Volume 20, No. 1
June, 1998

ALZA Corporation
Clarisa Peer, Editor

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special delivery

Leukemia Inhibitory Factor— *a cytokine for times of adversity?*

by Maxine Warren

Since its identification in the early 1980s, leukemia inhibitory factor (LIF) has been demonstrated to interact with numerous cell types and to elicit diverse biological responses. Rapid increases in plasma or tissue LIF concentration following a physical insult, or chemical challenge to the body, have led researchers to question whether the various actions of LIF are beneficial or detrimental in nature. Unfortunately, the very short half-life exhibited by LIF *in vivo* has complicated assessment of its biological effects in animals. Recent studies have overcome this difficulty by utilizing the ALZET® Osmotic Pump for the constant, controlled delivery of LIF, both systemically and directly into target tissues.[1,2] The ability to maintain effective levels of this cytokine has greatly facilitated evaluation of its actions, and such studies have indicated they are predominantly favorable in nature.

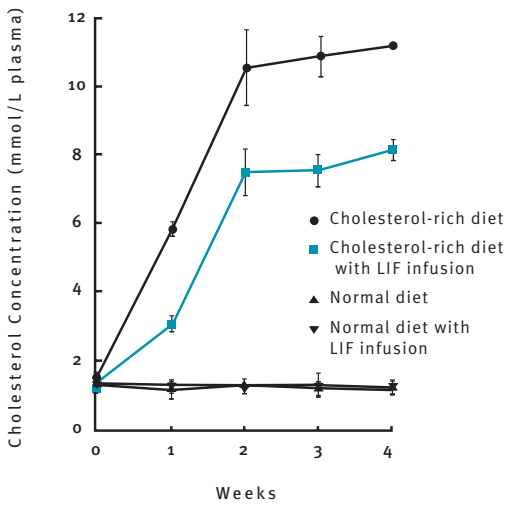


Figure 1. Continuous administration of LIF by ALZET pump over four weeks resulted in a reduced elevation in plasma cholesterol levels of rabbits fed a cholesterol-rich diet.
(Reproduced with permission from Moran CS *et al. Arterioscler Thromb Vasc Biol* 1997; 17:1267-1273.)

Muscle Regeneration

The stimulation of myoblast proliferation induced by LIF in cell culture led researchers to examine whether it conferred any beneficial effect on muscle regeneration *in vivo*. Barnard *et al.*, of Monash University, Australia, observed that LIF mRNA, undetectable in healthy skeletal muscle, rapidly appeared in increasing amounts following muscle injury.[1] The significance of this finding was further investigated by administration of exogenous LIF, or phosphate buffered saline (PBS) as a control, directly into the vastus lateralis muscle at the site of an inflicted crush trauma. Delivery was accomplished by connecting a subcutaneously implanted ALZET pump, Model 2001, to a polyethylene catheter, the tapered end of which was inserted into the injured region of the muscle. After 24 hours of LIF infusion, the diameter of regenerating fibers increased by 27% versus PBS controls ($p < 0.005$), leading *(continued on page 6)*

INSIDE

alzet® OSMOTIC PUMPS

Urokinase Plasminogen Activator –

a target for preventing the spread of cancer

by Maxine Warren

A crucial component in the local invasion of tissues by growing cancers is their ability to present a proteolytic interface to surrounding tissue matrices, which can be achieved through proteolytic enzymes such as urokinase plasminogen activator (uPA). This serine protease binds to receptors (uPAR) in the plasma membrane, then interacts with serum plasminogen to generate plasmin, which activates collagenases and metalloproteases, and stimulates production of uPA in a cyclic cascade reaction (Figure 2). The presence of plasmin at the cell surface provides the proteolytic interface essential for tumor invasion and metastasis.

As the uPA/uPAR system has been implicated in the progression of many malignancies, it presents a potential therapeutic target for controlling or preventing the spread of cancer cells. Antiproteolytic agents, specifically targeted at the plasmin proteolytic cascade, are currently being assessed for their antiproliferative and antimetastatic effects in animals. ALZET pumps have proved valuable in such investigations, including those presented here, enabling researchers to maintain effective concentrations of test agents, thereby facilitating evaluation of their anti-invasive capability.

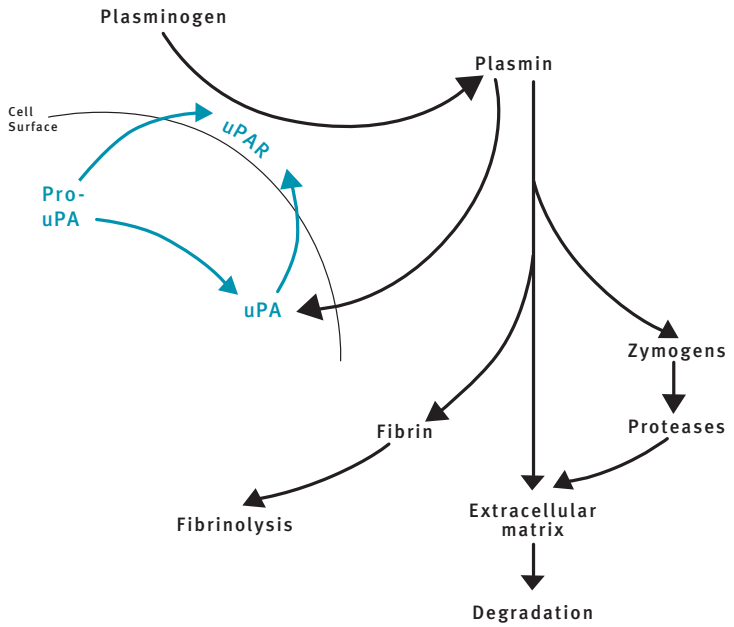


Figure 2. Inhibitors of the plasmin proteolytic cascade, delivered by ALZET pumps, interfered with the uPA/uPAR interaction. The resultant decrease in plasmin formation and the proteolytic interface of cancer cells significantly reduced local tissue invasion and the development of metastases. (Reproduced with permission from Evans DM and Lin PL. *The American Surgeon* 1995; 61:692-697.)

uPA Inhibitors

A study conducted by Evans and Lin, of Akron Medical Center, Ohio, examined the ability of the uPA inhibitor PAI-2 to prevent the metastasis of breast cancer cells into the lung.[1] Rats received an IV bolus of MatB rat mammary cancer cells, followed by an infusion of PAI-2 or saline, via an ALZET pump attached to an intravenous catheter, for 7 days. The control group, and eight of the ten receiving PAI-2, exhibited lung metastases, however, there were significantly fewer metastases in animals receiving the uPA inhibitor ($p < 0.033$).

In a study conducted by Xing *et al.*, of Royal Victoria Hospital, Montreal, MatB-III cells, or MatB-III cells engineered to overexpress uPA receptors (MatB-III-uPAR cells), were injected into the mammary fat pads of rats.[2] Animals received the urokinase inhibitor B-428, or vehicle, via intraperitoneally implanted ALZET pumps for up to two weeks, with or without daily injections of tamoxifen (TAM), and were subsequently assessed for tumor volume and the development of macroscopic metastases. Tumor volume decreased in all rats treated with either B-428 or TAM compared to untreated controls. Rats inoculated with the modified MatB-III-uPAR cells and infused with B-428 demonstrated the greatest reduction in tumor volume. The magnitude of the effect elicited by TAM was comparable in animals inoculated with either MatB-III or MatB-III-uPAR cells, and similar to that produced by B-428 following MatB-III cell injection. Combination therapy had a synergistic effect, and was more pronounced in those animals inoculated with the MatB-III-uPAR cells. TAM exerted no effect upon the development

Effective Administration of Leptin

by Laura Whitman

Recent studies have identified an important role for leptin in regulating food intake and body weight. Administration of leptin to animals can suppress appetite and increase energy expenditure as a means of maintaining body weight and adiposity at specific levels. Leptin has a short half-life unrelated to adiposity, with estimates ranging from 9 minutes in rats,[1] 36 minutes in mice[2] to 25 minutes in humans;[3] mainly due to high renal clearance. Providing leptin at optimum dosage levels and for extended periods of time increases agent activity; as Wade *et al.* commented, "the fact that leptin is so much more effective when infused continuously than when injected once every 12 hours is probably due to the rapid clearance of injected leptin."[4] ALZET pumps are designed to deliver short half-life compounds, and have been used to deliver leptin continuously to a variety of target sites for durations of 3 days to 3 weeks.

In each of the following studies, ALZET pumps provided a means to overcome the delivery difficulties associated with recombinant protein research. Harris *et al.* infused recombinant human leptin intraperitoneally in mice for 7 days via ALZET pumps to elucidate the physiological effects of leptin at various doses. These authors used continuous infusion because "one or two daily injections...cause excessive, intermittent elevations of the serum leptin concentration."[5] Dawson *et al.* used ALZET pumps to administer leptin to rats inflicted with damage to the arcuate nucleus of the hypothalamus (ANH).[6] These animals did not demonstrate the expected leptin-induced weight loss and decrease in fat depots seen in non-damaged animals, suggesting that certain physiological actions of leptin related to fat mobilization are mediated through receptors in the ANH. Boyer *et al.* examined the effects of recombinant murine leptin infused continuously into posthibernation arctic ground squirrels via subcutaneously implanted pumps.[7] Continuous leptin administration reduced food intake and prevented posthibernation weight gain without altering energy expenditure.

ALZET pumps offer researchers a distinct advantage in overcoming the difficulties associated with the administration of short half-life agents. A complete listing of leptin references is available on request (see page 7).

1 Zeng J, Patterson BW, Klein S, Martin DR, Dagogo-Jack S, Kohrt WM, Miller SB and Landt M. *Am J Physiol* 1997;273(6 Pt 1):E1102-E1106.

2 Harris RB, Zhou J, Weigle DS and Kuijper JL. *Am J Physiol* 1997;272(6 Pt 2):R1800-R1808.

3 Klein S, Coppack SW, Mohamed-Ali V and Landt M. *Diabetes* 1996;45(7):984-987.

4 Wade GN, Lempicki RL, Panicker AK, Frisbee RM and Blaustein JD. *Am J Physiol* 1997;272 (4 Pt 2):R1354-R1358.

5 Harris RBS, Zhou J, Redmann SM, Smagin GN, Smith SR, Rodgers E and Zachwieja JJ. *Endocrinology* 1998;139(1):8-19.

6 Dawson R, Pelleymounter MA, Millard WJ, Liu S and Eppler B. *Am J Physiol* 1997;273(36): E202-E206.

7 Boyer BB, Ormseth OA, Buck L, Nicolson M, Pelleymounter MA and Barnes BM. *Comp Biochem Physiol* 1997;118C(3):405-412.

of macroscopic tumor metastases, whereas, B-428 proved effective in reducing metastasis for both inoculation cell types. The more pronounced effectiveness of B-428 against cancer cells overexpressing uPAR supports mediation of its actions through the down-regulation of the proteolytic cascade.

These studies confirm that inhibition of the proteolytic cascade and the associated decrease in growth factor levels and proteolytic interface, provide a means of inhibiting local tissue invasion and metastasis. The development of such compounds, to be used alone or in combination with other agents, provides another possibility in the search for novel anticancer treatments.

Role of the ALZET Pump

Wide fluctuations in plasma levels, often associated with conventional dosing regimens, can result in cytotoxic or ineffective drug concentrations, complicating evaluation of potential anticancer compounds. The steady-state conditions achieved using ALZET pumps have led to their use in these, and many other studies, investigating the efficacy and mechanistic actions of a diverse range of anticancer agents.

If you would like more information on pump applications in this or other areas, please contact us as detailed on page 7.

1 Evans DM and Lin PL. *The American Surgeon* 1995;61:692-697.

2 Xing RH, Mazar A, Henkin J and Rabbani SA. *Cancer Res* 1997;57:3585-3593.

Animal Models of Pediatric Hearing Loss

by Clarisa Peer

Despite improvements in antimicrobial treatment regimens, many children suffer hearing loss due to either aminoglycoside-induced ototoxicity or chronic otitis media with effusion. Two recent studies describe the use of ALZET pumps in animal models designed to facilitate study of the mechanisms of, and potential treatments for, these very different causes of hearing deficit. These studies were enhanced by the ability of the ALZET pump to maintain a continuous level of drug in tissues either by constant systemic administration, or by using a catheter to perfuse the target site directly.

Targeted Delivery to the Middle Ear

About 5–10% of cases of acute otitis media, one of the most common pediatric infections, advance to chronic otitis media with effusion (OME). Contributing to OME is the persistence in the middle ear of bacterial cell wall components, such as lipopolysaccharide (LPS), and the ensuing inflammatory response. Effusions with more viscous fluid have a higher mucin content and are linked to increased auditory impairment, although the processes controlling mucin secretion are unclear. In what may be the first published study using ALZET pumps to infuse substances into the middle ear, Rose *et al.*, at the University of North Carolina, theorized a role in OME for nitric oxide, which has been implicated as a regulator of mucin production.[1]

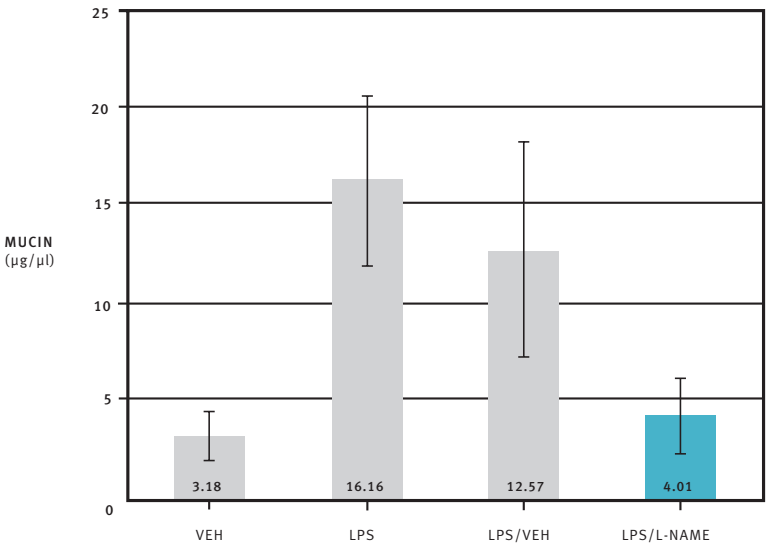


Figure 3. Mean mucin concentration in middle ear fluid was measured after 7 days. Rats underwent eustachian tube blockade and then instillation of either vehicle (VEH) or lipopolysaccharide (LPS). Experimental animals also received middle ear infusion of L-NAME (LPS/L-NAME) via catheter from a subcutaneously implanted ALZET pump. L-NAME infusion significantly ($p < 0.05$) decreased mucin content of middle ear effusions as compared with vehicle infusion alone (LPS/VEH).
(Reproduced with permission from Rose AS *et al. Otolaryngol Head Neck Surg* 1997;116:308-316.)

Rose *et al.* created a rat model of LPS-induced chronic OME by surgically obstructing the eustachian tube and then transtympanically injecting either LPS or vehicle (Krebs-Ringer solution) into the middle ear. After one week, middle ear fluid was removed and its volume and mucin content measured. Rats instilled with LPS had greater effusion volumes and mucin concentrations than those receiving vehicle alone. Further validating the animal model, immunostaining revealed inflammatory infiltration of both middle ear epithelia and lumina, and increased goblet cells and submucosal glands in LPS-exposed ears versus controls.

After LPS or vehicle instillation, experimental animals also received a transtympanic catheter which continuously infused N-nitro-L-arginine methyl ester (L-NAME) from a subcutaneously implanted ALZET pump, Model 2001. L-NAME is a competitive inhibitor of nitric oxide synthase, the enzyme which synthesizes the free radical, nitric oxide. L-NAME infusion lowered effusion volume, although not to a statistically significant extent, but significantly decreased mucin content ($p < 0.05$) as compared with infusion of vehicle alone (see Figure 3).

The work by Rose *et al.* demonstrates a role for LPS in inducing both OME and mucin

production. Suppression of mucin secretion by L-NAME infusion suggests a mediatory role for nitric oxide in this process. These authors' novel approach, directly infusing L-NAME into the middle ear, facilitated characterization of this agent's local bioactivity, without confounding systemic effects.

Hypothesizing a role for NMDA receptors in AG-induced excitotoxicity at the hair cell-afferent nerve synapse, Basile *et al.*, at the National Institutes of Health and Louisiana State University, created an animal model of AG-induced ototoxicity by chronically administering neomycin or kanamycin to separate groups of guinea pigs by daily injection for two or three weeks.[2] The experimental group also received either dizocilpine or ifenprodil, both NMDA antagonists, infused from a subcutaneously implanted ALZET pump, Model 2002. To achieve continuous infusion for three weeks, the pumps were surgically replaced with fresh pumps after two weeks.

After four weeks, animals were stimulated with trains of 5-10 kHz tones at specific intervals and sound pressures. The number of pinna flicks, a known reflex in guinea pigs to such sounds, were recorded. The sound levels required to elicit a 50% pinna flick rate in experimental animals treated with both AGs and an NMDA antagonist did not differ significantly from control animals at 5 kHz (Figure 4), and were only modestly increased at 7 and 10 kHz. In addition, measurement of hair cell function in the organ of Corti via distortion product otoacoustic emissions revealed that concurrent administration of NMDA antagonists with AGs preserved outer hair cell function to nearly that of control animals, whereas AG-treated animals showed emissions indistinguishable from the noise floor, signifying severe loss of hair cell function. The authors propose that overstimulation of NMDA receptors at afferent synapses may be responsible for the AG-induced auditory deficit, although they also speculate that direct activation of NMDA receptors on the hair cells themselves could be causative. Continuous infusion was key to maintaining stable receptor occupancy in this model.

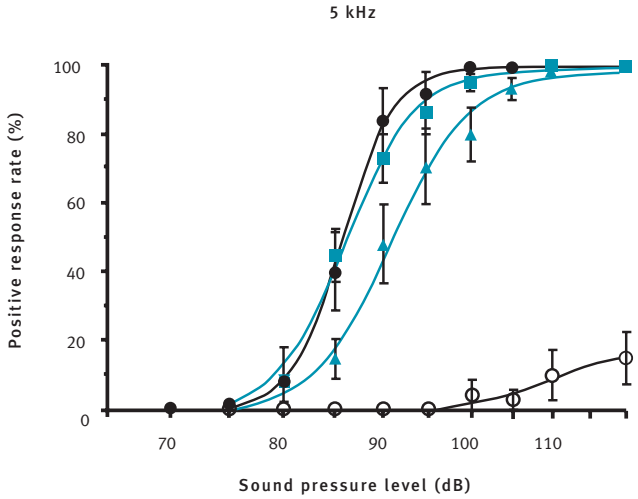


Figure 4. NMDA antagonists attenuate decreases in the pinna flick reflex due to aminoglycoside treatment in guinea pigs. Animals exposed to neomycin alone (○) showed minimal response to 5-kHz tones, even at high sound pressures. Concurrent treatment with an NMDA antagonist, either dizocilpine (■) or ifenprodil (▲), maintained response rates which did not significantly differ from animals with no aminoglycoside exposure (●).
(Reproduced with permission from Basile AS *et al. Nature Medicine* 1996;2(12):1338-1343.)

Role of NMDA Receptor in Aminoglycoside Toxicity

Aminoglycoside (AG) antibiotics have been in use, beginning with streptomycin, for more than 50 years. They remain the drugs of choice in numerous circumstances relevant to pediatrics, including septicemia and other serious infections due to Gram negative bacilli. Increasingly sophisticated dosing strategies have not eliminated the main adverse effects of nephro- and ototoxicity. Although the molecular mechanisms are poorly understood, the hearing loss results from degeneration of the cochlear hair cells and can occur suddenly and unpredictably.

Both Basile *et al.* and Rose *et al.* capitalized on the control over drug delivery afforded by ALZET pumps. In numerous other published studies, researchers have overcome obstacles to drug delivery presented by tissues which are difficult to penetrate or compounds with short half-lives. To request references on the delivery of agents to the ear, on NMDA antagonists, or other compounds relevant to your area of research, please contact us as outlined on page 7.

1 Rose AS, Prazma J, Randell SH, Baggett HC, Lane AP and Pillsbury HC. *Otolaryngol Head Neck Surg* 1997;116:308-316.

2 Basile AS, Huang J-M, Xie C, Webster D, Berlin C and Skolnick P. *Nature Medicine* 1996; 2(12):1338-1343.

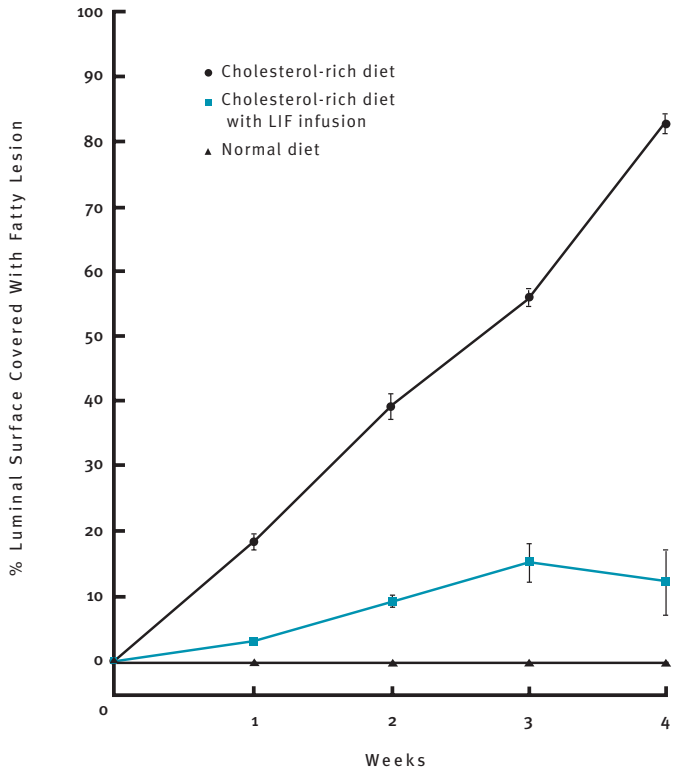
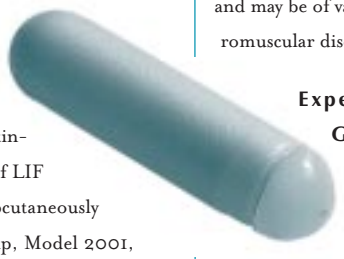


Figure 5. Continuous administration of LIF by ALZET pump over four weeks resulted in decreased fatty streak formation in the thoracic aorta.
(Reproduced with permission from Moran CS *et al. Arterioscler Thromb Vasc Biol* 1997;17:1267-1273.)

(*continued from page 1*) to the conclusion that LIF is capable of accelerating muscle regeneration following injury.

This putative beneficial action was further investigated in *mdx* mice, an animal model of human Duchenne muscular dystrophy. In this study by Kurek *et al.*, of St. Vincent's Hospital, Australia, continuous targeted delivery of LIF was achieved using a subcutaneously implanted ALZET pump, Model 2001, connected to a cannula inserted into the vastus lateralis muscle.[2] Seven days of LIF treatment resulted in a significant increase (22%, $p < 0.05$) in regenerating muscle fiber diameter over saline controls, which was still apparent seven days after cessation of treatment, together with a two-fold increase in the number of regenerating fibers. These findings led Kurek *et al.* to conclude that LIF did indeed stimulate muscle regeneration and may be of value in the treatment of neuromuscular diseases.



Experimental Glomerulonephritis

The observation that glomerular levels of LIF mRNA rapidly increased after treatment with anti-glomerular basement membrane antibody (anti-GBM Ab), prompted Tang *et al.*, of Amgen Inc., to investigate the possible role of LIF in the pathogenesis of the ensuing glomerulonephritis.[3] Animals implanted intraperitoneally with an ALZET pump, Model 1003D, were continuously treated with LIF for 24 hours prior to and following induction of anti-GBM Ab glomerulonephritis. LIF treatment attenuated the resultant glomerular inflammation, as evidenced by reduced proteinuria (85%) and glomerular macrophage infiltration (60%), together with a decreased expression of the inflammatory cytokines IL-1β and TNF-α, and the chemokine MCP-1. These protective effects of LIF led the researchers to suggest a potential role for this cytokine in the treatment of glomerular inflammation.

Cholesterol Lowering

Additional investigations by Moran *et al.*, of the University of Queensland, Australia, have indicated that beneficial cholesterol-lowering and antiatherosclerotic effects may be attributable to LIF.[4,5] These studies involved the continuous administration of LIF, via intraperitoneally implanted ALZET pumps, Model 2ML4, to rabbits over a four week period, during which time the animals were maintained on either a normal or cholesterol-rich diet. Plasma cholesterol levels and the formation of fatty streaks on the thoracic aortas of the animals were recorded throughout,[5] or on completion of the treatment.[4]

As expected, rabbits maintained on the cholesterol-rich diet had considerably higher plasma cholesterol levels and an increased incidence of aortic fatty streak formation compared to those on the normal diet. Administration of LIF to rabbits on the cholesterol-rich diet significantly reduced this elevation in plasma cholesterol levels and fatty streak formation compared to their non-treated counterparts (Figures 1 and 5). Moran *et al.* have suggested that

these beneficial effects of LIF are, in part, related to an upregulation of hepatic LDL receptors giving rise to improved clearance of lipoprotein-associated cholesterol from the circulation.

Delivery Dilemma Resolved

Conventional delivery methods, such as injection, can result in wide variations in the level of LIF achieved in the plasma and tissues, due to the very rapid rate at which it is eliminated.[2] The use of ALZET pumps in the above studies enabled effective systemic[3,4,5] or local tissue[1,2] levels of LIF to be maintained. This additional control over experimental variables greatly facilitated evaluation of the beneficial effects conferred by this cytokine in times of adversity.

Many other cytokines and growth factors have similar drawbacks associated with short half-life, poor bioavailability and the high costs of material. For information on how the osmotic pump has been successfully used for the delivery of a diverse range of such agents, please contact us as outlined below.

1 Barnard W, Bower J, Brown MA, Murphy M and Austin L. *J Neurol Sci* 1994;123:108-113.

2 Kurek J, Bower J, Romanella M and Austin L. *Neurosci Letts* 1996;212:167-170.

3 Tang WW, Qi M, Van GY, Wariner GP and Samal B. *Kidney Intl* 1996;50:1922-1927.

4 Moran CS, Campbell JH, Simmons DL and Campbell GR. *Arterioscler Thromb* 1994;14:1356-1363.

5 Moran CS, Campbell JH and Campbell GR. *Arterioscler Thromb Vasc Biol* 1997;17:1267-1273.

Overcoming Challenges in Neonatal Drug Delivery

by Laura Whitman

A review by Sapolsky, at Stanford University, published in Science stated that as little as 15 minutes each day of human handling of newborn rats during the first few weeks of life can profoundly affect maternal interaction and, later, adult behavior.[1] In order to study the effects of agents, while avoiding the physiological and behavioral changes associated with frequent handling required by repeated injections, researchers have used ALZET pumps. Thornton and Smith noted that "bolus injections require repeated handling and stress of neonatal rats and dams that could affect the development of tolerance... these concerns led us to begin using subcutaneously implanted ALZET osmotic minipumps to render neonatal rats tolerant and physically dependent on opioids."[2] The smallest ALZET pumps are designed for subcutaneous implantation in animals as small as 10 grams, the weight of a 6-day old rat pup. The surgical implantation takes about 5 minutes, following which the animals require no further handling for the duration of the infusion period.

While minimizing stress from frequent handling, these tiny implantable pumps also provide zero-order drug delivery in neonates for durations lasting from 3 to 14 days. Thornton and Smith found this aspect particularly beneficial: "mini-pumps have the advantage of delivering drug at a constant rate to provide stable plasma and tissue opioid concentrations for long periods of time. The constant infusion of opioid by minipumps may also reduce the toxicity often associated with bolus drug administration."[2] A neonatal information package, including surgical techniques and references on the use of ALZET pumps to administer agents to neonates, is available on request.

1 Sapolsky RM. *Science* 1997;277:1620-1621.

2 Thornton SR and Smith FL. *J Pharm and Exp Therapeut* 1997;281:514-521.

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, 650-962-2251, Fax: 650-962-2488, E-mail: alzet@alza.com*

New Agents in the ALZET Literature

Including more than 5,200 publications from the scientific literature, the ALZET pump bibliography is a valuable source of information on the controlled delivery of a wide variety of agents. Updates are frequent, and the most recent includes references on the delivery of the agents listed here. References on these and other agents are available on request.

Agent	Therapeutic Category
1400W	Nitric oxide synthase inhibitor
A802715	Immunosuppressant (methylxanthine derivative)
Adrenomedullin, human	Vasodilator
Ancrod	Anticoagulant
Antibody, anti-IL-1α	Immunologic
Antibody, monoclonal CC49	Immunologic
Bisoprolol	Antihypertensive
Bunazosin	Antihypertensive
Cloprostenol, calcium	Infertility agent (prostaglandin analog)
CP-98,113	NMDA antagonist
CP-101,606	NMDA antagonist
Etidronate	Calcium regulator
FK633	Platelet glycoprotein antagonist
GR144053	Fibrinogen receptor antagonist
Hepatocyte growth factor, recomb. human	Growth factor
HR 720	Angiotensin II antagonist
Interleukin-8	Cytokine
Leptin, recomb. human	Hormone
Metergoline	Prolactin inhibitor
Metolazone	Antihypertensive, diuretic
Monocyte chemotactic protein-1	Chemoattractant
Oxyntomodulin	Regulatory gut peptide
Parathyroid hormone, human 1-84	Hormone
R-95288	Antiviral oligonucleotide derivative
Ramiprilat	ACE inhibitor
RO-23-7553	Vitamin D analog
UCN-01	Protein kinase C inhibitor

ALZET Expert Seeks Infusion of Academia

With great regret, we announce the departure of Nigel Ray from ALZA Corporation, as Nigel pursues his education at the Anderson School of Business, UCLA. Nigel joined ALZA in 1986 as a Technical Information Associate for ALZET Osmotic Pumps. He ascended the ranks to create, and then become director of, ALZA Scientific Products. Under his leadership there were numerous innovations, including introduction of the ALZET Brain Infusion Kit, the Technical Information Manual, and the ALZET Web Site. In addition, Nigel spearheaded the development of several new pump models.

We would like to thank Nigel for his enormous contributions to ALZA. ALZA Scientific Products exists, and is vital with activity, because of his efforts. We wish him all the best in his future endeavors at business school and thereafter.

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