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Leptin Activity May be Schedule Dependent

It was recently reported that leptin, the protein product of the *ob* gene, causes sustained weight loss when administered to mice over several weeks.[1,2,3] This effect is most pronounced in grossly obese *ob/ob* mice which, due to a mutation in the *ob* gene, lack an endogenous source of the OB protein. Lean, wild-type mice exhibit less pronounced weight loss in response to leptin administration. Pelleymounter et al suggest that the activity of the OB protein in lean mice could be increased by changing its mode of administration. These authors found that constant infusion at low doses was as effective as high dose injections.[1] A continuous low dose infusion of leptin (0.3 mg/kg/day) from an ALZET® osmotic pump resulted in a small but significant weight loss. Despite a 30-fold increase in the dose administered (10 mg/kg/day), similar weight changes resulted from daily leptin injections. These results suggest that further study of the pharmacokinetics of leptin is warranted. Moreover, various dosing schedules should be considered in order to optimize the activity of leptin in animal models.

1. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, and Collins F. *Science* 1995;269:540-543.
2. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, and Friedman JM. *Science* 1995;269:543-546.
3. Campfield LA, Smith FJ, Guisez Y, Devos R, and Burn P. *Science* 1995;269:546-549.

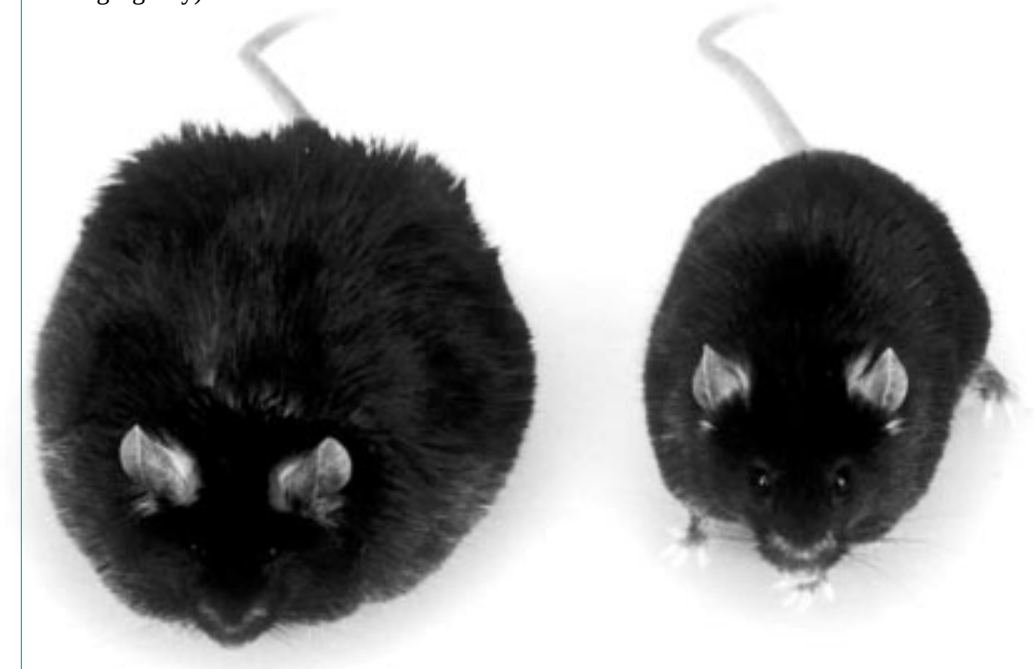

Figure 1. The OB protein, leptin, caused significant weight loss in an ob/ob *mouse (right) compared with an untreated* ob/ob *mouse (left). In lean, wild-type mice, continuous infusion of leptin via ALZET pumps achieved weight loss at a dose far below that required to produce the same effect by injection.*

Photo reprinted by permission from The Rockefeller University, New York, N.Y. Photo by John Sholtis.

Antisense Oligonucleotides Evaluated in Vivo

The therapeutic potential of antisense oligonucleotides has far-reaching implications for an impressive array of human diseases. This new class of drugs has an elegantly simple capacity to disrupt the transfer of genetic information selectively, a powerful mechanism if effectively developed for therapeutic application.

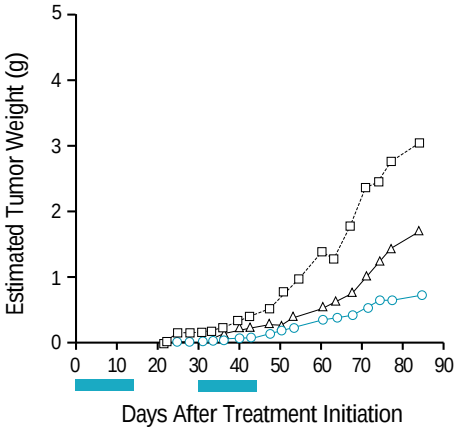

*Figure 2. Antisense oligonucleotide targeted to the c-*myb *gene was administered by ALZET pumps in a SCID mouse model of human melanoma. Treated animals (—○—) were infused on days 1-14 and 30-44 (▬), reducing mean tumor weight to about 23% that of untreated animals (··□··). Sense-treated animals (—△—) did not significantly differ from untreated controls.*

Reprinted with permission from Hijiya N, Zhang J, Ratajczak MZ, Kant JA, DeRiel K, Herlyn M, Zon G, & Gewirtz A. *Proc Natl Acad Sci* 1994;91:4499-4503.

Most antisense oligonucleotides are designed to arrest translation of a target mRNA, thereby transiently reducing or eliminating its gene product. Ongoing investigations of the efficacy, pharmacokinetics, distribution, and toxicity of oligonucleotides have used ALZET® osmotic pumps to surmount barriers to successful administration in vivo.

(Continued on page 3, column 3)

Very Special Delivery

Osmotic Pumps Deployed Among the Kelp

Imagine if in order to reach your study's subjects you had to drive to the coast, charter a boat, and navigate the open sea. Consider the challenges if each test sample had to be retrieved from an ocean depth of 3800 meters under variable weather conditions. These are but a few of the obstacles faced by chemical oceanographers at the Monterey Bay Aquarium Research Institute (MBARI) and Moss Landing Marine Laboratories (MLML) in Monterey, California, as they study ocean chemical cycles on large temporal and spatial scales.[1] To circumvent some of these difficulties, Jannasch, Johnson and Sakamoto adapted ALZET osmotic pumps in a unique application to perform reagent addition and sampling functions in a self-contained, continuous flow analyzer which determines nitrate concentrations in sea water during long-term, deep sea deployment.[2]

The pumping action of modified ALZET pumps draws sea water into a miniature manifold, past reagent-bearing ALZET pumps, a cadmium-reducing surface and a photodiode detector. The catalyzed reduction of nitrate to nitrite is followed by production of an azo dye, which is picked up by the detector. A datalogger, which powers the electronic components, converts the detector's signal to voltages and then amplifies, digitizes and stores the data.

Data from one month's operation of the analyzer in both a fresh water aquarium and the kelp tank at the Monterey Bay Aquarium agreed well with nitrate measurements made by standard methods. Barnacle and algae growth on the analyzer's external surface did not affect its operation, constituting an important victory over biofouling. Development of the analyzer continues at MBARI and MLML because of its many advantages over field sample collection and analysis. In fact, three analyzers are currently operating on a deep sea mooring off of Bermuda in the Atlantic Ocean.[3] Jannasch et al assert that "the inherent simplicity and small size of osmotic pumps allow the analyzers to be used to continually monitor dissolved chemicals at remote sites for limnological, estuarine, and oceanographic studies." [2]

1. Johnson KS, Coale KH & Jannasch HW. *Anal Chem* 1992;64(22):1065A-1075A.
2. Jannasch HW, Johnson KS & Sakamoto CM. *Anal Chem* 1994;66(20):3352-3361.
3. Jannasch HW, personal communication, August 9, 1995.

Figure 3. ALZET pumps release reagents and draw sea water into a nitrate analyzer used by chemical oceanographers at the Monterey Bay Aquarium Research Institute and Moss Landing Marine Laboratories to study ocean chemical cycles.

Photo provided courtesy of Dr. Hans Jannasch, Monterey Bay Aquarium Research Institute.

Local IGF-I Infusion Restores Wound Healing

Using growth factors to enhance wound healing offers great therapeutic potential. However, the complexity of the wound healing process and challenge of achieving adequate levels of these short-acting peptides in the wound necessitate novel means of growth factor administration. Recent work using an ALZET pump and a catheter to target delivery to the wound helped elucidate the role of IGF-I as a modulator of wound healing.

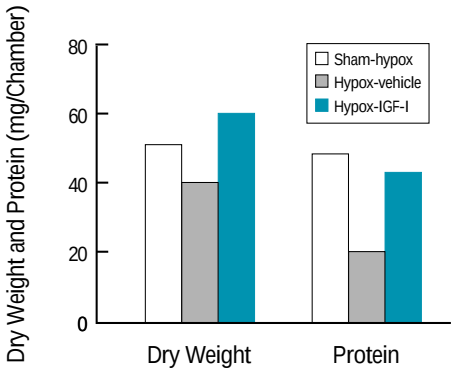

Figure 4. IGF-I was infused via ALZET pumps into subcutaneous wound chambers implanted in three groups of animals. IGF-I increased wound healing, as measured by tissue dry weight and protein, in hypophysectomized animals to levels nearly equaling or even exceeding those in sham-hypophysectomized controls.

Reprinted with permission from Mueller RV, Hunt TK, Tokunaga A, Spencer EM. *Arch Surg* 1994;129:262-265.

Normally after injury, fibroblasts, platelets and macrophages release IGF-I locally. IGF-I is a potent mitogen and also a mediator of normal growth and development.[1] Poor wound healing occurs in diabetes, malnutrition, and glucocorticoid therapy, all of which are IGF-I deficient states with reduced wound macrophage levels. Wound models exposed to reduced IGF levels, either by hypophysectomy or exogenous IGF binding protein, show significant impairment in cell replication and collagen deposition. Since IGF-I has a plasma half-life of less than 11 minutes, making systematic

Figure 5. Following hypophysectomy, IGF-I was infused directly into a wound for 14 days using an ALZET pump and a catheter. IGF-I replacement (—●—) increased wound macrophage levels throughout the treatment period as compared with sham-hypophysectomized animals (--◇--) and vehicle-infused, hypophysectomized controls (—◆—).

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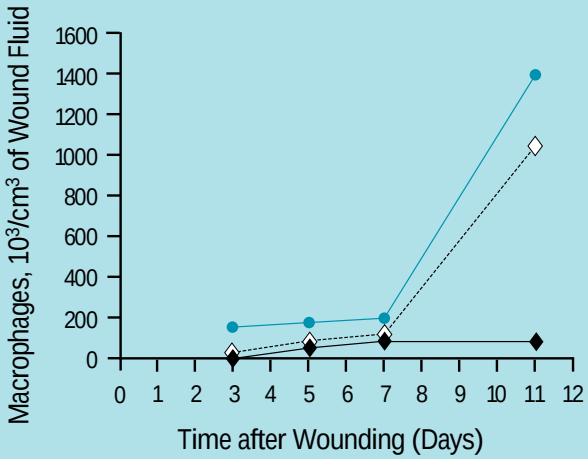

administration rather costly, continuous IGF-I delivery directly into the wound was investigated as a means for restoring wound healing.[3]

Restoring Local IGF-I Levels

Mueller et al implanted stainless steel, wire mesh wound chambers subcutaneously in hypophysectomized and sham-operated rats.[1] By means of a catheter, each chamber was connected to an ALZET pump filled with IGF-I or saline. The continuous action of the pump ensured stable levels of IGF-I in the microenvironment of the wound during the 14 day treatment period. Results were compared against vehicle infusion, hypophysectomy without infusion, and sham-hypophysectomy. Wound fluid and chamber tissue were analyzed for wound repair activity.

Hypophysectomy reduced all indices of wound healing by 27%-79%. Continuous infusion of IGF-I directly to the wound restored local IGF-I levels, resulting in normal or supranormal wound healing. Mueller et al report that "in every instance, hypophysectomy resulted in a decrease in wound healing variables and (local) IGF-I infusion resulted in an increase." [1] Variables examined included chamber hydroxyproline (a measure of wound collagen synthesis), dry weight, protein, and DNA (see Figure 4). In addition, wound macrophage concentrations were significantly increased in IGF-I-infused animals as compared with hypophysectomized, vehicle-infused controls (see Figure 5). These results demonstrate that local delivery of IGF-I promotes wound healing.

Using a similar experimental model, Suh et al examined whether corticosteroid therapy decreased wound IGF-I levels and if exogenous IGF-I infused directly into the wound could reverse this deficit.[2] Rats were injected with methylprednisolone and received four subcutaneous wire mesh chambers, each of which was connected via catheter to an ALZET pump infusing IGF-I or saline. Suh et al found that doses of methylprednisolone which significantly impaired wound healing also decreased IGF-I levels in the wounds by 32-56%. In addition, exogenous IGF-I infused directly into the wound for 14 days increased chamber DNA by 216%, chamber hydroxyproline by 41%, and chamber total protein by 109%, as compared with saline-infused controls, largely reversing the tissue repair deficit.

Both studies illustrate that direct infusion into a wound by an ALZET pump permits manipulation of the wound microenvironment without the confounding systemic effects of exogenous growth factor. ALZET pumps have been used to study the effects of other proteins, such as relaxin and tissue plasminogen activator, on wound healing. References on wound healing, growth factors, and other targeted delivery applications are available by contacting ALZET Technical Services or returning the attached reply card.

1. Mueller RV, Hunt TK, Tokunaga A, & Spencer EM. *Arch Surg* 1994;129:262-265.
2. Suh DY, Hunt TK, & Spencer EM. *Endocrinology* 1992;131(5):2399-2403.

(Continued from page 1, column 3)

Antisense

Whitesell et al identified several impediments to successful in vivo administration of oligonucleotides. These include rapid degradation of unmodified phosphodiester oligonucleotides within biologic fluids and rapid clearance from the circulation.[1] Addressing these difficulties, Whitesell et al describe a model in which "oligo solutions are continuously infused via subcutaneously implanted osmotic pumps. Such administration prevents degradation of bulk oligo while allowing the controlled, continuous release of relatively small quantities of drug into surrounding tissue...(demonstrating) successful in vivo application of antisense oligonucleotides as gene-targeted pharmacologic agents." [1]

Further complicating delivery is poor bioavailability in certain tissues. For example, phosphorothioate oligonucleotides, modified to prevent nuclease degradation, do not cross the intact blood-brain barrier.[2] To ensure exposure of brain tissue to the oligonucleotide, Zhang and Creese used ALZET pumps and catheters for cerebroventricular administration of an antisense oligonucleotide complementary to the rat dopamine D_2 receptor mRNA.[3] They successfully reduced D_2 receptor density by 48% without altering D_1 receptor density. This method could selectively block the production of any receptor subtype. This offers a distinct advantage over current therapies, such as pharmacological receptor blockade, which can result in unwanted activity by cross reaction of compounds with multiple receptors or receptor upregulation.

Another potential advantage of antisense-based therapy is that its unique mechanism may be less susceptible to drug resistance. This feature would be of particular benefit in treating leukemias, most of which ultimately develop resistance to chemotherapy. Applying antisense in chronic myelogenous leukemia, Gewirtz used ALZET pumps to administer an antisense oligonucleotide targeted to the c-*myb* protooncogene in SCID mice engrafted with human leukemic cells.[4] The c-*myb* gene has been implicated in the growth and survival of myeloid leukemic blast cells, and disruption

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of its expression can inhibit both normal and malignant hematopoiesis. Antisense administration increased survival by 3.5 to 8 times that of control animals, with reduced ovarian infiltration and little to no CNS involvement.

The c-*myb* gene has also been implicated in the pathogenesis of cutaneous melanoma, the most lethal form of skin cancer. Hijiya et al inoculated SCID mice with melanoma, then used ALZET pumps to infuse an antisense oligonucleotide complementary to the c-*myb* gene.[5] Two 14-day infusion periods effected the most dramatic reduction in mean tumor weight to about 23% that of the untreated group (Figure 2, page 1). Surprisingly, tumor growth suppression continued beyond the infusion period, despite the rapid rebound of mRNA levels which suggest that the interruption of gene expression was transitory. Hijiya et al hypothesize that c-*myb* suppression may have caused death in some cells and temporary cytostasis in others, suggesting a complex role for the c-*myb* gene product which warrants further study.

Although clinical trials have begun with ISIS 2922 for refractory CMV retinitis in AIDS patients, and ISIS 2105 for genital warts, antisense therapy is in its infancy.[2] ALZET pumps continue to be an important tool as researchers refine this emerging therapy. For more information on the use of ALZET pumps to deliver oligonucleotides, please contact ALZET Technical Services or return the attached reply card.

1. Whitesell L, Rosolen A & Neckers LM. *Antisense Res & Dev* 1991;1:343-350.
2. Crooke ST. *Antisense Res & Dev* 1994;4:145-146.
3. Zhang M & Creese I. *Neurosci Letters* 1993;161:223-226.
4. Gewirtz AM. *Leukemia & Lymphoma* 1993;11(1):131-137.
5. Hijiya N, Zhang J, Ratajczak MZ, Kant JA, DeRiel K, Herlyn M, Zon G, & Gewirtz A. *Proc Natl Acad Sci* 1994;91:4499-4503.

New Agents in the ALZET Literature

Including more than 4,000 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:

- Bendroflumethiazide
- BQ123 (endothelin antagonist)
- Caffeine
- Calphostin C (PKC inhibitor)
- Candoxatrilat
- Carbidopa
- Chlorodeoxyuridine
- CV-11974
- Diltiazem
- DuP E3800 (κ-opioid agonist)
- Etretinate (retinoid)
- ICI-118,551
- Leukemia Inhibitory Factor
- L-NAME (NOS inhibitor)
- LR3-IGF-I (IGF-I variant)
- Neurotrophin-3
- SKF-10,047 (NMDA agonist)
- Tetracycline

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.

Intracranial Peptide Delivery

Jeffrey White and Michael Schwartz have authored a well written review of methods for using osmotic pumps for delivering proteins and peptides to the central nervous system.[1] These authors contrast methods for microperfusing specific CNS tissue sites with those for intraventricular infusion into the cerebrospinal fluid (CSF) and discuss the relative advantages and disadvantages. Especially interesting is their review of brain interstitial and cerebrospinal fluid flow, brain endothelium structure, and the effects of both on drug distribution within the brain. In reviewing work from their labs and others, White and Schwartz conclude that "osmotic minipump systems can be designed to deliver virtually any compound into the CNS via indwelling cannulas for administration via the CSF or into specific tissue sites." For a copy of this review, contact ALZET Technical Services at (800) 692-2990 or (415) 962-2251.

1. White JD & Schwartz MW. Using Osmotic Minipumps for Intracranial Delivery of Amino Acids and Peptides. In Flanagan TR, Emerich DF, and Winn SR (eds). *Methods in Neurosciences, Volume 21: Providing Pharmacological Access to the Brain.* Academic Press, 1994. pp. 187-200.

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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 comparing injections and infusions. (IIC)

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

❑ A videotape of surgical techniques for implanting ALZET pumps. I understand that this tape is available for loan at no charge and that I may make copies of it. (VHS VIDEO)

❑ A reprint of the White & Schwartz review of intracranial peptide delivery. (CNSD)

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

❑ Antisense Oligodeoxynucleotides (ANTS)
❑ Peptide Growth Factors (GF)
❑ Cytokines (CYTO)
❑ Targeted Delivery (TARG)
❑ Brain Infusion (BRAI)
❑ Wound Healing (WOUN)
❑ Other (please specify)_____

_____

❑ I have used ALZET pumps.

❑ I have never used ALZET pumps.

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