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Manipulating the Local Nerve Environment through Targeted Delivery

Targeted delivery is especially useful when the desired drug effect is dependent on local events. Peripheral nerve regeneration following injury is one process where events at the injury site are critical to successful regeneration. Recent publications in the neuroscience literature demonstrate techniques for targeting peripheral nerves with neurotrophins. Targeted delivery can also be used to block nerve conductivity temporarily, a technique which may have utility in the study of neuromuscular development and reinnervation.

A Novel Method for Targeting Peripheral Nerves

Several neurotrophic factors stimulate nerve regeneration *in vitro*, but these agents have proven difficult to study *in vivo*. Hekimian et al. from the Lahey Clinic developed a method for manipulating the nerve environment during regeneration.[1] This group constructed a chamber to contain the distal and proximal stumps of a sectioned sciatic nerve and to guide regeneration (Figure 1). Via a catheter, neurotrophic agents were delivered from an osmotic pump into the lumen of the nerve chamber. A second catheter functioned as a vent to prevent pressure accumulation. Using this device, Hekimian et al. confirmed that continuous four-week delivery of drug solutions can be achieved in an environment which is conducive to nerve regeneration. The authors concluded that "the success of this model opens the door for a multitude of studies. With relative ease, any stable solution can be infused continuously into a nerve-guide chamber at a steady rate over a period of time." [1]

IGF-I Protects Against Diabetic Neuropathy

Neuropathy is a common complication of diabetes. It is accompanied by impaired nerve regeneration in experimental diabetes, a condition associated with reduced levels of

(Continued on Page 3, Column 1)

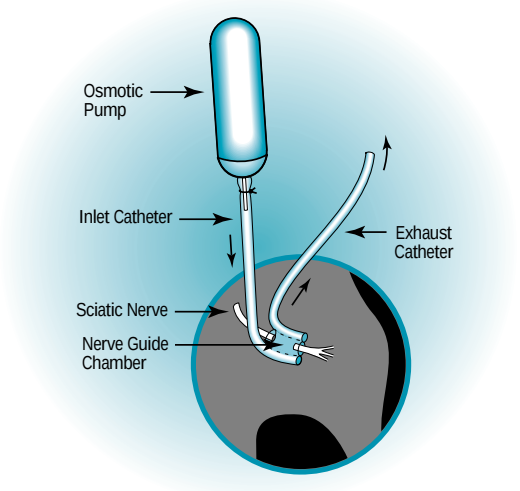


Figure 1: Schematic diagram of a nerve regeneration chamber. Drug solution from the pump continuously bathes the lumen of the nerve chamber, allowing the effect of neurotrophic agents on nerve regeneration to be determined.

Reprinted with permission from Hekimian KJ et al. *J Reconstruct Microsurg* 1995; 11(2):93-98.

L-NAME Elucidates the Role of Nitric Oxide

Nitric oxide (NO) is a free-radical gas that acts as a vasodilator. It is derived from L-arginine in endothelial cells, macrophages, neutrophils, platelets, and neurons. A shortage of nitric oxide may result in arterial hypertension and atherosclerosis. Abnormal nitric oxide levels can also contribute to infection and inflammation, neurodegenerative disorders, and the brain damage seen with stroke.[1]

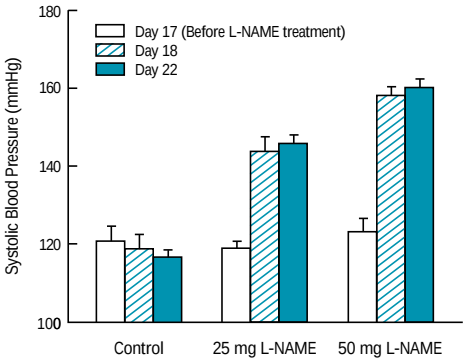


Figure 2: Changes in systolic blood pressure before L-NAME treatment (day 17), after 1 day of treatment (day 18), and after five days of treatment (day 22). Control animals received saline. Changes in blood pressure after 25 mg and 50 mg L-NAME infusion were significantly different from controls ($p < 0.01$).

Reprinted with permission from Yallampalli C, Garfield RE. *AM J Obstet Gynecol* 1993; 169:1316-1320.

Nitric Oxide Inhibition Simulates Preeclampsia

Preeclampsia, or pregnancy-induced hypertension, is a significant health problem which affects up to 30% of all women. It is the leading cause of fetal growth retardation, infant morbidity, and mortality associated with premature delivery and maternal death. Yallampalli and Garfield hypothesized that a causal factor in preeclampsia might be

(Continued on Page 4, Column 1)

Very Special Delivery

Chronic Heavy Metal Exposure

While the teratogenicity of cadmium (Cd^{2+}) exposure has been well documented, investigation into the mechanism of action has been impeded by difficulty in administering precise amounts of Cd^{2+} throughout gestation. Administration of cadmium in food or drinking water is complicated by a lack of control over dose. Gavage and injection offer greater control, but are stressful to animals, and hence can skew results.

Mahalik et al. used ALZET pumps to surmount some of these obstacles and to deliver Cd^{2+} successfully to pregnant mice for 14 days.[1] Their model resulted in a pattern of fetal anomalies and Cd^{2+} distribution which closely mirrored results obtained using other dosing routes. Mahalik et al. stated that "chronic teratogenic studies are often complicated by the requirement for daily drug administration and animal handling, which have been shown to produce significant fetal and/or postnatal effects. The osmotic minipump provides a convenient and dependable route of drug or toxicant administration that minimizes animal handling and maternal stress. Surgical implantation of the pumps is also well-tolerated and does not typically cause infection or other observable complications." [1] For references on using ALZET pumps to administer cadmium and other heavy metals, contact ALZET Technical Services at (800) 692-2990.

1. Mahalik MP, Hitner HW, and Prozialeck WC. *Toxicol Letters* 1995; 76:195-202.

Intrapericardial FGF May Salvage Ischemic Myocardium

In numerous instances, researchers have used ingenious surgery and an ALZET pump to deliver growth factors directly to a specific tissue. Targeted delivery can avoid confusing or toxic systemic effects, while ensuring that the growth factor reaches the target tissue and is maintained there at sufficient concentration to exert its effects. For example, Cuevas et al. used basic fibroblast growth factor (bFGF), a peptide with a very short half-life, to induce angiogenesis in the arterial adventitia.[1] Hayek et al. relied on intrasplenic FGF infusion to promote the angiogenesis needed to maintain pancreatic islet grafts.[2] Eppley et al. applied FGF directly to a bone graft to advance healing through angiogenesis.[3]

More recently, Landau et al. capitalized on bFGF's angiogenic enhancement of myocardial perfusion in a rabbit model of ischemia.[4] Increased coronary collateral vasculature might rescue jeopardized tissue in conditions of decreased perfusion. Landau et al. induced left ventricular hypertrophy (LVH) by infusing intravenous angiotensin II (AII). Rapidly-acquired LVH results in myocardial oxygen deficit due to inadequate capillary density to support hypertrophic muscle. Landau et al. hoped to offset this deficit by stimulating new capillary formation. After one week of AII treatment, bFGF was infused by ALZET pump for 11 to 28 days via a catheter advanced into the pericardial space using a transphrenic approach. Controls received albumin in place of bFGF or intravenous AII alone. Non-ischemic controls received saline instead of AII.

Histological analysis of subepicardial myocardium graded capillary density, ischemic necrosis, and fibrosis. Greater fibrosis in AII-treated animals was consistent with microinfarcts resulting from LVH-induced oxygen deficit. Capillary density was increased markedly in animals treated with both bFGF and AII, as compared with normal epicardial vascularity in animals receiving AII alone.

Myocardial angiogenesis has been demonstrated in other animal models. Yanagisawa et al. produced a canine model of ischemia caused by coronary artery stenosis and an artificial thrombus.[5] Slow injection of bFGF after occlusion increased the number of capillaries and arterioles versus controls. Left ventricular ejection fraction, a measure of cardiac performance, decreased post-occlusion, but rebounded significantly after one week of bFGF treatment. Battler et al. also increased microvessel counts with bFGF in a porcine ischemia model.[6] Stimulation of angiogenesis by locally-administered bFGF bears promise for ischemic disease treatment.

A wealth of knowledge about bFGF and other growth factors continues to accumulate as researchers systematically deliver individual factors to various tissues. To draw upon published research in these areas, request an updated literature search from ALZET Technical Services at (800) 692-2990 or (415) 962-2251.

1. Cuevas P, Gonzalez AM, Carceller F & Baird A. *Circ Res* 1991; 69:360-369.
2. Hayek A, Lopez AD & Beattie GM. *Transplantation* 1990; 50(6):931-933.
3. Eppley BL, Doucet M, Connolly DT, & Feder J. *J Oral Maxillofac Surg* 1988; 46:391-398.
4. Landau C, Jacobs AK, & Haudenschild CC. *Am Heart J* 1995; 129:924-931.
5. Yanagisawa-Miwa A, Uchida Y, Nakamura F, Tomaru T, Kido H, Kamijo T, Sugimoto T, Kaji K, Utsuyama M, Kurashima C, & Ito H. *Science* 1992; 257:1401-1403.
6. Battler A, Scheinowitz M, Bor A, Hasdai D, Vered Z, Segni E, Varda-Bloom N, Nass D, Engelberg S, Eldar M, Belkin M & Savion N. *J Am Coll Cardiol* 1993; 22:2001-2006.

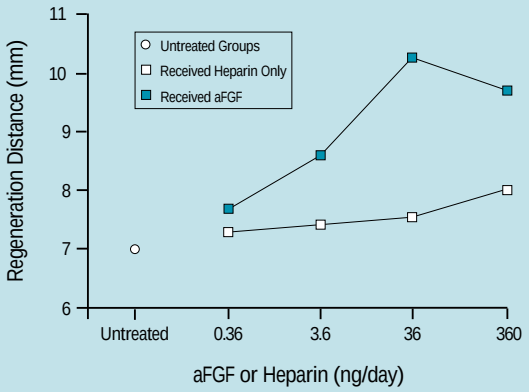
Direct Delivery to the CNS

In a recently-published review, Theo Hagg provides a comprehensive protocol for the infusion of agents into the brain using ALZET osmotic pumps.[1] Dr. Hagg outlines a variety of procedures and recommendations, including the construction of coiled tubing to serve as an external reservoir, preparation of a dental acrylic platform for securing the cannula to the skull, preimplantation advice, surgical tips, and post vivo analysis. Hagg concludes that "the ALZET osmotic pump has clearly facilitated and expanded the possibilities of experimental approaches in the investigation of the CNS".[1] For a copy of this review, contact ALZET Technical Services at (800) 692-2990 or at (415) 962-2251.

1. Hagg T. Continuous Central Nervous System Infusion with ALZET Osmotic Pumps. In Flanagan TR, Emerich DF, and Winn SR (eds). *Methods in Neurosciences, Volume 21: Providing Pharmacological Access to the Brain: Alternate Approaches.* Academic Press, 1994, pp. 201-213.

Figure 3: Effects of locally-infused aFGF on motor axon regeneration distance. Filled squares correspond to groups which received aFGF while open squares indicate groups which were given heparin only. Open circle indicates untreated groups. P < 0.05 (compared to untreated controls) for groups which were given 36 and 360 ng/day aFGF.

Reprinted with permission from Laird J.M.A. et al. *Neurosci* 1995; 65(1):209-216.



Nerve infusion *(Cont. from Page 1, Column 2)*

insulin-like growth factors (IGFs). IGFs stimulate neurite survival and outgrowth *in vitro*, and may play a role in peripheral nerve regeneration.

In a rat model of diabetic neuropathy, Ishii and Lupien compared the effects of systemic and targeted delivery of insulin-like growth factor-I (IGF-I) on sensory nerve regeneration.[2] Following crush injury of the sciatic nerve, rats made diabetic with streptozotocin were implanted with ALZET pumps containing IGF-I, IGF-II, or vehicle. IGF-I was delivered directly to the injured nerve by connecting the pumps to a fenestrated catheter, the tip of which was sutured to the epineurium and underlying muscle. For comparison, IGF-I and IGF-II were delivered systemically at a subcutaneous site distant from the nerve injury.

Ishii and Lupien concluded that "locally infused IGF-I significantly ameliorated the impairment of nerve regeneration in diabetic rats." [2] Similar results were achieved with systemic administration, but a 20-fold increase in the dose of IGF-I was required.

Laird et al. used a similar experimental model to determine the effects of local and systemic delivery of acidic fibroblast growth factor (aFGF) on regeneration following nerve crush in normoglycemic rats.[3] The administration of aFGF at the site of injury resulted in a 47% increase in regeneration distance (Figure 3). Similar results were achieved with systemic administration, but the dose required was 250-fold higher. These results may have therapeutic implications for the treatment of diabetic neuropathy.

Achieving Nerve Block Through Targeted Delivery

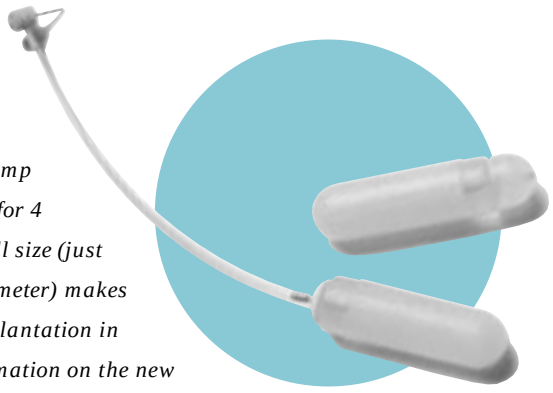
Targeted delivery can also be used to block conductivity in peripheral nerves. Barry and Ribchester used a temporary nerve block to study the effect of activity on reinnervation.[4] Partial denervation of the fourth deep lumbrical muscle (4LM) was created in the rat by crushing the lateral plantar nerve (LPN). Normally, muscle fibers in the 4LM are mononeuronally innervated by the LPN or the sural nerve (SN). When either nerve is injured, axons from the uninjured nerve sprout to innervate the denervated muscle fibers. During reinnervation, axons may establish connections with muscle fibers which are already innervated, resulting in dual innervation. The competitive elimination of these superfluous connections resembles events in neonatal muscle development and provides a model for understanding this process.

During reinnervation, Barry and Ribchester blocked conduction in the sciatic nerve by infusing tetrodotoxin (TTX) via a silastic cuff connected to an osmotic pump. Hind-limb paralysis confirmed effective nerve block by TTX. After two weeks of paralysis, the level of dual innervation in the 4LM was assessed. Dual innervation was found in 50% of 4LM muscle fibers in the limb subjected to temporary nerve block, compared with 20% in untreated, contralateral controls. Upon removal of the nerve block, conduction in the sciatic nerve resumed, as evidenced by normal use of the previously paralyzed hind limb. After 8 weeks of recovery, the limb subjected to temporary paralysis retained a greater level of dual innervation (35%) than the untreated, contralateral control (17%). Barry and Ribchester concluded that "paralysis produced an enduring, stable pattern of polyneuronal innervation." [4] These findings have therapeutic implications because muscles rarely recover full innervation following injury. Improvements in the extent of reinnervation may be achieved by prolonging the period of muscle disuse after injury.[4]

1. Hekimian KJ, Seckel BR, Bryan DJ, Wang KK, Chakalis DP, & Bailey A. *J Reconstruct Microsurg* 1995; 11(2):93-98.
2. Ishii DN and Lupien SB. *J Neurosci Res* 1995; 40:138-144.
3. Laird JMA, Mason GS, Thomas KA, Hargreaves RJ, & Hill RG. *Neurosci* 1995; 65(1):209-216.
4. Barry JA & Ribchester RR. *J Neurosci* 1995; 15(10):6327-6339.

Introducing the New Model 2004 ALZET Osmotic Pump

A new ALZET osmotic pump, the Model 2004, is now available. With a reservoir volume of 200 µl, this pump delivers agents continuously for 4 weeks at 0.25 µl/hr. Its small size (just 3 cm long, and 0.7 cm in diameter) makes it ideal for subcutaneous implantation in mice or rats. For more information on the new Model 2004, call (800) 692-2990 or (415) 962-2251.



L-NAME *(Cont. from Page 1, Column 3)*

impaired vascular nitric oxide synthesis.[2] ALZET pumps were implanted subcutaneously in pregnant rats to infuse two different doses of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) or nitroglycerin (a nitric oxide donor) from day 17 of gestation to term. While nitroglycerin had no effect, L-NAME was found to elevate blood pressure in a dose-dependent fashion (Figure 2, Page 1). It also decreased pup weight, increased pup mortality, and caused maternal proteinuria. However, length of gestation was not affected. These researchers concluded that NO synthesis inhibition during pregnancy caused maternal hypertension and fetal growth retardation. As these effects are similar to those of preeclampsia, a change in NO synthesis may be a factor in this disorder.[2]

L-NAME Increases Neointimal Formation

Atherosclerosis can be induced by hypercholesteremia. Endothelium-dependent vasodilation is impaired in both hypercholesteremic animals and in those which suffer from atherosclerosis. This decreased vasodilation may be related to reduced NO synthesis, as NO is thought to act as an "endothelium-derived relaxing factor". Since NO also has antiproliferative effects on vascular cells, the endothelial cell dysfunction seen with hypercholesteremia could contribute to atherosclerotic neointima formation. Indeed, administration of the NO precursor L-arginine limits the development of aortic atherosclerosis and improves endothelial cell function.[3]

Cayatte et al. examined the effects of NO inhibition on neointima formation in hypercholesteremic rabbits.[3] After five weeks of a high cholesterol diet, rabbits were treated with L-NAME for 4 weeks using subcutaneous ALZET pumps. Since increases in blood pressure can also accelerate atherosclerosis, the dose of L-NAME was adjusted so that it would not affect blood pressure. Compared to untreated hypercholesteremic animals, L-NAME-treated animals had significant increases in neointimal lesion area and impaired endothelial function. L-NAME did not induce atherosclerosis in rabbits fed a normal diet, indicating that it accelerates the atherosclerosis induced by hypercholesteremia.[3]

NO is involved in a wide range of pathophysiological processes, including many of cardiovascular origin. As these articles suggest, chronic modulation of NO synthesis *in vivo* may elucidate the role of NO and be used to create novel disease models. For a complete list of references on ALZET pump applications in NO research, contact the ALZET Technical Services desk at (800) 692-2990 or (415) 962-2251.

1. Chustecka Z. *Scrip Magazine* 1995; July/August:39-41.
2. Yallampalli C, Garfield RE. *AM J Obstet Gynecol* 1993; 169:1316-1320.
3. Cayatte AJ, Palacino JJ, Horten K, Cohen RA. *Arterioscler Thromb* 1994; 14:753-759.

New Agents in the ALZET Literature

With more than 4,500 publications from the scientific literature, the ALZET pump bibliography is a valuable source of information on the controlled delivery of proteins, peptides, and other agents. Updates are frequent, and the most recent includes references on the delivery of:

- ABT-418 (cholinergic channel activator)
- Alaproclate
- AMPA (excitatory amino acid)
- Barium Hydroxide
- CGS 22652 (thromboxane A_2 synthase inhibitor)
- Fluvoxamine
- Harman (ß-carboline)
- H7 (PKC inhibitor)
- LY-274614 (NMDA receptor antagonist)
- Memantine (NMDA receptor antagonist)
- Neomycin
- Pertussis Toxin
- Venlafaxine (5-HT reuptake inhibitor)

References on these and other agents are available to you through ALZET Technical Services at (800) 692-2990 or (415) 962-2251. A bibliography search customized to your research interests can be mailed or faxed to you at no charge (see attached reply card).

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American Association of Cancer Research	Washington, DC	April 21-23
ASBMB	New Orleans, LA	June 3-6
Endocrine Society	San Francisco, CA	June 12-14
American Association of Pharmaceutical Sciences	Seattle, WA	October 28-30
Society for Neuroscience	Washington, DC	November 17-20

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I am interested in receiving more information about ALZET® osmotic pumps. Please send me:

❑ General information on ALZET osmotic pumps. (TIM)

❑ Information on using ALZET pumps for delivering proteins and peptides. (BB)

❑ Information on using ALZET pumps for brain infusion. (BRAI, CNSI)

❑ A videotape of surgical techniques for implanting ALZET pumps. (VHS VIDEO)

❑ A reprint of the Hagg review on CNS infusion. (CNSI)

I would like to receive references on the use of ALZET pumps in the following areas:

❑ Antisense Oligodeoxynucleotides (ANTS)

❑ Cytokines (CYTO)

❑ Peptide Growth Factors (GF)

❑ ICV Delivery (ICV)

❑ Metal Infusion (META)

❑ Nerve Infusion (NERV)

❑ Nitric Oxide Research (NOSI)

❑ Targeted Delivery (TARG)

❑ Injection-Infusion Comparisons (IIC)

❑ Other (please specify)_____

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