A L Z A

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ALZET Pump Information Is Now Available via the Internet

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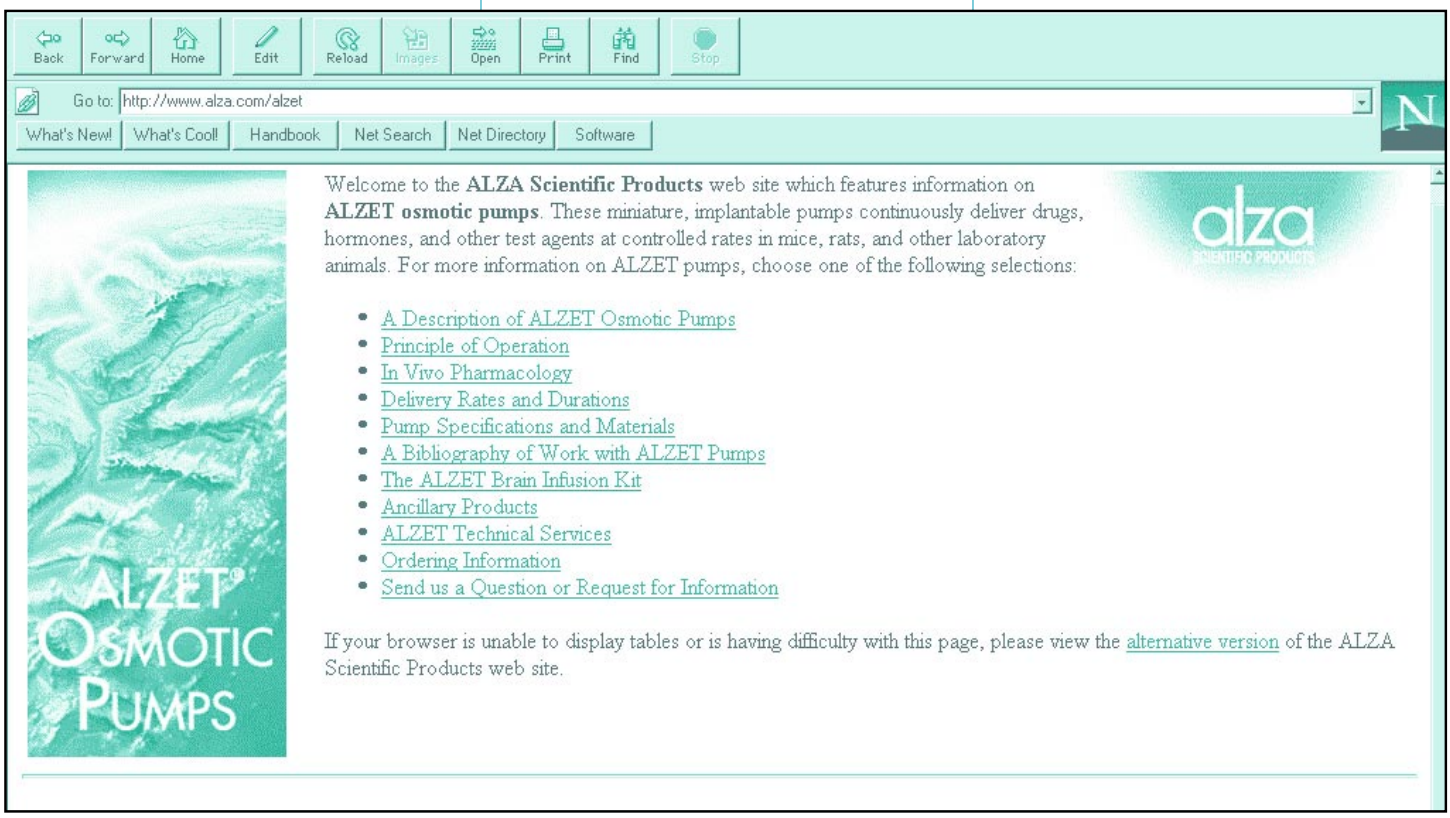
The ALZET web site can help you review past research or determine benchmark methods in an area of interest. It can also provide time-saving information on agent stability, solubility, pharmacokinetics, or techniques for optimum delivery.



In This Issue

Very Special Delivery

BDNF Suppresses Seizures in an Epilepsy Model

Epileptic disorders affect approximately 2.5 million people in the United States. Drugs which inhibit seizures are available, but there is no effective prophylaxis or cure. When investigating what distinguishes normal from epileptic tissue, Gall found that seizures stimulate pronounced changes in the expression of neurotrophic factors and their functional receptors in specific regions of the brain.[1]

The precise role of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) is uncertain. Larmet and his fellow researchers from Inserm 398 in Strasbourg modulated BDNF exogenously to understand its effect on seizure development.[2] This group used a kindling model in which daily electrical stimulation of the hippocampus induced epileptic seizures.[3,4]

Larmet et al. affixed a delivery cannula to the tip of a stimulating and recording electrode. This cannula/electrode was placed in the dorsal hippocampus of anesthetized rats (Figure 1). Stylets maintained cannulae patency during a one week recovery period, after which the cannulae were connected to Model 2001 ALZET osmotic pumps filled with either phosphate buffered saline or recombinant human BDNF (5 µg/µl/hr).

During the week of infusion, the animals were stimulated daily via the hippocampal electrode. Thereafter, animals were stimulated twice daily for 14 days. The behavioral and electrical components of the resulting seizure activity were scored, and the infusion of BDNF during kindling suppressed epileptogenesis significantly (Figure 2 on page 3). This effect continued for fifteen days after infusion ended.

Using targeted continuous infusion, Larmet and his group were able to establish the anti-epileptogenic effects of BDNF. The continuous delivery of BDNF to the portion of the brain where seizures originate showed that "BDNF is involved in a long term process which tends to protect hippocampal cells against the development of epileptic seizures".[2]

The ability to infuse agents directly and continuously to specific regions of the brain can elucidate the effects of neurotrophins and other growth factors on neurological disorders. For more information about cerebral or

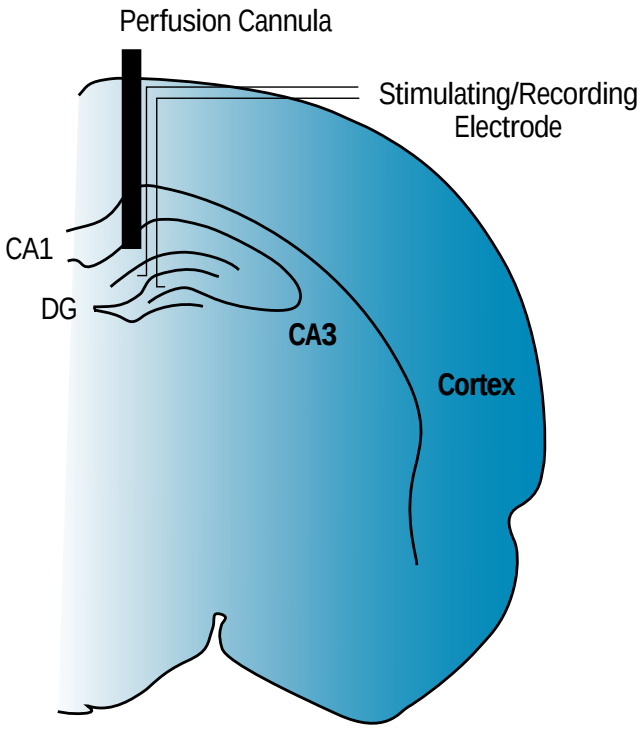


Figure 1:
Brain infusion cannula and stimulating/recording electrode placement relative to DG (dentate gyrus) and hippocampal pyramidal cell layer fields CA1 and CA3.[1]

targeted infusion, call ALZET Technical Services at (800) 692-2990. References on neurotrophic factor infusion are also available.

1. Gall CM. Seizure-induced changes in neurotrophin expression: Implications for epilepsy. *Exp Neurol* 1993; 124:150-166.
2. Larmet Y, Reibel S, Carnahan J, Nawa H, Marescaux C, Depaulis A. Protective effects of brain-derived neurotrophic factor on the development of hippocampal kindling in the rat. *NeuroReport* 1995; 6:1937-1941.
3. McNamara JO. Cellular and molecular basis of epilepsy. *J Neurosci* 1994; 14(6):3413-3425.
4. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969; 25:295-330.
5. Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalography & Clin Neurophysiology* 1972; 32:281-294.

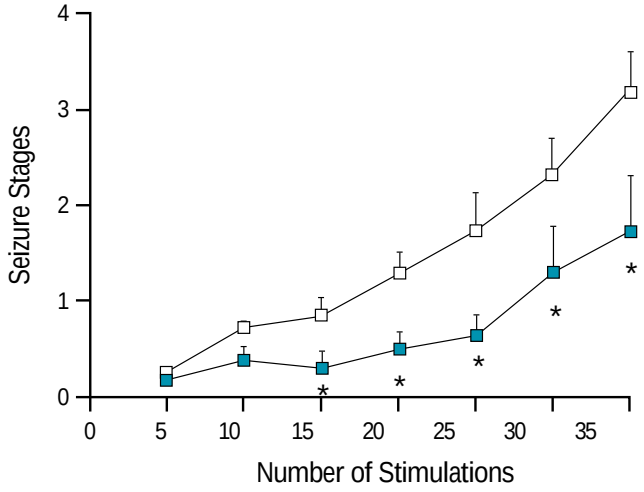


Figure 2:
Animals which received a continuous infusion of BDNF (■) exhibited less intense seizures than those which received PBS (□). Starred points differed from controls significantly ($p < 0.02$). Seizure stages as defined by Racine are: 1, mouth and facial movements; 2, head nodding; 3, forelimb clonus; 4, rearing.[5]

New Agents in the ALZET Literature

AGENT	THERAPEUTIC CATEGORY
CI-959	Anti-inflammatory
CTOP	μ-Opioid Receptor Antagonist
DENSPM	Polyamine Spermine Analog
DMPO	Spin Trapping Compound
HS-142-1	Non-Peptide ANP Receptor Antagonist
Imipromidine	H_2 Agonist
Platelet Factor 4	Neurotoxin
SNX-111 & 239	φ-Conopeptides; Calcium Channel Blockers
Tianeptine	Tricyclic Antidepressant
TIPP	δ-Opioid Receptor Antagonist
Trihexyhenidyl HCl	M_1 Receptor Agonist
Valsartan	AT_1 Receptor Antagonist
Vascular Endothelial Growth Factor	Growth Factor

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).

Antisense Inhibits Tumor Formation in Burkitt's Lymphoma

The first antisense therapeutic agents are now entering phase III clinical trials, as the race to realize the full potential of this technology charges ahead. For over five years, researchers in this field have used ALZET pumps for the in vivo administration of oligonucleotides in order to broaden their understanding of a technology optimistically dubbed the "magic bullet". Synthetic oligonucleotides can regulate the expression of specific genes in vivo. Not only is this useful for clarifying the role of the targeted gene, but it also allows intervention when the gene is over-expressed or has undergone mutation. A review of select antisense citations can be found in Table 1 on page 5.

Burkitt's lymphoma results from genetic error. In this B-cell tumor, the c-*myc* gene is translocated from its normal position on band q24, at the distal end of chromosome 8, into the immunoglobulin (Ig) heavy chain region of chromosome 14.[1] This translocation deregulates expression of the c-*myc* oncogene, which is the critical component of Burkitt's pathogenesis, and results in over-proliferation of pre-B lymphocytes. In 1988, Harris et al. created an animal model for this disease by introducing a normal *myc* gene, designated Eμ-*myc*, into the Ig heavy chain enhancer region of a mouse.[2] In this transgenic model, the inserted gene is expressed exclusively in the B-cell lineage, causing proliferation which begins before birth, and typically results in malignant lymphoma.

Hoping to down-regulate c-*myc* expression before tumor formation, Huang, Snyder, Kligshteyn, and Wickstrom subcutaneously implanted Model 2002 ALZET pumps containing anti-c-*myc* DNA phosphorothioate into weanling Eμ-*myc* mice.[3] By sequentially implanting ALZET pumps, Huang et al. were able to administer antisense DNA for 6 weeks. At 15 weeks of age, 18/24 control animals given either saline or scrambled DNA had palpable tumors. Only 3/12 mice treated with antisense DNA developed tumors. Furthermore, the tumor-free mice remained so for as long as 16 weeks (Figure 3). Thus, anti-c-*myc* DNA dramatically inhibited tumor formation, a chemoprotective effect not demonstrated previously.

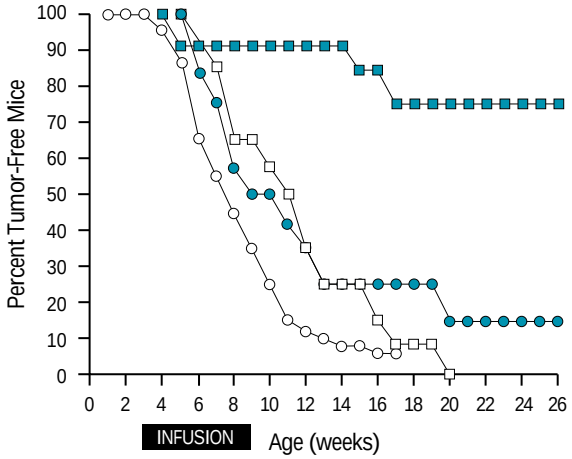


Figure 3:
Transgenic Eμ-myc mice carrying a gene translocation responsible for Burkitt's lymphoma were treated with antisense DNA for 6 weeks by ALZET pumps. Treated animals (■) were less likely to develop tumors during and after treatment as compared with untreated (○), saline-treated (□), and scrambled DNA-treated animals (●).

Several other antisense delivery methods were used by this group. Semi-weekly subcutaneous injections proved equally effective at preventing tumor formation, but caused skin damage at the injection site. The authors postulated that since the MYC protein is an important regulator of keratinocyte growth and differentiation in mature epidermis, down-regulation of c-*myc* initiated apoptosis and inhibited cell proliferation, resulting in the local toxicity observed. The authors also attempted antisense delivery using commercial pellets containing DNA. However, they reported that "...the total amount of DNA in the pellets and the rate of release from each pellet varied widely and unpredictably."[3] Tumor formation in the group that received pellets did not differ from controls after 4 weeks of treatment. "Pellet administration was discarded following these unpromising results. Inconsistent, poor delivery of antisense DNA may have resulted in a low effective dose, obscuring any antisense effects."[3] In sharp contrast, ALZET pumps provided the continuity of exposure necessary for effective treatment.

Huang et al. continue to study the effects of anti-c-*myc* DNA, having initiated dose-response experiments to determine the lowest effective dose. Meanwhile, ALZA has introduced a 4-week pump equal in size to that used in the work described above. For more information on this new model, or to obtain a customized list of references from our extensive bibliography of ALZET publications, please contact ALZET Technical Services at (800) 692-2990 or (415) 962-2251.

1. Magrath I. *Adv Cancer Res* 1990; 55: 133-270.
2. Harris AW, Pinkert CA, Crawford M, Langdon WY, Brinster RL, & Adams JM. *J Exper Medicine* 1988; 167:353-371.
3. Huang Y, Snyder R, Kligshteyn M, Wickstrom E. *Molecular Medicine* 1995; 1(6):647-658.

Summary of Antisense Research Using ALZET® Pumps

Table 1

REFERENCE	GENE TARGET	ANIMAL MODEL	DURATION; PUMP MODEL	DISEASE TARGET
Liebsch et al. Regulatory Peptides 1995;59:229-239	CRH_1 Receptor	Rat	4 days; 1007D	Anxiety
Landgraf et al. J Neurosci 1995;15(6):4250-4258	V_1 Vasopressin Receptor	Rat	4 days; 1007D	Anxiety; Social Discrimination
Plata-Salaman et al. Mol Brain Res 1995;33:72-78	G-Protein α-Subunit Subclasses	Rat	2001	Eating Disorders
Hijiya et al. Proc Natl Acad Sci USA 1994;91:4499-4503	c-*myb*	SCID Mice	7, 14 days	Cutaneous Melanoma
Iversen et al. Antisense Res Dev 1994;4:43-52	*rev* of HIV Type I	Rat	28 days	HIV
Spampinato et al. Proc Natl Acad Sci USA 1994;91:8072-8076	ß-Endorphin mRNA	Rat	60 hours; 1003D	N/A
Stepkowski et al. J Immunol 1994;153:5336-5346	ICAM-1	Mice	7, 14 days	Heart Allograft Rejection
Gewirtz. Leuk Lymphoma 1993;11(1):131-137	c-*myb*	SCID Mice	3, 7, 14 days	Chronic Myelogenous Leukemia
Higgins et al. Proc Natl Acad Sci USA 1993;90:9901-9905	p65 Subunit of NF-kB	Nude Mice	Unknown	Fibrosarcoma, Melanoma
Zhang & Creese. Neurosci Lett 1993;161:223-226	Dopamine D_2 Receptor	Rat	3 days; 1003D	Schizophrenia, Parkinson's Disease
Ratajczak et al. Proc Natl Acad Sci USA 1992;89:11823-11827	c-*myb*	SCID Mice	3, 7, 14 days; 1003D, 2001	Leukemia
Whitesell et al. Antisense Res Dev 1991;1:343-350	N-*myc*	Nude Mice	14 days; 1007D	Cancer

Matrix Metalloproteinase Inhibitor Testing in Novel Arthritis Models

Arthritis results from the progressive destruction of articular cartilage. Common treatments for arthritis include NSAIDs, gold, immunosuppressants, and other types of agents. Unfortunately, no current therapy is both well-tolerated and effective at preventing cartilage destruction. Though the mechanisms of cartilage degradation are not yet known, collagenase is the most likely matrix metalloproteinase to mediate this process. Further investigation into this theory has been hampered by a lack of specific inhibitors and the difficulty of measuring collagen degradation in vivo. To address these issues, Karran et al. developed two in vivo models of cartilage degradation which allowed the testing of novel metalloproteinase inhibitors.[1,2]

In one of Karran's models, type I collagen was prepared from rat skin, acetylated with [^{3}H] or [^{14}C], and embedded in 14 mg cotton buds. After collagen fibrils formed, the cotton buds were implanted subcutaneously into rats to elicit an inflammatory response. The resulting granuloma progressively invaded the cotton bud, degrading the radiolabelled collagen. The key assumption in this model is that this granuloma is similar to the invading pannus which causes cartilage erosion in arthritis. Using standard scintillation techniques, the degree of radiolabelled collagen breakdown was assessed upon cotton bud removal.[1]

Several agents were infused into the center of the cotton buds via a catheter connected to an ALZET pump. The pump was used to guarantee site-specific delivery to the point of collagen degradation. Of the 13 agents infused, only the synthetic collagenase inhibitors CI-A and CI-C proved effective at inhibiting collagen degradation. CI-A inhibited the degradation of radiolabelled collagen by 26%, while CI-C inhibited collagen degradation completely (Figure 4).

In a second model, Karran et al. implanted intact articular cartilage in place of the cultured collagen fibrils used in the previous model. Degradation of intact cartilage mimics the arthritic process more closely. In this model, CI-C inhibited collagen removal by 65% ($p < 0.05$).[2]

The work of Karran et al. illustrates how novel targeted delivery methods can be used to generate data critical to the drug development process. The authors concluded that the "collagen-gelled cotton bud can be cannulated and agents delivered directly to the site of collagen degradation using subcutaneously implanted osmotic mini-pumps: this permits the testing of agents of unknown systemic toxicity and bioavailability. This is a simple, sensitive and adaptable in vivo model of collagen degradation."[1] For more information about using targeted delivery methods in drug development, contact ALZET Technical Services at (800) 692-2990.

1. Karran EH, Dodgson K, Harris SJ, Markwell RE, Harper GP. A simple in vivo model of collagen degradation using collagen-gelled cotton buds: the effects of collagenase inhibitors and other agents. *Inflamm Res* 1995; 44:36-46.
2. Karran EH, Young TJ, Markwell RE, Harper GP. In vivo model of cartilage degradation - effects of a matrix metalloproteinase inhibitor. *Ann Rheum Dis* 1995; 54:662-669.

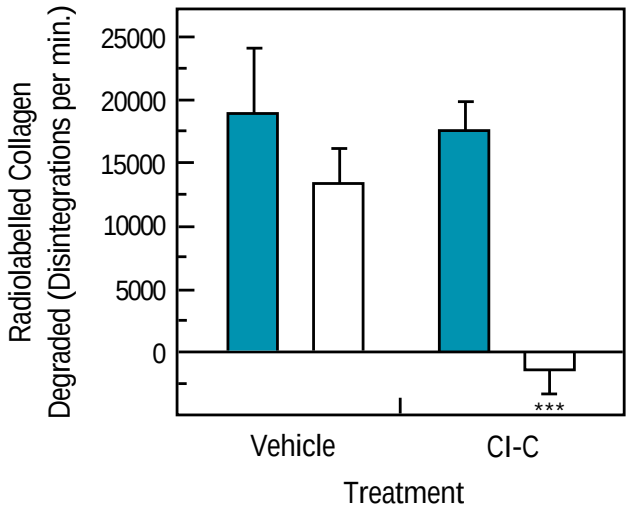
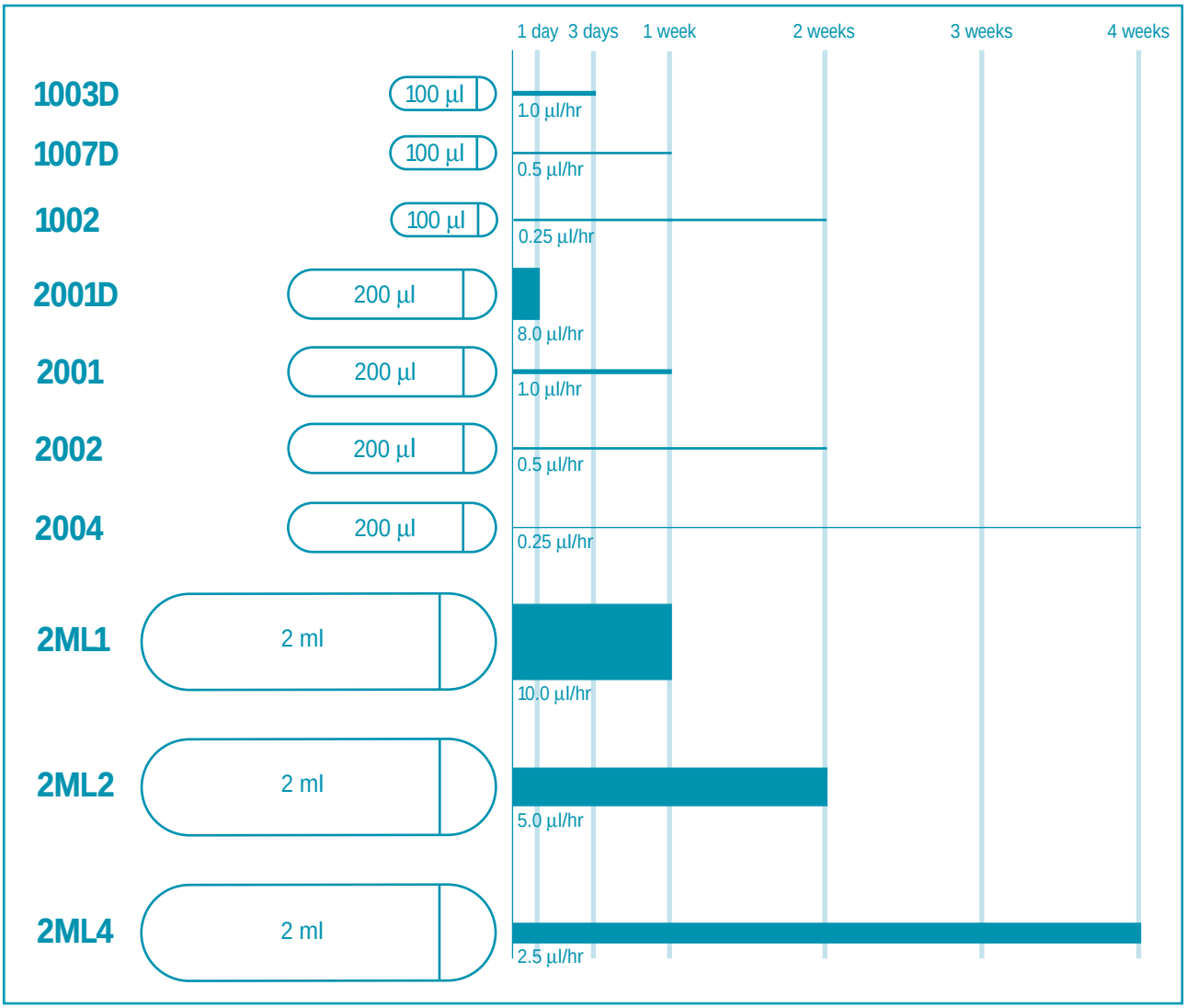


Figure 4:
*The infusion of 80 nmoles/hr CI-C inhibited collagen degradation completely as compared to vehicle-infused controls. White bars indicate cotton buds infused with agent while dark bars represent contralateral controls (***p < 0.001).*

Delivery Rates and Reservoir Capacities of ALZET® Osmotic Pumps



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ALZET Technical Services

If you have questions about ALZET pumps or need assistance planning your experiments, please contact ALZET Technical Services. Catherine Oyler, our Technical Information Associate, can help you determine the correct model of pump to use or assess whether previous ALZET pump research has been done in your field of interest. To reach Catherine with your technical inquiries, please call ***(800) 692-2990*** *or* ***(415) 962-2251****. She can also be reached at fax number (415) 962-2488, or via e-mail address* ***alzet@alza.com****.*

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