Effect of the antiprogesterone RU-38486 on meningioma implanted into nude mice

JEFFREY J. OLSON, M.D., DAVID W. BECK, M.D., JANET A. SCHLECHTE, M.D., AND PAO-MIN LOH, PH.D.

Division of Neurosurgery and Departments of Internal Medicine and Surgery, University of Iowa Hospitals, Iowa City, Iowa

Meningiomas have been shown to have steroid-binding proteins. In vitro, estradiol, progesterone, and the antiestrogen tamoxifen stimulate tumor growth. However, incubation of tumor cells with an antiprogesterone agent results in tumor inhibition. In this investigation, a human meningioma was implanted subcutaneously in athymic nude mice. Two treatment groups were established, one receiving the antiprogesterone agent RU-38486 (10 mg/kg/day in suspension) and the other receiving only vehicle. After 3 months, the tumor growth index (defined as the tumor volume at 3 months divided by the initial tumor volume) was $0.25 \pm 0.46$ (mean $\pm$ standard deviation) in the group receiving antiprogesterone and was $1.54 \pm 0.58$ in the control group ($p = 0.041$). Further investigation of the effect of antiprogestational agents on the growth and hormone-binding proteins of other meningiomas will better define the mechanism of their effects.

KEY WORDS: meningioma, antiprogesterone, tumor growth index, nude mice

It is well established that meningiomas contain steroid-binding proteins.\textsuperscript{4,5,8,20,21,25,28,31} Subsequent investigation has shown that progesterone, estradiol, and the antiestrogen tamoxifen modulate growth of meningioma cells \textit{in vitro}.\textsuperscript{11,19} Further evidence that these tumors are hormone-responsive has been deduced from the predominance of the tumor in females and the observation that the tumors may cause symptoms during pregnancy that resolve after delivery.\textsuperscript{2,6,7,10,12,13,22,23,29} In previous work, it has been demonstrated that there are high concentrations of progesterone-binding protein present in meningioma tissue and that the antiprogesterone agent RU-38486 decreased growth of meningioma cells in tissue culture.\textsuperscript{15} In this report, these studies have been expanded to examine the effect of this antiprogestational agent on the growth of meningioma tissue implanted into nude mice.

Materials and Methods

A freshly resected human meningioma was divided into three portions: one for histological study, one for binding assays, and the third for implantation in athymic nude mice.* For histological evaluation, the tissue was paraffin-embedded, stained with hematoxylin and eosin, and studied by light microscopy.

For estrogen receptor determinations, cytosol was incubated with 1 nM tritiated (\textsuperscript{3}H) estradiol (101 Ci/mM) and with increasing concentrations of unlabeled estradiol (0 to 500 pg/tube) at 4°C for 18 hours.\textsuperscript{7} Progesterone receptor determinations were made by incubation of cytosol with 1 nM \textsuperscript{3}H Promegestone (87 Ci/mM) and with increasing concentrations of unlabeled progesterone (0 to 1000 pg/tube) at 4°C for 4 hours.\textsuperscript{3} Bound and free hormones were separated by dextran-coated charcoal, and radioactivity was determined in a liquid scintillation counter.\textsuperscript{3} Receptor concentration and binding affinity were determined by Scatchard analyses.\textsuperscript{24}

The tumor to be implanted into the mice was divided into six equal fragments each measuring $3 \times 3 \times 4$ mm. Six male athymic, nu/nu Balb C mice, each weighing 35 to 45 mg, were anesthetized with intraperitoneal chloral hydrate. After the region was prepared with 70% ethyl alcohol, a 5- to 6-mm incision was made over the

* Mice obtained from Harlan Sprague Dawley, Inc., Indianapolis, Indiana.

† Estradiol obtained from Amersham, Arlington Heights, Illinois.

‡ Progesterone obtained from New England Nuclear, Boston, Massachusetts.

§ Liquid scintillation counter, Model 4530, manufactured by Hewlett Packard Co., Palo Alto, California.
Effect of antiprogesterone on meningioma growth in mice

**TABLE 1**

<p>|Meningioma growth index measurements in antiprogesterone-treated and control mice|
|---|---|---|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Mouse No.</th>
<th>Growth Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>control mice</td>
<td>1</td>
<td>1.18</td>
</tr>
<tr>
<td>2</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>mean ± standard deviation</td>
<td></td>
<td>1.54 ± 0.58</td>
</tr>
<tr>
<td>antiprogesterone-treated mice</td>
<td>4</td>
<td>0.79</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>mean ± standard deviation</td>
<td></td>
<td>0.25 ± 0.46*</td>
</tr>
</tbody>
</table>

* Significantly different from control findings (p = 0.041).

left scapula and a subcutaneous pocket was formed. One tumor fragment was placed in each animal and the wound was closed with a silk suture. The suture was removed after 3 days. On the 7th day following implantation, subcutaneous tumor was measured with a caliper. The volume of tissue was defined as \( V = W^2 \times L / 2 \), where \( V \) represented total volume, \( W \) represented width, and \( L \) represented length. The nodule in each animal was measured weekly by an investigator blinded as to the treatment regimen. After baseline measurements were made on Day 7, the animals were divided into two groups. The experimental group began receiving 10 mg/kg/day of RU-38486. This was administered orally each day in a suspension containing 25 mg of carboxymethyl cellulose and 20 μl of Tween 80 in 10 cc of water. The control animals received the same volume of the suspension vehicle orally on a daily basis. The animals were kept in standard filtered-air hoods and fed clean food and water *ad libitum*. At the end of 3 months, the animals were sacrificed and autopsied; the tumor nodules were fixed in formalin for histological evaluation. Comparative tumor growth was determined by a growth index (GI) calculated such that \( GI = V_f/V_i \), where \( V_f \) represented nodule volume after 3 months of drug treatment and \( V_i \) represented nodule volume measured 1 week after implantation. The results were compared using Student's t-test for unpaired data.

**Results**

Histological evaluation of the original neoplasm revealed primarily meningothelial components with little fibrous tissue (Fig. 1 left). The concentrations of specific estrogen-binding and progesterone-binding protein in the original tumor were 3.99 fM/mg cytosol protein and 89.4 fM/mg cytosol protein, respectively. The growth data are summarized in Table 1. The average growth index for the control group was significantly higher than for the group receiving RU-38486. Light microscopic evaluation of tumor nodules in the control animals showed a core of meningothelial cells in the nodule of each animal (Fig. 1 right). A small number of cells resembling those of the primary tumor were found in one mouse of the experimental group, but only scar tissue was present in the other two experimental mice. The nodules in two of the three experimental mice disappeared within 8 to 10 weeks of treatment. Over the 3-month period, no overtly detrimental effects on body habitus, feeding, or growth were noted in either group.

---

**Fig. 1.** *Left:* Photomicrograph of the original meningioma showing regularly spaced vesicular nuclei in a syncytial background. This appearance was representative of the majority of the meningothelial components of the tumor. H & E, × 175. *Right:* Photomicrograph of a tumor nodule in a control animal 3 months after implantation. The meningothelial character of the specimen has persisted. H & E, × 280.
Discussion

Recurrent meningiomas are usually treated by reoperation or by radiation therapy. Unfortunately, these tumors are not clearly radiosensitive, and reoperation can be fraught with complications. The development of a hormonal agent that could decrease tumor size in patients with recurrent meningioma would be a major therapeutic advance.

Meningiomas have been shown to contain specific binding proteins for estrogen and progesterone, raising the possibility that these tumors might respond to hormonal manipulation. Jay, et al. showed in vitro growth stimulation with estradiol, progesterone, and the antiestrogen tamoxifen. Although lower concentrations of binding protein were measured, these results have been confirmed in a separate investigation in addition to demonstrating that the antiprogestational agent RU-38486 inhibits the growth of cell populations in vitro. This study suggests that this antiprogestational compound may be efficacious in decreasing tumor size and may potentially be important in the treatment of recurrent meningioma.

The growth and study of other tumors, such as bladder carcinoma and glioblastoma, in nude mice are well accepted. This technique offers a physiological milieu for the long-term maintenance of tumor cell lines. Nevertheless, the physiological and immunological parameters present in the subcutaneous tissue of a nude mouse are quite different from those in the human cranial vault. The effects on hormone-binding proteins, tumor growth rate, and physiological response to hormonal manipulation in mice may not parallel the tumor response in humans, and these results must be interpreted in light of these factors. Although these are preliminary data, our findings justify a larger in vivo study of the effect of antiprogestational agents on the growth of meningiomas as well as an investigation of the effects of the antiprogestational agent on hormone-binding proteins in these tissues. These proposals are aimed at defining possible alternative methods of therapy for meningiomas.

Acknowledgments

We acknowledge R. Deraedt, M.D., of the Roussel-Uclaf Company, Paris, France, for providing the antiprogestrone RU-38486. In addition, we express our gratitude to Teri Saunders for technical assistance and to Mary Yoder for assistance in preparation of the manuscript.

References

6. Cushing H, Eisenhardt L: Meningiomas arising from the tuberculum sella with the syndrome of primary optic atrophy and bitemporal field defects combined with a normal sella turcica in a middle-aged person. Arch Ophthalmol 1:1--41, 168--206, 1929
Effect of antiprogesterone on meningioma growth in mice


______________________________
Manuscript received June 5, 1986.
Accepted in final form August 26, 1986.
Address reprint requests to: Jeffrey J. Olson, M.D., Division of Neurosurgery, University of Iowa Hospitals, Iowa City, Iowa 52242.