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Medicine-Based Evidence: Reverse Translational Ear Research Recommendations

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Abstract: Presented here is a first-person account of the evolution of the practice of surgical neurootology to that of medical neurootology shaped mainly by results of treatment directed at underlying otosclerosis-like lesions of the otic capsule and metabolic factors. With new technologies and rapidly evolving concepts, the changing treatment algorithms did not remain constant to provide the usual evidence-based outcome analyses. However, the majority of the patients presenting with neurootological symptoms had undergone previous medical or surgical treatment before undergoing the medical management herein described. The underlying ongoing basic science findings over this period were linked to the clinical observations. On the basis of the more effective results of treating neurootological disorders, recommendations are made for future areas of investigation—mostly basic science—into developing an investigative foundation for future effective management of patients with a variety of neurootological disorders.

Key Words: hyperinsulinemia; impaired glucose tolerance; Ménière's syndrome; osteoclast; osteoprotegerin; otosclerosis-like

For the last 40 years, I have been immersed in the field of clinical neurootology. My roots were in two distinct disciplines. The first discipline is the study of the underlying mechanisms of clinical problems in neurootology. The second discipline is surgical neurootology.

After finishing a surgically oriented neurootology fellowship in 1968, my next pursuit was to find surgical cases, almost to the exclusion of any nonsurgical cases. I was particularly focused on the surgical treatment of dizziness and Ménière's syndrome [1–4]. At that time, no treatment for progressive sensorineural hearing loss or tinnitus was available. As my clinical surgical experience grew, I was fortunate to be influenced by others to return to my roots and explore the underlying mechanisms of clinical disease. My treatments began to change in response to my continuing observations and to new developments in basic science and clinical practice.

Two main treatment pathways emerged as a basis for my medical (nonsurgical) treatment of neurootological disorders [5–8]. One treatment pathway was based on the recognition of an inner-ear otosclerosis-like disorder that was discovered while searching for an explanation for an etiology underlying the clinical findings. The other treatment pathway focused on the metabolism of the inner ear. In time, my experience with these treatment algorithms made me realize that some medical solutions are more effective than surgical solutions. In fact, the medical managements that evolved were disease-modifying, far more efficient, more cost-effective, and less morbid than the invasive surgical alternatives and previous and current medical management standards of practice.

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PATIENTS, METHODS, AND RESULTS

The decisive factor in achieving successful outcomes in patients with neurootological symptoms is the manner in which hair cell function is modified throughout the duration of treatment. Normal hair cell function is dependent on the muscle or actin component of the hair cell. Current hair cell research is focused on the motor protein prestin (which is found within the actin), on repopulating or replacing hair cells, and on actin elements in general.

Prestin research has given us a greater understanding of the cochlear amplifier of the outer hair cells [9–13]

and the underlying movement of calcium in the changed polarization of the cochlear hair cells [14,15] at the synaptic junction. Repopulation has focused on the conversion of supporting cells or on the unlocking of the genetic code observed in lower species that allows for repopulation. Hair cell replacement is investigated in stem cell research.

Impaired function of hair cells is attributable to a disorder in the behavior of the actin elements. When hair cell function is impaired, as demonstrated clinically by otoacoustic emissions testing, the dysfunction appears to be the source of the otological and neurootological symptoms we treat in practice. If hair cell function can be improved, the symptoms that go along with impaired function can abate or disappear. In some of my patients, hearing loss has been significantly reversed. With the inclusion of routine otoacoustic emission testing in clinical practice, reversible otoacoustic emission results infer regeneration of hair cell function along with parallel clinical improvement. Though the thrust in current hair cell research is the repopulation of hair cells or their replacement with stem cells, little research has been directed at recovery of impaired hair cell function.

Analysis of clinical observations along with the results of evolving basic research could explain the clinical findings in patients with neurootological symptoms and lead to novel clinical treatments. Thus, this article serves as my proposal for future ear research.

Clinical Treatment Observations

The results of my medical treatments led me to develop further innovations, which in turn prompted more questions. Some of the answers to these questions were found in the domain of basic science. I have been awed, at meetings of the Association for Research in Otolaryngology, by the presentations of basic science methodologies used to investigate the processes of inner-ear function. However, as basic science techniques continue to progress, it is important that we not overlook some of the older investigative techniques that could help explain the underlying mechanisms of some of my treatment successes. Though it is true that some of the newer investigative techniques have validated some of my observations, more research using both older and newer techniques is required to further advance the successful management of these patients presenting with neurootological symptoms.

Clinical Observations of Otosclerosis

As mentioned, my first treatment pathway was based on my understanding that underlying otosclerosis or otosclerosis-like lesions of the inner ear can produce a variety of symptoms, including Ménière's syndrome, dizziness, sensorineural hearing loss, tinnitus, hyperacusis, and some hemicranial headaches [16]. When I was in training, those who studied the influence of otosclerosis on the inner ear were divided into two camps. One camp believed that the otosclerotic lesion must impinge on the endosteal membrane [17,18] to alter inner-ear function [19,20], whereas the other camp believed that the mere presence of otosclerosis in the temporal bone could influence the inner ear [21–25]. By 2005, we found the existence of lacunar and canalicular channels that lead from the perilymphatic space into the otic capsule [26]. The presence of these channels explains how the byproducts of the otosclerotic process can enter the perilymph to exert their potential effect on the sensory structures of the inner ear without the otosclerotic focus physically impinging on the endosteal membrane. By 2008, we found that temporal bone histopathology explained some of the variety of clinical findings [27–30].

When I finished my fellowship in 1969, sodium fluoride was the only medical otosclerosis treatment that was recognized by some authors [31–40]. However, its use was controversial at that time because of general nutritional considerations and a divided clinical community [41,42]. During my training, I was not firmly convinced of the value of sodium fluoride in the treatment of otosclerosis. However, my fellowship lasted only 1 year, and this may not have been enough time for me to arrive at any conclusion about the efficacy of sodium fluoride in the medical treatment of the neurootological symptoms of otosclerosis.

In my search for candidates for acoustic tumor surgery in the early 1970s, I obtained tomographic scans of the temporal bones to more accurately examine the size of the internal auditory canals. Though most of these acoustic neuroma candidates did not demonstrate findings that would suggest an acoustic tumor, many of them had polytomographic evidence of otosclerosis [43,44]. Those who did demonstrate polytomographic evidence of otosclerosis were placed on sodium fluoride to treat the neurootological symptoms. As a result of the sodium fluoride treatment, many patients showed evidence that their Ménière's syndrome had stabilized or that their vertigo or progressive sensorineural hearing loss had been arrested. Some patients also demonstrated a reduction in their tinnitus and hearing loss [45].

On the basis of these observations, I began treating my otosclerosis patients with etidronate after the bisphosphonate group of drugs became available (around 1990) [16]. Etidronate was approved for the treatment of Paget's disease of the bone and demonstrated its effect on the hearing loss in Paget's disease [46]. Etidronate was subsequently studied as a treatment for osteoporosis. It seemed logical that this compound could also be

considered for the treatment of otosclerosis. Indeed, etidronate quickly demonstrated that it was more effective than sodium fluoride alone for the treatment of otosclerosis. My success with etidronate led me to use other bisphosphonates to treat the inner-ear symptoms of otosclerosis [16]. Newer generations of bisphosphonates became available, and their effectiveness became predictable. They were better than sodium fluoride alone and better than any surgical procedure directed at the inner-ear symptoms of otosclerosis.

Basic Science of Otosclerosis

Our understanding of the active phase of otosclerosis is that it involves an osteoclast-driven dystrophy of the otic capsule bone. In the basic science world, the field of osteoclastogenesis developed around the turn of the twenty-first century. The inner ear was found to produce a cytokine called *osteoprotegerin* (OPG) [26]. OPG acts as a decoy for the receptor activator of nuclear kappa B ligand, another cytokine that attaches at its receptor site to a macrophage, transforming it into an osteoclast. In addition, channels that were identified connect the perilymphatic space with the otic capsule, a finding that could explain how OPG diffuses into the otic capsule [26] and how the byproducts of the breakdown of bone (osteoclast activity) could diffuse in a direction opposite from that of the otic capsule and into the perilymph to produce inner-ear symptoms. Without a membrane barrier, these channels provide a conduit for OPG to diffuse out into the otic capsule bone and prevent or reduce osteoclast production. This effect results in very low bone turnover, which makes the normal otic capsule the hardest bone in the body [47]. In the past, some believed that the otosclerotic area had to impinge on the endosteal membrane for neurootological symptoms to occur [42]. The cytokines that are breakdown products of bone enter into the perilymph through the same channels that the OPG uses to diffuse out into the otic capsule.

Tumor necrosis factor alpha (TNF-α) is one of the cytokine byproducts of osteoclastic activity that has been shown to be toxic to the inner ear, presumably to the hair cells [48–57]. An investigation of mice that were genetically altered so as not to produce OPG (OPG knockout mice) found that hearing was lost shortly after birth; histopathology of the temporal bones revealed lesions similar to those seen in humans with otosclerosis [58]. A recent investigation revealed that intraperitoneal bisphosphonate (risedronate) in OPG knockout mice prevented the development of otosclerosis-like lesions and hearing loss [59]. Because bisphosphonates target osteoclasts and because otosclerosis or otosclerosis-like disorders are osteoclast-driven, the use of bisphosphonates could truly produce a disease-modifying effect.

For the last 3 years, my laboratory evaluations have included the identification of 25-hydroxy vitamin D. I look at vitamin D_3 and parathyroid hormone (PTH) levels in particular. This has allowed me to identify deficient ($<$20 ng/ml) and insufficient ($<$40 ng/ml) vitamin D levels and primary and secondary hyperparathyroidism even in patients whose serum calcium levels are normal. In some patients, hyperparathyroidism occurred secondary to insufficient vitamin D and was reversible by appropriate vitamin D replacement [6,60–62]. Vitamin D and PTH abnormalities may have an effect on osteoclastogenesis, on the neurootological symptoms of otosclerosis [60–62], and on the efficacy of bisphosphonates. Patients who present with a radiological finding of a superior semicircular canal fistula (with or without symptoms) have been found to have abnormalities of vitamin D or PTH levels or both. When these abnormalities are identified and treated, many patients experience a regression of symptoms or an improvement of otoacoustic emissions. My management of superior semicircular canal dehiscence suggests that it may represent a metabolic disorder related to insufficient vitamin D or to primary or secondary hyperparathyroidism. The fact that it may not be a surgical disorder may explain why it can recur in patients who have undergone surgery to replace the bone. It would also explain why canal plugging is a more effective surgical treatment. In my experience, some patients who undergo surgery for chronic otitis media with cholesteatoma have been found to have an eroded and exposed dehiscent lateral semicircular canal. The cholesteatoma matrix is peeled off the lateral semicircular canal fistula and, after an interval when a second operation for hearing restoration has been performed, the bone has usually regrown over the lateral semicircular canal. If superior semicircular canal dehiscence is a metabolic disorder of bone, the bony dehiscence may in fact spontaneously close, given the correct conditions of vitamin D or PTH (or both).

Moreover, carbonic anhydrase, an enzyme in the stria vascularis [63–77], has been speculated to play a role in electrolyte homeostasis in the inner ear. Acetazolamide is a carbonic anhydrase inhibitor that is used as a diuretic; in fact, it is frequently the diuretic of choice for treating otological symptoms [78–81]. Carbonic anhydrase also plays a role in osteoclastogenesis. Osteoclasts express an mRNA for carbonic anhydrase [82–89]. Carbonic anhydrase is found at the ruffled border of the osteoclast. Presumably, its function is to provide the hydrogen ion required to produce the acid needed to break down bone. PTH stimulates carbonic anhydrase activity, thereby producing the acidity necessary for osteoclastic activity. It is possible that carbonic anhydrase production may play a reciprocal role with the production of OPG by the inner ear.

The molecular biology of bone in general and otosclerosis-like lesions in the experimental animal and in humans explains how patients may demonstrate radiographic evidence of otosclerosis and respond to bisphosphonate treatment without experiencing the typical symptoms associated with otosclerosis.

Clinical Observations of Metabolic Aspects of Inner-Ear Disorders

The evolution of my second treatment pathway had to do with the energy requirements of the inner ear. During the late 1960s and mid-1970s, information was developing about an effect that blood glucose and lipids had on the inner ear [90–104]. Other lines of thought included concepts of thyroid disease [105,106] and evidence of insulin disorders that could influence blood glucose levels and their effect on inner-ear function. In 1992, published criteria for considering abnormally elevated insulin levels on a glucose tolerance test were developed [102]. Patients presenting with clinical neurootological symptoms were evaluated for thyroid disease, lipid disorders, and blood glucose and insulin disorders with the use of a 5-hour glucose tolerance test with simultaneous insulin levels [107]. The most common abnormalities were seen on the glucose tolerance test. Though only a few patients exhibited evidence of diabetes or hypoglycemia, many of the remainder showed abnormal fluctuations in blood glucose levels; in rare cases, a flat curve in the blood glucose level was seen [103]. These glucose levels were sufficiently abnormal to adversely affect the function of an already impaired inner ear, but they did have a high probability of responding favorably to dietary management.

Coincident with these blood glucose findings were findings of hyperinsulinemia [108–110]. During this time, endocrinologists identified the insulin resistance syndrome, which was first called *syndrome X* and, later, *metabolic syndrome* [111]. By virtue of the highly metabolic nature of the inner ear, especially the hair cells, subtle changes in blood glucose or elevations in insulin could explain the genesis of some inner-ear symptoms. In addition, in patients so identified, the adversely functioning inner ear acts as the "canary in the coal mine" by identifying patients who are at risk of developing insulin resistance syndrome and its sequelae. Untreated, the insulin resistance syndrome can progress to such diseases as non-insulin-dependent (type 2) diabetes [112,113], hypertension [104], cardiovascular disease, and nonalcoholic fatty liver disease.

Recent literature has suggested a possible convergence of my two treatment pathways. Research in bone metabolism and regulation has demonstrated an overlap with insulin regulation. One has to do with a plasma cell membrane glycoprotein-1 (PC-1) [114,115] that is involved in bone metabolism and may also be involved in regulating the entry of insulin into cells [116–127]. Other findings suggest that bone in general and osteocalcin in particular may be potent insulin regulators [128–136].

Research has also demonstrated that insulin and blood glucose have an effect on inflammatory markers and OPG and a possible effect on otic capsule bone. Fetuin-A is a hepatic secretory protein that binds to the insulin receptor and inhibits insulin action. High levels are associated with insulin resistance and with incident diabetes independent of physical activity, inflammatory markers, and other common markers of insulin resistance [137–140]. Fetuin-A may play a role in hair cell function, and it is known to be an inhibitor of extraosseous calcification. In addition, a relationship between fetuin-A and OPG has been described [141,142]. These insulin and osseous effects may bring together a concept of the convergence of carbohydrates in the diet and their effect on blood glucose, insulin, and osteoclast function as evidenced by otosclerosis-like lesions. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist belongs to the nuclear receptor superfamily composed of ligand-activated transcription factors. It is highly expressed in adipose tissue and involved in insulin sensitization. It has also been shown to inhibit many inflammatory mediators, including TNF-α and interleukin-6. It has been shown to be an important regulator of airway inflammation and allergic rhinitis. Rosiglitazone is a PPAR-γ agonist used primarily to decrease insulin resistance in type 2 diabetes. Some animal experiments showed that it has a neuroprotective effect in experimental stroke [143]. Because of this effect on inflammatory mediators, rosiglitazone may inhibit the triggers of osteoclast production and could be considered as a replacement for corticosteroids in the treatment of autoimmune disorders of the inner ear.

Molecular Biology of Otosclerosis and Effects of Inflammatory Cytokines on the Inner Ear

Specimens obtained from patients who had previously undergone stapedectomy to treat the conductive hearing loss of otosclerosis showed that they clearly had otosclerosis. These specimens from stapedectomy procedures have shown the presence of TNF-α in addition to strong evidence of prior measles virus exposure [52]. Another study in experimental animals demonstrated that the use of a TNF-α-neutralizing agent could prevent the sensorineural hearing loss secondary to induced pneumococcal meningitis [48]. Other authors have reported the role of inflammatory cytokines, particularly TNF-α, in inner-ear inflammation [49,54,55]. The field of osteoclastogenesis informs us that one of the byproducts of osteoclast

production and the breakdown of bone is TNF-α [50, 57,144–146].

Vestibulocollic Headache and Vestibular "Migraine"

I became interested in a reflex that, in a number of patients, was initiated by the vestibular portion of the inner ear and resulted in muscular contraction primarily focused in the cervical area. Many of these patients had evidence of spasm in the muscles of the neck behind the mastoid, usually on the same side as the vestibular abnormality. Some of these patients also had headache, usually hemicranial. Some patients had only hemicranial headache and no vestibular symptoms, yet they showed signs of abnormal vestibular function [147].

Review of the pertinent literature related to migraine reveals that its fundamental mechanism is still not fully understood. It is possible that in a large number of migraineurs, migraine may be a manifestation of an abnormally functioning vestibular system that does not necessarily cause vestibular symptoms. It is only by evaluating the vestibular system in these headache patients that an abnormality could be identified.

RECOMMENDATIONS FOR FUTURE RESEARCH

I make the following recommendations for research, which are based on my many years of clinical observations and my review of the basic science and other clinical literature relevant to these findings.

Otosclerosis

We should continue to investigate the effects of the bisphosphonates on OPG knockout mice. If we can identify a genetic line of experimental animal that develops progressive sensorineural hearing loss, we can measure the levels of OPG over time to determine whether the inner ear produces less OPG and whether this could be a factor in progressive sensorineural hearing loss that, in the past, was attributed to aging or another process.

We should also experiment with mouse monoclonal antibody to OPG. By selectively blocking or reducing the production of OPG, this antibody could be used experimentally to determine whether hearing loss or histopathological evidence of otosclerosis-like lesions can be induced by progressive alterations in this antibody.

We need to develop imaging guidelines for radiologists to use in the early diagnosis of otosclerosis-like lesions. In addition to measuring otoacoustic emissions in OPG knockout mice and performing postmortem histopathology of hair cells (including mitochondria and prestin), clinicians should obtain computed tomographic scans of the temporal bones. Studies of OPG knockout mice have revealed that they have histopathological lesions similar to those seen in human otosclerosis. This finding suggests that otosclerosis-like lesions may be triggered in humans by something other than the measles virus [51,58,148,149].

Practitioners and researchers should investigate the effect of PTH on the otic capsule to determine whether it can be used as a treatment for otic capsule disorders or plays any role in otic capsule disorders. Likewise, otoconia should be investigated in relation to OPG, vitamin D, and PTH. Investigations into vitamin D deficiency or insufficiency may help us determine whether this vitamin has any effect on the otic capsule in terms of osteoclastogenesis, inner-ear OPG, or hyperparathyroidism.

By inducing a vitamin D deficiency or hyperparathyroid state in experimental animals, we may be able to determine whether superior semicircular canal dehiscence will develop. If so, we can investigate whether the dehiscence will close once vitamin D or PTH levels (or both) are restored to normal.

Finally, we should investigate the effect of acetazolamide alone, with PTH, and with bisphosphonates on otoacoustic emissions in wild mice and then in OPG knockout mice.

METABOLIC ABNORMALITIES OF THE INNER EAR

The viscosity of endolymph and perilymph warrants investigation. Knowledge of the viscosity can lead to further understanding of the tuning of the inner ear to sound. In addition, it is likely that the viscosity of the endolymph is such that otoconia may not freely move in endolymph with a change in the position of the head, which would contradict the current explanation for benign paroxysmal positional vertigo and nystagmus.

The production of endolymph also warrants study. Is there a feedback mechanism between the hair cells (particularly with regard to prestin) and the secretory mechanism of endolymph that creates the proper pressure medium for the most efficient hair cell function? If inefficient functioning reduces the rigidity of hair cells, is there a reflex stimulus to secrete more endolymph to overcome the necessary pressure? Would this explain the increase in the endolymphatic volume in Ménière's syndrome without an alteration in the sodium and potassium components of the endolymph?

Investigators should produce hypoglycemia in experimental animals and test for otoacoustic emissions, for changes in the endocochlear potential, and for postmortem changes in the mitochondria of the hair cells and supporting cells. They might also evaluate and compare

otoacoustic emissions and mitochondria size in the hair cells of Zucker rats and wild rats. Zucker rats produce type 2 diabetes, and they have been found to undergo changes in the mitochondria of skeletal muscle. Possibly the effects of diabetes on the inner ear may, in turn, have an effect on the mitochondria of the hair cells.

Researchers should induce blood glucose abnormalities in OPG knockout mice and measure any additional effect they may have on mitochondria and prestin function. A baseline investigation of the mitochondria before the blood glucose abnormalities are induced could also show a possible effect of TNF-α on the hair cells. Research projects should be integrated to include investigations of PC-1, fetuin-A, and osteocalcin, as there is a convergence of both metabolic and osteoclast functions. In turn, these findings should be integrated with what we know about mitochondria, hair cell actin, endocochlear potential, endolymph viscosity, and actin (prestin) of hair cells and the role of TNF-α in inner-ear function.

SUMMARY

It is my enthusiastic hope that the information contained herein will spark a team or teams of scientists to further investigate the observations as yet unexplained. Finding expertise in basic science investigation in one laboratory appears unlikely. Regenerating hair cell function through the use of clinical management algorithms built on a basic research foundation could lead to the development of effective treatments for a variety of inner-ear disorders. Effective treatment of inner-ear disorders will likely stimulate more questions that can be answered only through basic science investigations.

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