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Interferon-Containing Controlled-Release Polymers for Localized Cerebral Immunotherapy

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ABSTRACT

Controlled-release ethylene-vinyl acetate copolymers (EVAc), which were used previously for the *in vivo* intracerebral delivery of chemotherapeutics, were evaluated as a possible route of localized intracerebral delivery of interferon (IFN). Natural mouse IFN-α/β (Mu-IFN-α/β) was incorporated into polymers at 5% or 10% by weight with 2×10^4 U or 4×10^4 U, respectively. *In vitro* and *in vivo* studies of the release of Mu-IFN-α/β from EVAc polymers showed the released IFN to be biologically active, as determined by the inhibition assay of viral cytopathic effect (CPE). Evaluation of the *in vitro* kinetics of release showed that most of the IFN activity was released in the first 4 days, with the rest being released thereafter. The *in vivo* kinetic release of Mu-IFN-α/β from intracerebrally implanted polymers showed that most of the IFN activity was released within 24 h after polymer implantation in the hemisphere ipsilateral to the polymer. This IFN activity gradually decreased over the next 72 h, with a significant linear trend ($p < 0.0001$). The hemisphere contralateral to the implanted polymer showed no significant levels of IFN activity throughout the 4 days of evaluation. By contrast, blood levels of IFN increased from day 1 to day 4, showing a significant linear trend ($p = 0.0125$), with IFN levels on day 4 being significantly higher ($p < 0.05$) than on day 1 after polymer implant. This study demonstrates the feasibility of intracranial controlled local delivery of IFN using a polymer delivery device.

INTRODUCTION

CONTROLLED-RELEASE POLYMERS have been used to obtain localized, continuous delivery of various chemotherapeutic agents, including proteins and macromolecules.[1–3] Specific therapeutic agents locally released from polymers can achieve high regional levels that could affect neoplastic cells, for example, glioma.[4–9] Malignant glioma is a devastating disease that despite the benefits of surgery, radiation, and chemotherapy, has a poor prognosis with median survival times <1 year for some types of glioma (e.g., glioblastoma multiforme).[10,11] The blood-brain barrier (BBB) limits the entry of certain therapeutic agents into the brain. Therefore, systemic administration of these agents relies on either their passive diffusion through the BBB, the formation of lipid-soluble prodrugs, or the use of monoclonal antibodies (mAb) as carriers for delivery into the CNS.[12] Although the BBB is partially disrupted in brain tumors, the physiologic importance of these changes and estimation of possible benefits for brain tumor therapy are not known.[13] Moreover, very high levels of drugs are required systemically, causing unacceptable toxic side effects.

Malignant gliomas rarely metastasize systemically, and their treatment with localized therapy has been shown to be beneficial. Localized delivery of chemotherapeutic agents after incorporation into biocompatible polymers *in vivo* in a glioma model[5–9] and in clinical trials[6,14–16] showed limited toxicity and increased survival. Consequently, evaluation of other therapies delivered in this manner that would further extend survival of glioma patients is warranted. For example, patients with primary glioblastoma and metastatic tumors might receive additional benefits from localized immunotherapy with cytokines, for example, with interferon (IFN) or combined immunochemotherapy or both.

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Immunotherapeutic agents, such as IFNs, have a broad range of activities in addition to their antiproliferative activity, including local stimulation of the immune response against glioma[17,18] and multiple effects on extracellular matrix and glioma invasion.[19–22] IFNs are potent immunomodulators that show antiproliferative, antiglioma activity *in vitro*[19,23,24] and *in vivo*.[24–26] In clinical trials, treatment with IFN administered systemically to patients with glioma has had modest effects.[27] This relatively low response to systemically administered IFN could be due, in part, to insufficient IFN delivery into the tumor across the BBB. However, if IFNs were delivered in sufficient levels to the glioma, for example, via biocompatible polymers, the resultant higher IFN levels at the tumor may inhibit neoplastic cell growth, enhance depressed immune responses, or inhibit glioma invasion. The effect of combined chemotherapy and immunotherapy, with IFN being delivered via polymers, may also produce additional benefits in the treatment of glioma.[28,29] To our knowledge, this study provides the first report on the biologic activity of IFN incorporated into ethylene-vinyl acetate (EVAc) polymers, in addition to data on the kinetic release of IFN from these polymers *in vitro* and *in vivo* following their intracerebral implantation.

MATERIALS AND METHODS

The first question being addressed is if IFN can be incorporated into the EVAc polymers and if this procedure would allow the IFN biologic activity to be retained. The second question is if IFN concentration in the polymer, 5% or 10%, will affect the duration of IFN release from the polymer. If *in vitro* data show that IFN can retain biologic activity in EVAc polymers and if increasing concentration of incorporated IFN into the polymers would result in a longer time of IFN activity release from polymers, these polymers will be evaluated *in vivo*.

Polymer preparation

EVAc (40% vinyl acetate by weight, Elvax 40P) was obtained from Dupont Company (Wilmington, DE). The glass transition temperature for this polymer is −30°C to −40°C, and the molecular weight is expressed by the following values: weight average = 76,000, and number average = 25,000. The polymer was washed extensively in absolute ethyl alcohol, with total volume changes every 24 h to extract the inflammatory antioxidant butylhydroxytoluene, which was monitored in the washes spectrophotometrically at 230 nm. When the absorbance fell below 0.03 U, the polymers were dried in a vacuum desiccator for 5 days.[1]

A lyophilized preparation of mouse IFN-α/β (Mu-IFN-α/β) (1.7×10^6 U/ml, specific activity 5×10^6 U/mg protein) (Lee Biomolecular Inc., San Diego, CA) was incorporated into the polymer matrix by a modification of the fabrication procedure described by Rhine et al.[30] To facilitate release, Mu-IFN-α/β was coloaded with ficoll. Ficoll was ground with a glass pestle through a 200-mesh (74 μm) screen in a Cellector tissue sieve (Bellco Glass, Inc., Vineland, NJ). Mu-IFN-α/β and ficoll were suspended in a 10% solution of EVAc and methylene chloride (a solvent for pharmaceuticals and in food processing). The total load (of the ficoll and IFN) by weight in the polymer was 40%. The Mu-IFN-α/β load was either 5% or 10%, with the ficoll load being either 35% or 30%. Polymers 40% loaded with ficoll were prepared as controls. The EVAc solution with IFN was poured into glass cylindrical molds (5 × 27 mm) and cooled to −70°C. After 20 min, the solidified polymers were removed, transferred to a −30°C freezer, and allowed to dry for 2–3 days. The polymers were then dried in a vacuum desiccator for 3–4 days (to remove any residual methylene chloride) and stored at −20°C. (The activity of Mu-IFN-α/β that was used in this polymer preparation is indefinitely stable at 4°C and −80°C when lyophilized. When in suspension form, it is stable for several months at 4°C and indefinitely stable at −80°C).

Animals

Male C57BL/6 mice (Charles River Labs, Wilmington, MA) weighing 20–26 g were housed in plastic cages, 10 mice per cage, and given food and water *ad libitum*.

In vitro *kinetics of IFN release*

The 10-mg fragments of EVAc polymers containing by weight 5% (equal to approximately 2×10^4 U) or 10% (approximately 4×10^4 U) of Mu-IFN-α/β were incubated at 37°C and 5% CO_2 for various times (short-term release studies: incubation for 15 and 30 min, as well as 1, 2, 3, and 6 h; long-term release studies: incubation for 1–7 days) in 1 ml of growth medium containing Eagle's modified essential medium (EMEM), 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine (all from Bio-Whittaker, Walkersville, MD), and 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc., Logan, UT). Growth medium was collected at each time point for the assay of IFN activity and replaced with fresh medium. The biologic activity of IFN released into the medium was assessed by the semimicrotiter assay of viral cytopathic effect (CPE) inhibition in six experiments, with two or three measurements at each point.

In vivo *intracerebral kinetics of IFN release*

Groups of 6 C57BL/6 mice at each time point (on days 1–4, a total of 24 mice) were implanted intracerebrally (i.c.) with EVAc polymers containing 10% Mu-IFN-α/β. Also, a group of 4 mice was implanted with control polymers containing 40% ficoll. The i.c. implantation of EVAc polymers was performed in mice anesthetized with sodium pentobarbital (100 mg/kg) by using a stereotaxic device. An incision was made in the scalp at the midline, and using the tip of an 18-G needle, a hole was drilled in the calvarium overlying the right parietal cortex 2 mm caudal to the coronal suture and 2.5 mm lateral to the midline. The hole was then enlarged to 3 mm long and 1 mm wide. An incision 3 mm deep was made in the cortex, and the polymer (2–4 mg) containing approximately 8×10^3–1.6×10^4 U was inserted. A piece of saline-saturated Gelfoam was placed over the exposed cortex. The incision was sutured with 9-10 prolene (Ethicon, Somerville, NJ), and the mice were placed under a heating lamp for recovery before they were returned to their cages. Starting on either day 1, 2, 3, or 4 after polymer (IFN-containing or control) implant, groups of mice were anesthetized with sodium pentobarbital, and cardiac blood was col-

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lected for assessment of IFN level. Blood was centrifuged at 1000 rpm, and serum was collected. Mice were sacrificed and brains were removed, weighed, and rinsed with 0.9% NaCl solution. The left and right brain hemispheres were separated, and the polymer was removed from the right hemisphere. Both hemispheres were suspended separately in 1 ml of growth medium (EMEM with 10% FBS) and analyzed for IFN levels.

IFN assay

Left and right brain hemispheres (average weight 0.257 ± 0.03 g) obtained from *in vivo* experiments on IFN kinetic release were homogenized in 1 ml of growth medium (EMEM and 10% FBS) and centrifuged at 25,000*g* for 15 min, and the supernatant fractions were collected for IFN assay as described previously.[24,26,31,32] The incubation medium obtained from *in vitro* experiments on the kinetics of release of IFN, as well as supernatant fluids from left and right hemispheres and serum, were assayed for Mu-IFN-α/β activity. The semimicrotiter assay (detection level ≥ 10 U/ml) measuring the inhibition of viral CPE caused by vesicular stomatitis virus (VSV) in mouse L_{929} cells[24,26,31,33] was used. All IFN titers were expressed in reference units or $\log_{10}$ U/ml based on an NIH standard reagent. In this laboratory, the intraassay error for repeated measurements using this technique is approximately 5%. The data for each time point were presented either as representative of three experiments for 5% and 10% IFN loaded polymers (*in vitro* study) or as the means and standard error of six experiments (*in vivo* study).

Data analysis

For statistical evaluation, logarithmic ($\log_{10}$) transformation of the data was performed, followed by linear regression analysis by using INSTAT2 Software (GraphPad, San Diego, CA). Statistical significance of differences between the groups was evaluated using analysis of variance (ANOVA), followed by the Duncan multiple range test, with $p < 0.05$ being considered as significant.

RESULTS

In vitro *experiments*

Incubation of biocompatible IFN-containing polymers in EMEM culture medium caused the release of IFN biologic activity into the medium, which was evaluated by the semimicrotiter assay of IFN inhibition of viral CPE. The time points for *in vitro* kinetic release were chosen to evaluate whether all IFN activity is released from polymer within the first few minutes or hours or whether any of the IFN activity still remains in the polymer and is released at the later times, that is, 1–7 days. A relatively large amount of IFN activity (1.5×10^3–1×10^4 U/ml) was released into the growth medium from both 5% and 10% IFN-loaded polymers within the first 15 min of culture (Figs. 1 and 2). During the next 15 min, IFN activity continued to be detected in the growth medium, but on average, this was about 20%–30% of the amount released during the first 15 min. Release of IFN activity continued through 5 or 7 days of incubation of 5% or 10% IFN-loaded polymers, respectively. Further analysis showed that the growth medium samples collected during *in vitro* incubation of 5% IFN-loaded polymers at points between 15 min and 5 days had an initial release of IFN activity (1.5×10^3 U/ml) during the first 15 min of incubation, followed by a steady decline during the first 2 h of in-

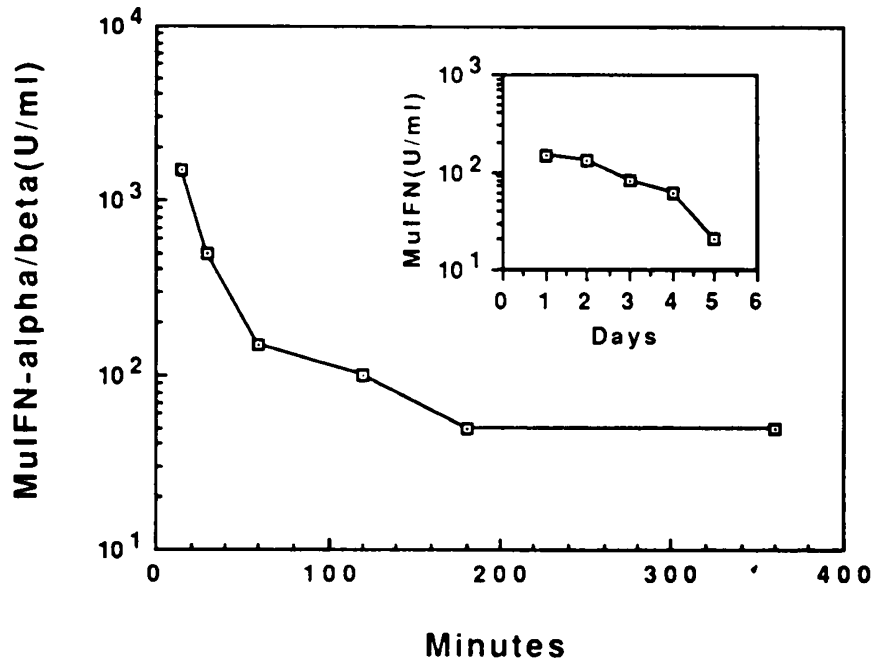

FIG. 1. The kinetics of *in vitro* release of Mu-IFN-α/β from 5% IFN-loaded biocompatible polymers containing approximately 2×10^4 U. The data are expressed as U/ml and are representative of three experiments.

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cubation (Fig. 1). After the first 2 h of incubation, the IFN levels in medium fell to a plateau at lower levels. These levels were only a small percentage (approximately 7%) of the initial activity, that is, the IFN activity released during the first 15 min. These low levels were still detectable on days 1 and 2. On days 3 and 4, IFN levels further decreased, with a clearance of all IFN activity by days 5 of incubation.

The 10% IFN-loaded polymers released relatively higher levels of IFN activity when compared with the 5% IFN-loaded polymers. During first 15 min, there was an initial release of 1×10^4 U/ml from the 10% IFN polymer (approximately 25% of the estimated total 4×10^4 U IFN activity of the implanted polymer). Subsequent release of IFN throughout the rest of day 1 was equivalent to approximately $1–2 \times 10^3$ U/ml, which was approximately 2.5%–5% of the estimated IFN activity initially present in the polymer. In addition, IFN activity was released from the 10% IFN polymer for a longer period of time than occurred with the 5% IFN polymer (steady release of 1×10^3 U/ml from day 2 to day 4), with IFN levels relatively decreased later in the incubation (days 5–7) (Fig. 2). Total recovery of approximately 60% (2.4×10^4 U) of the IFN activity initially incorporated into the polymer was detected in the growth medium collected from day 0 to day 7. In addition, *in vitro* incubation of 10% IFN-loaded polymer at 37°C for 7 days in the same volume of growth medium (with only occasional samples being tested for IFN activity) showed that IFN activity released during the first 15 min was stable for 7 days (data not shown).

In vivo *experiments*

Evaluation of the kinetic release of IFN *in vivo* from i.c. implanted mice with the 10% IFN-loaded polymers was performed in homogenates of brain hemispheres and serum samples obtained on days 1–4 after polymer implantation. The control group of mice implanted with control polymers containing only 40% ficoll (no IFN) was also evaluated for the presence of IFN activity in the brain and blood on days 1–4. IFN was undetectable (<10 U/ml) in the brain tissue (left and right hemisphere) and in the blood of control animals implanted with polymers containing only 40% ficoll at all times. IFN activity in the brain was presented as units per milliliter, which corresponded to a total IFN activity eluted from a homogenate of a brain hemisphere (left or right) into the 1 ml volume of growth medium. The IFN activity in the serum was presented as units per 1 milliliter volume (Fig. 3). Total IFN activity in mouse blood was calculated based on a total blood volume of 1.55 ml in 20-g mice.[34]

Analysis of the total daily recovery of IFN (from brain tissue and blood) released from the 10% IFN-containing polymers *in vivo* showed that on day 1 approximately 2%–4% (3×10^2 U) of the IFN activity was accounted for after i.c. implantation of 2–4 mg polymers containing an estimated 8×10^3–1.6×10^4 U, respectively. Similar observations were made for day 2 (1%–2% activity released), day 3 (1.25%–2.5% activity released), and day 4 (2%–4% activity released) after the implant. However, because multiple sampling of the brain was not possible, the data obtained on days 2–4 from the *in vivo* studies actually represent cumulative data: day 3 represents days 1–3 after the implant, day 4 represents days 1–4 after the implant, and so on.

The IFN activity in the brain was found in the right cerebral hemisphere, ipsilateral to the implanted polymer, whereas only baseline levels of IFN (10–30 U) were detected in the left brain hemisphere contralateral to a polymer implant (Fig. 3). In addition, it was observed that the highest amount of IFN activity detected in the right brain hemispheres assayed on days 1–4 af-

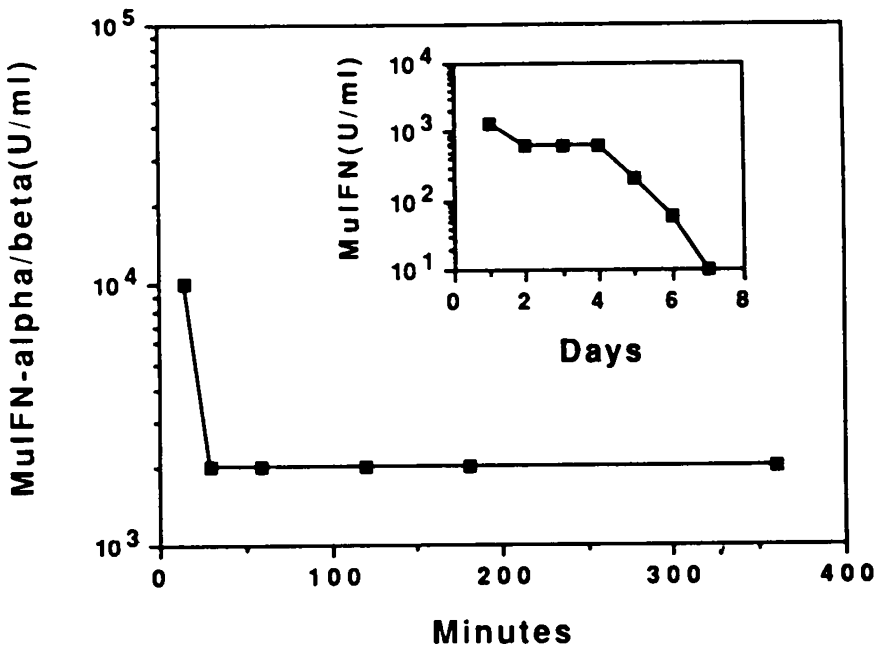

FIG. 2. The kinetics of *in vitro* release of Mu-IFN-α/β from 10% IFN-loaded biocompatible polymers containing approximately 4×10^4 U. The data are expressed as U/ml and are representative of three experiments.

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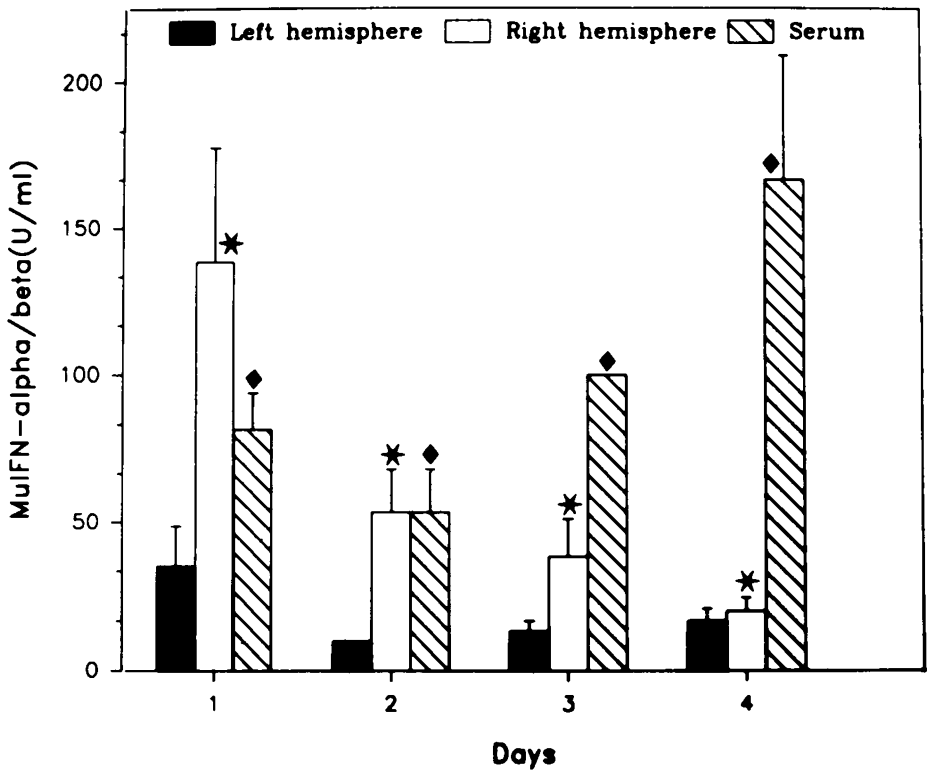

FIG. 3. The kinetics of *in vivo* release of Mu-IFN-α/β from 10% IFN-loaded biocompatible polymers containing approximately 4×10^4 U implanted into the mouse right brain hemisphere. The data are expressed as U/ml and represent the means and standard error of six experiments. (✶) The linear trend and a significant exponential decrease across the 4 days ($p < 0.0001$, $F_{3,23} = 23.669$) of IFN levels in the right brain hemisphere. (◆) The linear trend and a significant exponential increase across 4 days ($p = 0.0125$, $F_{3,23} = 7.396$) of IFN levels in the serum across the 4 days.

ter polymer implant was found on day 1. On days 2 and 3, the level of IFN activity in the brain gradually decreased, and on day 4, intracerebral levels of IFN decreased to the baseline level.

Statistical analysis of a linear regression of these IFN activity changes by using $\log_{10}$ values of the biologic activity of IFN levels detected on days 1–4 after the implant in the right cerebral hemisphere showed a linear trend and a significant exponential decrease across the 4 days ($p < 0.0001$, $F_{3,23} = 23.669$). With day 1 values as 100%, the following percentage decreases were noted on days 2 (18.5%), 3 (26.8%), and 4 (39%) after the implant. In addition, the level of IFN in the right cerebral hemisphere on day 1 was significantly higher ($p < 0.05$) than on days 2, 3, and 4 when evaluated by ANOVA (using $\log_{10}$ values of IFN activity), followed by Duncan multiple range test. The IFN biologic activity in the left cerebral hemisphere (contralateral to the implanted polymer) was relatively low, and no significant changes were observed on days 1–4 after the implant.

In contrast, the IFN levels measured in the serum on days 1–4 after polymer implant increased, with the highest level being detected on day 4 after polymer implant while cerebral IFN levels in the right hemisphere were decreasing (Fig. 3). Statistical analysis of a linear regression by using $\log_{10}$ values of biologic activity of serum IFN levels per milliliter detected on days 1–4 showed a linear trend and a significant exponential increase across 4 days ($p = 0.0125$, $F_{3,23} = 7.396$). By using day 1 values (U/ml) as 100%, the following percentage changes after the implant were observed: on days 3 and 4, increases of 6.3% (106.3%) and 15.4% (115.4%), respectively, were observed. ANOVA (using $\log_{10}$ values of IFN activity, followed by the Duncan multiple range test) showed that between day 1 and day 2, there was no statistically significant difference between serum IFN concentrations. However, a significant ($p < 0.05$) increase of IFN levels in serum was found on days 3 (6.3%) and 4 (15.4%) when compared with IFN levels in the serum (U/ml) on day 1 after intracerebral implant. In addition, the level of IFN in the serum on day 4 after the implant was significantly higher ($p < 0.05$) than on day 2 but did not differ significantly from the day 3 level.

Total IFN content in both brain hemispheres and blood (per 1.55 ml) on each day of the study (days 1–4) was also evaluated using ANOVA, followed by the Duncan multiple range test. Analysis of total IFN content showed that on day 1, there was a significantly higher ($p < 0.05$) IFN content in both the right hemisphere and the blood that was found in the left hemisphere. On day 2, there were also significant differences ($p < 0.05$) between IFN levels in the left and right cerebral hemispheres and between the left hemisphere and the blood, whereas levels of IFN in the blood and the right hemisphere did not differ significantly. On day 3, there were significant differences ($p < 0.05$) in IFN levels among all three samples (left and right hemispheres and blood). On day 4, there were no significant

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differences between the low, but still detectable, IFN levels in the two brain hemispheres. However, there was a significantly greater amount of IFN in blood ($p < 0.05$) when compared with the whole brain (left and right hemisphere) IFN concentrations.

When the total IFN activity present in both left and right cerebral hemispheres plus blood on day 1 was combined and compared with categorically similar total activity on day 4, most (98%) of the total IFN activity (present in brain and blood) found on day 1 was also found in these fractions on day 4. However, the blood level of IFN on day 4 represented approximately 88% of the total IFN activity present in brain and blood *in toto* on that day.

DISCUSSION

To our knowledge, this report demonstrates for the first time that IFN can be incorporated into a biocompatible EVAc polymer and still retain its biologic activity. Any loss of IFN activity calculated from the estimated initial IFN activity incorporated into the polymer and compared with the resultant IFN activity recovered after polymer processing could have been due to this polymer preparation. It is known that IFN sensitivity to temperature (for example, prolonged storage at 4°C or freezing and thawing of an IFN suspension) may affect the activity of an IFN preparation, especially if it is highly purified IFN. However, the preparation of IFN that was used in this study was not highly purified and had a specific activity of 5×10^6 U/mg protein.

As expected, evaluation of the release of IFN from EVAc polymers *in vitro* over time revealed that 10% IFN-loaded polymers released relatively higher amounts of IFN activity over a longer period of time (7 days) when compared with 5% IFN-loaded polymers (5 days). Consequently, 10% IFN-loaded polymers were chosen for the *in vivo* kinetic study. This evaluation was performed in groups of 6 mice, with a different set of mice for each time point, to permit the collection of brain samples and blood on each day of the study. Consequently, a total of 24 mice were tested. Despite this requirement for using different sets of mice at each chronologic interval, the variance of the results obtained at a particular time was acceptable. Moreover, the brain tissue and blood obtained from control mice (implanted with polymers containing only 40% ficoll) showed no IFN activity at any of the study intervals, demonstrating that polymers alone and the procedure of polymer implantation did not induce IFN in the brain, and IFN remained undetectable as in the normal brain.[31]

The *in vivo* evaluation of IFN release from polymers into the brain showed that biocompatible polymers released relatively higher amounts of activity on the first day after implantation, with a gradual decrease during days 2–4. The total IFN activity (in the brain and blood) released on day 1 was approximately 2%–4% of the total estimated IFN activity contained in the polymer. This *in vivo* finding obtained on day 1 closely resembled results obtained *in vitro*, where the amount of IFN released on day 1 was 2.5%–5%. In addition, the total amount of IFN released on each subsequent day between days 2 and 4 was similar to the amount on day 1 and constituted 1%–4% of total estimated IFN activity contained in the polymer. Calculation of the percentage of the total recovery of IFN from polymer *in vivo* during these 4 days was not possible because early time points that were studied *in vitro* (15 and 30 min) were not evaluated here and data from days 2–4 were cumulative. In contrast, data from *in vitro* experiments were not cumulative and included the early times of 15 or 30 min, which showed the highest levels of released IFN. It is possible that the highest IFN level *in vivo* was released in the early intervals, such as 15 min, but this was not investigated. Consequently, IFN activity found *in vivo* on day 1 would represent cumulative IFN levels from time 0 through day 1. The results showing that the total recovered activities of IFN on day 2 (1%–2%), day 3 (1.25%–2.5%), and day 4 (2%–4%) were similar to activity recovered on day 1 (2%–4%) may be explained by the fact that the majority of IFN activity had been released early on day 1, inasmuch it was found *in vitro* at 15 and 30 min.

Our observation that blood levels of IFN were rising with increasing time after the implant may be explained by the fact that IFN could have gradually passed from the brain tissue compartment to the blood compartment; 88% of the total IFN activity (together in blood and brain) was found in the blood on day 4. The fact that the BBB may have been compromised after implantation of the polymer into the brain may have contributed to this effect.

Under normal conditions, the BBB is not permeable to large proteins, such as IFN,[26,32,35] and systemic administration of IFN does not permit accumulation of significant cerebral levels of IFN.[24] Previously, this laboratory reported that after mechanical disruption of the BBB in mice (intracerebral sham injection), reconstitution of the BBB occurred within 10 days after this procedure.[24] Therefore, in the present study, evaluation of cerebral and blood levels of IFN on days 1–4 after polymer implant was performed while the BBB was still compromised, which would enable IFN to move from the CNS to the blood compartment.

It is important to note that total activity of IFN (measured collectively in the blood and brain compartments) released from the polymer at the end of 4 days and representing approximately 2%–4% of IFN activity contained in the implanted polymer did not differ significantly from the IFN level released on day 1. For example, 98% of total IFN activity released on day 1 still remained in these tissues at the end of day 4. Previous studies from this laboratory showed that although IFN injected systemically, either intravenously or via the carotid artery, achieved high levels in the blood instantly. However, the IFN activity recovered during the first 2 min in those studies represented only approximately 6% of the amount of injected IFN. Subsequently, most of the IFN activity was cleared from the blood within a few hours (4–8 h), and only approximately 0.04% remained at 24 h.[31,32] In general, IFN injected into the bloodstream clears rapidly, whereas it remains much longer in the blood after intramuscular injection.[32] It was calculated by Ho[36] that the half-life for mouse IFN *in vivo* following intravenous injection is 10 min. After an initial rapid fall of circulating IFN within 1 h after intravenous injection, the clearance rate decreases, and levels are not detectable after 6 h. When injected intramuscularly, it is likely that IFN binds locally and, thus, sets up a local IFN pool that leaches into the circulation over a prolonged period.

It was speculated that the rapid disappearance of IFN from blood after intravenous injection could be caused by a number

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of factors: dilution in body fluids, catabolism by the liver and kidney or plasma proteinases, and body temperature.[31] However, previous study from this laboratory showed that activity of an IFN preparation added to aliquots of blood obtained from 6 individual donors and incubated 1 h or 24 h at 37°C was stable.[32] In the present study, IFN activity was present in the blood throughout 4 days of evaluation, with increasing levels on day 4. This suggested that during these 4 days, IFN was moving from the CNS to the blood compartment, and its activity was somehow protected in both compartments. IFN released from polymers may have bound to the brain tissue and subsequently was released slowly into the blood compartment. However, significant observations were the shift of IFN activity from the CNS tissue compartment to the blood compartment in these 4 days and the presence of IFN activity through day 4 after implant that was equivalent to the amount detected on day 1 after implant. It would be important to evaluate IFN release from polymers that contain higher initial IFN activity. Thus, the release of IFN might continue for a longer time, possibly after BBB reconstitution. For example, the distribution of IFN in CNS tissue and the systemic blood compartment could be further characterized after 10 days, when the BBB is reconstituted. It would be interesting to determine if the level of IFN in the CNS compartment would continue to remain high after BBB reconstitution.

It was shown in this study that by using cytokine-impregnated biocompatible polymers, high local levels of an immunotherapeutic factor, such as IFN, can be achieved. Moreover, after 4 days of IFN delivery, 98% of the activity initially detected on day 1 was recovered. We have shown that IFN can inhibit glioma proliferation[19,24] and invasion[21,22] *in vitro*. Local cerebral delivery of IFN *in vivo* to a glioma may also have similar antiproliferative activity. Finally, this laboratory has reported that IFN delivered intravenously *in vivo* activates the hypothalamic-pituitary axis (HPA), upregulating systemic levels of glucocorticoids.[37] Glucocorticoids may affect phagocytosis and antibody-dependent cell-mediated cytotoxicity, and they may also act synergistically with IFN in a dose-dependent and time-dependent mechanism.[38] Therefore, the report showing that IFN treatment of glioma patients, which affected their lymphocyte populations by decreasing suppressor/cytotoxic T cells and increasing the helper T cell population (also correlated with an increase in patient survival vs. control),[18] may be related to a consequent activation of the HPA axis and the related increase in blood glucocorticoid levels.

These polymers have been used clinically for administration of birth control medications and for introduction of therapeutic agents in the treatment of glaucoma.[39] They have been shown to be noninflammatory in a rabbit cornea assay.[40] Biocompatible polymers containing various chemotherapeutic agents have been used successfully *in vivo*[6,7] and in clinical trials.[15,16] For example, it was reported that survival of glioma-bearing animals treated with cerebrally implanted biocompatible polymers containing 4-hydroperoxycyclophosphamide (4HC) was significantly increased up to 9 weeks when compared with control animals (median survival 77 days for the treated group and 14 days for the nontreated group).[41] Clinical trials in glioma patients implanted with biocompatible polymers containing carmustine showed encouraging results, with increased survivals up to 8 weeks when compared with a placebo group (median survival 31 weeks for the treated group and 23 weeks for the nontreated group).[16] It is hypothesized that in the treatment of glioma, the use of polymers containing IFN or both IFN and a chemotherapeutic agent could further increase survival. A number of reports have shown a synergistic antiproliferative effect of IFN and chemotherapeutic agents when investigated *in vitro* in various tumor cell lines.[42] For example, 5-fluorouracil (5-FU) and IFN-α in glioma cells[28,29] and in glioma patients had a synergistic effect in increasing the helper T cell levels and downregulating suppressor T cell levels.[18] This synergistic activity *in vitro* occurred because IFN enhanced the cytotoxicity of antimetabolites and alkylating agents against cultured human tumors *in vitro* and *in vivo*.[43]

Clinical application of such combined therapy in various cancers, however, is associated with high toxicity when these agents are administered systemically,[44] with less pronounced side effects when administered subcutaneously,[45] and combined therapy is reported to be highly successful when applied externally and locally.[46] Therefore, it is hypothesized that combined therapy with IFN and chemotherapeutic agents delivered intracerebrally through biocompatible polymers could have strong antitumor activity and low toxicity. This would require further *in vivo* evaluation in a glioma model.

This article presents a study evaluating just one type of biocompatible polymer that may be considered for the future application of immunotherapeutic agents. However, different polymers releasing immunotherapeutics at a more predictable, constant rate over a longer time should also be a focus for future evaluation.

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