MICROSphere BASED TAXOL DRUG DELIVERY FOR THE TREATMENT OF EWING'S SARCOMA

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Relevance to Musculoskeletal Conditions

Ewing’s sarcoma is a highly malignant tumor usually afflicting children during the second decade of life. The overall goal of this work is the development of a new treatment method for Ewing’s sarcoma based on controlled delivery of the radiosensitizer taxol. Utilizing an in vitro Ewing’s sarcoma model, this study examines the ability of this system to effectively deliver taxol in a bioactive form.

Introduction

Current methods for treatment of Ewing’s Sarcoma involve surgery, chemotherapy, and radiation, alone or in combination. Another approach to the treatment of Ewing’s sarcoma could be the specific modification of tumor radiosensitivity by the use of chemical radiosensitizers. Radiation sensitizers are drugs which have been shown to enhance the effects of both single and fractionated radiation therapies. One such drug, taxol, has demonstrated both independent cytotoxicity and the ability to radiosensitize cells. Our theorized approach to this problem has been the local delivery of taxol utilizing a biodegradable polymeric microsphere delivery system implanted at the tumor site. Our previous studies have shown that taxol can be successfully encapsulated in degradable polymer microspheres and released in a control manner. This study focuses on the ability of released taxol to effectively radiosensitize Ewing’s sarcoma cells.

Materials and Methods

The delivery system consisted of taxol loaded microspheres composed of a 50:50 poly[pyromellitylimidoalanine 1,6-bis (carboxyphenoxy)hexane] [PMA-CPH]. Preparation of this copolymer is described elsewhere. Briefly, taxol microspheres were fabricated using a solvent emulsion technique. Taxol was mixed with PMA-CPH (50:50) to obtain taxol concentrations of 1, 5, and 10 percent by weight. This mixture was dissolved in methylene chloride in a 1:5 w/v ratio. The taxol/polymer solution was added dropwise with stirring to a 1% aqueous solution of poly(vinyl alcohol). The suspension was mixed continually for one hour. The cells were then centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were fixed according to the dual-color staining procedure. Briefly, the cells were centrifuged at 100 rpm for 5 minutes. The supernatant was aspirated and the pellet was resuspended in 1ml of fetal bovine serum and 1ml of RPMI-1640 media (Sigma Co. St. Louis, MO). The suspension was mixed (containing all cells) for 2 hours. The cells were washed with deionized water, and allowed to dry in a vacuum. Individual microspheres were isolated by vacuum filtration, washed with deionized water, and allowed to dry in a vacuum. Microsphere Morphology:

Results and Discussion

Microsphere Morphology: Microsphere morphology can be used as an indicator of encapsulation. Figure 1 shows the electron micrographs of the 1% and 10% taxol loaded microspheres. As seen from these images, the surface of the microspheres changed as the concentration of taxol increased. Microsphere surface changed from a smooth, rounded morphology with relatively few pores to a roughened surface with an increase in the size and number of the surface pores. These results agree with the findings of Bodmeier and McGinity who found that at higher drug concentrations, the microsphere surface changed from a relatively smooth texture with few pores to a microporous “honeycomb” structure. Flow Cytometry: A flow cytometry study was conducted in order to assess the radiosensitivity of taxol released from the poly(anhydride-co-imide) microspheres. The results from the flow cytometry study are shown in table below:

<table>
<thead>
<tr>
<th>Taxol Concentration (weight %)</th>
<th>Mitosis Cycle (Cell %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>S</td>
</tr>
<tr>
<td>0 %</td>
<td>66.0 %</td>
</tr>
<tr>
<td>1 %</td>
<td>16.3 %</td>
</tr>
<tr>
<td>5 %</td>
<td>26.6 %</td>
</tr>
</tbody>
</table>

These results indicated that active taxol was released from the loaded polymeric microspheres. With the addition of 1% and 5% taxol loaded microspheres to the Ewing’s sarcoma culture, the number of cells in the G2/M phase increased compared to the non-taxol control. The data also showed that the number of cells in the G2/M phase did not increase with increasing taxol dose suggesting that the radiosensitizer effect was not dose dependent. This was consistent with the findings of Zanelli.

Conclusions

In these studies we examined the release of taxol from a degradable, poly(anhydride-co-imide) microsphere delivery system. Bioactive taxol was released from the microsphere as demonstrated by the ability of the released taxol to effectively block cells in the G2/M phase of mitosis. Based on these results and our previous work, the poly(anhydride-co-imide) microsphere system shows promise as a carrier for Taxol. The advantages of a microsphere based carrier system is that localized doses of drug can be delivered to tumor sites to radiosensitize sarcoma cells. Additionally, the delivery of a radiosensitizing agent such as taxol may enable a lower dose of radiation to be used.

References


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