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Clarisa Peer, Editor

ALZET special delivery

Chronic Infusion of Rotenone Results in New Model of Parkinson's

by Clarisa Peer

Neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's affect millions of Americans. Parkinson's disease (PD) is one of the most common, affecting about 1% of people over the age of 65 with its characteristic rigidity, bradykinesia and tremors. Critical to studying these slowly progressing diseases is the availability of accurate animal models in which to study disease mechanisms and potential therapies.

The established models of Parkinson's disease have successfully reproduced the loss of dopamine-producing neurons in the substantia nigra, which is a hallmark of the disease. The MPTP primate model is typical. When injected systemically, the neurotoxin MPTP is metabolized into the neurotoxin MPP^+ which is selectively taken up by dopaminergic neurons. MPP^+ inhibits mitochondrial respiration at the level of complex I of the electron transport chain, thereby causing extensive cell death in areas with high concentrations of dopamine-producing neurons such as the substantia nigra.[1] This effect is prominent in primates but not rodents.

The primary murine model of PD involves a slow intrastriatal injection of 6-hydroxydopamine (6-OHDA), resulting in the delayed and progressive loss of dopaminergic terminals and neurons and striatal dopamine.[2] This method provides a relevant model for studying the neuropathology of PD, despite its limited ability to predict efficacy of a potential therapy in either MPTP-lesioned primates or in patients.[3]

While useful for their ability to reproduce the nigral damage associated with PD, none of these animal models causes accumulation of the cytoplasmic inclusions called Lewy bodies, which are a histopathological hallmark of the disease. Betarbet *et al.* at Emory University showed that systemically administered rotenone, a common, organic garden pesticide, produces a more complete model of PD with both the progressive and selective loss of dopaminergic neurons (see Figure 1) and presence of cytoplasmic inclusions quite like Lewy bodies.[4]

Initial work to investigate rotenone's pattern of central nervous system damage was done by Ferrante *et al.*, who used ALZET pumps to deliver the compound intravenously to rats over a 7-9 day period in high doses.[5] After this period of exposure, Ferrante *et al.* observed nigral sparing, despite lesions throughout the striatum and globus pallidus. Higher doses were associated with increased systemic side effects.[4] A dose- and time-ranging study by Greenamyre *et al.* illustrated the specific time course of damage and its progression throughout the CNS.[1]

INSIDE

alzet® OSMOTIC PUMPS

Using ALZET pumps to infuse rotenone intravenously, Greenamyre *et al.* showed that the neurotoxin produced lesions initially in the central striatum, the extent and timing of which were dose-dependent. Higher doses produced earlier lesions. Greater striatal damage was associated with retrograde loss of neurons in the substantia nigra.

A subsequent study at Emory University was conducted to refine the model by optimizing the dose.[4] The histopathology results demonstrated the unique value of this model: staining of nigral neurons in rats with rotenone-induced dopaminergic degeneration revealed the presence of cytoplasmic inclusions with biochemical and morphological similarities to the Lewy bodies characteristic of PD. For rotenone infusion, Betarbet *et al.* used both 1-week ALZET pumps (model 2ML1) and 4-week pumps (Model 2ML4), implanted subcutaneously and connected to tubing placed in the jugular vein. After 4 weeks, some animals had the pumps removed and replaced with fresh pumps to extend the treatment period. Controls received vehicle administration alone.

In a disease like PD, which progressively robs affected individuals of normal motor function, this animal model which more accurately represents the human pathology provides a promising research platform to support evaluation of potential neuroprotective therapies. This work is ongoing and includes a grant to Dr. Greenamyre from the National Institute of Neurological Disorders and Stroke to further develop the rotenone model, understand its specific mechanisms, and modify it for use in primates.[6]

ALZET pumps have also been used to either create or test many other models of neurodegenerative disease, including Alzheimer's, Huntington's and neurodegenerative dementia.[7,8,9] Continuous infusion of many short half-life agents under evaluation for their neuroprotective activity, including neurotrophic factors such as glial-derived neurotrophic factor and brain-derived neurotrophic factor, have also been made possible through the use of ALZET pumps to provide continuous administration. For more information on the use of ALZET pumps for systemic or intracerebral infusion, or in one of these animal models, please contact ALZET Technical Support.

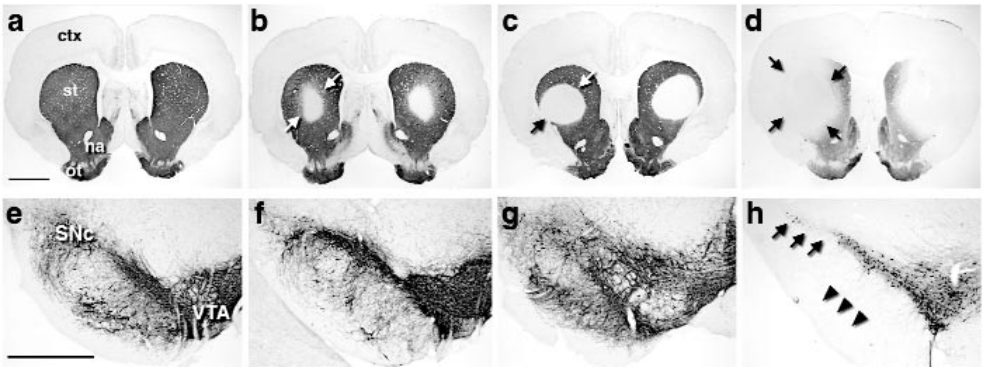


Figure 1: Rat coronal brain sections show nigrostriatal degeneration in rats following rotenone administration for 7 days (b, f), 36 days (c, g) or 33 days (d, h), but not in control animals infused with vehicle alone (a, e).[4] Rats were implanted with ALZET pumps attached to catheters placed intravenously. Tyrosine hydroxylase immunostaining is shown in the striatum (a–d) and substantia nigra (e–h). Progressive striatal damage (arrows) ranged from partial (b, c) to almost complete (d); the latter resulting in significant damage in the lateral portion (arrows) and ventral tier (arrowheads) of the substantia nigra pars compacta (SNc). ctx, cortex; st, striatum; na, nucleus accumbens; ot, olfactory tubercle. Reprinted with permission from Dr. Greenamyre and Nature Publishing Group.

1. *Greenamyre JT, MacKenzie G, Peng, T-I & Stephans SE. Biochem Soc Symp 1999;66:85-97*
2. *Kirik D, Georgievska B, Rosenblad C & Björklund A. Eur J Neurosci 2001;13:1589-1599.*
3. *Gulwadi AG, Korpinen CD, Mailman RB, Nichols DE, Sit S-Y & Taber MT. J Pharmacol Exper Ther 2001;296(2):338-344.*
4. *Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV & Greenamyre JT. Nature Neurosci 2000;3(12):1305-1306.*
5. *Ferrante TJ, Schulz JB, Kowall NW & Beal MF. Brain Res 1997;753:157-162.*
6. *Greenamyre JT; Fiscal Year 2001. Grant # 5P50NS038399-040001; National Institute of Neurological Disorders & Stroke.*
7. *Bartolomeo AC, Morris H & Boast CA. Neurobiol of Learning & Memory 1997;68:333-342.*
8. *Rieke GK. Exp. Neurol. 1989;104:147-154.*
9. *Misztal M, Frankiewicz T, Parsons CG & Danysz W. Eur J Pharmacol 1996;296:1-8.*
10. *Budavari S, O'Neil MJ, Smith A, Heckelman PE & Kinneary JF (Eds.) Merck Index 1996. Whitehouse Station: Merck & Co, Inc.*

Dissolving Rotenone for Chronic Infusion

The Merck Index describes rotenone as being practically insoluble in water.[10] This characteristic presents a significant challenge to researchers desiring to concentrate rotenone in solution for chronic administration. According to the Merck Index, rotenone is soluble in various organic solvents. Published studies on the use of ALZET pumps to administer rotenone have used a combination of dimethylsulfoxide (DMSO) and polyethylene glycol or DMSO and polyethylenimine.[4-5] Beyond the good results of these published studies, DURECT Corporation has limited information on the compatibility of these vehicle combinations with the ALZET pumps. When evaluating vehicle combinations which do not appear on the list of vehicles compatible with ALZET pumps available from DURECT Corporation, DURECT recommends that researchers consider testing the combination using an ALZAID® Chemical Compatibility Test Kit to be certain that the vehicle combination does not degrade the reservoir material inside the ALZET pump and interfere with accurate pumping. For more information on compatible vehicles or the ALZAID Test Kit, please contact ALZET Technical Support.

Advances in Gene Therapy Research for Inner Ear Disorders

by José R. Gadea

The feasibility of in vivo gene transfer to the inner ear as potential treatment for hearing disorders has been a subject of intense investigation in recent years. The goal of these studies is to express genes that may protect inner ear cells from trauma or enhance repair and regeneration following ototoxic insults. The inner ear, consisting of the cochlea and vestibular organs, has several features that make it an attractive target for gene transfer research. First, inoculation with gene vectors is technically feasible, and the enclosed anatomy of the inner ear limits the spread of these vectors to adjacent tissues. Second, the fluid-filled spaces allow for easy dissemination of vectors within the inner ear.[1] Finally, the cochlea contains several cell types that can be precisely quantified, simplifying assessment of gene expression in the target cells.[2]

Animal model of cochlear gene therapy

An animal model for the chronic infusion of drugs into the inner ear was first developed by Brown *et al.* and Davies *et al.*[3,4] The guinea pig was chosen for its relatively large, and therefore more surgically accessible, cochlea compared with mice and rats. ALZET Osmotic Pumps were selected to facilitate the slow, continuous delivery of agents directly into the cochlea. Brown *et al.* initially demonstrated the effectiveness of this animal model by depressing auditory nerve responses via tetrodotoxin infusion.[3] Davies *et al.* confirmed the validity of this model by inducing neuronal degeneration via neomycin infusion.[4] Recently, this chronic infusion model has proved to be an efficient way of introducing genetic vectors into the mammalian cochlea to study gene expression.

Gene vectors used in cochlear gene therapy research

A variety of viral and non-viral vectors are available to facilitate the transfer of genetic material into target cells. Each of these vectors possesses unique characteristics that make it useful for particular experimental applications (see Table 1).

Adeno-associated virus (AAV) is usually the vector of choice for gene transfer studies because of its non-pathogenicity, broad host range, and capacity to infect and integrate into dividing and non-dividing cells with high frequency. A series of studies by Lalwani *et al.*, at the University of California in San Francisco, demonstrated successful *in vivo* expression of foreign genes in the inner ear of guinea pigs following AAV vector infusion with ALZET Osmotic Pumps.[5,6,7,8] Steady-state, intracochlear infusion of AAV vectors containing reporter genes, such as ß-galactosidase (ß-gal) and recombinant human green fluorescent protein (rhGFP), resulted in transfection and gene expression in a variety of tissues within the cochlea.[5,6] Subsequent studies demonstrated the ability of AAV to transfer genes into the vestibular hair cells as well as the supporting cells within the vestibular neuroepithelia, confirming the ability of AAV to transfect a broad range of dividing and non-dividing cells.[8] Further research by Lalwani *et al.* uncovered the potential of AAV to establish long-term transgene expression within the cochlea, where the ß-gal gene product was detected for up to six months after initial gene vector infusion.[7]

(continued on page 7)

Gene Vector	Advantages	Limitations
Adeno-associated virus	• Nonpathogenic in humans and animals • Incapable of autologous replication without the aid of a helper virus • Broad host range • Capable of transducing dividing and non-dividing cells • Capable of stable integration and long-term gene expression	• Small capacity for foreign genes
Adenovirus	• High level of transgene expression • Capable of transducing dividing and non-dividing cells • Large capacity for foreign genes • Wide range of target tissues • Most do not cause serious disease • Can be engineered to become replication defective and less immunogenic	• Highly immunogenic • Capable only of transient integration; does not integrate into the genome
Lentivirus	• Capable of transducing dividing and non-dividing cells • Capable of stable integration and long-term gene expression	• Risk of generating replication-competent virus • Random integration of genes (might disrupt host genes)
Cationic Liposomes	• Do not cause disease (safe) • Non-immunogenic • Easy to prepare in large amounts • Capable of carrying genes of any size	• Lower transfection efficiency • Capable of transient gene expression • Lack tissue specificity

Table 1: Summary of advantages and limitations associated with various vectors used for gene delivery to the inner ear.

Very Special Delivery

A Method for Chronic Drug Delivery in Neonates

by José R. Gadea

Teratology studies aimed at investigating neonatal learning, memory, behavior or development present unique challenges to scientists because they require manipulation of young, very small animals during a critical developmental period. Authors of numerous rodent studies have concluded that environmental manipulations occurring early in life result in physiological and behavioral changes that persist into adulthood.[1,2,3] Some studies have shown that human handling of newborn rats for as little as 15-20 minutes daily during the first few weeks of life produces neuroendocrine, neurochemical and behavioral alterations in the adult.[2,4,5] Some methods of drug administration impose severe experimental conditions, such as repetitive handling and injections, which have more dramatic implications for neonatal development. Furthermore, such experimental artifacts are likely to confound and compromise research results.

Effects on Physical and Neurobehavioral Development

ALZET Osmotic Pumps have long been used as an alternative to repeated injections for chronic administration of experimental agents in unrestrained laboratory animals. These small, implantable pumps present an attractive alternative for use in neonates as well. Doucette *et al.*, at the University of Prince Edward Island, demonstrated the value of these miniature infusion pumps as a method for sustained drug delivery in neonatal rats.[6] These investigators used 8-day old Sprague-Dawley rat pups that were randomly assigned to one of three treatment groups: ALZET pump implantation, sham surgery, or no surgery. Saline filled ALZET pumps (Model 1003D) were aseptically implanted under the skin of rat pups under isofluorane anesthesia. The pups were allowed to recover from the anesthesia before being returned to their cages. The entire surgical procedure took an average of 10 minutes and was never longer than 20 minutes. Rats in the sham surgery control group received identical treatment except pump insertion. The rats not operated on were left undisturbed. Animals were evaluated at various times over a 72-day period using a standard battery of tests designed to measure physical and neurobehavioral development, such as weight gain, fur development, incisor eruption, startle, visual placing, and others. With the exception of transient decreases in weight gain during the first 24 to 48 hours following pump implantation, no significant differences were found in rats implanted with pumps compared to the control and sham treated rats on any of the parameters evaluated.

Doucette *et al.* attributed their experimental success, in part, to the careful use of good laboratory procedures to help minimize surgical stress. They emphasized the importance of following strict aseptic technique during the pump implantation in order to decrease the risk of infection. Additionally, they found the use of an inhaled anesthetic, isofluorane, which permits rapid induction and recovery, to be preferable to slower acting inhaled or injectable anesthetics. The authors concluded that the implantation of ALZET pumps, under carefully controlled surgical conditions, does not significantly affect neurobehavioral development in rat pups, thus they represent a viable alternative to repeated injections for sustained drug delivery.

Experimental Model of Neonatal Opioid Tolerance and Dependence

Many experimental models of opioid tolerance and dependence use repeated drug administration by bolus injection with a variety of dosing schedules. Such dosing schedules lead to wide fluctuations of opioid concentrations in the central nervous system that may affect the development of tolerance. Additionally, the added stress from repeated handling and injections could affect the development of tolerance as well. Investigators at the Medical College of Virginia have used ALZET pumps successfully to establish an animal model of neonatal opioid tolerance and physical dependence. Using this experimental model, Thornton *et al.* have characterized tolerance and dependence to fentanyl and morphine in neonatal rats.[7,8] Their studies indicate that continuous subcutaneous delivery using ALZET pumps is particularly useful since it closely mimics the intravenous route by which opioids are continuously administered to human neonates.[7] Another key benefit of the ALZET pumps in these experiments was their ability to maintain stable plasma and

tissue opioid levels, thus reducing toxicity associated with widely fluctuating plasma levels typical with conventional dosing methods. Furthermore, the pumps provided a means of chronic opioid delivery that minimized neonatal handling and the stress commonly seen with repeated injections.

Since these initial studies were published in 1997, the Virginia research group has produced a number of publications describing further research on the long-term consequences of opioid tolerance and dependence established during the neonatal stage.[9,10,11,12] Thornton *et al.* have now incorporated ALZET pumps as their standard method for chronic opioid administration in neonatal rats. To obtain a complete list of references on the use of ALZET pumps in neonates, a package of information on surgical techniques for neonates, or additional information about ALZET Osmotic Pumps please contact ALZET technical services.

1 Meany MJ, Aitken DH, van Berkel C, Bhatnagar S & Sapolsky RM. Science 1988;239(4841):766-768.

2 Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ & Sarrieau A. Psychoneuroendocrinol 1991;16(1-3):85-103.

3 Smythe JW, McCormick CM, Rochford J & Meaney MJ. Physiol Behav 1994;55(5):971-974.

4 Sapolsky RM. Science 1997;277:1620-1621.

5 Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM & Meaney MJ. Science 1997;277:1659-1662.

6 Doucette TA, Ryan CL & Tasker RA. Physiol Behav 2000;71:207-212.

7 Thornton SR & Smith FL. J Pharmacol Exp Ther 1997;281:514-521.

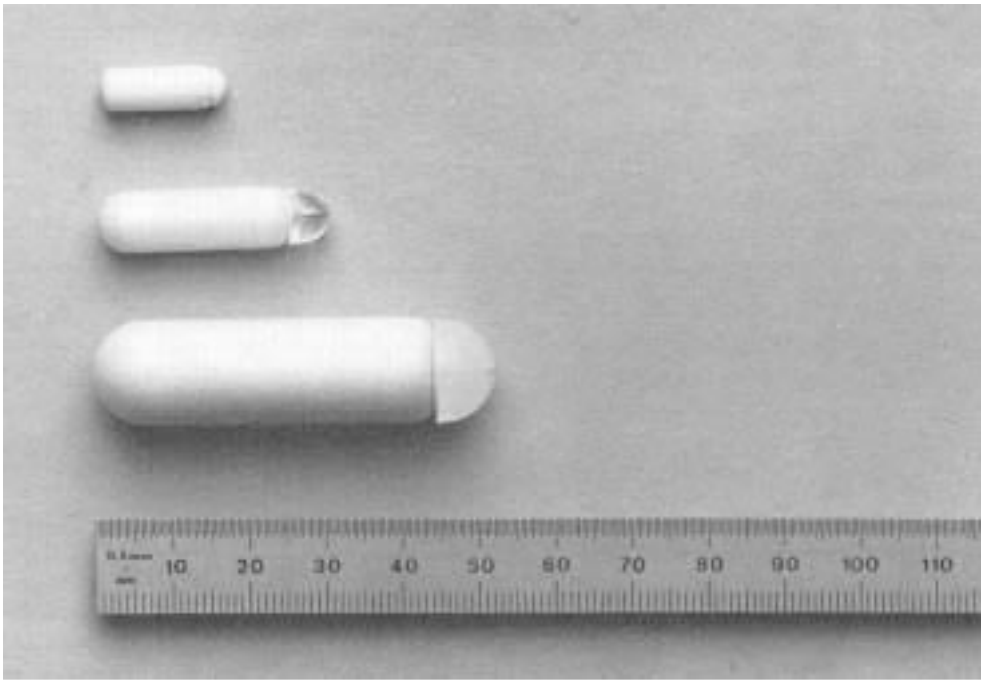
8 Thornton SR, Wang AF & Smith FL. Eur J Pharmacol 1997;340:161-167.

9 Thornton SR & Smith FL. Eur J Pharmacol 1998;363:113-119.

10 Choe CH & Smith FL. Pediatr Res 2000; 47(6):727-735.

11 Thornton SR, Lohmann AB, Nicholson RA, & Smith FL. Pharmacol Biochem Behav 2000;65(3):563-570.

12 Lohmann AB & Smith FL. Pediat Res 2001;49(1):50-55.



ALZET Osmotic Pumps come in the three sizes shown above and in a variety of rates and durations. The smallest pump can be implanted subcutaneously in animals weighting at least 10 grams, including mice and very young rats.

Cyclodextrin Derivatives: Improving Delivery of Hydrophobic Compounds

by Amy Kerfoot

Beta-cyclodextrin derivatives have emerged as a useful tool for improving the stability and solubility problems of hydrophobic molecules. With steroid hormones, for example, as much as a 50-fold increase in solubility and bioavailability can be achieved through the use of ß-cyclodextrin derivatives (ß-CDs) as carrier molecules.[1] Given the space limitations inherent in an implantable drug delivery system, ß-CDs are a viable option for use in ALZET Osmotic Pumps.

Cyclodextrins are conjoined glucose molecules forming a "donut" structure, or torus. The non-polar interior of the torus encapsulates hydrophobic molecules to increase solubility in aqueous solutions. The morphology of cyclodextrins encourages complexation, yet aqueous dilution elicits complete dissociation.[2] ß-cyclodextrins occur naturally, but derivatives formed by substitution with hydroxyalkyl groups have reduced toxicity and optimized solvent action. One popular derivative is 2-hydroxypropyl-ß-cyclodextrin, which is sold as Trappsol® by CTD, Inc., in either a powder or a pre-mixed solution.[1,3]

Optimizing Delivery

A drug's ability to form an inclusion complex with ß-CDs depends upon its size, shape, and lipid partition co-efficient.[4] Even a polar molecule can be complexed if it contains non-polar side chains. An optimal cyclodextrin:drug ratio maximizes both dissolution and redistribution speed. Dr. Tony Yaksh *et al.* of the University of California at San Diego dissolved capsaicin, a lipid-soluble agent, in 20% 2-hydroxypropyl-ß-cyclodextrin. A "dramatic shift into the aqueous phase was observed," with the lipid partition coefficient of capsaicin, inherently less than 0.01, rising to 2.05 when complexed with cyclodextrin.[3]

Cyclodextrin derivatives are considered a relatively safe, benign vehicle. Natural cyclodextrins may form precipitable cholesterol complexes and should not be used parenterally. However, nephrotoxicity studies on the hydroxypropyl-ß derivative have shown it to be well tolerated.[2] No production of unusual metabolites has been observed under normal physiological conditions.

Value in CNS Studies

The experimental utility of ß-CDs is evident when delivering agents to the central nervous system (CNS). ALZET pumps are frequently used for CNS delivery of agents that do not cross the blood-brain barrier in systemic delivery. Intrinsically, this delivery route demands a stable, bioavailable solution with minimal volume and no vehicle effect. For these reasons, Dr. Tony Yaksh and his group highlighted the superior ability of ß-CDs for CNS administration. Their results demonstrated normal motor function, nociception, EEG and general behavior in rats injected intrathecally with 20% hydroxypropyl-ß-cyclodextrin.[4] Unlike DMSO, the ß-CD allowed the test agent to exert full biological effect without reducing the endogenous peptides being studied. Yaksh *et al.* concluded that cyclodextrin "represents a benign vehicle for solubilizing relatively insoluble materials and retains these agents in a bioavailable form after intracerebral and intrathecal administration."[4]

Versatility for Agent Delivery

Cyclodextrins have been used in ALZET pumps to effectively deliver steroid hormones and other lipophilic molecules in a stable, bioactive form. Complexation "protects the guest molecule from loss by evaporation, from attack by oxygen, visible and uv light, and from intra- or intermolecular reactions."[5] Zhu *et al.* supplemented testosterone in hypogonadal rats using 45% 2-hydroxypropyl-ß-CD for up to eight weeks.[6] Additionally, Shrimpton *et al.* infused a cortisol:cyclodextrin complex to salmon in a receptor down-regulation study.[7] Interestingly, only the continuous administration method was capable of reducing the number and affinity of corticosteroid receptors without inducing stress. Lastly, Backensfeld *et al.* administered indomethacin, a non-steroidal anti-inflammatory agent with a water saturation solubility of 0.4 mg/ml that undergoes hydrolysis to form various acids.[8] The ß-CD vehicle augmented solubility by 175-875% depending on the pH and protected the indomethacin from chemical degradation. This finding, corroborated by NMR proton shift and HPLC data, led Backensfeld to conclude that, "ß-CDs stabilize the indomethacin considerably better [than other vehicles]."[8]

Cyclodextrins are fully compatible with ALZET pumps and are an appropriate option for some researchers trying to dissolve an agent for continuous infusion. Additional technical information can be found at the CTD, Inc. website: www.cyclodex.com. Information on additional solvents appropriate for use in ALZET pumps is available from ALZET Technical Support.

1. Pitha, J. Neurotransmissions 1989:5(1):1-4. Research Biochemicals Inc., Natick, MA.

2. Pitha, J. J Control Release 1987;6:309-313.

3. www.cyclodex.com CTD, Inc. corporate website.

4. Yaksh TL, Jang J, Nishiuchi Y, Braun KP, Ro S & Goodman M. Life Sci 1991;48(7):623-633.

5. Pagington JS. Chemistry in Britain 1987;23:455-458.

6. Zhu L-J, Hardy MP, Inigo IV, Huhtaniemi I, Bardin CW & Moo-Young AJ. Biol Reprod 2000;63(2):368-376.

7. Shrimpton JM & Randall DJ. Am J Physiol 1994;267(36):R432-R438.

8. Backensfeld T, Muller BW, Wiese M & Seydel JK. Pharm Res 1990;7(5):484-490.

(Continued from page 3)

The use of adenovirus as a gene delivery vector is usually hampered by safety concerns due to its immunogenicity, which can destroy recipient cells and limit gene expression. To overcome this limitation, adenoviral vectors are engineered to be both less immunogenic and replication defective by removing specific gene regions from the viral genome.[10] In a recent study, Luebke *et al*. reported the use of ALZET Osmotic Pumps for the continuous infusion of a replication-defective adenovirus vector carrying the ß-gal gene sequence to assess gene expression in the inner ear. The modified adenovirus vector proved to be effective at introducing the reporter gene into cochlear hair cells, with reduced toxicity, minimal inflammatory response and preservation of cochlear function.[10]

The utility of lentivirus as an intracochlear gene delivery vector has also been explored.[11] The lentivirus vector allows researchers to transduce both dividing and non-dividing cells. Furthermore, its larger size compared to AAV allows it to accommodate larger or multiple gene sequences. Lentivirus-mediated rhGFP gene expression was found to be limited to the periphery of the perilymphatic space, suggesting limited dissemination of the viral vector. This restricted transduction capacity, coupled with its potential for establishing long-term gene expression, makes lentivirus an ideal vector for delivering therapeutic genes into cell types lining the perilymphatic space. However, a potential drawback of using lentivirus in gene therapy research is the risk of generating replication-competent virus that may compromise the health of the recipient host.

A safer alternative to viral vectors is the use of non-viral vectors, such as cationic liposomes. Although non-immunogenic and easy to prepare on a large scale, cationic liposomes have low transfection efficiency and lack tissue specificity. Nevertheless, Wareing *et al.* demonstrated the feasibility of using cationic liposomes to introduce the ß-gal gene into the mammalian cochlea. Gene expression was detected for up to two weeks after infusion, and a range of cell types within the cochlea were shown to contain the gene product.[12]

Role of ALZET pumps

In conjunction, these studies illustrate the feasibility of introducing and expressing foreign genes in the peripheral auditory system of guinea pigs using a variety of gene vectors. The ALZET pumps have played an important role in the success of these studies, allowing localized delivery of gene vectors directly into the cochlea while eliminating most side effects commonly associated with systemic administration. Additionally, maintaining a slow, continuous infusion of vector over time minimizes local tissue trauma and preserves cochlear fluid homeostasis when compared to other microinfusion techniques. Furthermore, a slow infusion increases the contact time between the virus and the cell, thus enhancing transduction efficiency.[10]

Improvements to the model

This model provides an excellent means for introducing genes and other therapeutic agents into the inner ear, however, Lalwani *et al.* reported technical difficulties resulting from the surgery. Analysis of the cochlea from infused animals revealed a relatively intact cochlear cyto-architecture in most animals, with the exception of surgical trauma limited to the site of catheter insertion.[7,8,12] Hearing tests demonstrated loss at the mid- and high frequencies, which was attributed to the trauma from the cochleostomy and catheter insertion.[13] Interestingly, other research groups performing related procedures have not reported similar complications. In fact, Leubke *et al.* demonstrated preservation of cochlear function following pump implantation and infusion of substances into the cochlea.[10] Similarly, Yamasoba *et al.* showed that osmotic pump implantation did not influence or contribute to the noise-induced cochlear damage reported in their study.[14] Furthermore, it may be possible to avoid surgical trauma in the inner ear by using less invasive ear catheters, such as the specialized IntraEAR® catheters which have been used to deliver a variety of agents (i.e., gentamicin and leupeptin) across the round window membrane. For more information about ALZET Osmotic Pumps, please contact ALZET Technical Services. Additional information about IntraEAR catheters can be found at www.intraear.com.

1. Yamasoba T, Yagi M, Roessler BJ, Miller JM & Raphael Y. Hum Gene Ther 1999;10:769-774.

2. Weiss MA, Frisancho JC, Roessler BJ & Raphael Y. Int J Devl Neuroscience 1997;15(4/5):577-583.

3. Brown JN, Miller JM, Altschuler RA & Nuttall AL. Hear Res 1993;70:167-172.

4. Davies E, Gladstone HB, Williams H, Hradek G, Shah SB & Schindler RA. Am J Otol 1994;15(6):757-761.

5. Lalwani AK, Walsh BJ, Reilly PG, Muzyczka N & Mhatre AN. Gene Ther 1996;3:588-592.

6. Lalwani AK, Han JJ, Walsh Bj, Zolotukhin S, Muzyczka N & Mhatre AN. Hear Res 1997;114:139-147.

7. Lalwani AK, Walsh BJ, Reilly PG, Carvalho GJ, Zolotukhin S, Muzyczka N & Mhatre AN. Gene Ther 1998;5:277-281.

8. Lalwani AK, Walsh BJ, Carvalho GJ, Muzyczka N & Mhatre AN. Am J Otol 1998;19(3):390-395.

9. Jolly D. Cancer Gene Ther 1994;1(1):51-64

10. Luebke AE, Steiger JD, Hodges BL & Amalfitano A. Gene Ther 2001;8:789-794.

11. Han JJ, Mhatre AN, Wareing M, Pettis R, Gao WQ, Zufferey R, Trono D & Lalwani AK. Hum Gene Ther 1999;10:1867-1873.

12. Wareing M, Mhatre AN, Pettis R. Han JJ, Haut T, Pfister HFP, Hong K, Zheng WW & Lalwani AK. Hear Res 1999;128:61-69.

13. Carvalho GJ & Lalwani AK. Am J Otol 1999;20:87-90.

14. Yamasoba T & Dolan DF. Hear Res 1998;120:143-151.

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ALZET Technical Information

For technical information about ALZET Osmotic Pumps, or for a complimentary custom search of our extensive bibliography, please return the attached business reply card or contact us:

1-800-692-2990
408-367-4036
Fax: 408-865-1406
E-mail: alzet@durect.com

New Agents in the ALZET Literature

For over 25 years, the ALZET Osmotic Pump bibliography has been a valuable resource for scientists seeking to improve *in vivo* delivery of therapeutic agents. We update our database periodically with the latest citations referencing the successful use of ALZET pumps in research studies. Our database now comprises more than 6600 references. The following table lists some of the new agents recently added.

Agent	Therapeutic Category
Adenovirus	Gene Therapy Vector
Angiostatin	Antiangiogenic Agent
Azaline B	LHRH Antagonist
Betamethasone	Glucocorticoid
BMS-191563	RAS Farnesyltransferase Inhibitor
BQ-485	Endothelin Receptor Antagonist
Cart (42-89)	Hypothalamic Neuropeptide
Chlorgyline hydrochloride	Monoamine Oxidase Inhibitor
Cicaprost	Prostacyclin Analog
CP–154,526	Corticotropin-Releasing Hormone Receptor Antagonist
Dimethylthiourea (DMTU)	Antioxidant
Dinapsoline	D_1 Dopamine Receptor Agonist
DWH–146e	Selective A_{2A} Adenosine Receptor Agonist
Eph B2, Ephrin–B1-2	Receptor Tyrosine Kinases
Exendin	GLP-1 Receptor Antagonist
FPT III	RAS Farnesyl Transferase Inhibitor
Ghrelin	Novel Growth Hormone-Releasing Peptide
Huperzine	Acetylchlolinesterase Inhibitor
Irbesartan	Antihypertensive; Angiotensin II Receptor Blocker
L–744,832	RAS Farnesyltransferase Inhibitor
L–754,142	Endothelin A and B Receptor Antagonist
LU 135252	Endothelin Receptor Antagonist
S-Methylisothiourea	Nitric Oxide Synthase Inhibitor
Moclobemide hydrochloride	Antidepressant; Monoamine Oxidase Inhibitor
MPQX	Glutamate AMPA Receptor Antagonist
MRZ 2/579	NMDA-Receptor Antagonist
Neural endopeptidase	Cell Surface Enzyme (degrades Substance P)
Olvanil	Anti-Inflammatory Agent
PD-98059	MAP Kinase (MEK) Inhibitor
PR39	Inflammatory Peptide; Antibacterial
3,4–Diaminopyridine (3,4-DAP)	Potassium Channel Blocker
Reboxetine	Antidepressant
RO 20-1724	Phosphodiesterase Inhibitor
RO 43-0440	5-HT_{2C} Antagonist
RO 60-0175	5-HT_{2C} Agonist
Rotenone	Pesticide
Saporin	Potent Ribosome–Inactivating Protein
SB 239063	Protein Kinase Inhibitor
SR 140333	NK1R Antagonist
Thimerisol	Antibacterial
TrkA-IgG	Immunologic; Nerve Growth Factor Inhibitor
Urocortin	Neuropeptide Hormone
Xanomeline	Muscarinic M_1 Agonist
ZM-241385	Adenosine A_{2A} Receptor Antagonist
zVAD-fmk	Caspase Inhibitor

References on these and other agents are available to you as a complementary service. Contact the ALZET Technical Services Department at (800) 692-2990, or at alzet@durect.com to request a free bibliography search customized to your area of interest.

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