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Managing Local Swelling Following Intratumoral Electro-Chemo-Gene Therapy

Jeffrey Cutrera¹, Glenn King², Pamela Jones², Elias Gumpel², Xueqing Xia¹, and Shulin Li¹

¹Anderson Cancer Center, Houston, TX 77030

²GCVS, Houston, TX

Summary

Delivering genes and other materials directly into the tumor tissue causes specifically localized and powerfully enhanced efficacy of treatments; however, these specific effects can cause rapid, drastic changes in the appearance, texture, and consistency of the tumor. These changes complicate clinical response measurements which can confound the results and render recurring treatments difficult to perform and clinical response measurements nearly impossible to accurately obtain. One of these complicating issues is local swelling. Here, we will demonstrate how swelling caused by intratumoral gene treatments can confound the clinical results and impede further treatments, and we will demonstrate an easy technique to help to overcome this potential hurdle.

Keywords

canine; oral tumor; intratumoral; electroporation; gene therapy; chemotherapy; chemogene therapy; immune therapy; IL12

1. Introduction

The use of direct intratumoral administration of agents followed by electroporation (EP) is continuously being studied, and specific applications of intratumoral chemotherapy with EP have been approved for use in humans in the European Union [1]. The benefits of sequestering the activity of these agents in the tumor microenvironment vastly outweigh the minimal side effects, such as local inflammation, swelling, bleeding, and pain. Although these side effects are minor, they can easily affect tumor measurement, clinical assessment, treatment enactment, and ultimately the patient's response. In an ongoing veterinary trial investing intratumoral gene and chemotherapy with EP, termed Electro-Chemo-Gene Therapy (ECGT) [2, 3], local swelling not associated with an infection following treatments has occurred in two patients. If this swelling is not controlled and corrected the tumor measurements will be inflated causing improper evaluation of clinical response and potentially harmfully elevated doses. For these reasons, a safe and effective method for determining the presence of swelling and reducing the intratumoral fluid level.

The ECGT with intratumoral Interleukin 12 (IL12) plasmid DNA (pDNA) and bleomycin procedure for treating head and neck tumors in canines has been published previously [2–4]. BLM is metabolized mainly through the liver and kidneys, and 45–70% is excreted through urine in the first 24 hours; therefore, dogs with elevated renal values (1.5 times normal) or hepatic values (2.0 times normal) determined by blood chemistry profiles are excluded. Prior to and after treatment, radiographs and CT scans should be performed to monitor the effect of the treatment and the dog's health as necessary based on tumor location [5, 6].

As part of an ongoing study, one particular case developed incessant swelling at the tumor site after treatment. This 9-year-old, male (neutered) Chihuahua presented with a 5.1 cm by 3.4 cm SCC located on the left maxilla (Fig. 1). Due to the location of the tumor and involvement of the orbital bone, surgery was not an option, and the only pretreatment interventions were pain medicine and antibiotics. As per the trial protocol, the patient received 0.5 mg bleomycin and 150 µg IL12 pDNA per centimeter of diameter directly into the tumor followed by 1 application of EP at each injection site (5 injections and 5 EP) on Day 0. During the initial exam for the second treatment on Day 7, the tumor appeared to have slightly increased in volume (Fig 2A). Upon closer inspection, a difference in consistency was noted as part of the tumor remained firm while the upper portion was evidently filled with fluid. Delicately, the fluid was drained from the tumor, and the reduction in tumor volume was evident (Figure 2B). At this point, the previous treatment was performed as scheduled. The swelling continued to occur, and at each visit a large volume was removed in the method described below. The difference in measured tumor volume before and after draining were significantly different, and only by measuring the volume after draining were we able to note the drastic reduction in tumor volume and clinical response from the ECGT treatments with IL12 pDNA and bleomycin (Figure 2C).

2. Materials

2.1. General Anesthesia

1. 23 GA Winged Infusion Set (Terumo Medical, Somerset, NJ)
2. 4 mm endotracheal tube (Rüsch, Research Triangle Park, NC)
3. Propofol (Abbot Laboratories, Abbot Park, IL)
4. Isoflurane (Abbot Laboratories, Abbot Park, IL)
5. Surgivet Lingual Pulse Oximeter (Smiths Medical, Dublin, OH)

2.2. Prescreening and Histopathological Diagnosis of Malignancy

1. 22 GA (0.7 mm × 25 mm) needles
2. 6 ml syringes
3. 3 ml BD Vacutainer containing the anticoagulant K₂EDTA 367835
4. 4 ml BD Vacutainer with SST Gel and Clot Activator 366514
5. IDEXX ProCyt Dx Hematology Analyzer and Strips

6. 22 GA (0.7 mm × 38.1 mm) needles
7. 10 ml BD Vacutainer 364979
8. IDEXX VetLab UA Analyzer
9. Shimadzu E7823FX (*Radiograph*)
10. GE HiSpeed R2xT
11. MD-76r contrast medium
12. Supercore Biopsy Instrument
13. 10 % Formaldehyde

2.4. Draining of Fluid to Reduce Swelling

1. Latex-free Sterile Examination Gloves
2. Vernier Caliper
3. 70% isopropyl alcohol
4. 22 GA (0.7 mm × 25 mm) needles
5. 25 ml syringes (*see* Note 1)

2.5. Intratumoral Injection and Electroporation

1. 70% isopropyl alcohol (generic)
2. IL-12 gene construct (Valentis, Inc., Burlingame, CA). The construct contains DNA fragments which encode for both the p35 and p40 subunits in the same backbone. Both subunits are driven by independent CMV promoters and terminated by independent bovine growth hormone polyadenylation signal[7][6][4][4][2].
3. Bleomycin
4. Electroporator BTS EC830
5. ½-cm 6-Needle electrode (Inovio, San Diego, CA)

3. Methods (see Note 2)

3.1. General Anesthesia

1. Insert catheter (from Winged Infusion Set) into cephalic vein.
2. Anesthetize using propofol induction into the catheter at a dose of 6mg/kg.
3. Insert endotracheal tube.
4. Maintain anesthesia using isoflurane (1.0–3.0 %) inhalation through the endotracheal tube.

¹Syringe size is dependent on the estimated volume of fluid as determined in **Section 3.4.1**.

²A licensed veterinarian or approved veterinary technician is required to perform all procedures detailed here.

5. Apply lingual sensor to of Surgivet Lingual Pulse Oximeter to the patients tongue to monitor the patient during anesthesia.

3.2. Prescreening and Histopathological Diagnosis of Malignancy

1. CBC/Chemistry Profile. Insert needle attached to syringe into jugular vein. Acquire 6 ml of blood in the syringe. Inject 3 ml of blood into the K₂EDTA vacutainer. Inject 3 ml of blood into the SST gel and Clot Activator vacutainer. Invert both vacutainers 5 times. Place the K₂EDTA vacutainer in the IDEXX ProCyt Dx Hematology Analyzer to measure the number of red blood cells, number of white blood cells, amount of hemoglobin, hematocrit (fraction of blood composed of red blood cells), platelet count, and the mean corpuscular volume (size of red blood cells). Let the SST gel and Clot Activator vacutainer sit for 30 minutes. Centrifuge at full speed for 15 minutes. Place SST gel and Clot Activator vacutainer in IDEXX ProCyt Dx Hematology Analyzer to measure the levels of chemicals released from tissues.
2. Urinalysis. Insert needle through the abdominal wall and into the bladder. Acquire 10 ml of urine. Inject urine into the 10 ml vacutainer. Remove rubber stopper from 10 ml vacutainer. Insert an IDEXX VET UA Strip into the tube and insert the strip into the IDEXX VetLab UA Analyzer.
3. Radiograph. Take right lateral, left lateral, and dorsal ventral radiograph views.
4. CT Scan. Administer general anesthesia as previously described. Place dog in sternal recumbency, head-first into the gantry of GE HiSpeed. Obtain initial scout images and subsequent 2 mm contiguous transverse images. Administer intravenous bolus (0.5mg/kg) of MD-76r contrast agent. Acquire contrast images using same imaging technique. Window width and level are manipulated before and after contrast administration to best visualize the tumor measurement and evaluation.
5. Biopsy. Administer general anesthesia as previously described. Insert Supercore Biopsy Instrument into the tumor. Gently press down the plunger until the instrument releases. Remove instrument. Remove the specimen from the instrument and place in 10% formaldehyde. Specimens should be delivered to a pathologist for microscopic examination and diagnosis.

3.4. Draining of Fluid to Reduce Swelling

1. Physical Exam. If the affected area is painful, administer general anesthesia as previously described. Gently palpate the tumor area to determine firmness of the tumor and identify potential areas of swelling. Mark the margins of swelling and diagram in chart. Using vernier calipers, estimate the volume of the fluid (see Note 3).

³The volume of the fluid can be estimated using the formula $V = (\pi/8) \times (ab^2)$, where V = tumor volume, a = longest diameter and b = diameter perpendicular to a . If the location of the tumor makes measurement with calipers impossible, a CT scan as described in Section 3.2.4 should be performed to determine volume.

2. Fluid draining. If the patient is not under anesthesia, administer general anesthesia as previously described. Based on the physical exam and the estimated volume of fluid, determine the safest area in respect to sensitive tissues and organs and most centrally located to the area of swelling (*see* Note 4). Disinfect area with 70% isopropyl alcohol. Gently insert the syringe needle into the predetermined safest area and allow the internal fluid pressure to force fluid into the syringe. Making sure to keep the needle near the surface and away from any tumor or normal tissue, slowly pull back the plunger filling the syringe with fluid (*see* Notes 5 and 6).

3.5. Intratumoral Injection and Electroporation

1. Use the CT images or prescreening measurements to determine the size of tumor and best injection site.
2. Administer general anesthesia as previously described.
3. Disinfect area with 70% isopropyl alcohol.
4. Inject 150 µg of IL-12 gene construct and 0.5 units of BLM per cm² of tumor.
5. Immediately after injection, place caliper electrode tight around tumor and apply 450 V/cm for 2 pulses of 25 ms.

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⁴At the discretion of the attending veterinarian.

⁵This procedure needs to be performed with delicacy to ensure that the needle does not contact any normal tissue which may result in a little fluid remaining in the tumor as long as the injection needle and needle-electrode can enter the tumor.

⁶If the attending veterinarian believes that an infection is present, post-pone ECGT until antibiotics can eradicate the infection.

Figure 1. Pretreatment Analysis of Maxillary Squamous Cell Carcinoma
CT Scan (A) and photographs (B, C) of the maxillary squamous cell carcinoma prior to first treatment.

Figure 2. Local Swelling can create Severe Differences in Tumor Volume Measurements

During the physical exam prior to the second treatment, a large area of the tumor appeared to be enlarged (**A**). Closer examination revealed that a significant portion of the tumor area was only fluid. After performing the draining procedure detailed here, the tumor volume was reduced (**B**). The pre- and post-draining volumes were measured at each visit, and the tumor volumes were significantly different as determined with an unpaired Student's T Test (**C**). Black arrows represent ECGT treatments with IL12 pDNA and bleomycin.

Figure 3. Proper draining of local swelling allows for continued treatment and proper evaluation of tumor response

After draining the fluid from the tumor, ECGT with IL12 pDNA and bleomycin (Black Arrows, Days 0 and 7) or IL12 pDNA alone (Blue Arrows, Days 35 and 42) were continued (A). After the first treatment, the tumor volume decreased by 60%, and only progressed to less than half of the initial volume. The tumor growth remained relatively stable from Day 14 (B) through 28 (C). By day 56 (D), the owner's elected for euthanasia.