Local delivery of OncoGel delays paresis in rat metastatic spinal tumor model

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Object. Spinal column metastatic disease clinically affects thousands of cancer patients every year. Local chemotherapy represents a new option in the treatment of metastatic disease of the spine. Despite the clinical impact of metastatic spine disease, the literature currently lacks an accurate animal model for the effective dosing of local chemotherapeutic agents within the vertebral column.

Methods. Female Fischer 344 rats, weighing 150 to 200 g each, were used in this study. After induction of anesthesia, a transabdominal approach to the ventral vertebral body of L-6 was performed. A small hole was drilled and 5 μL of ReGel (blank polymer), OncoGel (paclitaxel and ReGel) 1.5%, OncoGel 3.0%, or OncoGel 6.0% were immediately injected to determine drug toxicity. Based on these results, efficacy studies were performed by intratumoral injection of 5 μL of ReGel, OncoGel 3.0%, and OncoGel 6.0% on Day 6 in a CRL-1666 breast adenocarcinoma metastatic spine tumor model. Hind limb function was tested pre- and postoperatively using the Basso-Beattie-Bresnahan rating scale. Histological analysis of the spinal cord and vertebral column was performed when the animal died or was killed.

Results. There were no signs of toxicity observed in association with any of the agents under study. No increased benefit was seen in the blank polymer group compared with the control group (tumor only). OncoGel 3.0% and OncoGel 6.0% were effective in delaying the onset of paralysis in the respective study groups.

Conclusions. These findings demonstrate the potential benefit of OncoGel in cases of subtotal resections of metastatic spinal column tumors. OncoGel 6.0% is the most efficacious drug concentration and offers the best therapeutic option in this experimental model. These results provide promise for the development of local chemotherapeutic means to treat spinal metastases. (DOI: 10.3171/SPI-07/08/194)

KEY WORDS • local chemotherapy • metastatic spinal tumor • rat

It has been estimated that cancer is diagnosed in 1.2 million individuals in the US each year. Of those individuals, approximately 5% will develop symptomatic spinal metastases at some point in their disease course. The most common primary cancers to metastasize to the spinal column are lung and breast lesions. With increasing survival times for patients with cancer, the burden of metastatic disease, particularly metastases to the spinal column, will undoubtedly increase in the coming years. Given the severe morbidities associated with spinal metastases—pain, paralysis, numbness, and incontinence—enhancing the efficacy of treatments for this disease is important in ensuring quality of life for an enlarging population of patients with advanced oncological disease.

Current treatment modalities for metastatic spinal tumors center around focused radiotherapy and excision. In a recent trial conducted by Patchell et al. excision followed by radiotherapy was superior to radiotherapy alone in maintaining ambulatory status in patients treated for metastatic spinal tumors (84% compared with 57%). As yet, chemotherapy has not been a major modality for the treatment of metastatic spinal tumors due to the advanced stages of the tumors involved, although rare reports of successful systemic chemotherapeutic regimens have appeared in the literature. Although the addition of effective chemotherapeutic regimens to the current scheme for the management of metastatic spinal tumors is largely unexplored, it could be a powerful treatment modality.

OncoGel is a novel chemotherapy delivery system developed by MacroMed, Inc. We investigated it in this study as a possible treatment for metastatic spinal tumors. The product consists of paclitaxel dissolved in a thermosensi-
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tive, water-soluble, biodegradable polymer (poly dl-lactide-co-glycolide) called ReGel (MacroMed). This polymer has a relatively low viscosity at temperatures below 13.62°C but transforms quickly into a gel state at temperatures of 17.8°C or greater. The results of in vitro studies have shown a steady, linear release of paclitaxel from ReGel over a period of 50 days. OncoGel’s steady release of paclitaxel in its gel state makes it an intriguing drug choice, as it could be easily injected and applied to the entire surface area of a tumor resection cavity to eliminate residual or adjacent tumor. Current commercially available drug concentrations include 1.5, 3.0, and 6.0%.

Materials and Methods

Tumor Cell Line

The tumor cell line used was the CRL-1666 mammary adenocarcinoma (also known as the 13762 MAT B III); the cell line was initially established at the EG&G Mason Research Institute from a transplantable rat ascites tumor derived from the 13762 solid mammary adenocarcinoma. The tumor cells used in this study were purchased from the American Type Culture Collection. The cells, which are only loosely adherent, are maintained in cell culture in Dulbecco modifed Eagle medium with 10% fetal bovine serum, streptomycin (80.5 pg/ml), penicillin (base, 80.5 U/ml), and 1% l-glutamine (all products from Gibco Laboratories) in a humidified atmosphere of 5% CO₂ at 37°C. The cells are grown to a concentration of 1 million cells per ml and then resuspended in medium (approximately every 3 days).

Solid tumor was established by injection of the cell suspension into the flanks of female Fischer 344 rats. To transfer the tumor, the flank of the carrier was shaved and prepared with povidone-iodine solution. Under sterile conditions, the tumor was excised and viable tissue was minced into approximately 5-mm³ pieces. A recipient female Fischer 344 rat flank was similarly prepared, and a single tumor piece was implanted into a subcutaneous pocket in the recipient flank via a 1-cm horizontal incision in the skin. The tumor was passaged once after 2 weeks of incubation in the initial carrier flank before implantation into a VB.

Anesthetic Agents

Female Fisher 344 rats were anesthetized with an intraperitoneal injection of 3 ml/kg of a stock solution composed of ketamine hydrochloride (Abbott Laboratories) 25 mg/ml, xylazine (Phoenix Pharmaceutical) 2.5 mg/ml, and 14.25% ethyl alcohol in 0.9% NaCl (Pharmaceuticals, Inc.).

Toxicity Study

Following induction of anesthesia and shaving of the animals’ abdomens, the skin of the abdomens was prepared by application of a povidone-iodine solution, and 0.15 ml of 0.25 g/ml ceftriaxone (Roche Pharmaceuticals) was administered intraperitoneally. A 3-cm midline abdominal incision was made through the skin and underlying peritoneum, with care taken to avoid injuring the abdominal contents. The abdominal viscera, including the large and small intestines, were then identified and retracted from midline. Small sprays of 0.9% NaCl were occasionally applied to the viscera throughout the rest of the procedure to prevent desiccation. The vascular bundle containing the aorta and the vena cava was then located. Once the aorta was visualized, a 2-cm diameter circular retractor was placed in the abdominal cavity to protect the abdominal contents from injury during the rest of the procedure.

A surgical drill and 1-mm bur (dental drill, Aseptico) were used to drill a hole into the inferior border of the L-6 VB, approximately 0.5 mm superior to the intervertebral disc. Once the desired depth of 1 mm was achieved, the bur was rotated so as to widen the cavity while preserving the initial hole size.

Twelve animals were allocated into four groups, and 5 ml of either ReGel or one of the three OncoGel formulations under investigation was injected into the cavity in the VB of each rat using a 10 μl Gaslight (Hamilton Co.) removable blunt-tipped syringe. The control animals (three rats) received 5 μl of ReGel, and the remaining nine animals received 5 μl of OncoGel 1.5% (three rats), 5 μl of OncoGel 3.0% (three rats), or 5 μl of OncoGel 6.0% (three rats). (ReGel and OncoGel were provided by MacroMed, Inc.)

After drug injection the hole was sealed with polymethylmethacrylate that was allowed to dry. Subsequently, the retractor was removed, and the abdominal muscles were sutured with 3-0 Vicryl (Ethicon) using a running suture technique. Once the abdominal muscles were sutured, the skin was closed with surgical autoclips.

The animals were allowed to recover. Hind limb function was assessed using the BBB locomotor rating scale before the tumor implantation procedure to obtain a pretreatment value.

Hind limb function was assessed using the BBB locomotor rating scale after the tumor implantation procedure. Following induction of anesthesia, the abdomen of the animal was shaved and prepared with a povidone-iodine solution and the anterior L-6 VB was approached as described in the previous section of this article. The hole was made at a depth of 1 mm, and once the hole achieved the desired depth, the bur was rotated so as to widen the cavity while preserving the initial hole size. A tumor section was then chosen such that it could fit entirely inside the hole in the VB, without any protuberances (approximately 1 mm²). After tumor implantation the hole was sealed with a small amount of polymethylmethacrylate that was allowed to dry. Subsequently, the retractor was removed, and the surgical wound was closed as described previously.

The animals were allowed to recover, and hind limb function was assessed and recorded using the BBB locomotor rating scale as described for the toxicity study.

On Day 6 post–tumor implantation, the animals were anesthetized again according to the previously outlined protocol and their abdomens were prepared as previously described. A 0.15-ml injection of 0.25 g/ml ceftriaxone was administered intraperitoneally prior to reoperation, and any remaining abdominal autoclips were gently removed. The entire length of the previous vertical abdominal incision was opened. The underlying peritoneal suture was identified and released, with care taken to lift the peritoneum up and away from the viscera while reopening the abdomen. Once the abdominal compartment was opened, the viscera were gently retracted from midline with the use of blunt lysis of adhesions.

The vascular bundle containing the aorta and the vena cava was then located. Once the aorta was visualized, a 2-cm-diameter circular retractor was placed in the abdominal cavity in order to protect the abdominal contents from injury during the reoperation procedure.

When necessary, the remaining polymethylmethacrylate cement cap overlying the previous tumor implantation site was then identified carefully freed of any adhesions to the viscera or great vessels using blunt dissection, and removed. More commonly the tumor mass had grown so much that the polymethylmethacrylate cap had been completely engulfed by tumor. Using a 10 μl Gaslight removable blunt-tipped Hamilton syringe, the tumor mass was penetrated and 5 μl ReGel (control group, eight rats), 5 μl OncoGel 3.0% (eight rats), or 5 μl OncoGel 6.0% (eight rats) was administered intratumorally.

Subsequently, the retractor was removed, and the abdominal muscles were sutured with 3-0 Vicryl using a running suture. Once the abdominal muscles were sutured, the skin was closed with the use of surgical autoclips.
The animals were allowed to recover, and hind limb function testing using the BBB locomotor rating scale was resumed. According to institutional review board criteria, the animals were killed if their BBB functional score fell below 7; otherwise they were allowed to die naturally. Histological analysis of the spinal cords and vertebral columns was performed after death.

Results

Toxicity Study

None of the animals showed signs of poor grooming, and at 28 days postoperatively, all surviving animals demonstrated BBB locomotor scores of 21 in all limbs. Notably, animals in the ReGel and OncoGel 3.0% groups did experience transient, minimal decreases in hind limb motor function but, with one exception, they recovered within 3 days. This transient decline was thought to be related to the surgical technique during the intravertebral injections. One rat treated with OncoGel 3.0% showed a decline in hind limb function immediately after surgery and died on Day 9. All other animals were alive without signs of distress on Day 120. Histological analysis demonstrated no evidence of toxicity to the spinal cord in any animal, including the one that died on Day 9.

Efficacy Study

Twenty-four female Fischer 344 rats were included in the efficacy portion of this experiment, eight in each of three groups. Based on the results of the toxicity studies, OncoGel 3.0% and OncoGel 6.0% were used for this portion of this study.

The functional status of the animals was tracked using the BBB locomotor rating scale (Fig. 1). Before tumor implantation, all animals displayed BBB locomotor scores of 21 (normal hind limb function). Immediately after tumor implantation surgery, on Day 1, there was no significant difference in BBB locomotor rating scores between the experimental groups. Using the Wilcoxon signed-rank test, the probability values for between-group differences were 0.2019 for the control group compared with the OncoGel 3.0% group; 0.9165 for control compared with OncoGel 6.0%; and 0.7150 for OncoGel 3.0% compared with OncoGel 6.0%. The average pretreatment BBB scores were as follows: control animals, 19.00; OncoGel 3.0% animals, 20.00; and OncoGel 6.0% animals, 19.56. At 10 days after tumor implantation, the average BBB scores for the control, OncoGel 3.0%, and OncoGel 6.0% animals were 9.00, 16.80, and 16.86, respectively. There was a significant difference between hind limb performance of the control animals and that of the OncoGel-treated animals, as measured using the BBB locomotor rating scale (controls compared with OncoGel 3.0%, p = 0.0431; controls compared with OncoGel 6.0%, p = 0.0117). No statistically significant difference was found between the BBB scores of the OncoGel 3.0% group and the OncoGel 6.0% group (p = 0.1380). By postimplantation Day 16 all surviving control animals had complete hind limb paresis (BBB locomotor rating score 0). Animals in the OncoGel 3.0% treatment group reached total hind limb paresis by postimplantation Day 19, whereas surviving animals in the OncoGel 6.0% treatment group became totally paralyzed in their hind limbs by Day 24.

Survival Analysis

The animals were monitored daily for death or extensive morbidity (BBB locomotor score < 7, poor grooming, and weight loss). Animals were killed if their hind limb function declined below a BBB score of 7 as required by the authors’ institution’s animal care and use committee guide-
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Figure 2 shows Kaplan–Meier curves illustrating animal survival in each group. There was a significant extension of animal survival in the OncoGel 3.0% and OncoGel 6.0% groups compared with the control animals (control compared with OncoGel 3.0%, p = 0.0229; control compared with OncoGel 6.0%, p = 0.0048). All control animals died by Day 17 post–tumor implantation as the study criteria were met. The OncoGel 3.0% and OncoGel 6.0% animals had all died by Days 19 and 24 post–tumor implantation, respectively. Median survival time was 14, 18, and 18 days post–tumor implantation for the control, OncoGel 3.0%, and OncoGel 6.0% animals, respectively. There was no significant difference in animal survival between the 3.0% and 6.0% OncoGel-treated groups (p = 0.0706).

Histological analysis universally demonstrated extensive tumor growth and infiltration of the VB with encroachment on the spinal cord. There were patchy regions of necrosis present in animals from the treatment and control groups, with an observed trend towards more patches of necrosis and less infiltration in the OncoGel 6.0% group. There was no significant difference in the size of the tumor mass or the degree of spinal cord impingement between the control and treatment groups at the time of histological analysis.

Discussion

The excessive morbidities associated with metastatic breast cancer greatly limit the treatment modalities available to physicians combating the disease. Limited bone marrow reserves (due to radiation and tumor invasion), advanced age, tumor-induced osteoporosis, and multiorgan involvement leave patients severely ill and unable to tolerate systemic cytotoxic chemotherapeutic agents. Standard systemic chemotherapeutic agents for the treatment of metastatic breast cancer, such as the anthracyclines and taxoids, are often poorly tolerated, and the better-tolerated hormonal therapies often take too long to demonstrate efficacy to be of practical use. Locally implanted paclitaxel therefore offers particular promise in allowing patients to avoid the terrible side effects of systemic paclitaxel while still providing a rapid cytotoxic effect on nearby tumor. This effect would be particularly relevant in patients with metastatic disease in bone only or spine only.

Previous experiments in nude mice have demonstrated minimal toxicity when OncoGel is delivered intratumorally. Using carbon-14–radiolabeled paclitaxel in OncoGel (6 mg/ml), the authors of previous studies have shown that intratumoral OncoGel does not significantly concentrate in the bloodstream or organs when injected intratumorally. These past experiments also failed to demonstrate toxicity in mice at paclitaxel concentrations up to 60 mg/kg. When ReGel was examined using the Food and Drug Administration Modified Biocompatibility Tests, it did not induce immune responses greater than those observed with surgical suture material highlighting the benign nature of the carrier polymer.

In the toxicity portion of the current study, 5 μl of ReGel, OncoGel 1.5%, OncoGel 3.0%, or OncoGel 6.0% was injected into the L-6 VB of rats. Minor reductions in BBB locomotor scores were observed in the hind limbs of animals in the ReGel and OncoGel 3.0% treatment groups. These reductions in scores occurred immediately postoperatively and were completely resolved within 7 days. Such decreases in hind limb function are consistent with surgical trauma related to the depth and pressure of the drilling within the L-6 VB. Notably, one animal in the OncoGel 3.0% group experienced declining hind limb function and then died 8 days postoperatively. All other animals were alive and healthy 120 days postoperatively, suggesting little to no toxicity associated with local OncoGel therapy, even at 6.0% concentrations of paclitaxel in ReGel. The single death appeared on postmortem examination to be due to postoperative complications.

Well-tolerated, local paclitaxel treatment in the form of OncoGel 3.0% and OncoGel 6.0% also demonstrated significant prolongation of hind limb function (mean 19 and 24 days, respectively, compared with 16 days in the control group). Previous rat metastatic spinal tumor models have been difficult to interpret with regard to the onset of hind limb paresis. However, the rat spinal tumor model used here has demonstrated remarkable consistency in the onset of paresis (total paresis by 16 days after tumor implantation).
It is therefore unlikely that the difference in BBB locomotor scores between the controls and OncoGel-treated animals was due to variability in the spinal tumor model.

OncoGel treatment did not completely salvage hind limb function over time or eliminate the breast adenocarcinoma. Average BBB locomotor scores were significantly higher in OncoGel-treated animals, regardless of paclitaxel dose, than in controls up to the point of death, but hind limb ambulatory status among treated animals maintained a slow but consistent decline in both the OncoGel 3.0% and the OncoGel 6.0% treatment groups.

In addition to demonstrating delayed onset of paresis, OncoGel-treated animals also survived significantly longer than controls, with a trend toward a significant dose-related extension of survival (OncoGel 3.0% group compared with OncoGel 6.0% group, p = 0.0706). Consistent with a dose-related cytotoxic effect against this metastatic spinal tumor model, the histopathological findings in sections obtained shortly after death showed a trend toward more foci of necrosis and fewer instances of bony invasion in the OncoGel 6.0% group than in the controls or the OncoGel 3.0% group. These findings were not consistent among all the animals in the respective groups, however, and there was no significant between-group difference in the mean size of the tumor mass at the time of histological analysis. Further studies are necessary to determine if the histopathological findings represent a real effect of the treatment or an artifact. It is possible that with higher doses of paclitaxel or the addition of other treatment modalities (for example, resection or radiotherapy) that the antitumor effects of OncoGel could be enhanced.

Conclusions

The morbidity associated with metastatic spinal column tumors is significant, and there remains significant ground to be gained against this disease process. Unfortunately, most patients tolerate systemic chemotherapeutic treatments poorly. OncoGel is a promising local chemotherapeutic agent that avoids systemic toxicity while delivering high local concentrations of paclitaxel. In a rat model for metastatic spinal tumor disease, OncoGel demonstrated little toxicity and was associated with a significant delay in paresis. Though even the highest commercially available doses of OncoGel (6.0% paclitaxel in ReGel) did not eliminate the tumors in this animal model, there was a significant extension in survival in the treated groups. Further refinement of the drug (higher paclitaxel concentrations, larger injections, and so forth) alone or in combination with other treatment modalities holds promise for increasing OncoGel’s efficacy in this model. Nonetheless, the delay in the onset of paresis demonstrated in this rat model is encouraging with respect to the possibility of one day minimizing the terrible effects of metastatic spinal column disease on patients’ quality of life.

Disclaimer

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References
