成した。この徐放電極について、電極特性の基礎実験および動物実験を実施し海外（オーストラリア）の人工内耳製造会社と商品化に向けた協議を進めた。

分担研究者
坂本　幸士　東京大学医学部附属病院　耳鼻咽喉科・頭頸部外科　講師
狩野章太郎　東京大学医学部附属病院　耳鼻咽喉科・頭頸部外科　特任講師
松本　有　東京大学医学系研究科　臨床医工学部門　特任研究員
樫尾　明憲　東京大学医学部附属病院　耳鼻咽喉科・頭頸部外科　助教

A．研究目的

新世代人工内耳の発展

人工内耳は 20 世紀最高の発明とも言われ、最も成功した人工臓器のひとつである。1970 年代の実用化以降、単チャンネルからマルチチャンネルへ、スピーチプロセッサの IC 化といった様々な改良がなされてきたが、近年になり大きなパラダイム・シフトが起きている。それは、人工内耳自体の性能向上に沿った中等度難聴への適応拡大・両耳装用、さらには人工内耳と補聴器の併用（EAS）などに代表される「人工内耳治療の普遍化」である（Van de Heyning 2010）。EAS は欧州ではすでに 10 年前より臨床での使用が始まっているが、日本でも昨年 12 月 9 日に厚生労働省

「高度医療評価会議」で承認され、普及への第1歩を踏み出した。

しかしながら、こうした新世代人工内耳に対応した内耳保護技術は必ずしも充分ではない。EASを行うためには残存聴力の温存が必須であるが、電極挿入により起こる内耳組織破壊・繊維化などのために不可逆的に喪失してしまうことが多い(Nadol 1997 など)。したがってより安全で低侵襲な人工内耳手術術式を開発するとともに、手術時には繊維化を防ぎ、蝸牛細胞を保護・再生する薬剤を内耳局所に投与することが望まれている。

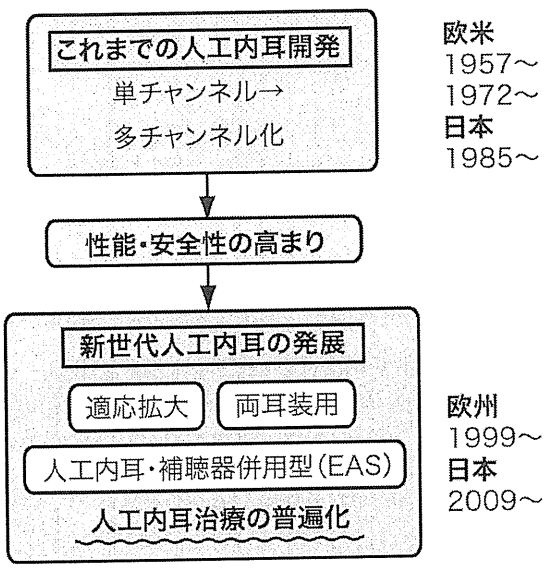


図1　人工内耳の発展

図1　人工内耳の発展

人工内耳を利用した薬剤投与技術の開発が急務である

人工内耳からの薬剤投与に関しては現在世界的に熾烈な競争が繰り広げられているが、浸透圧ポンプなどを使うと装置が巨大化してしまうことや、効果の高い薬剤が入手できないといった理由からいずれも学会報告レベルに留まっている。

研究代表者はこれまで所属していたテキサス大学、京都大学で人工内耳の感染予防加工や低侵襲手術法の開発を行い(Med-El 社との共同研究)、内耳薬剤徐放の種々の技法を習得した。IGF-1・ハイドロゲル徐放製剤は動物実験で高い効果が得られ、突発性難聴に対する臨床第 I/II 相試験では5割に効果を認めた（BMC Medicine 2010, 8:76）。また、東京大学ではアポトーシス予防に関して基礎研究(Someya 2009 PNAS)、全身投与 (Kashio 2007, J Neurosci Res) を通して技術を確立しており、本研究ではこの両者の技術を統合して内耳薬剤徐放機能を備えた低侵襲型人工内耳を開発、人工内耳埋込時に起きる組織損傷を極限まで抑える技術を開発する。

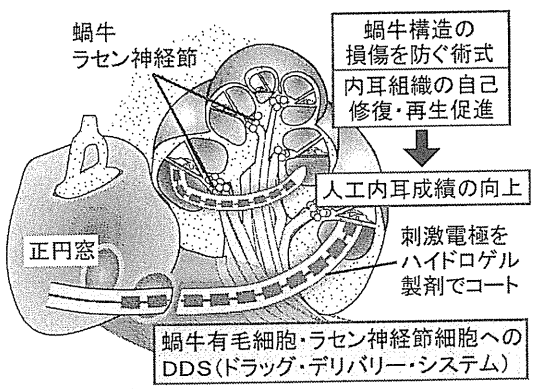


図2　本計画の概念図

さらにこの技術が完成すれば、人工内耳治療に限らず突発性難聴や進行性難聴などの様々な内耳疾患の普遍的な治療法として応用が期待できると考えられた。

B．研究方法

1）人工内耳電極コート技術の開発

ハイドロゲルコート電極のプロトタイプを作成し、*in vitro* 徐放試験やIGF-1、HGFを使った動物実験により薬剤徐放機能・人工内耳挿入時損傷防止効果を測定する。シリコン電極の親水化コート方式としてはプラズマ放電を用いるが、通電試験（5-30mA）により電極性能の低下やゲル剥脱などの問題が認められた場合にはＵＶ照射あるいは O_3（オゾン）処理を検討する。

・ゼラチン担体からの *in vitro* 薬物徐放試験

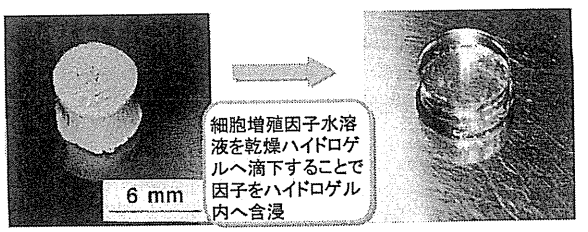
1．コート済みダミー電極をサンプリングチューブに分け入れ、I^{123} ラベル IGF-1 溶液 1ml を滴下して電極と触れる状態にし、室温 3 時

間（もしくは 37℃ 1 時間）静置して含浸させる。（n=3 程度）

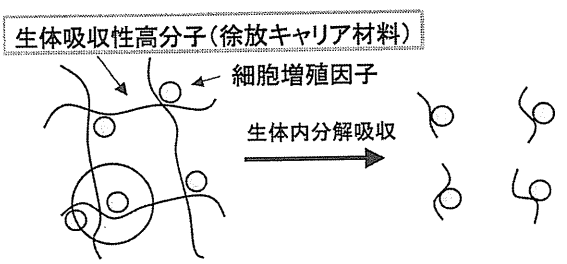
2. PBS を 1ml ずつ加え、37℃恒温槽で浸とうしながら薬物を拡散放出させる。

3. 0.5, 1, 2, 4, 8, 12, 24 hr 後に、PBS を全量抜き取り、サンプル溶液とする。PBS 1ml を新たに加え、引き続き 37℃で浸とうする。

4. それぞれの時間に採取したサンプル溶液中の薬物濃度を算出し、累積して放出量を計算する。全てサンプリング後、可能であれば残存量を測定して合計量の確認を行う。



凍結乾燥ハイドロゲル　　細胞増殖因子を含浸したハイドロゲル



2）齧歯類モデルでの動物実験

開発した人工内耳電極を内耳に挿入して、実際の薬物徐放を行い、薬物の生物学的有効性を齧歯類モデルで解析する。内耳機能の解析方法としては、聴力検査（ABR）、神経反応テレメトリ（NRT）、凍結切片を用いた組織学的検査を行う。

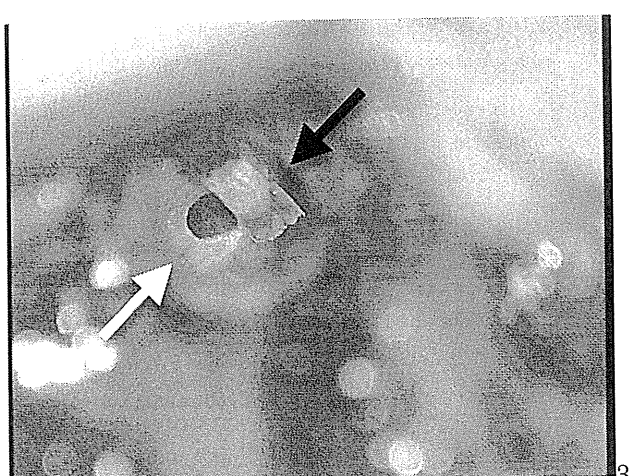


図　蝸牛開窓（白矢印）を行い、ダミー電極（黒矢印）を挿入

・モルモット蝸牛損傷予防実験

1. コート済みダミー電極をサンプリングチューブに分け入れ、IGF-1 または HGF 溶液 0.5ml を滴下して電極と触れる状態にし、4℃で一昼夜静置して含浸させる。（n=5 程度）

2. モルモット側頭部に耳後切開を置いて耳胞を開放、蝸牛開窓を置きそこから薬剤含浸ダミー電極を挿入する。

3. 術前、術直後、3、7、14、21、28 日後に ABR を測定する。

4. 28 日後に蝸牛を回収し中耳および内耳の組織学的検査を行う。

3）最適材料の選定

ゼラチン・ハイドロゲルの他に、以下の３つの材料に関して検討を加え、人工内耳コートに最適な材料を選出した。

① Tetra-PEG gel（Kurakazu 2010、東大工学部（マテリアル工学専攻）酒井崇匡助教）

② ナノミセル型DDS （Nishiyama 2003、東大医工連携　片岡一則教授）

③MPCポリマーゲル（Kihara 2003、東大工学部（先端バイオデバイス工学）石原教授、金野准教授）

① ナノミセル型DDS

ダハプラチン内包ナノミセル抗がん剤（ナノプラチン）として Phase III試験中、②は軟骨再生材料として前臨床試験中、③は国産で初めて承認された埋込型補助人工心臓「エバハート」の血栓予防のための表面コーティングとして市販され、それぞれ臨床応用されている。

4）徐放性能・安全性・耐久性テスト

上の選定作業で選んだ材料を用いてダミー人工内耳を作成、以下の試験を行って内耳薬剤徐放材料としての性能を検証した。ベースとなる人工内耳にはコクレア社（オーストラリア）Nucleus シリーズを使用した。

電極コーティングの特性検査

光学顕微鏡・電子顕微鏡検査

通電検査

音響インピーダンス検査

サイクリックボルタンメトリー

電気化学インピーダンス

スペクトロスコピー

繰り返し通電検査

一過性電位測定

耐損傷検査（電子顕微鏡）

埋め込み術後検査

（倫理面への配慮）

動物実験に関しては、本学の動物実験に関する倫理委員会の承認のもとに、動物愛護に十分配慮した上で行う。ヒト側頭骨を使用する場合は、人権擁護上の配慮を十分に行った上で研究を実施する。

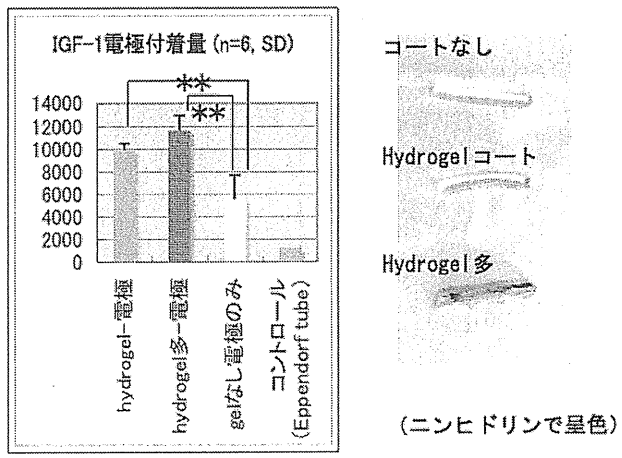
C．研究結果

1）人工内耳電極コート技術の開発

ゼラチン・ハイドロゲルを用い、コート電極のプロトタイプを作成した。プラズマ放電を用いてシリコーン表面を親水化することで、ハイドロゲル膜の形成が可能であった。

・ *in vitro*薬剤電極吸着試験

I^{123}ラベルIGF-1を用いて測定したところ、ハイドロゲル付着ダミー電極は、ゲル層を持たない電極に比べて有意にIGF-1を多く吸着していた。



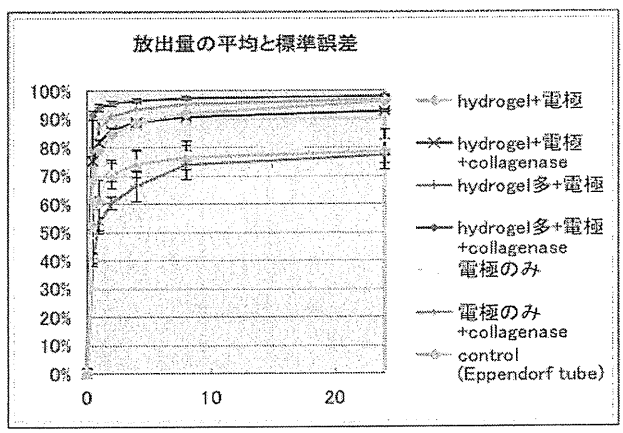


図　*in vitro* 薬剤放出試験

2）齧歯類モデルでの動物実験

モルモットを使った電極挿入実験では（未公開データ）、HGF含浸電極、IGF-1含浸電極ともに無薬剤電極に比べて有意に電極挿入時以降のABRの回復が早かった。（ダミー電極　vs　HGF/ハイドロゲル：$P=0.00018$、ダミー電極　vs　IGF-1/ハイドロゲル：$P=0.0000065$）

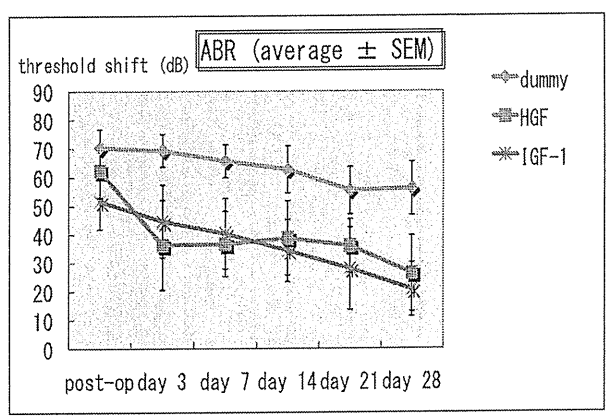


図　モルモット蝸牛開窓実験

組織学的解析では、内耳及び中耳に特に強い炎症反応は認められなかった。

なお、薬剤を含んだゼラチン・ハイドロゲル層が電極挿入時に電極から剥奪する現象が高い割合で発生した。また、ハイドロゲル原料が豚皮由来であり生物学的製剤であるため潜在的な感染症リスクがあること、この２点を考慮して人工内耳コート材料としては新たな薬剤徐放物質の探索が必要となった。

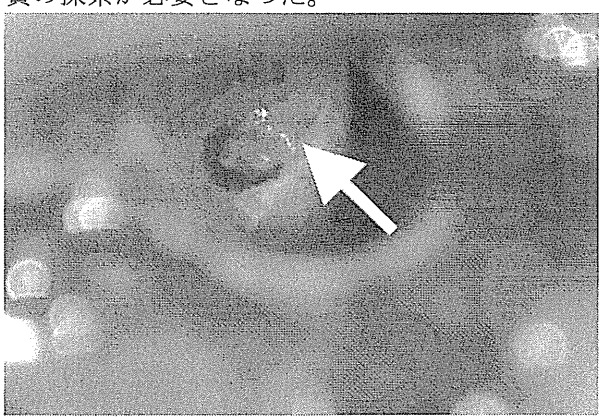
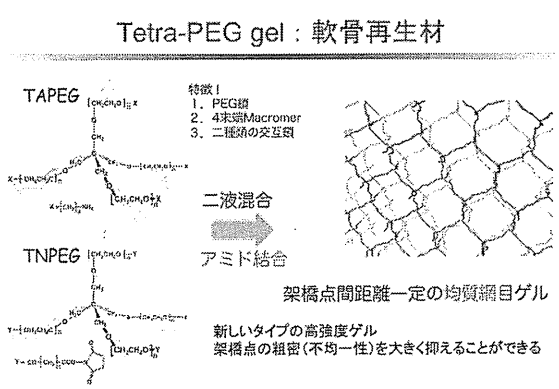
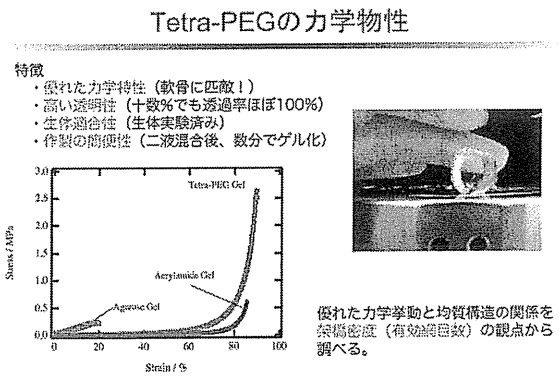


図　薬剤徐放層（ゼラチン・ハイドロゲル）の剥脱（白矢印）

3）最適材料の選定

・Tetra-PEG gel



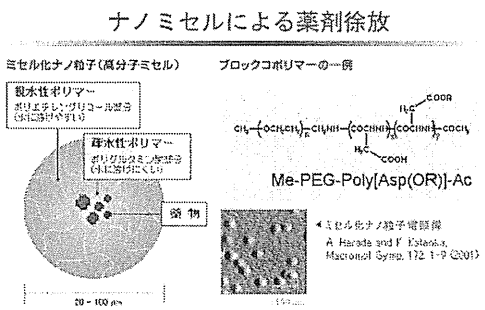


・硬度が高く、人工内耳電極コートに適さない一断念

Tetra-PEG ゲルは軟骨の代替材料になりうる優れた力学特性を持ち、作成も簡便であるが、硬度が高すぎるために人工内耳電極被覆剤としては不適であった。

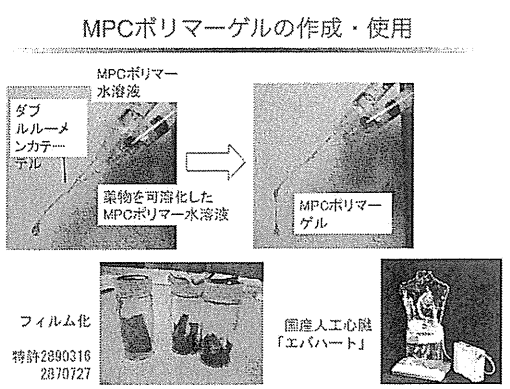
・ナノミセル

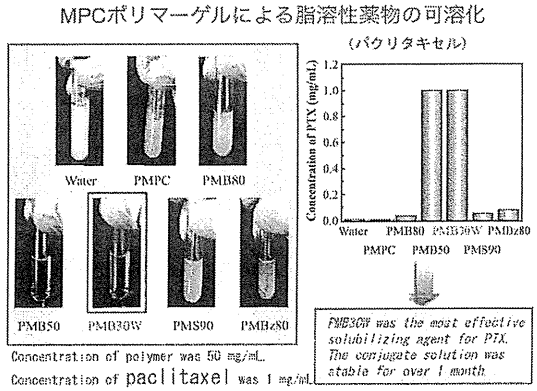
ナノミセルを用いた薬剤徐放は、疎水性ポリマーを内包するためステロイドなどの疎水性薬剤の徐放に最適と考えられたが、粒子状のため人工内耳の電極コートには使うことができなかった。



・粒子状のため人工内耳の電極コートに適さない

・MPCポリマーゲル





MPC polymer は国産補助人工心臓「エバハート」の表面被覆素材として承認済みであり、平成２３年４月からは国内の市販も始まっている。また、MPC ポリマーをコーティングすることにより血小板等の蛋白質の吸着を防ぐことができるため、コンタクトレンズケア製品、抗血栓性を主目的にした人工透析やカテーテル等の医療デバイスのコーティング材料（リピジュア®-CM）として注目されている。ポリマー水溶液と重合剤の２液を混合させて作るため、薄膜フィルム化および薬剤含浸がきわめて容易である。

以上よりゼラチン・ハイドロゲルに代わる新たな人工内耳徐放コート剤として MPC ポリマーを選定し、以降の実験を行った。

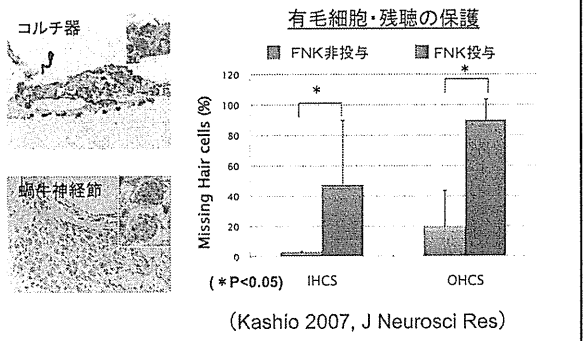
4）徐放性能・安全性・耐久性テスト

動物用ダミー電極を用い、MPC polymer を使用した内耳挿入実験を実施。MPC polymer でコートした電極がモルモット内耳に強い炎症反応を惹起しないこと、また、非コート電極に比べて外装が親水性であるため電極挿入が容易になることを確認した。(前臨床試験用の基礎データ)。

D．考察

本研究では、ゼラチン・ハイドロゲルで被覆した人工内耳ダミー電極が内耳用徐放製剤として使用可能で、ゲル量に応じて薬剤徐放速度と徐放時間を制御できることが確認できた。動物実験では本電極が人工内耳埋込術時の蝸牛損傷予防に大きな効果を発揮することがわかり、実用化に向けて人工内耳メーカーを含め協議中であったが、実験中、ダミー電極を挿入した際に電極からハイドロゲル層が剥奪する事故が発生しうることが判明した。O_3（オゾン）処理によりゲル層とシリコンの結合強化を図ったが剥奪例が減らず、薬剤徐放電極の実用化は困難かと思われた。

そのためハイドロゲルに代わる新たな徐放材料を検討し、MPC（2-メタクリロイル オキシエチルホスホリルコリン）ポリマーを選定した。MPC



(Kashio 2007, J Neurosci Res)

polymerはすでに医療用コーティング材料として厚労省より承認、国内外で市販されているため実用化・臨床応用が容易であり、本研究でも動物実験で性能を確認することができた。

今後は徐放型人工内耳電極として、知的財産化・特許申請および実用化を進めていく予定である。

E．結論

本研究課題では、研究代表者らが有するバイオマテリアル技術および内耳アポトーシス予防技術を統合し、薬剤徐放機能付き人工内耳などの新たな内耳治療手技の開発を行った。今後、徐放性能の最適化および臨床試験への準備を進めて行く。

F．健康危険情報

現時点では、ヒトにおける健康危険に関する情報は得られていない。

F．研究発表

１．論文発表

Nakagawa T, Sakamoto T, Hiraumi H, Kikkawa YS, Yamamoto N, Hamaguchi K, Ono K, Yamamoto M, Tabata Y, Teramukai S, Tanaka S, Tada H, Onodera R, Yonezawa A, Inui K, Ito J. Topical insulin-like growth factor 1 treatment using gelatin hydrogels for glucocorticoid-resistant sudden sensorineural hearing loss: a prospective clinical trial. *BMC Medicine* 8:76-81, 2010.

Taura A, Kikkawa YS, Nakagawa T, Ito J. Hydrogen protects vestibular hair cells from free radicals. *Acta Oto-Laryngologica*, 2010; 130: 95–100.

Matsumoto Y, Nomoto T, Cabral H, Matsumoto Y, Watanabe S, Christie J, Miyata K, Oba M, Ogura T, Yamasaki Y, Nishiyama N, Yamasoba T, Kataoka, K. Direct and instantaneous observation of intravenously injected substances using intravital confocal micro-videography. *Biomedical Optics Express*, 1(4): 1209–1216, 2010.

Lin Y, Kashio A, Sakamoto T, Suzukawa K, Kakigi A, Yamasoba T. Hydrogen in drinking water attenuates noise-induced hearing loss in guinea pig. *Neurosci Lett.* 3;487(1):12-6, 2011.

Sekiya T, Matsumoto M, Kojima K, Ono K, Kikkawa YS, Kada S, Ogita H, Horie RT, Viola A, Holley MC, Ito J. Mechanical stress-induced reactive gliosis in the auditory nerve and cochlear nucleus. *J Neurosurg.* 114(2):414-25, 2011.

Sakamoto T, Nakagawa T, Horie RT, Hiraumi H, Yamamoto N, Kikkawa YS, Ito J. Inner ear drug delivery system from the clinical point of view. *Acta Otolaryngol Suppl.* (563):101-4, 2010

狩野章太郎：知っておきたい生理・病態の基礎 聴覚末梢．耳鼻咽喉科・頭頸部外科　82 巻 9 号 631-635, 2010.

樫尾明憲，山岨達也：最新の治療デバイス 人工内耳．月刊臨床神経科学　29(4):397-400, 2011.

Kashio A, Ito K, Kakigi A, Karino S, Iwasaki S, Sakamoto T, Yasui T, Suzuki M, Yamasoba T. Carhart notch 2-kHz bone conduction threshold dip: a nondefinitive predictor of stapes fixation in conductive hearing loss with normal tympanic membrane. *Arch Otolaryngol Head Neck Surg.* 137:236-240,2011

Karino S, Philip H. Smith , Tom C. T. Yin, Philip X. Joris. Axonal Branching Patterns as Sources of Delay in the Mammalian Auditory Brainstem: A Re-Examination. *J. Neurosci.* 31: 3016-3031, 2011

Sakamoto T, Kakigi A, Kashio A, Kanaya K, Suzuki M, Yamasoba T. Evaluation of the Carhart effect in congenital middle ear malformation with both an intact external ear canal and a mobile stapes footplate. *ORL J Otorhinolaryngol Relat Spec.* 73:61-7,2011

Suzukawa K, Kondo K, Kanaya K, Sakamoto T, Watanabe K, Ushio M, Kaga K, Yamasoba T, Age-related changes of the regeneration mode in the mouse peripheral olfactory system following olfactotoxic drug methimazole-induced damage. *J Comp Neurol.* 519:2154-2174,2011

松本　有、野本貴大、藤加珠子、片岡一則．生体内リアルタイム共焦点顕微鏡．　*Drug Delivery System* 26(5)：535-539，2011

２．学会発表

林穎 Lin Ying, 樫尾明憲, 山岨達也: Hydrogen in drinking water attenuates noise-induced temporary threshold shifts、第 111 会日本耳鼻咽喉科学会総会・学術講演会、10 年 5 月 21 日、仙台

狩野章太郎：内側上オリーブ核への求心性神経支配（両耳間時間差を検出するために）　第 111 会日本耳鼻咽喉科学会総会・学術講演会、10 年 5

月 22 日、仙台
狩野章太郎：左右の耳からの情報を統合して得られる脳磁場活動　第 10 回東京大学生命科学シンポジウム、10 年 5 月 1 日、東京
吉川　弥生：培養液への水素添加による蝸牛活性酸素の除去　第 4 回聴覚アンチエイジング研究会、10 年 7 月 2 日、東京
吉川　弥生：培養蝸牛における水素の保護効果東京大学夏期症例検討会、10 年 7 月 17 日、東京
堀江　理恵、吉川　弥生、中川　隆之、伊藤　壽一：水素は活性酸素から内耳を保護する　第 28 回頭頸部自律神経研究会　10 年 9 月 11 日、大阪
狩野章太郎：シンポジウム I 言語の生物学的基礎のために:誘発電位および認知関連電位による聴覚認知の発達　第 55 回日本音声言語医学会総会・学術講演会、10 年 10 月 14-15 日、東京
樫尾明憲、安井　卓也、狩野章太郎、坂本　幸士、柿木　章伸、岩崎　真一、山岨達也：先天性一側高度難聴例のＣＴ画像所見について　第20回日本耳科学会総会　10年10月9日、松山市
Kikkawa YS : Generation and Reduction of Reactive Oxygen Species in the Cochlea　Sixth International Symposium on Meniere's Disease and Inner Ear Disorders 2010/11/14, Kyoto
Karino S : Neuromagnetic Responses to Binaural Beat in Human Cerebral Cortex. 第 3 回ＧＣＯＥリトリート及び国際シンポジウム、10 年 12 月 11-12 日、幕張
Kashio A, Sakamoto T, Kakigi A, Kondo K, Yamasoba T : Urokinase-type plasminogen activator attenuates hair cell damage induced by aminoglycoside.　ARO　2011.2.19-23　Baltimore
馬場美雪、松本有、 カブラルオラシオ、 西山伸宏、片岡一則、山岨達也：シスプラチン内包高分子ミセルによる内耳障害軽減効果。第 27 回日本 DDS 学会　2011.6.9-10　東京
樫尾明憲、安達のどか、安井拓也、尾形エリカ、赤松裕介、坂田英明、山岨達也：当科における先天性サイトメガロウイルス感染症児に対する人工内耳の成績、第 6 回日本小児耳鼻咽喉科学会　2011.6.16-17　さいたま市
斉藤真紀、樫尾明憲、狩野章太郎、尾形エリカ、赤松裕介、安達のどか、浅沼聡、坂本幸士、柿木章伸、山岨達也：再手術を要した小児人工内耳症例の検討、第 6 回日本小児耳鼻咽喉科学会　2011.6.16-17　さいたま市
赤松裕介、尾形エリカ、樫尾明憲、安井拓也、安達のどか、浅沼聡、山岨達也：当科における重複障害児に対する人工内耳成績、第 6 回日本小児耳鼻咽喉科学会　2011.6.16-17　さいたま市
Kim HJ, Oba M, Pittella F, Nomoto T, Cabral H, Matsumoto Y, Miyata K, Nishiyama N, Kataoka K. : PEG-detachable polyaspartamide derivative block copolymer bearing stearoyl moieities for in vivo siRNA delivery.第 60 回高分子討論会　2011.9.28-30　岡山市
Kashio A, Akamatsu Y, Ogata E, Adachi N, Yasui T, Karino S, Sakamoto T, Kakigi A, Iwasaki I, Yamasoba T. : Cochlear implant in children with GJB2 gene mutation. APSCI　2011　10.25-28　Daegu
狩野章太郎、赤松裕介、越智篤、山岨達也：雑音負荷時の子音聴取－信号音源と雑音音源の空間的配置との関連．日本聴覚医学会　2011.10.28　福岡
Nomoto T, Matsumoto Y, Miyata K, Oba M, Fukushima S, Cabral H, Murakami M, Nishiyama N, Kataoka K : In Situ Monitoring of Drug Delivery Systems Using Intravital Real-time Confocal Laser Scanning Microscopy. Seoul Nanohealth 2011 Symposium　2011.11.17-18　Seoul, Korea
樫尾明憲：アポトーシス抑制蛋白 PTD-FNK を用いた内耳タンパク治療　<公募シンポジウム>　第 21 回 日本耳科学会総会・学術講演会　2011.11.24-26　宜野湾
松本有、狩野章太郎、吉川弥生、奥野妙子、片岡一則、山岨達也：生体内リアルタイム共焦点顕微鏡と蝸牛イメージングへの展開．第 21 回 日本耳

科学会総会・学術講演会　2011.11.24-26　宜野湾

坂本幸士、樫尾明憲、安井拓也、狩野章太郎、柿木章伸、山岨達也：外耳道閉鎖症の術後聴力に影響を及ぼす因子に関する多重ロジスティッ回帰分析による検討. 第21回 日本耳科学会総会・学術講演会　2011.11.24-26　宜野湾

Yamasoba T, Kashio A, Baba M. : Symposium: Novel technologies to prevent from inner ear damage. Nanotechnology and PTD technology. 11th Japan-Taiwan Conference on Otolaryngology-Head and Neck Surgery　2011.12.8-9　Kobe

Matsumoto Y, Arimoto T, Oda K, Kawana K, Yano T, Taketani Y, Matsumoto Y, Nomoto T, Toh K, Kataoka K. : Clinical Course and Features of Fatal Intersititial Pneumonitis Induced by PEGylated Liposomal Doxorubicin (PLD) and Analysis of PLD-Induced Lung Injury Using Real-Time Intravital Microscope. The 11th US-Japan Symposium on Drug Delivery Systems　2011.12.19-20　Hawaii,USA

Matsumoto Y, Nomoto T, Toh K, Miyata K, Cabral H, Christie RJ, Matsumoto Y, Nishiyama N, Yamasoba T, Kataoka K. :

Intravital Real-Time Confocal Laser Scanning Microscopy for In Situ Evaluation of Nanocarriers. The 11th US-Japan Symposium on Drug Delivery Systems　2011.12.19-20　Hawaii,USA

G. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakagawa T, Sakamoto T, Hiraumi H, Kikkawa YS, Yamamoto N, Hamaguchi K, Ono K, Yamamoto M, Tabata Y, Teramukai S, Tanaka S, Tada H, Onodera R, Yonezawa A, Inui K, Ito J.	Topical insulin-like growth factor 1 treatment using gelatin hydrogels for glucocorticoid-resistant sudden sensorineural hearing loss: a prospective clinical trial.	*BMC Medicine*	8	76-81	2010
Taura A, Kikkawa YS, Nakagawa T, Ito J.	Hydrogen protects vestibular hair cells from free radicals.	*Acta Oto-Laryngologica*,	130	95–100	2010
Matsumoto Y, Nomoto T, Cabral H, Matsumoto Y, Watanabe S, Christie J, Miyata K, Oba M, Ogura T, Yamasaki Y, Nishiyama N, Yamasoba T, Kataoka, K	Direct and instantaneous observation of intravenously injected substances using intravital confocal micro-videography.	*Biomedical Optics Express*, 1	4	1209–1216	2010
Sakamoto T, Nakagawa T, Horie RT, Hiraumi H, Yamamoto N, Kikkawa YS, Ito J.	Inner ear drug delivery system from the clinical point of view.	Acta Otolaryngologica Suppl.	563	101-4	2010
Lin Y, Kashio A, Sakamoto T, Suzukawa K, Kakigi A, Yamasoba T.	Hydrogen in drinking water attenuates noise-induced hearing loss in guinea pig.	*Neurosci Lett*	3;487(1)	12-6	2011
Sekiya T, Matsumoto M, Kojima K, Ono K, Kikkawa YS, Kada S, Ogita H, Horie RT, Viola A, Holley MC, Ito J.	Mechanical stress-induced reactive gliosis in the auditory nerve and cochlear nucleus.	*J Neurosurg*	114(2)	414-25	2011

狩野章太郎	知っておきたい生理・病態の基礎 聴覚末梢.	耳鼻咽喉科・頭頸部外科	82巻9号	631-635	2010
樫尾明憲，山岨達也	最新の治療デバイス 人工内耳.	月刊臨床神経科学	29(4)	397-400	2011
Kashio A, Ito K, Kakigi A, Karino S, Iwasaki S, Sakamoto T, Yasui T, Suzuki M, Yamasoba T.	Carhart notch 2-kHz bone conduction threshold dip: a nondefinitive predictor of stapes fixation in conductive hearing loss with normal tympanic membrane.	*Arch Otolaryngol Head Neck Surg.*	137	236-240	2011
Karino S, Philip H. Smith , Tom C. T. Yin, Philip X. Joris.	Axonal Branching Patterns as Sources of Delay in the Mammalian Auditory Brainstem: A Re-Examination.	*J. Neurosci*	31	3016-3031	2011
Sakamoto T, Kakigi A, Kashio A, Kanaya K, Suzuki M, Yamasoba T	Evaluation of the Carhart effect in congenital middle ear malformation with both an intact external ear canal and a mobile stapes footplate.	*ORL J Otorhinolaryngol Relat Spec.*	73	61-7	2011
Suzukawa K, Kondo K, Kanaya K, Sakamoto T, Watanabe K, Ushio M, Kaga K, Yamasoba T	Age-related changes of the regeneration mode in the mouse peripheral olfactory system following olfactotoxic drug methimazole-induced damage.	*J Comp Neurol.*	519	2154-2174	2011
松本　有、野本貴大、藤加珠子、片岡一則.	生体内リアルタイム共焦点顕微鏡.	*Drug Delivery System*	26	535-539	2011

Nakagawa et al. BMC Medicine 2010, 8:76
http://www.biomedcentral.com/1741-7015/8/76

BMC Medicine

RESEARCH ARTICLE Open Access

Topical insulin-like growth factor 1 treatment using gelatin hydrogels for glucocorticoid-resistant sudden sensorineural hearing loss: a prospective clinical trial

Takayuki Nakagawa[1*], Tatsunori Sakamoto[1], Harukazu Hiraumi[1], Yayoi S Kikkawa[1,2], Norio Yamamoto[1], Kiyomi Hamaguchi[1], Kazuya Ono[1,3], Masaya Yamamoto[4], Yasuhiko Tabata[4], Satoshi Teramukai[5], Shiro Tanaka[5], Harue Tada[5], Rie Onodera[6], Atsushi Yonezawa[7], Ken-ichi Inui[7], Juichi Ito[1]

Abstract

Background: Sudden sensorineural hearing loss (SSHL) is a common condition in which patients lose the hearing in one ear within 3 days. Systemic glucocorticoid treatments have been used as standard therapy for SSHL; however, about 20% of patients do not respond. We tested the safety and efficacy of topical insulin-like growth factor 1 (IGF1) application using gelatin hydrogels as a treatment for SSHL.

Methods: Patients with SSHL that showed no recovery to systemic glucocorticoid administration were recruited. We applied gelatin hydrogels, impregnated with recombinant human IGF1, into the middle ear. The primary outcome measure was the proportion of patients showing hearing improvement 12 weeks after the test treatment. The secondary outcome measures were the proportion of patients showing improvement at 24 weeks and the incidence of adverse events. The null hypothesis was that 33% of patients would show hearing improvement, as was reported for a historical control after hyperbaric oxygen therapy.

Results: In total, 25 patients received the test treatment at a median of 23 days (range 15-32) after the onset of SSHL, between 2007 and 2009. At 12 weeks after the test treatment, 48% (95% CI 28% to 69%; $P = 0.086$) of patients showed hearing improvement, and the proportion increased to 56% (95% CI 35% to 76%; $P = 0.015$) at 24 weeks. No serious adverse events were observed.

Conclusions: Topical IGF1 application using gelatin hydrogels is well tolerated and may be efficacious for hearing recovery in patients with SSHL that is resistant to systemic glucocorticoids.

Background

Sudden sensorineural hearing loss (SSHL) is a condition in which an individual experiences hearing loss of at least 30 dB over at least three test frequencies in one ear within a period of 3 days [1]. Some patients recover completely without medical intervention, often within the first 3 days. Others get better slowly over a 1-week or 2-week period, which is known as 'spontaneous recovery' [1]. Although a good recovery is likely, 15% of patients with SSHL experience hearing loss that worsens over time. Approximately 40,000 new cases of SSHL occur each year in the US [1], and 35,000 patients with SSHL consult a doctor each year in Japan [2]. SSHL can affect anyone; however, for reasons that so far remain unknown, it is most often reported in people aged between 30 and 60 years. The most common therapy for SSHL is the systemic application of glucocorticoids. Unfortunately, about 20% of patients do not respond to this treatment [3].

Based on these findings, researchers have sought alternative therapeutic options for SSHL. Protecting auditory hair cells and primary neurons from irreversible

* Correspondence: tnakagawa@ent.kuhp.kyoto-u.ac.jp
[1]Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
Full list of author information is available at the end of the article

degeneration is a practical strategy, as inner ear cells have limited regeneration capacity [4]. Recent improvements in our understanding of the role of growth factors in the maintenance of mature peripheral auditory systems have led to numerous attempts to define ways to reduce auditory hair cell and neuron degeneration, which have indicated that some growth factors have potential for the treatment of SSHL [5-8]. However, growth factors have not yet been used for this purpose in a clinical setting, as several obstacles have hindered their progress. Safe and effective methods for the sustained delivery of growth factors to the inner ear need to be developed to facilitate their clinical application. As a solution to this problem, we used gelatin hydrogels as a vehicle to deliver growth factors to the inner ear [9]. Gelatin hydrogels consist of gelatin polymers that are electrostatically complexed with growth factors [10]. The growth factors are released by the enzymatic degradation of the gelatin polymers after application. Our focus was on insulin-like growth factor 1 (IGF1), which has been approved for clinical application. We conducted a series of animal experiments, which revealed that topical IGF1 application via gelatin hydrogels significantly improved hearing by protecting auditory hair cells against damage caused by intense noise exposure [11] or ischaemic injury [12]. Moreover, no adverse events were observed in animals following the local application of IGF1 via gelatin hydrogels [11].

Here, we report on a prospective clinical trial of topical IGF1 application through gelatin hydrogels for the treatment of glucocorticoid-resistant SSHL, which was intended to provide preliminary estimates of variables for generating hypotheses for more specific studies using randomised trials when appropriate. Systemic glucocorticoid application has been regarded as a primary treatment of choice for SSHL. We recruited patients with SSHL that showed no recovery to systemic glucocorticoid administration as subjects in the present study.

Methods

Patients

Patients were eligible for inclusion in the study if they met the following conditions: they had been diagnosed between December 2007 and July 2009 at the Department of Otolaryngology, Head and Neck Surgery of Kyoto University Hospital, Japan as having definite or probable SSHL within 29 days of onset; they presented with an abnormality in evoked otoacoustic emission, which indicated dysfunction of the auditory hair cells; no recovery was determined according to the criteria for hearing improvement as set by the Sudden Deafness Research Committee of the Japanese Ministry of Health, Labour and Welfare in 1984 [13] (Table 1) more than 7 days after systemic glucocorticoid treatment; and they were aged over 20 years. We excluded patients with active chronic otitis media, acute otitis media, otitis media with effusion or dysfunction of the auditory tube, a history of previous treatments (except for systemic application of glucocorticoids or prostaglandin E1), malignant tumours, severe liver dysfunction (aspartate aminotransferase (AST) >100 IU/L and alanine aminotransferase (ALT) >100 IU/L), 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. Magnetic resonance imaging (MRI) was performed on all patients to rule out acoustic neurinoma.

This study was single arm, non-randomised and open. Placebo applications and blinding were not used, as it was anticipated that they would have reduced compliance.

The primary outcome measure was the proportion of patients showing hearing improvement, which was defined as better than slight recovery according to the criteria shown in Table 1, 12 weeks after the test treatment. The secondary outcome measures were the proportion of patients showing hearing improvement 24 weeks after the test treatment and the incidence of adverse events during the observation period.

Ethics approval

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.

Trial registration

This trial was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) on 6 December 2007 under trial registration number UMIN-CTRR000000936.

Procedures

The test treatment was performed within 4 days of registration. 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, according to the method described previously [14], and were preserved at temperatures below 4°C before use. Procedures for topical IGF1 treatment were performed in the Day-Surgery Unit of Kyoto University Hospital. Mecasermin (recombinant human IGF1 (Somazon), 10 mg injection; Astellas Pharma Inc., Tokyo, Japan) was dissolved in physiological saline at a final concentration of 10 mg/ml. A 30 μl sample of

Table 1 Criteria for hearing improvement determined by the Sudden Deafness Research Committee of the Japanese Ministry of Health, Labour and Welfare in 1984

Improvement	Criteria
Complete recovery	Recovery of a hearing level within 20 dB at all five frequencies tested (0.25, 0.5, 1.0, 2.0 and 4.0 kHz) or recovery to the same level as the opposite side in pure tone auditometry
Marked recovery	More than 30 dB recovery in the mean hearing level at the five frequencies tested
Slight recovery	Recovery of 10 to 29 dB in the mean hearing level at the five frequencies tested
No recovery	Less recovery than 10 dB in the mean hearing level at the five frequencies tested

mecasermin solution was mixed with 3 mg of gelatin hydrogels 60 min before application. The hydrogel containing 300 μg of mecasermin was placed in the round-window niche of the middle ear following tympanostomy under local anaesthesia with 1% lidocaine. A single application was used. Patients were hospitalised for 4 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. Pure-tone audiometry and evoked otoacoustic emission were measured on the day of registration, at 3 days after the test treatment, and at 1, 2, 4, 12 and 24 weeks after the test treatment. During the observation period, all adverse events were recorded.

Statistical analysis

The threshold improvement (33%, 66/199) was based on a historical control of hyperbaric oxygen therapy (19 times in total; range 5-55) for 199 patients with glucocorticoid-resistant SSHL at Kyoto University Hospital between October 2000 and September 2006 [15]. The null hypothesis was that the proportion of patients with hearing improvement at 12 or 24 weeks after the test treatment would be equivalent to the proportion of patients with hearing improvement reported in a historical control administered hyperbaric oxygen therapy. The sample size was based on binominal distribution with a one-sided significance level of 0.05 and a power of 0.90 (expected proportion of 63%). The required sample size was 25 after considering 10% (3 samples) of patients who would be excluded from the analysis. The null hypothesis was rejected at the 0.05 level of probability (one-sided) based on a binominal distribution. Statistical analyses were performed using SAS v.9.2 (SAS Institute Inc. Cary, NC, USA).

Results

In all, 26 patients fulfilled the inclusion criteria, 1 of whom was excluded before the test treatment because of a diagnosis of functional hearing loss. In total, 25 patients (13 women and 12 men) were treated in accordance with the study protocol, and data for assessment of the primary and secondary outcomes were available for all patients. The median age at registration was 49 years (range 23-72 years). Comorbidities were found in 22 of the 25 patients (88%), and 18 of the 25 patients (72%) had a history of previous diseases. None of the comorbidities or previous diseases presented were directly associated with SSHL. None of the patients had family histories of SSHL. All 25 patients complained of associated symptoms: 22 (88%) complained of tinnitus, 19 (76%) had a feeling of ear fullness and 14 (56%) complained of dizziness. The median interval between the onset of SSHL and the initiation of the test treatment was 23 days (range 15-32 days). The mean hearing level at registration was 81.2 dB (95% confidence interval (CI), 71.2 to 91.1).

A summary of the hearing recovery according to pure-tone audiometry for all of the patients is shown in Table 2. At 12 weeks after the test treatment, 48% (95% CI 28% to 69%; $P = 0.086$) of the patients showed hearing improvement. The null hypothesis for the primary outcome was not rejected. Of the 25 patients, 0 showed complete recovery, 1 (4%) showed marked recovery, 11 (44%) showed slight recovery and 13 (52%) showed no recovery at 12 weeks. None of the patients who were treated more than 26 days after the onset of SSHL showed hearing improvement. At 24 weeks after the test treatment, the proportion of patients showing hearing improvement was 56% (95% CI 35% to 76%; $P = 0.015$), showing that the null hypothesis was rejected for the data at 24 weeks. Of the 25 patients, none showed complete recovery, 1 (4%) showed marked recovery, 13 (52%) showed slight recovery, and 11 (44%) showed no recovery. Two patients showed a hearing improvement of less than 10 dB at 12 weeks after the treatment, but an improvement of 10 dB at 24 weeks.

No serious adverse events associated with the test treatment occurred, although any adverse events were recorded in all of 25 patients to be evaluated. Adverse events with an incidence rate of more than 20% included dizziness (44%), nausea (24%), otitis externa (32%), common cold (20%) and otitis media (28%). All adverse events disappeared within the observation period. Except for two patients, the dizziness appeared either on the day of local IGF1 application or on the next day, and continued for a mean of 6.4 days (range

Table 2 Hearing recovery according to pure-tone audiometry

				Averaged hearing level (dB)			Hearing improvement	
Patient	Age	Gender	Days from onset	Before registration	12 weeks	24 weeks	12 weeks	24 weeks
1	54	M	19	88	77	75	SR	SR
2	36	F	31	62	55	60	NR	NR
3	46	M	21	107	81	86	SR	SR
4	29	F	24	107	95	95	SR	SR
5	38	M	19	65	64	62	NR	NR
6	72	M	29	98	97	97	NR	NR
7	49	M	17	111	105	105	NR	NR
8	49	F	26	47	42	42	NR	NR
9	55	M	21	104	78	75	SR	SR
10	55	F	29	52	57	57	NR	NR
11	60	F	27	37	33	32	NR	NR
12	35	F	21	76	68	66	NR	SR
13	59	M	23	90	79	78	SR	SR
14	58	M	32	60	81	77	NR	NR
15	60	F	26	63	40	39	SR	SR
16	36	M	19	56	51	46	NR	SR
17	33	F	18	88	88	87	NR	NR
18	61	F	25	92	72	74	SR	SR
19	42	F	15	111	89	92	SR	SR
20	23	F	18	79	22	18	MR	MR
21	45	F	26	95	82	77	SR	SR
22	45	M	28	87	84	85	NR	NR
23	60	F	23	108	84	86	SR	SR
24	26	M	20	109	92	86	SR	SR
25	55	M	21	37	34	35	NR	NR

Average hearing level was the mean hearing level according to pure-tone audiometry at the five frequencies tested (0.25, 0.5, 1.0, 2.0 and 4.0 kHz). Hearing improvement was determined by the criteria shown in Table 1.

MR = marked recovery; NR = no recovery; SR = slight recovery.

1-20 days). In all patients, the dizziness appeared after the test treatment. In one patient, dizziness appeared 2 months after the test treatment and continued for 4 months. In another patient, dizziness appeared 7 days after the application and disappeared 2 days later. Otitis media was found in 7 of the 25 (28%) patients, and was cured within a mean of 9.4 days (range 2-17 days). Exacerbation of tinnitus appeared in two patients at 29 and 33 days after the test treatment, respectively. None of the patients showed residual perforation of the tympanic membrane or additional hearing loss over 10 dB.

Discussion

Hearing loss is common, affecting about 5% to 6% of the population of the USA [1]. SSHL is one of the most common clinical conditions encountered by otolaryngologists, although it is less common than age-related hearing loss. National surveys have demonstrated the incidence of SSHL to be 5-30 per 100,000 per year [2,16,17]. Systemic application of glucocorticoids has been used as a standard therapy, although the supporting evidence is weak. Although systemic glucocorticoid application results in hearing recovery in some patients with SSHL, approximately 20% show no recovery [3]. Alternative therapeutic treatment options for SSHL have thus been eagerly sought. Against this background, we began developing topical IGF1 treatments using gelatin hydrogels in animal models [5,11,12], followed by a clinical trial to investigate their safety and efficacy for use in patients with SSHL. Some studies have indicated that SSHL develops when the inner ear does not receive a sufficient oxygen supply [18]. Consequently, hyperbaric oxygen treatment has been used as an alternative option for the treatment of SSHL [19,20]. At Kyoto University Hospital, hyperbaric oxygen therapy has been used as a secondary treatment of choice for glucocorticoid-resistant SSHL [14]. We thus used the proportion of patients with glucocorticoid-resistant SSHL showing hearing

recovery following hyperbaric oxygen therapy as a historical control.

Here, we report hearing recovery according to pure-tone audiometry and incidence of adverse events following topical IGF1 application using gelatin hydrogels in patients with SSHL enrolled in a single arm, non-randomised and open trial. Topical IGF1 treatment resulted in hearing recovery in approximately half of the patients with SSHL that had not responded to systemic glucocorticoid application, although the null hypothesis was rejected at 24 weeks after the test treatment but not at 12 weeks. In addition, no serious adverse events were observed during the 24-week observation period. The results indicated that the topical IGF1 application using gelatin hydrogels was safe, and had equivalent or superior efficiency to the hyperbaric oxygen therapy that was used as a historical control; this suggests that the efficacy of topical IGF1 application using gelatin hydrogels for SSHL that is resistant to systemic glucocorticoid treatments should be evaluated using randomised clinical trials.

Spontaneous recovery occurs in 40% to 65% of patients with SSHL [21,22], which makes it difficult to examine the exact therapeutic effects of interventions. It is therefore important either to eliminate patients with spontaneous recovery from such trials or to include a placebo control. In the present study, the test treatment was initiated in all patients more than 14 days (mean 23 days; range 15-32 days) after the onset of SSHL. In most cases, spontaneous recovery occurs within 14 days of onset [21]. We therefore consider spontaneous recovery to have had a negligible influence on the present results.

As a secondary treatment of choice for SSHL, intratympanic injection of glucocorticoids has gained considerable attention, because it seems to deliver a high concentration of glucocorticoids to the inner ear [23]. In addition, local application can reduce the total amount of glucocorticoids that needs to be applied, leading to a reduced risk of adverse events [24]. However, this approach remains controversial, because the criteria used to judge its efficacy differ in the literature. Haynes *et al.* [25] reviewed the literature on the intratympanic injection of glucocorticoids for SSHL after the failure of systemic treatment, and re-estimated the hearing recovery based on their own criteria, according to which a 20 dB improvement as indicated by pure-tone audiometry or a 20% improvement in discrimination was considered to be a successful therapeutic intervention. The recovery rates according to their criteria were 0% to 40%. When these criteria for successful intervention were applied to the data from the present study, the recovery rate was 24%, suggesting that the efficacy of topical IGF1 treatment using gelatin hydrogels might be equivalent to that of the intratympanic injection of glucocorticoids. We therefore recommend that the efficacy of topical IGF1 treatment using gelatin hydrogels should be evaluated in a randomised clinical trial, and its effectiveness for SSHL should be compared with that of the intratympanic injection of glucocorticoids.

Conclusions

The present results indicate the safety and efficacy of the use of topical IGF1 treatment using gelatin hydrogels for SSHL resistant to systemic glucocorticoid treatments. A double-blinded, randomised clinical study could clarify these findings. However, there are ethical obstacles to the use of double-blinded, randomised clinical trials for SSHL. For instance, the time from the onset of SSHL to the start of treatment has been regarded as important for the outcome, with prompt treatment preventing the development of irreversible auditory pathological changes. In addition, systemic glucocorticoid treatments have widely been accepted as a standard therapy for SSHL, and have led to improvement in some patients [26]. Hence, there would be ethical difficulties in not offering patients treatment with systemic glucocorticoids. Moreover, topical IGF1 application using gelatin hydrogels requires the use of surgical procedures, which would make it difficult to test in a double-blinded study. Therefore, as a next step, we will conduct a randomised clinical trial to compare the efficacy of topical IGF1 treatment using gelatin hydrogels with that of the intratympanic injection of glucocorticoids in patients with SSHL that is resistant to systemic glucocorticoids; it is hoped that this might clarify the efficacy of topical IGF1 treatment using gelatin hydrogels.

Acknowledgements

This study was supported by grants for Research on Sensory and Communicative Disorders from the Japanese Ministry of Health, Labour and Welfare. We thank Akira Shimizu, Masayuki Yokode, Syuji Higuchi and Masanori Fukushima for help in the design of the study. We also thank Toshinori Murayama, Manabu Minami, Toshiko Ihara, Erika Hirata, Tomoko Yokota, Kazumi Miura and Chika Toyo-oka for support in trial protocol enforcement and clinical trial coordination. We are grateful for support from all clinical staff in the Department of Otolaryngology, Head and Neck Surgery at Kyoto University Hospital and the Translational Research Centre at Kyoto University Hospital.

Author details

[1]Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [2]Department of Otolaryngology, Faculty of Medicine, University of Tokyo, Tokyo, Japan. [3]RIKEN Center for Developmental Biology, Kobe, Japan. [4]Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan. [5]Department of Clinical Trial Design & Management, Translational Research Center, Kyoto University Hospital, Kyoto, Japan. [6]Collaboration Center for Community and Industry, Sapporo Medical University, Sapporo, Japan. [7]Department of Pharmacy, Kyoto University Hospital, Kyoto, Japan.

Authors' contributions

TN, RO, SaT and JI planned the study. TS, HH, YSK and NM performed surgical treatment and collected the data. KH, KO, AY, KI, MY and YT prepared the gelatin hydrogels. SaT, ShT and HT analysed the data. TN wrote the article. JI edited the article.

Competing interests
The authors declare that they have no competing interests.

Received: 16 August 2010 Accepted: 25 November 2010
Published: 25 November 2010

References

1. National Institute of Health: *Sudden Deafness* NIH publication 00-4757. Bethesda, MD: National Institutes of Health; 2000.
2. Teranishi M, Katayama N, Uchida Y, Tominaga M, Nakashima T: **Thirty-year trends in sudden deafness from four nationwide epidemiological surveys in Japan.** *Acta Oto-Laryngologica* 2007, 127:1259-1265.
3. Wilson WR, Byl FM, Laird N: **The efficacy of steroids in the treatment of idiopathic sudden hearing loss. A double-blind clinical study.** *Arch Otolaryngol* 1980, 106:772-776.
4. Roberson DW, Rubel EW: **Cell division in the gerbil cochlea after acoustic trauma.** *Am J Otol* 1994, 15:28-34.
5. Malgrange B, Rigo JM, Van de Water TR, Staecker H, Moonen G, Lefebvre PP: **Growth factor therapy to the damaged inner ear: clinical prospects.** *Int J Pediatr Otorhinolaryngol* 1999, 49(Suppl 1):S19-25.
6. Romand R, Chardin S: **Effects of growth factors on the hair cells after ototoxic treatment of the neonatal mammalian cochlea in vitro.** *Brain Res* 1999, 825:46-58.
7. Iwai K, Nakagawa T, Endo T, Matsuoka Y, Kita T, Kim TS, Tabata Y, Ito J: **Cochlear protection by local IGF-1 application using biodegradable hydrogel.** *Laryngoscope* 2006, 116:526-533.
8. Inaoka T, Nakagawa T, Kikkawa YS, Tabata Y, Ono K, Yoshida M, Tsubouchi H, Ido A, Ito J: **Local application of hepatocyte growth factor using gelatin hydrogels attenuates noise-induced hearing loss in guinea pigs.** *Acta Otolaryngol* 2009, 129:453-457.
9. Endo T, Nakagawa T, Kita T, Iguchi F, Kim TS, Tamura T, Iwai K, Tabata Y, Ito J: **A novel strategy for treatment of inner ears using a biodegradable gel.** *Laryngoscope* 2005, 115:2016-2020.
10. Young S, Wong M, Tabata Y, Mikos AG: **Gelatin as a delivery vehicle for the controlled release of bioactive molecules.** *J Control Release* 2005, 109:256-274.
11. Lee KY, Nakagawa T, Okano T, Hori R, Ono K, Tabata Y, Lee SH, Ito J: **Novel therapy for hearing loss: delivery of insulin-like growth factor-1 to the cochlea using gelatin hydrogel.** *Otol Neurotol* 2007, 28:976-981.
12. Fujiwara T, Hato N, Nakagawa T, Tabata Y, Yoshida T, Komobuchi H, Takeda S, Hyodo J, Hakuba N, Gyo K: **IGF1 treatment via hydrogels rescues cochlear hair cells from ischemic injury.** *Neuroreport* 2008, 19:1585-1588.
13. Kanzaki J, Inoue Y, Ogawa K, Fukuda S, Fukushima K, Gyo K, Yanagihara N, Hoshino T, Ishitoya J, Toriyama M, Kitamura K, Murai K, Nakashima T, Niwa H, Nomura Y, Kobayashi H, Oda M, Okamoto M, Shitara T, Sakagami M, Tono T, Usami S: **Effect of single-drug treatment on idiopathic sudden sensorineural hearing loss.** *Auris Nasus Larynx* 2003, 30:123-127.
14. Marui A, Tabata Y, Kojima S, Yamamoto M, Tambara K, Nishina T, Saji Y, Inui K, Hashida T, Yokoyama S, Onodera R, Ikeda T, Fukushima M, Komeda M: **A novel approach to therapeutic angiogenesis for patients with critical limb ischemia by sustained release of basic fibroblast growth factor using biodegradable gelatin hydrogel: an initial report of the phase I-IIa study.** *Circ J* 2007, 71:1181-1186.
15. Miura M, Sakamoto T, Hiraumi H, Kanemaru S, Ito J: **Evaluation of hyperbaric oxygen therapy for the treatment of sudden hearing loss in both primary and secondary cases.** *Practica Oto-Rhino-Laryngologica* 2008, 101:749-757.
16. Wu CS, Lin HC, Chao PZ: **Sudden sensorineural hearing loss: evidence from Taiwan.** *Audiol Neurootol* 2006, 11:151-156.
17. Nosrati-Zarenoe R, Arlinger S, Hultcrantz E: **Idiopathic sudden sensorineural hearing loss: results drawn from the Swedish national database.** *Acta Otolaryngol* 2007, 127:1168-1175.
18. Castro NP Junior, Almeida CI, Campos CA: **Sudden sensorineural hearing loss and vertigo associated with arterial occlusive disease: three case reports and literature review.** *Sao Paulo Med J* 2007, 125:191-195.
19. Ragab A, Shreef E, Behiry E, Zalat S, Noaman M: **Randomised, double-blinded, placebo-controlled, clinical trial of ozone therapy as treatment of sudden sensorineural hearing loss.** *J Laryngol Otol* 2009, 123:54-60.
20. Muzzi E, Zennaro B, Visentin R, Soldano F, Sacilotto C: **Hyperbaric oxygen therapy as salvage treatment for sudden sensorineural hearing loss: review of rationale and preliminary report.** *J Laryngol Otol* 2010, 124:e2.
21. Mattox DE, Simmons FB: **Natural history of sudden sensorineural hearing loss.** *Ann Otol Rhinol Laryngol* 1977, 86:463-480.
22. Guyot JP, Thielen K: **Evolution of sudden deafness without treatment.** *Schweizerische Medizinische Wochenschrift* 2000, 116:93S-96S.
23. Parnes LS, Sun AH, Freeman DJ: **Corticosteroid pharmacokinetics in the inner ear fluids: an animal study followed by clinical application.** *Laryngoscope* 1999, 109:1-17.
24. Free RH, Smale ND, De Kleine E, Van Der Laan BFAM: **Side effects of oral dexamethasone pulse therapy for idiopathic sudden sensorineural hearing loss.** *Otol Neurotol* 2009, 30:691.
25. Haynes DS, O'Malley M, Cohen S, Watford K, Labadie RF: **Intratympanic dexamethasone for sudden sensorineural hearing loss after failure of systemic therapy.** *Laryngoscope* 2007, 117:3-15.
26. Chen CY, Halpin C, Rauch SD: **Oral steroid treatment of sudden sensorineural hearing loss: a ten year retrospective analysis.** *Otol Neurotol* 2003, 24:728-733.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1741-7015/8/76/prepub

doi:10.1186/1741-7015-8-76
Cite this article as: Nakagawa *et al.*: Topical insulin-like growth factor 1 treatment using gelatin hydrogels for glucocorticoid-resistant sudden sensorineural hearing loss: a prospective clinical trial. *BMC Medicine* 2010 8:76.