Analgesia induced by transplantation of encapsulated tumor cells secreting β-endorphin

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The purpose of this study was to assess whether xenogeneic tumor cells immunologically isolated in polymer capsules could survive and continue to reduce pain when transplanted into the cerebrospinal fluid (CSF) of rats. The mouse tumor cell lines AtT-20 and gene-transfected Neuro2A, which secrete β-endorphin, were enclosed in polymer capsules at a density of $5 \times 10^6$ cells/ml and transplanted into the spinal CSF space of the occipitoatlantal junction in male Sprague-Dawley rats. The analgesiometric tests (tail pinch, hot plate, and electrical stimulation) showed that the five rats with encapsulated AtT-20 or Neuro2A (eight rats) were significantly less sensitive to pain after transplantation than the eight control animals (analysis of variance; $p < 0.05$). The analgesia induced by encapsulated cells secreting β-endorphin could be attenuated by the opiate antagonist naloxone, which suggested the involvement of opiate in mediating this response. Morphological study revealed that the cells in polymer capsules survived 1 month after transplantation in the CSF space. In vitro experiments with cultured capsules showed that both encapsulated AtT-20 and Neuro2A secrete peptide for 1 month. The results of this study suggest that immunologically isolated xenogeneic tumor cells can secrete opiate in the CSF space, and this method may be applied to the treatment of cancer pain.

KEY WORDS • AtT-20 • Neuro2A • polymer capsule • pain • opiate

Opiate infusion into the cerebrospinal fluid (CSF) space affects the opiate receptor in the spinal cord and reduces sensitivity to pain. In clinical treatment, this theory has been applied to the treatment of the pain caused by cancer. Various methods have been used for epidural or intrathecal infusion of morphine. Semipermeable membranes used for capsules allow transport of low-molecular-weight substances, such as nutrients and neurotransmitters, but prevent the inward diffusion of both the humoral and cellular elements of the immune system. Therefore, the cells in the capsules are isolated from the host immune system, which facilitates xenograft survival. In addition, the ability to remove an implant is an advantage in the event of infection, overdose, or idiopathic side effects. In the central nervous system, this technique has been used in the treatment of Parkinson’s disease in animal models and for reduction of pain.

In the present study, AtT-20 and proopiomelanocortin gene–transfected Neuro2A, both of which secrete ACTH and β-endorphin, were encapsulated and transplanted into the CSF space of rats. The rats were expected to exhibit analgesic effects because the secreted β-endorphin from encapsulated cells binds opiate receptors in the spinal cord. Three measures (tail pinch test, hot-plate test, and electrical stimulation) were used to evaluate possible analgesic effects associated with the β-endorphin–secreting cells. This experiment is the first in which encapsulated tumor cell lines secreting opiate were transplanted into the CSF space. This study was approved by the Institutional Care and Use Committee.

Materials and Methods

Cell Encapsulation

The mouse pituitary tumor cell line AtT-20 and the mouse neuroblastoma cell line proopiomelanocortin gene–transfected Neuro2A (gift from Dr. Boileau), both of which secrete ACTH and β-endorphin, were used for encapsulated transplantation. The experimental animals were divided into three groups, AtT-20 (five rats), Neuro2A (eight), and control (eight). Polymer capsules (PM30; inner diameter, 500 μm; length, 15 mm) were used for transplantation. This capsule was permeable to molecules below 30 kD. The tumor cells were suspended in phosphate-buffered saline (PBS) at a density of $5 \times 10^6$ cells/ml. After injection of tumor cell suspension into the polymer capsule, both ends were heat sealed. Polymer capsules for control implants (control group) were filled with the conditioned culture medium.
Analgesia induced by β-endorphin–secreting cells

In Vitro Experiments

Cell line AtT-20 was maintained in Ham’s F10 medium containing 15% horse serum and 2.5% fetal bovine serum, and Neuro2A was maintained in Dulbecco’s modified Eagle’s medium containing 5% horse serum, 10% fetal bovine serum and 100 μg/ml geneticin. Simultaneously with the implantation of polymer capsules in the CSF space of experimental animals, both tumor cell lines were suspended in PBS at a density of 5 × 10^6 cells/ml and encapsulated in 3-cm long polymer capsules (five pieces of each tumor cell). These capsules were then incubated in each conditioned culture medium (3 ml). On Days 3, 10, 17, 24, and 31 after encapsulation, the medium was changed, and the capsule was incubated for 24 hours. The concentrations of ACTH secreted from the tumor cells as they incubated in the media were measured by radioimmunoassay.

Transplantation Surgery

Young adult male Sprague-Dawley rats each weighing 300 g were anesthetized by intraperitoneal injection of 60 mg/kg of sodium pentobarbital. Under an operative microscope, the occipitotemporal junction was exposed, dura mater and arachnoid membrane were incised, and a capsule was implanted into the subarachnoid space of the spinal cord. The animals in the control group received the polymer capsules without cells. In some animals, subarachnoid bleeding occurred at the time of insertion of the capsule. The animals that had massive bleeding in the CSF space or showed motor abnormalities following the surgical procedures were eliminated from the study. The remaining animals were returned to their cages and allowed free access to food and water. They were tested for pain sensitivities at 2 and 4 weeks after the transplantation.

Analgesiometric Tests

The tests included the tail-pincho test, hot-plate test, and electrical stimulation. For the tail-pincho test, a homeostatic clip (1 kg constant pressure) was applied to the root of the rat’s tail.12 Nociceptive responses were measured by latency of the biting response to the clip. In the absence of a response, the clip was removed after 10 seconds to prevent tissue damage and assigned a tail-pincho latency of 10 seconds. Hot-plate test response was determined by placing the rat on a 55˚C plate enclosed in a plexiglass cylinder. The interval between placement on the hot plate and the response of either licking the hind paws or jumping off the plate is defined as the “hot-plate latency.”21 Neuromuscular electrical stimulation* was performed on the hind leg. The voltage was increased sequentially until the animal reacted with a withdrawal response. The animals were initially screened for baseline pain sensitivities. The six Neuro2A-implanted animals received 1 mg/kg body weight of naloxone, given intramuscularly 1 day after the test for pain sensitivity at 4 weeks. One-half hour after the naloxone injection, pain sensitivity was assessed again using the three analgesiometric tests.

Morphological Examination and Radioimmunoassay

After the analgesiometric tests, the animals with polymer capsules were killed with an overdose of sodium pentobarbital, and the polymer capsules were removed. The encapsulated tumor cells were embedded in paraffin. Sections 5 μm thick were cut and mounted on glass slides, stained with H & E, and studied under light microscopy. The concentrations of ACTH and β-endorphin in the media were measured by Allegro kit†. Anti-β-endorphin immunoglobulin G was originally raised in rabbits by several subcutaneous injections of synthetic human β-endorphin.

Statistical Analysis

Mean and standard deviation or standard error of the mean were calculated for each time point and group in the analgesiometric tests. Data were subjected to analysis of variance corrected for repeated measures on two factors (treatment and time), followed by Newman–Keuls’ test for multiple comparisons. The paired t-test was used to compare the responses to analgesiometric tests before and after naloxone injection.

Results

In vitro data are summarized in Fig. 1. The ratio of ACTH and β-endorphin secretions was consistent in each cell line throughout the study. The ratio was 1.012 ± 0.02 in AtT-20 (five capsules) and 1.082 ± 0.09 in Neuro2A (five capsules). A few weeks after encapsulation, tumor cells continued to secrete peptides. Ten days after encapsulation, the concentrations of the peptides in the media of both tumor cell lines were maximum. As late as 31 days after encapsulation, the peptide secretion was as high as that of Day 3 in Neuro2A.

In the in vivo study, rats in both groups (AtT-20, five animals; Neuro2A, eight animals) revealed significant

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* Neuromuscular stimulator Model DPS-07 provided by DTA Medical System Co., Tokyo, Japan.
† Allegro kit supplied by Nichols Diagnostics, San Juan Capistrano, California.
analgesia in comparison with the control group and pretransplanted animals, except for the hot-plate test and electrical stimulation at 4 weeks after implantation in the AtT-20 groups (Table 1, Fig. 2). There was no significant difference between pain sensitivity at 2 weeks and 4 weeks in each group. The intramuscular injection of naloxone produced the same pain sensitivity as in control animals at 4 weeks in the Neuro2A group (Fig. 3).

In preliminary study, two rats in the AtT-20 group had massive bleeding in the CSF space at the time of capsule implantation. These two revealed a poor response to analgesiometric tests (hot-plate test: 5.1, 3.6 seconds, tail pinch test: 1.7, 1.0 seconds, electrical stimulation: 20, 20 V, respectively) at 2 weeks after the transplantation. At autopsy, the capsules were found to be covered with connective tissue (Fig. 4). Therefore, the animals with bleeding in the CSF space at the time of implant were prospectively removed from the study.

Morphological study revealed that the tumor cells in the capsules survived but aggregated, and the core of the spheroid was necrotic. The tumor cells in outer layers of the spheroid in the capsules survived 1 month after the implant (Fig. 5). Analgesic effects were provided to these animals by the capsules.

**Discussion**

In this study, rats that had received implants of encapsulated β-endorphin–secreting cells (AtT-20 and Neuro2A) into the CSF space revealed significant analgesia at 2 and 4 weeks after implantation. This effect was reversed by the opiate antagonist, naloxone. The viability of encapsulated tumor cells was confirmed by morphological examination after the testing of pain sensitivity given at 4 weeks. The *in vitro* study revealed that the encapsulated tumor cells kept secreting peptides for 1 month. Analgesic effects were provided to these animals by the capsules.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Hot-Plate Test (sec)</th>
<th>Tail-Pinch Test (sec)</th>
<th>Electrical Stimulation (V)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuro2A</td>
<td>8</td>
<td>6.3 ± 1.5</td>
<td>1.3 ± 0.7</td>
<td>15.6 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>preop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wks</td>
<td>10.3 ± 2.1*</td>
<td>9.6 ± 0.7*</td>
<td>38.1 ± 7.0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wks</td>
<td>9.5 ± 3.2*</td>
<td>9.3 ± 1.4*</td>
<td>32.5 ± 4.6*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtT-20</td>
<td>5</td>
<td>6.1 ± 1.3</td>
<td>1.6 ± 0.7</td>
<td>14.0 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>preop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wks</td>
<td>11.3 ± 3.0*</td>
<td>9.8 ± 0.4*</td>
<td>30.0 ± 11.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wks</td>
<td>7.4 ± 2.2</td>
<td>8.9 ± 2.5*</td>
<td>23.0 ± 5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8</td>
<td>6.5 ± 1.7</td>
<td>1.0 ± 0.3</td>
<td>19.4 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>preop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 wks</td>
<td>4.7 ± 1.5</td>
<td>1.5 ± 0.9</td>
<td>16.9 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wks</td>
<td>5.2 ± 1.3</td>
<td>2.0 ± 2.4</td>
<td>13.8 ± 4.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The responses to the analgesiometric tests were significantly different from those at the preimplantation (p < 0.05). The values represent the mean ± SD.*

Injection of opiate into the CSF space at the lumbar level is more beneficial than injection into ventricles, because intraventricular injection of opiate induces activation of brain opiate receptors, and the patients develop drowsiness, mental clouding, and changes in mood. Therefore, various alternative methods for delivery of opiates into the CSF were tried for the treatment of pain. Some studies have compared the continuous infusion of opiates and bolus injection into the intrathecal space. They concluded that continuous infusion is more effective than bolus injection for long-term pain control. Long-term administration of low doses of opiate to the patient’s spinal cord caused analgesia without the development of tolerance or the side effects that occur with activation of brain opiate receptors. In the present study, encapsulated β-endorphin–secreting tumor cells were implanted in the CSF space and induced an analgesic effect on the host animals for 1 month. This transplant system may be
called a “biological pump” system of β-endorphin. The mechanical delivery systems have a limited capacity to store the drug and are also limited to use with agents that are chemically stable for long periods. Therefore, encapsulated tumor cells may be superior in many respects.

In the hot-plate test and electrical stimulation, the AtT-20 group at 4 weeks after the implantation failed to reveal significant analgesia in comparison with tests prior to transplantation. From the in vitro study, the secretions of β-endorphin from the AtT-20 cells line at 2 weeks after implantation were expected to be higher than those at 4 weeks. Therefore, the animals might fail to show significant analgesia in these tests at 4 weeks.

It was reported that β-endorphin is a potent antinociceptive agent against both thermal and nonthermal stimuli through β and ε receptors. Also, metenkephalin has an antinociceptive effect on both stimuli through µ and δ receptors. On the other hand, κ-receptor agonists (such as dynorphin) are, in general, relatively feeble agents against thermal stimuli in comparison with their pronounced activity on nonthermal stimuli. From this evidence, our system is expected to be effective for both thermal and nonthermal stimuli.

There were some problems associated with our encapsulation implants. The tumor cells formed spheroids in the capsules. One month after transplantation, morphological investigation showed that only the surface layers of the spheroid of tumor cells survived (Fig. 5). The survival rate of tumor cells was not quantified and did not seem to be high. Other researchers have used a method that enclosed cells within alginate spherical droplets which have an average volume of 500 to 600 µm; these droplets are then encapsulated. The implanted cells survived and appropriately secreted neurotransmitters, apparently because there was no formation of a spheroid of cells within the capsules after 1 month. This method is under consideration for application to our cell lines. The other problem of encapsulation was that connective tissue would often cover the polymer capsules after transplantation. This was seen particularly when the operation was associated with heavy bleeding, and little analgesia was achieved in these animals. However, this problem may be avoided in large animals or humans.

From the in vitro data, we concluded that the maximum concentrations of peptides from tumor cells 10 days after the encapsulation are due to the release of large amounts.
of peptides from cytoplasm, caused by the collapse of cells located deep within the spheroids. One month after encapsulation, secretion from the encapsulated cells was almost the same as that just after the encapsulation (see Fig. 1). Adrenocorticotrophic hormone and b-endorphin are processed from proopiomelanocortin (precursor peptide), and the ratio of the production of these substances is thought to be consistent in AtT-20 and Neuro2A. In our study, the ACTH/b-endorphin ratio was 1:0.12 ± 0.02 in AtT-20 and was 1:0.82 ± 0.09 in Neuro2A. Confirmation of peptide secretion from the tumor cells in the capsules was performed by measuring the level of ACTH in the culture media.

Sagen and colleagues21 reported that transplants of chromaffin cells to the spinal CSF space provided analgesia in rats. They suggested that the combination of catecholamines and enkephalin secreted from chromaffin cells was the basis for analgesia. Some researchers are using encapsulated bovine chromaffin cells to evaluate their effectiveness for the treatment of cancer pain (Lysaght, et al., unpublished data). It will be very important to compare the effects on pain control of chromaffin cells and our tumor cell lines secreting b-endorphin. In the near future, these implant systems may be applied to the clinical treatment of cancer pain.

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References


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