Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4665–4669, May 1993
Medical Sciences

Stability, clearance, and disposition of intraventricularly administered oligodeoxynucleotides: Implications for therapeutic application within the central nervous system

(antisense/phosphorothioate oligonucleotide/brain tumor/chemotherapeutic)

LUKE WHITESELL*†, DANIEL GESELOWITZ*, CHRISTINE CHAVANY*, BRIGID FAHMY*, STUART WALBRIDGE‡, J. R. ALGER§, AND L. M. NECKERS*

*Tumor Cell Biology Section, Clinical Pharmacology Branch, National Cancer Institute, and ‡Surgical Neurology Branch and §Neuroimaging Branch, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD 20892

Communicated by Gilbert Stork, February 8, 1993

ABSTRACT **We report experiments in the rat demonstrating the feasibility of intraventricular administration of oligodeoxynucleotides (ODNs) as a regional treatment approach to disorders within the central nervous system (CNS). Although we find little intrinsic nuclease activity in cerebrospinal fluid (CSF), phosphodiester ODNs are rapidly degraded by brain-associated α-exonuclease activity. Phosphorothioate ODNs, however, appear resistant to degradation in the CNS and, after intraventricular administration, we find they are cleared in a manner consistent with CSF bulk flow. Continuous infusion of ODN at 1.5 nmol/hr by miniosmotic pump can maintain micromolar concentrations of intact phosphorothioate ODN in CSF for at least 1 week without obvious neurologic or systemic toxicity. After infusion, extensive brain penetration and marked cellular uptake, especially by astrocytic cells, is demonstrated.**

In the laboratory, antisense oligodeoxynucleotides (ODNs) have repeatedly demonstrated efficacy in modulating the expression of various genes, thus providing important insights into their roles in tumorigenesis or normal growth and development (1–3). Although attention has been focused recently on the development of antisense ODNs as therapeutics for a variety of diseases (4), data on systemic application of ODNs have been slow to accumulate.

To avoid obstacles associated with systemic administration, we and others have focused on regional therapeutic strategies to evaluate ODN actions *in vivo* (5, 6). We now describe the stability, disposition, and clearance of ODNs within a clinically important, physiologically well-defined biologic compartment—namely, the cerebrospinal fluid (CSF) space of the rat. We report that potentially therapeutic concentrations of intact phosphorothioate ODN can be maintained within the CSF without gross toxicity to the animal. Extensive penetration into brain parenchyma can be demonstrated. These findings support the feasibility of ODN-based therapeutic approaches to viral and neoplastic disorders within the central nervous system (CNS).

MATERIALS AND METHODS

ODN Synthesis, Modification, and Purification. Lyophilized preparations of several unmodified 15-base phosphodiester ODNs were obtained from Gilead Sciences (Foster City, CA) or Synthecell (Gaithersburg, MD). The sequences used in this study were as follows: 5′-ATG CCG AGC TGC TCC-3′ (ODN 1) and 5′-GGA GCA GCT CGG CAT-3′ (ODN 2). Both ODNs were used interchangeably and behaved identically in all cases. These ODNs were synthesized by standard cyanoethyl phosphoramidite chemistry and were chromatographically purified. Corresponding 15-base phosphorothioate ODNs were prepared on an Applied Biosystems 380B automated synthesizer by using phosphoramidite chemistry and Beaucage sulfurization reagent (Glen Research, Sterling, VA) in the oxidation step (7).

The 5′ radiolabeling of phosphodiester ODNs was performed with polynucleotide kinase (BRL) according to the manufacturer's instructions. The 3′ radiolabeling of sequences was performed with terminal deoxynucleotidyltransferase (BRL) according to the manufacturer's instructions. Labeled material was added as tracer ($1–5 \times 10^7$ cpm per μmol of total DNA) to ODN infusions.

Internal fluorescein labeling of both phosphodiester and phosphorothioate ODNs (ODN 1) was accomplished at the 8th base position (from the 3′ end) during automated synthesis with a fluorescein amidite reagent (Glen Research). Fluorescein-labeled ODNs were used either undiluted or diluted up to 1:3 with nonlabeled ODN of the same sequence.

Analysis of ODN Stability *in Vitro*. Solutions of 20 μM fluoresceinated ODN (both phosphodiester and phosphorothioate) were prepared in human CSF containing 100 units of penicillin per ml and 100 μg of streptomycin per ml (Biofluids, Rockville, MD). A vol of 0.75 ml was added per well in standard 24-well tissue culture plates (Costar). To some wells a 5-mm-thick axial cross-section of freshly isolated rat brain weighing 20–30 mg was added to the ODN/CSF solutions. The plate was incubated at 37°C in a humidified incubator. At 1, 6, and 24 hr postplating, CSF was harvested for analysis. A 50-μl aliquot was analyzed directly on an automated fluorescence plate reader (Fluoroskan 2; Flow Laboratories). Readings were compared to a standard curve prepared on the same plate by serial dilution of fluoresceinated ODN in CSF. The remainder of the CSF was extracted with an equal volume of water-saturated phenol followed by phenol/chloroform (1:1), chloroform, and ethanol precipitation. After a 70% ethanol wash, the pellet was dissolved in urea loading buffer [8 M urea/1× TBE (100 mM Tris/90 mM boric acid/1 mM EDTA)], heated to 90°C, and electrophoresed on a 15% polyacrylamide/8 M urea/1× TBE gel. After electrophoresis, fluorescein-containing bands were identified by long-wave UV transillumination.

Tissue accumulation of ODN was analyzed by digestion of rinsed brain sections in 1 ml of lysis buffer (200 mM Tris, pH 8.5/100 mM EDTA/1% SDS/1 mg of proteinase K per ml) at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; IVC, intracerebroventricular catheter; ODN, oligodeoxynucleotide.

†To whom reprint requests should be addressed at: Clinical Pharmacology Branch, National Cancer Institute, Building 10, Room 13N262, Bethesda, MD 20892.

56°C overnight. After digestion, lysates were spun at 12,000 × *g* for 10 min and 50-μl aliquots of supernatant were analyzed on the Fluoroskan 2 as described above except that a standard curve was prepared in lysis buffer instead of CSF. The remainder of tissue lysate supernatant was ultrafiltered through a 1-kDa cut-off microconcentrator (Filtron Technology, Northborough, MA). The retained solution was recovered in 0.5 ml of water and refiltered through a 3-kDa cut-off microconcentrator (Amicon). Aliquots of the retained material after ultrafiltration were analyzed for the presence and integrity of fluoresceinated ODN by gel electrophoresis and UV transillumination as described above.

Intraventricular ODN Administration. Female 250-g Sprague–Dawley rats with indwelling 22-gauge stainless steel cannulae stereotactically implanted in the lateral ventricle were obtained from Zivic–Miller. Fifty microliters of either ^{32}P- or fluorescein-labeled ODN/[^{3}H]inulin (2.84 Ci/mmol; 1 Ci = 37 GBq; Amersham) mixture was infused into cannulae over 1 min with a 100-μl Hamilton syringe. At various intervals after injection, 20- to 30-μl CSF samples were obtained via cisterna magna puncture and aspiration. Samples were analyzed by scintillation counting with crossover correction. Samples containing fluorescein-labeled material were analyzed on a fluorescent plate reader as described above. Integrity of ODN within CSF samples was analyzed by gel electrophoresis and transillumination or autoradiography as indicated.

For continuous infusion experiments, a miniosmotic pump designed to deliver 1 μl/hr for 7 days (Alzet 2001; Alza) was filled with ODN/[^{3}H]inulin solution, and a 5-cm length of polyethylene tubing (PE 50; Clay Adams) was inserted onto the flow moderator. After overnight priming at 37°C, pump and tubing were implanted subcutaneously. Tubing was then fed through a subcutaneous tunnel rostrally to emerge at the site of intracerebroventricular catheter (IVC) placement. It was fitted to the stainless steel cannula and fixed in place with dental cement. At intervals after pump implantation, CSF was sampled via cisternal puncture and analyzed as described above.

Clearance $t_{1/2}$ values for inulin and phosphorothioate ODNs were determined from steady-state concentrations by the formula

$$\frac{D \times f}{T} = C_{ss} \times V_d \times \frac{0.693}{t_{1/2}},$$

where D is dose, f is fraction absorbed, T is interval of administration, C_{ss} is concentration at steady state, and V_d is volume of distribution.

All animal procedures involving pain/discomfort were performed under general anesthesia induced by Metofane (Pitman–Moore, Mundelein, IL) inhalation or intraperitoneal injection of 0.2 mg of ketamine/0.01 mg of xylazine per kg of body weight. All procedures were performed under an animal study protocol approved by the National Cancer Institute/Division of Cancer Treatment-Animal Care and Use Subcommittee.

Fluorescence Microscopy. After fluoresceinated ODN administration, animals were sacrificed and brains were rapidly removed. Cryostat sections were prepared by standard techniques. After paraformaldehyde fixation, sections were incubated with mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) (Boehringer Mannheim) (8). Phycoerythrin-conjugated goat anti-mouse antibody (Tago) was applied as a secondary detecting antibody. After coverslipping with Pro-texx mounting medium (Lerner Laboratories, Pittsburgh), slides were photographed under UV epi-illumination with fluorescein and rhodamine filters on a Zeiss Axiophot microscope.

RESULTS

***In Vitro* ODN Stability and Tissue Uptake.** Both phosphodiester and phosphorothioate ODNs are quite stable in CSF after up to 24 hr of incubation at 37°C (Fig. 1 *B* and *C*, lanes 5–8). The total concentration of fluorescein label in CSF also remains relatively constant during incubation independent of the type of ODN labeled and of whether or not tissue is present (data not shown). However, addition of brain tissue results in near complete loss of intact phosphodiester ODN by 24 hr (Fig. 1*C*, lanes 1–4). Phosphorothioate ODN stability, on the other hand, is insensitive to the presence of tissue and ODN remains intact for at least 24 hr (Fig. 1*B*, lanes 1–4). Consistent with these degradation findings, very little fluorescein label can be recovered from the brain slice when phosphodiester ODN is used (Fig. 1*D*) and no intact phosphodiester ODN can be seen on gel electrophoresis (data not shown). When phosphorothioate ODN is used, however, a significant accumulation of label by the slice (almost 10% of the total incubated material) can be detected by 24 hr (Fig. 1*D*). Moreover, as Fig. 1*A* demonstrates, the label recovered represents intact phosphorothioate material.

Intracerebroventricular Bolus Kinetics. To standardize experiments and allow for meaningful comparison among ODN modifications, we coinjected [^{3}H]inulin, a well-recognized marker of extracellular space sharing the same approximate molecular mass (5 kDa) as the ODNs under investigation (9). The level of [^{3}H]inulin marker measured in CSF after bolus injection behaves quite consistently in this rat model. Generally, ≈5 min after completion of a 50-μl injection, inulin

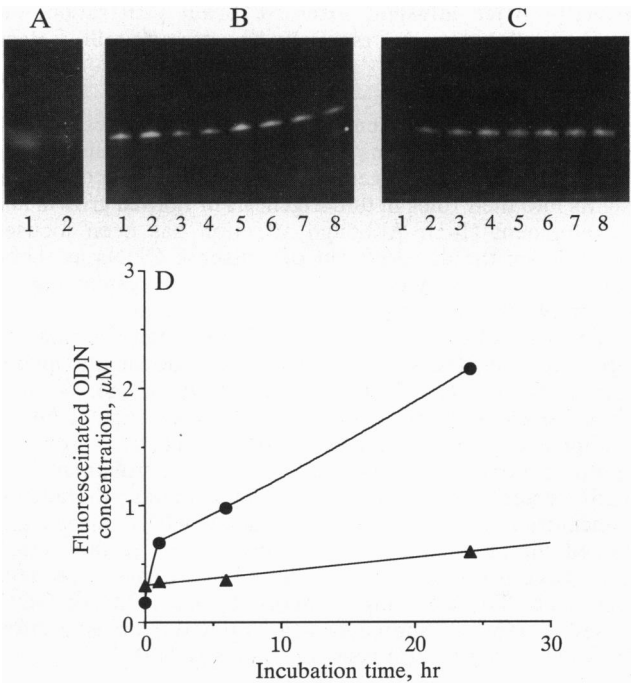

FIG. 1. ODN stability and tissue accumulation *in vitro*. (*A*) Electrophoretic analysis of fluorescent ODN extracted from brain sections after incubation in CSF containing 20 μM phosphorothioate ODN. Lanes: 1, 24-hr incubation; 2, 6-hr incubation. (*B*) Electrophoretic analysis of phosphorothioate ODN integrity after incubation in CSF. Lanes: 1–4, incubation with brain tissue present for 24, 6, 2, and 0 hr, respectively; 5–8, incubation without brain tissue present for 24, 6, 2, and 0 hr, respectively. (*C*) Same conditions and time points as in *B* except phosphodiester ODN was used. (*D*) Quantitation of total fluorescence extractable from brain tissue after incubation of slices in CSF containing 20 μM phosphodiester ODN (▲) or phosphorothioate ODN (●). This experiment was performed once with independent brain slices at each time point and for each ODN type.

concentration in a CSF sample is 20–30% that of the injected material. During the next 2 hr, concentration falls with non-first-order kinetics (Fig. 2*A*). Inulin is thought to be cleared mainly by bulk flow; we believe the initial apparent $t_{1/2}$ observed reflects clearance of inulin from the ventricle, while the secondary $t_{1/2}$ represents dispersion and bulk flow from the total CSF. However, for several reasons determination of clearance $t_{1/2}$ values from such bolus data is subject to experimental artifact. First, distortion of CSF volume can occur due to an initial administration of 10% of total volume, followed by repeated sampling of up to 4% of that volume. Second, the level of radioactivity sampled at later time points is quite low and subject to error. Third, normal circulation of CSF in the rat depends in part on animal movement (data not shown) and the animals are anesthetized during the course of the bolus experiments. Therefore, we have not used bolus kinetic data to estimate clearance $t_{1/2}$ values. Instead, these values have been estimated from the steady-state concentration of ODN and inulin obtained during Alzet pump infusion (see below).

When phosphodiester ODNs end-labeled with ^{32}P at the 5′ or 3′ end are coinjected with inulin, the ratio of ^{32}P in the CSF relative to injected solution is reduced compared to inulin by ≈20% at 5 min, and this relative depletion of ^{32}P typically increases during the course of experiments. For both labeling schemes, the mean ratio of inulin to ^{32}P compared to injected material is 7:1 after 2 hr (0.078 ± 0.008 vs. 0.011 ± 0.001; $P = 0.001$). Systemic administration of a dose of probenecid reported to completely inhibit the choroid plexus small anion efflux pump (10) has no effect on the kinetics of label disappearance (data not shown). When phosphodiester ODN internally labeled with fluorescein is used and total CSF fluorescence is monitored, the ratio of ^{3}H to fluorescence is typically reduced to ≈2:1 after 2 hr. This variant behavior is readily apparent in Fig. 2*A*, which plots the various inulin/ODN ratios obtained in a representative experiment with dual-labeled ODN carrying both internal fluorescein and 5′ ^{32}P. Regression analysis confirms that the ODN disappearance curves generated by monitoring either ^{32}P or fluorescence are significantly different from each other ($P < 0.05$).

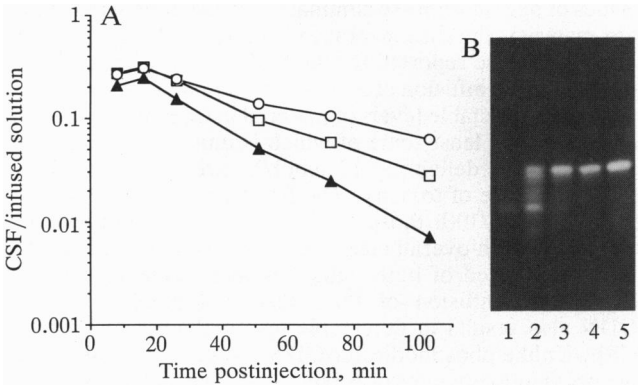

FIG. 2. Phosphodiester ODN clearance and degradation after IVC bolus injection of fluorescein/^{32}P dual-labeled ODN and [^{3}H]inulin. Concentrations are presented relative to injected material. (*A*) ○, Concentration of inulin determined by liquid scintillation counting of ^{3}H cpm in CSF samples obtained at intervals after injection; □, concentration of ODN as determined by fluorescence intensity of CSF samples; ▲, concentration of ODN as determined by ^{32}P cpm in CSF samples. (*B*) Electrophoretic analysis of ODN degradation after bolus injection. Lanes 1–5, CSF obtained at 105, 72, 50, 22, and 12 min, respectively, after injection of dual-labeled material as described above. ODN visualized by UV transillumination. This dual label experiment is representative of six animals in which ^{32}P-labeled ODN was used and three animals in which fluoresceinated ODN was used to measure the kinetics of bolus phosphodiester ODN clearance.

Both patterns of apparent clearance are observed depending on which label is monitored.

This apparent discrepancy in clearance rates is most readily explained by degradation of phosphodiester ODN within the CSF space after injection. Electrophoresis of CSF samples after injection of fluorescein-labeled material demonstrates extensive degradation of material recovered after 100 min (Fig. 2*B*). Such a finding is consistent with the behavior of phosphodiester material observed *in vitro* in the presence of brain tissue. When 3′-labeled ODN is used, no size ladder of degradation products is seen on autoradiographs, although intact ODN rapidly disappears (when equivalent cpm are loaded per lane; data not shown). When a 5′ label is used, a ladder similar to that seen with internal fluorescein-labeled material (Fig. 2*B*) can be seen. These results suggest that degradation of phosphodiester ODN is initiated primarily at the 3′ end. The more rapid apparent clearance of 5′ ^{32}P label shown in Fig. 2*A* compared to fluorescein label also suggests the presence of significant brain-associated phosphatase activity in addition to nuclease activity.

When phosphorothioate ODN with internal fluorescein label is injected as an IVC bolus, the level of fluorescence in CSF closely matches the ^{3}H level over 100 min (Fig. 3*A*). In contrast to the findings with phosphodiester material, electrophoretic analysis of CSF samples after phosphorothioate bolus injection reveals only intact ODN even after 100 min (Fig. 3*B*). Again, these findings are consistent with the pattern of stability observed *in vitro* in the presence of fresh brain slices.

Continuous IVC Infusion. Given the poor distribution (data not shown) and relatively rapid clearance demonstrated for ODNs after bolus administration, we explored the potential of a continuous infusion technique to maintain steady-state levels of ODN within the CSF of free-roaming rats. Concentrations up to 15 mM phosphodiester ODN are tolerated at a rate of 1 μl/hr without obvious neurologic or systemic toxicity after 7 days. Using 5′-end-labeled material, a total ^{32}P steady-state level of ≈0.01% of the concentration of the infused solution is seen (data not shown). This level is ≈1/10th the [^{3}H]inulin level we generally observe during

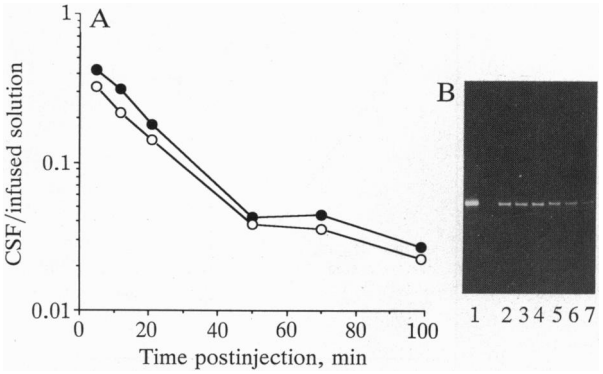

FIG. 3. Phosphorothioate ODN clearance and integrity following IVC bolus injection of fluorescein-labeled ODN and [^{3}H]inulin. Concentrations are presented relative to injected solution. (*A*) ●, Concentration of inulin determined by liquid scintillation counting of ^{3}H cpm in CSF samples obtained at intervals after injection; ○, concentration of ODN as determined by fluorescence intensity of CSF samples. (*B*) Electrophoretic analysis of ODN degradation after bolus injection. Lanes: 1, 1 μl of injected solution as standard; 2–7, CSF samples obtained at 5, 12, 20, 48, 68, and 100 min, respectively, after injection. ODN was visualized by UV transillumination. This experiment was performed in three rats in which independent samples were drawn from CSF at the time points indicated. No significant differences were observed (by regression analysis) between the ODN and inulin clearance kinetics.

infusion and correlates well with the depletion of ^{32}P relative to ^{3}H we describe for late time points after bolus injection. Due to the extensive tissue-associated degradation of phosphodiester ODN we observe in bolus experiments and *in vitro*, we have not attempted to rigorously quantitate the level of intact phosphodiester material maintained during infusions. Gel electrophoresis of CSF samples, however, does reveal detectable, intact ODN. In light of degradation issues, we next examined the feasibility of continuous IVC infusion of fluorescein-labeled phosphorothioate ODN. Concentrations of infused solution that were >3 mM administered at 1 μl/hr are toxic. Global CNS depression followed by death occurs within 24 hr of infusion initiation. Concentrations up to 1.5 mM, however, are well tolerated during a 1-week period. ODN levels in the CSF of 0.1% of the infusion concentration can be readily maintained during infusion periods (Fig. 4). As might be expected from bolus observations, ODN levels correlate almost identically with the fractional level of [^{3}H]inulin observed in a particular animal. The material recovered in CSF appears completely intact by gel electrophoresis (data not shown). We have calculated a clearance $t_{1/2}$ for phosphorothioate ODN based on steady-state values ($n = 3$) to be 17.2 ± 4.7 min. The corresponding clearance $t_{1/2}$ calculated for inulin ($n = 9$) is 23 ± 7.5 min. This value is consistent with previously published data on inulin clearance out of CSF (9).

Tissue Penetration. Given that phosphorothioate ODN appears stable in the presence of brain tissue, we examined phosphorothioate ODN penetration into brain by fluorescence microscopy. Paraformaldehyde-fixed frozen sections prepared from rat brains 30 min after bolus injection of 40 nmol of a fluorescein-labeled phosphorothioate ODN demonstrate readily detectable tissue signal (Fig. 5*A*). A gradient distribution is apparent with the highest signal noted at the ependymal surfaces, consistent with diffusion in from the surrounding CSF. The endothelium of small vessels also appears highly fluorescent, suggesting uptake of material that has been cleared into the vasculature. After continuous infusion of phosphorothioate ODN for 1 week (1.5 nmol/hr), a gradient distribution of ODN is less apparent, but marked uptake of fluorescent material is now observed in many cell bodies scattered throughout the tissue section (Fig. 5*B*). GFAP immunostaining of sections prepared from such a phosphorothioate-perfused brain demonstrates colocalization of ODN uptake and GFAP positivity within astrocytes (Fig. 5 *C* and *D*). The identity of non-GFAP-positive cells that accumulate ODN during infusion remains to be determined.

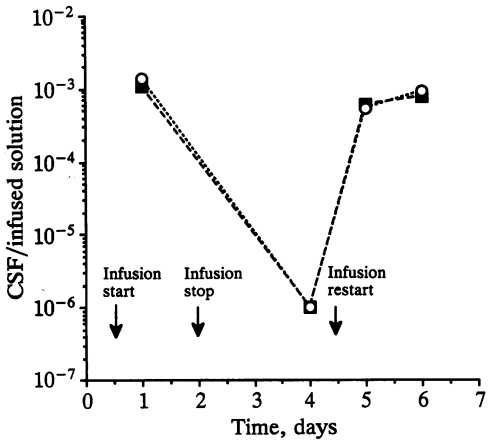

FIG. 4. Phosphorothioate ODN level in CSF during continuous IVC infusion of fluoresceinated ODN/[^{3}H]inulin solution. Concentrations are presented relative to injected solution. ○, Concentration of inulin determined by liquid scintillation counting of ^{3}H cpm in CSF samples obtained during infusion period; ■, concentration of ODN as determined by fluorescence intensity of CSF samples. Miniosmotic pump was disconnected from catheter at the infusion stop point indicated and reconnected at the infusion restart point. This is a representative experiment depicting data obtained at the independent time points shown from one animal. The experiment was repeated using the same time points (without pump shut off) in a total of three animals.

DISCUSSION

We have sought to overcome many of the current obstacles associated with *in vivo* application of ODNs by avoiding the requirement for systemic administration. Because many drugs including ODNs show very poor penetration into the CNS after systemic administration (11), regional therapeutic strategies are particularly attractive in this organ system (12). The CSF space of the rat provides a practical, clinically relevant, physiologically well-defined compartment in which to systematically examine ODN actions *in vivo*.

Unlike blood and serum, CSF possesses little intrinsic nuclease activity. Unmodified phosphodiester ODNs remain undegraded after incubation in CSF *ex vivo* for up to 24 hr at 37°C. This finding holds for both rat and human CSF, but care must be taken in small animals to avoid contamination with blood during the collection process or serum-associated nuclease activity can be inadvertently introduced. However, brain tissue *ex vivo* or in the living animal possesses abundant nuclease and phosphatase activity. The predominant mode of degradation as determined from end-label experiments is consistent with 3′ attack by α-exonuclease(s). Phosphorothioate-modified linkages, however, are resistant to this form of attack both *ex vivo* and after IVC administration. Our findings underline the potential for significant artifacts in the study of ODNs *in vivo* that can be associated with common labeling strategies. In our hands, internal fluorescein has been the most convenient and robust label for *in vivo* studies.

After acute bolus administration, ODNs readily flow throughout the ventricular system as demonstrated by the rapid appearance of material in CSF sampled from the extracranial cisterna magna. CSF bulk flow is central to the distribution and clearance of IVC-administered ODN. When issues of degradation are eliminated by use of phosphorothioate material, the disappearance kinetics of ODNs are very similar to those reported for the bulk flow marker inulin (9).

Continuous infusion of 5′ ^{32}P-labeled phosphodiester ODN results in detectable levels of material in CSF over the course of infusion, at least some of which is intact. Concentrations up to 15 mM delivering 15 nmol/hr are tolerated without gross evidence of toxicity. The finding that steady-state ^{32}P levels are ≈1/10th those of [^{3}H]inulin during coinfusion is consistent with overall clearance of phosphodiester material being composed of both bulk flow and tissue degradation. Continuous infusion of fluoresceinated phosphorothioate ODN also results in detectable levels of intact material in CSF. Unlike phosphodiester ODN, however, lethal toxicity occurs at infusion rates of >3 nmol/hr. The steady-state level of phosphorothioate achieved relative to infused solution can be maintained at ≈0.1%, closely matching the steady-state concentration of inulin. Consequently, the well-tolerated concentration of 1.5 mM results in steady-state phosphorothioate ODN concentrations of >1 μM. Unlike phosphodiester material, intact phosphorothioate can be recovered from brain tissue after both *ex vivo* incubation and IVC injection. Fluorescence microscopy also reveals extensive brain uptake of phosphorothioate ODN. Although readily detected, the tissue uptake of phosphorothioate ODN during continuous infusion probably accounts for only a small fraction of the total material administered because it does not appreciably decrease ODN steady-state level in CSF compared to inulin. The striking gradient distribution seen in

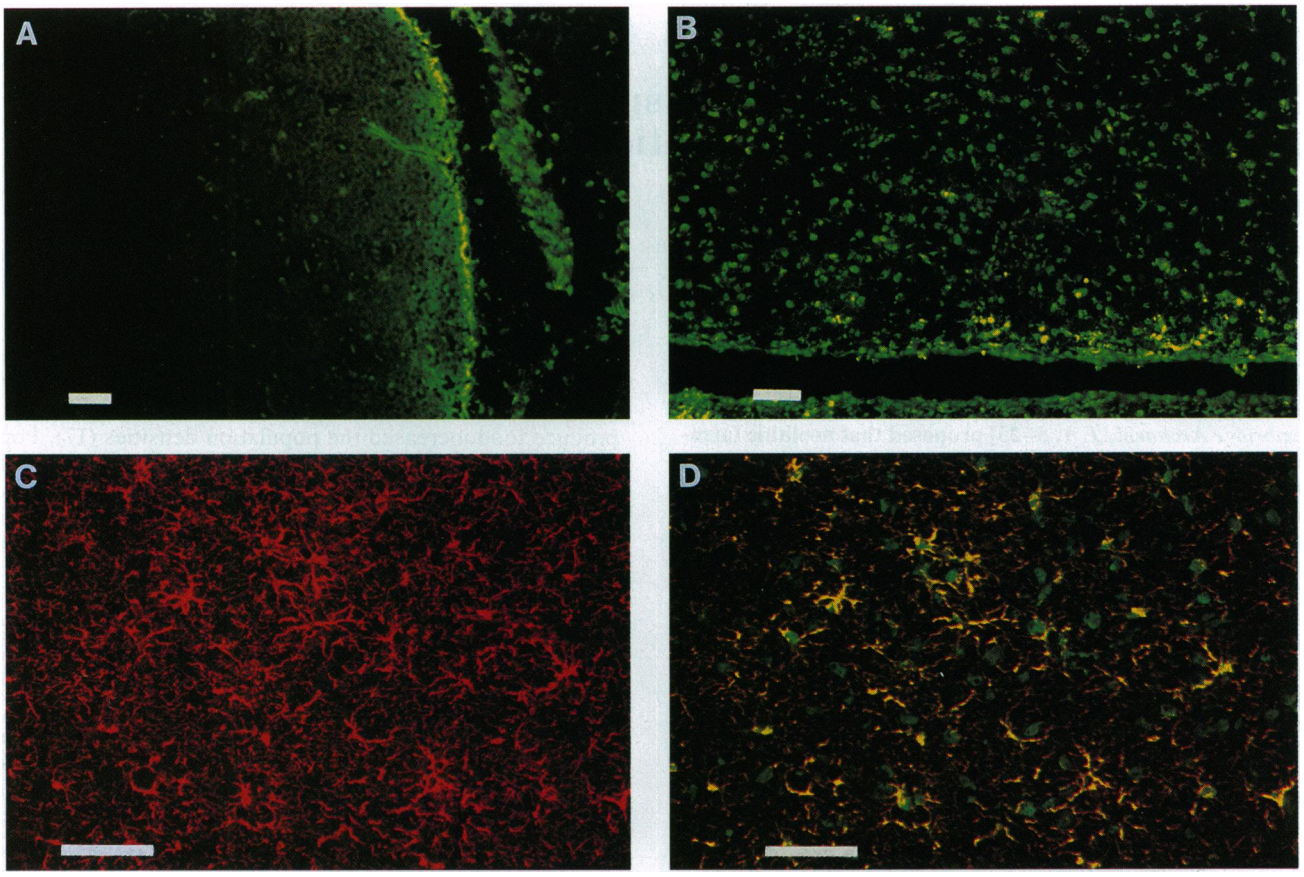

FIG. 5. Fluorescein-labeled phosphorothioate ODN penetration into brain and uptake by cells. (*A*) Thirty minutes post-IVC bolus injection. Green signal demonstrates tissue distribution of ODN. (*B*) Seven days of continuous IVC infusion. Green signal demonstrates tissue distribution of ODN. (*C*) Same rat as in *B*. Brain section was immunostained with anti-GFAP antibody. Red signal demonstrates cells of presumed glial origin. (*D*) Same tissue section as in *C*, but dual wavelength fluorescence filters were used. Green signal demonstrates ODN distribution and uptake. Yellow-orange signal demonstrates ODN–GFAP colocalization. (Bars = 50 μm.)

subependymal brain shortly after bolus injection is probably related to very high transient concentrations of ODN in CSF. After continuous infusion, a more uniform distribution of material is seen with prominent uptake noted in the soma of many cells, especially those of presumed glial (GFAP positive) origin.

The rat model described in this report demonstrates the feasibility of continuously perfusing the CNS with potentially therapeutic concentrations of intact ODN. It clearly indicates that, although CSF itself is low in nuclease activity, ODN modifications that protect at least the 3′ base linkage from exonuclease attack are required to stabilize ODN in the CSF space and achieve tissue penetration of intact material. The penetration into brain we now report suggests the possibility of targeting both leptomeningeal and intraparenchymal disease processes.

We thank Dr. P. Henkart for assistance with fluorescence quantification. We also thank R. Dreyfuss for fluorescent photomicrography assistance. This work was supported in part by a Cooperative Research and Development Agreement with Gilead Sciences.

1. Cohen, J. S. (1991) in *Gene Regulation: Biology of Antisense RNA and DNA*, eds. Erickson, R. P. & Izant, J. G. (Raven, New York), Vol. 1, pp. 247–260.
2. Goodchild, J. (1989) in *Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression*, ed. Cohen, J. S. (Macmillan, London), Vol. 12, pp. 53–77.
3. Neckers, L., Whitesell, L., Rosolen, A. & Geselowitz, D. A. (1992) *CRC Crit. Rev. Oncogenesis* **3**, 175–231.
4. Zamecnik, P. C. (1991) in *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, ed. Wickstrom, E. (Wiley-Liss, New York), Vol. 1, pp. 1–6.
5. Whitesell, L., Rosolen, A. & Neckers, L. M. (1991) *Antisense Res. Dev.* **1**, 343–350.
6. Simons, M., Edelman, E. R., DeKeyser, J.-L., Langer, R. & Rosenberg, R. D. (1992) *Nature (London)* **359**, 67–70.
7. Iyer, R. P., Phillips, L. R., Egan, W., Regan, J. B. & Beaucage, S. (1990) *J. Org. Chem.* **55**, 4693–4698.
8. Debus, E., Weber, K. & Osborn, M. (1983) *Differentiation* **25**, 193–203.
9. Bass, N. & Lundborg, P. (1973) *Brain Res.* **52**, 323–332.
10. Curzon, G., Hutson, P. H., Kantamaneni, B. D., Sahakian, B. J. & Sarna, G. S. (1985) *J. Neurochem.* **45**, 508–513.
11. Agrawal, S., Temsamani, J. & Tang, Y. T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7595–7599.
12. Poplack, D. G. & Riccardi, R. (1987) in *The Role of Pharmacology in Pediatric Oncology*, eds. Poplack, D. G., Massimo, L. & Cornaglia-Ferraris, P. (Nijhoff, Boston), pp. 137–156.