Antitumor activity of the growth hormone receptor antagonist pegvisomant against human meningiomas in nude mice

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Object. The authors have previously demonstrated that modulation of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis can significantly affect meningioma growth in vitro. These studies were performed to evaluate the efficacy of GH receptor blockade in vivo.

Methods. Primary cultures from 15 meningioma tumors obtained in humans were xenografted into athymic mice. Approximately 1.5 million cells from each of the 15 tumors were implanted into the flanks of two female mice, one pair for each tumor. One animal from each of the 15 pairs was then treated with the GH receptor antagonist pegvisomant and the other with vehicle alone for 8 weeks. The tumor volume was measured using digital calipers three times per week. The mean tumor volume at the initiation of injections was 284 ± 18.8 mm³ in the vehicle group and 291.1 ± 20 mm³ in the pegvisomant group. After 8 weeks of treatment, the mean volume of tumors in the pegvisomant group was 198.3 ± 18.9 mm³ compared with 350.1 ± 23.5 mm³ for the vehicle group (p < 0.001). The serum IGF-I concentration in the vehicle group was 319 ± 12.9 µg/L compared with 257 ± 9.7 in the pegvisomant group (p < 0.02). A small but significant decrease was observed in circulating IGF binding protein (IGFBP)-3 levels, whereas slight increases occurred with respect to serum IGFBP-1 and IGFBP-4 levels. In the placebo group the tumor weight was 0.092 ± 0.01 g compared with 0.057 ± 0.01 g in the pegvisomant group (p < 0.02). The IGF-I and IGF-II concentrations were measured in the tumors by using a tissue extraction method. These human-specific immunoassays demonstrated that there was no autocrine production of IGF-I in any of the tumors, either in the pegvisomant or vehicle group. The IGF-II levels were highly variable (0–38.2 ng/g tissue) and did not differ significantly between treatment groups.

Conclusions. In an in vivo tumor model, downregulation of the GH/IGF-I axis significantly reduces meningioma growth and, in some instances, causes tumor regression. Because the concentrations of IGF-II in tumor did not vary with pegvisomant treatment and there was no autocrine IGF-I production by the tumors, the mechanism of the antitumor effect is most likely a decrease of IGF-I in the circulation and/or surrounding host tissues. Because the authors have previously demonstrated that the GH receptor is ubiquitously expressed in meningiomas, direct blockade of the GH receptor on the tumors may also be contributing to inhibitory actions.

Key Words • meningioma • insulin-like growth factor • growth hormone • antagonist • mouse

Growth hormone, which is secreted from the anterior pituitary gland, stimulates the synthesis of IGF-I in the liver and in many other tissues. The direct effects of GH and the indirect effects of IGF-I combine to produce the changes associated with normal growth. In at least 20 years of both basic and clinical studies, growth hormone (GH) and IGF-I have also been identified as potent inducers of cell growth in many types of neoplasms. Examples of malignancies responsive to IGF-I include primary tumors of the breast, colon, and prostate. Previously, in a series of in vitro experiments, we demonstrated that the GH/IGF-I axis is also important in modulating meningioma growth. Specifically, we performed a series of experiments in which the following were demonstrated: 1) that the GH receptor is ubiquitously expressed in meningiomas; 2) that blockade of the GH receptor by using the GH receptor antagonist pegvisomant (formerly B2036-PEG) significantly decreases the growth rates of many primary meningioma cultures; and 3) that administration of IGF-I significantly increases primary culture growth rates. These findings indicated that further experiments would be warranted to evaluate the therapeutic efficacy of GH receptor blockade by using an in vivo meningioma model, as is described in this manuscript.

A number of different methods have been developed to study meningioma growth in vivo, including models in

Abbreviations used in this paper: GH = growth hormone; IGFBP = insulin-like growth factor binding protein; SEM = standard error of the mean.
TABLE 1
Characteristics of 15 patients from whom tumors were obtained and lesion grade and location*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tumor Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign (10 tumors)</td>
</tr>
<tr>
<td>M/F</td>
<td>3:7</td>
</tr>
<tr>
<td>age (yrs)</td>
<td>mean ± SEM</td>
</tr>
<tr>
<td>men</td>
<td>52 ± 7.34</td>
</tr>
<tr>
<td>women</td>
<td>62 ± 5.54</td>
</tr>
<tr>
<td>location</td>
<td></td>
</tr>
<tr>
<td>frontal</td>
<td>1</td>
</tr>
<tr>
<td>petroclival</td>
<td>1</td>
</tr>
<tr>
<td>convexity</td>
<td>3</td>
</tr>
<tr>
<td>tentorial</td>
<td>3</td>
</tr>
<tr>
<td>parasagittal</td>
<td>2</td>
</tr>
<tr>
<td>olfactory groove</td>
<td>0</td>
</tr>
</tbody>
</table>

* NA = not applicable.

which human tumors were implanted into the subrenal capsule of immunocompromised mice. Jensen, et al., recently described a model in which meningioma cells obtained from patients were grown in primary culture and then xenografted into the subcutaneous space in the flank of athymic (“nude”) mice. By including Matrigel (a mixture of basement membrane proteins and growth factors derived from a mouse sarcoma line) in the injection material, they produced tumor development in 100% of animals. Histological and immunohistochemical studies revealed that the xenografted tumors demonstrated many of the characteristics of the parent tumors. A modified version of this methodology was used in the experiments detailed here.

The primary rationale for pursuing these studies is the current clinical requirement for a more effective adjuvant therapy in the management of patients with meningioma. Although most meningiomas (80–85%) are histologically benign, they are often located in areas where they surround important structures such as venous sinuses, cranial nerves, or the major arteries at the skull base, making complete resection impractical. The risk of relapse after incomplete resection has been reported to be 90% at 15 years; for tumors thought to be completely resected, the recurrence rate has been reported to be 32% at 15 years. Although adjuvant radiation therapy appears to offer some benefit, relapse rates, particularly in patients with residual disease, are still greater than 50%. Experience with biological response modifiers and chemotherapy agents is quite limited.

Materials and Methods

Specimen Collection and Primary Culture Establishment

Fifteen meningioma specimens were included in this study. The mean ages, sex ratio, histological classification, and tumor location for each of the patients from whom a surgical specimen was obtained are listed in Table 1. Ten tumors were benign, four were atypical, and one was malignant; five specimens were obtained in men and 10 in women. The ages of the patients ranged from 34 to 78 years (mean age 58 years).

The processing of tumor specimens has been described previously. Briefly, after resection, a portion of each tumor was sent to the neuropathology laboratory for routine analysis. The remainder of all samples was immediately used to establish primary cultures. To establish primary cultures, tumor fragments were dispersed into individual cells by treatment with dispase I for 15 to 30 minutes at 37°C. One million cells from each tumor were then plated in a 100 × 20-mm tissue culture dish in low-glucose Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, penicillin, and streptomycin. The cells were grown to confluence and then harvested, aliquoted and stored in liquid nitrogen for future use.

Meningioma Xenografts

Frozen tumor cells were thawed and grown in tissue culture dishes under the aforementioned conditions. For each tumor, at least 3 million cells were required. Approximately 1.5 million of the cells, in a total volume of 0.4 ml of culture media, were injected subcutaneously into one flank of each of a pair of athymic mice (female Swiss nu-nu/Ncr, 8–10 weeks old). Prior to injection, an equal volume of Matrigel was added to the cells, bringing the total volume at the time of injection to 0.8 ml per animal. Tumor volume (width × depth × height) was measured using digital calipers. No treatment was initiated for 11 to 12 days because we and others have observed that there is an initial period (7–10 days) in which there is tumor shrinkage, presumably due to absorption of some of the components of the injection mixture. The volume measured at the 11- to 12-day time point served as the baseline tumor size.

Pegvisomant or Vehicle Treatment

A total of 15 pairs of animals was used in these experiments. One animal from each pair was randomized to the pegvisomant group and the other to the vehicle-only group. The animals in the pegvisomant group were treated with daily subcutaneous injections of 0.2 ml of the compound (4.5 mg/ml); the total dose was 315 mg/kg/wk. Because pegvisomant is a variant form of human GH, a higher dose must be administered to mice on a milligram per kilogram basis than is required in humans to achieve the same degree of reduction in circulating IGF-I levels. The vehicle-only group received 0.2-ml injections of the formulation buffer for pegvisomant (18 g/L mannitol, 0.68 g/L glycine, 5 mM sodium phosphate monobasic, pH 7.4). Injections were administered on the flank opposite the side of the tumor xenograft by using a 25-gauge needle attached to a tuberculin syringe. Pegvisomant or vehicle treatment was continued for 8 weeks. Tumor volume measurements were made three times weekly with digital calipers.

Tumor Harvesting

At the completion of the study the animals were killed. Whole blood was collected from each mouse and placed on ice. A flank incision was made and the tumors were carefully dissected away from normal subcutaneous tissues. The harvested tumors were weighed, frozen, and stored at −80°C until processing. The whole blood was centrifuged at 13,000 G for 15 minutes at 4°C, and the serum was collected and stored at −80°C until it was assayed.

Serum Assays

Mouse serum IGF-I was measured by radioimmunoassay after acid–ethanol extraction as previously described. Briefly, the mixture was incubated at room temperature for 2 hours, centrifuged, and the 1:200 dilution of the supernatant was made for analysis. Concentrations of IGF-I were then measured by radioimmunoassay by using a polyclonal rabbit antibody; recombinant human IGF-I was also used. Serum IGFBPs were measured by Western ligand blotting as previously described. Briefly, 2 μl of serum was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10% polyacrylamide gel) under nonreducing conditions. Proteins were transferred to nitrocellulose and incubated at 4°C with 500,000 counts per minute of [125I]-IGF-I (2,000 Ci/mmole) in a 10 mmol/L Tris-HCl buffer (TBS) containing 1% bovine serum albumin and 0.1% Tween 20. The nitrocellulose membrane was washed with TBS and exposed to x-ray film in the presence of an enhanc-

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ing screen for 3 to 7 days. On Western ligand blots in which 125I-IGF-I was used as a ligand, IGFBP-3 appears as a 38- to 42-kD doublet band corresponding to the intact acid-stable IGF-binding subunit of IGFBP-3, IGFBP-2 appears as a 34-kD band, IGFBP-1 as a 29-kD band, and IGFBP-4 as a 24-kD band. Specificity of the IGFBP bands was ensured by competitive coincubation with unlabeled IGF-I. Densitometric analysis was performed to quantitate individual signals. Intra- and interassay coefficients of variation were less than 5% and less than 10%, respectively.

Tumor Assays

The IGF-I was extracted as previously described.5,8 Briefly, 50 mg of tissue was homogenized on ice in 1 M acetic acid (5 ml/g tissue) and further disrupted using another homogenizer. This extraction process was repeated twice for each sample. The supernatant was lyophilized and redissolved in 40 mM phosphate buffer, pH 8. For IGF-II extraction, 50 mg of tissue was homogenized on ice in 3.3 M formic acid–0.5% Tween 20 (5 ml/g tissue) and centrifuged at 40,000 G for 10 minutes at 4°C. A 150–µl aliquot was heated to 90°C for 30 minutes. Reagent-grade acetone (350 µl) was added and the samples were vortexed and centrifuged at 3000 G. As with IGF-I, the supernatants were lyophilized and redissolved in 40 mM phosphate buffer, pH 8. The tissue extracts were stored at −20°C until IGF-I and IGF-II concentrations were measured in duplicate in diluted extracts by a noncompetitive time-resolved immunofluorometric assay for human IGF-I and IGF-II as previously described.5,8 The cross-reactivity of IGF-I and IGF-II was less than 0.0002% and the detection limits were 0.0025 µg/L and 0.0010 µg/L for the IGF-I and IGF-II assays, respectively. Circulating mouse IGF-I and IGF-II were undetectable in these assays; accordingly, tumor IGF levels were not influenced by entrapped serum. Intraassay and interassay coefficients of variation were less than 5% and less than 10%, respectively.

Data Analysis

All results are expressed as the mean ± SEM unless otherwise noted. The data for the pegvisomant and vehicle groups were compared using a paired t-test calculated using commercially available software. Probability values are reported throughout the text; values of less than 0.05 were considered to represent significant differences between the two groups.

Sources of Supplies and Equipment

The dispase I was acquired from Boehringer Mannheim, Indianapolis, IN. The Dulbecco’s modified Eagle’s medium was purchased from Gibco BRL, Gaithersburg, MD. The polyclonal rabbit antibody was supplied by Nichols Institute, San Juan Capistrano, CA, and the recombinant human IGF-I by Amersham International, Bucks, UK. The mono-iodinated IGF-I (125I-[Tyr31]-IGF-I) was obtained from Novo-Nordisk A/S, Bagsvaed, Denmark. Unlabeled IGF-I was acquired from Bachem, Bubendorf, Switzerland.

Results

Tumor volume was measured using digital calipers three times per week. The mean tumor volume at the initiation of injections (Days 11–12 after xenografting) was 284 ± 18.8 mm³ in the vehicle group and 291.1 ± 20 mm³ in the pegvisomant group. The volume of tumors in the pegvisomant group was significantly less than those in the vehicle group after 1 week of drug administration and became progressively more so throughout the remainder of the study (Fig. 1). After 8 weeks of treatment, the mean volume of the tumors in the pegvisomant group was 198.3 ± 18.9 mm³ compared with 350.1 ± 23.5 mm³ for the vehicle group. For the pegvisomant group, the mean tumor volume at the completion of the study was 31.8% less than the volume at the initiation of treatment (p < 0.001). For the vehicle group, there was a 23.2% increase in tumor volume over the 8 weeks (p < 0.005). These data indicate that the reason that tumors in the two groups differed so significantly in volume was that pegvisomant treatment resulted not only in inhibition of tumor growth, but also in some tumor regression.

Because primary cultures of human tumors were used as the source material for these xenografts, each pair of tumors essentially represents a separate cell line. As can be
grades (benign, atypical, malignant) for each specimen. Also shown are the tumor weights in grams of meningioma responding to pegvisomant administration, including benign and atypical tumors, as well as the sole malignant tumor.

After 8 weeks of therapy, the animals were killed and the tumor xenografts were harvested and weighed. The animals were killed approximately 24 hours after the last injection of vehicle or pegvisomant. Tumor weight in the placebo group was 0.092 ± 0.01 g compared with 0.057 ± 0.01 g in the pegvisomant group (p < 0.02). Because the animals were mature at the initiation of pegvisomant or vehicle injections, there was no difference in body weight, as might be expected if they had experienced GH deficiency during the normal period of growth and development. Core blood was collected and assayed for serum IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-4. These values, which demonstrate that IGF-I and IGFBP-3 concentrations were significantly depressed in the pegvisomant group, are shown in Table 2. The IGFBP-1 and IGFBP-4 levels were modestly but significantly elevated, whereas no significant change was observed in IGFBP-2 levels.

The IGF-I and IGF-II were also measured in tissue extracts from eight pairs of randomly chosen tumors. No detectable IGF-I was present in the tumor samples, indicating that there is no autocrine production of IGF-I by the tumor. The IGF-I assay used to measure concentrations does not crossreact with mouse IGF-I. Thus, any IGF-I in the circulation or from surrounding tissues would not be detected. On the other hand, IGF-II concentrations in the tissue extracts varied substantially between the individual tumors but did not change appreciably with pegvisomant therapy. Again, this assay does not crossreact with mouse IGF-II. As shown in Fig. 3, there was no significant relationship between the concentration of IGF-II in the tumor and the amount of tumor growth, although there was a trend toward more robust growth with higher concentrations of IGF-II. There was a statistically significant relationship between the amount of IGF-II in the tumor and its response to pegvisomant, with the tumors expressing the most IGF-II being the most responsive.

Discussion

The experiments outlined in this manuscript indicate that downregulation of the GH/IGF-I axis with the GH receptor antagonist pegvisomant significantly decreases the growth rate of human meningiomas in nude mice. In previous studies conducted by us and other investigators, meningiomas have been shown to respond to modulation of the GH/IGF-I axis in vitro. This is the first study in which the effect of downregulation of the GH/IGF-I axis in vivo has been evaluated, and it shows that a modest (20%) decrease in total IGF-I concentration in serum has a significant antitumor effect. Our findings are consistent with those reported by a number of investigators who have noted a clinical relationship between excess GH (acromegaly) and the development of meningiomas.2,13,15,23

The GH receptor antagonist used in this study, pegvisomant, represents the first therapeutic agent that can potentially downregulate the GH axis over long periods of time.4 In most patients with acromegaly, the signs and symptoms of excess GH can be controlled with pegvisomant doses of 15 to 20 mg/day.29 Although similar to natural human GH, pegvisomant has eight amino acid substitutions at binding site 1 that significantly increase affinity. A single amino acid substitution in binding site 2 significantly decreases affinity for the GH receptor at that site. Because binding at sites 1 and 2 is necessary to initiate signal transduction, pegvisomant essentially acts as a competitive antagonist with natural GH for binding to the GH receptor. Although

![Figure 2](https://via.placeholder.com/150)

**Fig. 2.** Graph showing percentage of change in tumor volume after 8 weeks of vehicle or pegvisomant administration for each of the 15 pairs of tumors used in this study. Also shown are the tumor grades (benign, atypical, malignant) for each specimen.
the half-life of naturally occurring GH is approximately 20 minutes, pegvisomant has several covalently attached polyethylene glycol molecules that significantly prolong its half-life to approximately 100 hours. Conjugation with polyethylene glycol does not significantly alter the specificity of the compound or increase its toxicity.

The vast majority of IGF-I in the circulation is bound to IGFBPs, whereas only a small percentage is believed to be unbound, and therefore available for binding to the IGF-I receptor. In the present study, we observed a modest but significant decrease in IGFBP-3 and total IGF-I in the pegvisomant group, along with corresponding modest increases in IGFBP-1 and IGFBP-4. With the aforementioned changes in IGFBPs, the amount of decrease in bioavailable IGF-I is likely to be at least equal to the changes observed in total IGF-I.

None of the meningioma specimens examined in this study was found to have autocrine IGF-I production after implantation. As stated previously, several investigators have identified IGF-I receptors on these tumors. The functional importance of these receptors has also been clearly demonstrated. For instance, in previous studies we found that the addition of 10 ng/L of IGF-I to culture medium increased the mean growth rates of meningiomas in primary culture by 176%. The concentrations of IGF-II varied substantially between individual specimens but did not change significantly with pegvisomant administration. This finding indicates that modulation of the IGF-I axis can affect tumor growth despite the existence of autocrine IGF-II production by these tumors. In fact, in our limited series, those tumors containing the highest concentrations of IGF-II appeared to be the most responsive to pegvisomant therapy.

Because the tissue concentrations of IGF-II did not vary with pegvisomant therapy and there was no autocrine IGF-I production, the most plausible mechanism of the antitumor effect of pegvisomant is a decrease of IGF-I in the circulation and/or surrounding host tissues. Because expression of the GH receptor by meningiomas is ubiquitous, it is also possible that direct blockade of the GH receptor contributed to the antitumor effect we observed, especially because our previous in vitro studies have demonstrated that pegvisomant inhibits tumor growth modestly (by approximately 20%) in the absence of changes in IGF-I concentrations.

As noted previously, the primary rationale for pursuing these studies is the current clinical need for a more effective adjuvant therapy for controlling residual tumor in patients with meningioma. Resection, although clearly the primary therapy for these tumors, is frequently not curative, even when coupled with radiation therapy. Meningiomas are a logical choice for pegvisomant therapy because their vasculature lacks a blood–tumor barrier despite their intracranial location. These tumors derive most of their vascular supply from the external carotid artery circulation and so are exposed to systemic levels of therapeutic agents, including pegvisomant.

Conclusions

In the in vivo studies presented here, we have demonstrated that downregulation of the GH/IGF-I axis with pegvisomant is an effective antitumor therapy in an animal model of meningioma growth. Analysis of the efficacy of this approach for patients with residual or recurrent meningiomas will, of course, require evaluation of the effects on tumor growth in the clinical setting.

References


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