



NOTE

Surgery

Surgery and electrochemotherapy treatment of incompletely excised mammary carcinoma in two male pet rats (*Rattus norvegicus*)

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ABSTRACT. Two male rats (*Rattus norvegicus*; 18 and 24 months old), were referred for treatment of large masses located in the axillary area. Following total body radiography and hematological and serum biochemical analysis, the rats were anesthetized, and the masses were surgically removed. Both lesions were diagnosed as mammary carcinoma based on histopathological diagnosis. The tumor beds were treated with two sessions of electrochemotherapy (ECT), two weeks apart. ECT involved cisplatin administration in the tumor bed, followed by a series of eight biphasic electric pulses. The treatment was well tolerated, and the rats were disease-free after 10 and 14 months. Therefore, adjuvant ECT resulted in good local control of mammary carcinoma and can potentially be used for adjuvant treatment of pet rats with cutaneous and adnexal tumors.

KEY WORDS: biphasic pulses, cisplatin, electrochemotherapy, rat

Mammary tumors are the most commonly reported form of neoplasm in rats [6]. Mammary cancer has been increasingly reported in female rats. Well-differentiated fibroadenomas comprise 80–90% of all tumor cases, whereas carcinomas comprise the remaining 10–20% [6]. Mammary tumors in rats are locally invasive and show well-defined margins, and metastasis is generally rare [6]. Malignant mammary tumors can reach considerable dimensions in female rats and sometimes in male rats [6]. Considering that mammary tissues on both sides of the ventral midline extend from the axillary region up to the inguinal region, mammary tumors can extend anywhere from the submandibular region to the base of the tail [6]. Current studies report that up to 16% of old rats develop mammary tumors, most of which are fibroadenomas [10]. Fibroadenomas are benign tumors that usually have well-defined borders. On the other hand, mammary carcinomas, although very uncommon, can be locally invasive and metastasize [6, 10]. Early detection and surgical excision can lead to the best prognosis for fibroadenomas. However, surgical excision of mammary carcinomas could be challenging, and the tumors are prone to recurrence [3, 6, 10].

An owner of a colony of pet store rats (*Rattus norvegicus*) referred two male rats (4 months apart) for evaluation of rapidly growing axillary masses. The rats were bright, alert and responsive, and had body weights of 350 g (0.77 lbs) and 380 g (0.83 lbs). The tumor masses had maximum diameters of 3 cm and 3.5 cm, respectively, and were located in the left axillary area and grew in a span of 20 days. The masses were firm and non-ulcerated, and manual palpation did not elicit pain response. Fine needle aspirate cytology was compatible with mammary neoplasia for both lesions, which displayed clusters and sheets of atypical epithelial cells with altered nucleus:cytoplasm ratios and large prominent nucleoli. For general health evaluation, CBC, serum biochemical profile, and urinalysis were performed. Blood samples were obtained via venipuncture [4, 7, 14]. Total body radiography (three projections) was performed to complete staging. Laboratory analysis and whole body radiography showed no abnormal findings. Rats were anesthetized with butorphanol (Dolorex, Intervet, Aprilia, Italy) [1 mg/kg (0.45 mg/lb), intramuscularly (IM)] and medetomidine (Sedator, Dechra, Turin, Italy) [0.08 mg/kg (0.036 mg/lb), IM], followed by mask maintenance with 2% isoflurane [9]. Masses were excised, and each wound was closed by performing a continuous subcutaneous and intradermal suture using absorbable sutures. Histological sections of the masses were stained with H&E. Rats were hospitalized for 24 hr and received fluid therapy (subcutaneous administration of saline solution) and antimicrobial treatment with enrofloxacin (Baytril injectable solution 5%, Bayer, Milan, Italy) [5 mg/kg (2.3 mg/lb) subcutaneously (SC), q 24 hr].

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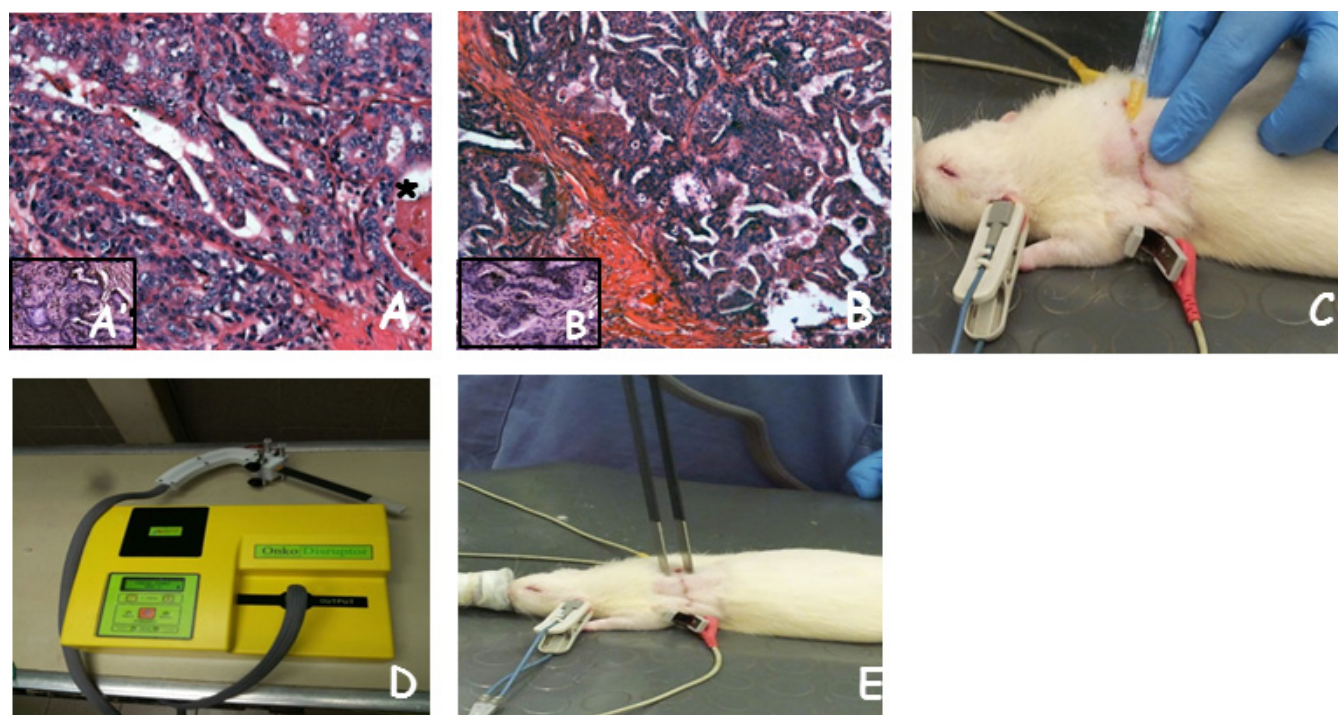


Fig. 1. a) Ductal mammary carcinoma. Region of central necrosis is indicated with the asterisk. b) Papillary mammary carcinoma. Residual displastic areas of mammary parenchyma are depicted in the insets A' and B' (H&E; original magnification, 20×). c) Cisplatin was locally injected along the surgical scar and within the tumor bed. d) Clinical electroporator used for electrochemotherapy treatment. e) Permeabilizing electrical pulses were delivered using plate electrodes.

The owner was instructed to continue enrofloxacin administration for 9 days after discharge from the hospital. Meloxicam (Metacam, Boehringer Ingelheim, Ingelheim, Germany) [0.5 mg/kg (0.23 mg/lb), PO, 3 days] was prescribed for analgesia.

Both tumors histopathologically diagnosed with moderately differentiated carcinoma of the mammary gland. One tumor had a prevalence of ductal aspects, while the other had a prevalence of papillary pattern (Fig. 1). The mitotic index was five active mitotically active cells for each high-power field (40× magnification) for both cases. Histological analysis of the surgical margins showed that the excisions for both cancers were incomplete. The owner elected to treat the residual masses with ECT, because this method has been previously documented in exotic and in laboratory animals [1, 2, 5, 8, 12, 15]. Two weeks after tumor resection, the surgical scar and a 1-cm margin of unaffected tissue were injected with cisplatin (Cisplatino 1% solution, Teva, Milan, Italy) at a concentration of 0.5 mg/ml (total volume, 2 ml) (Fig. 1B). No macroscopic evidence of tumor recurrence was observed at the surgical site at the time of administration. Five minutes after cisplatin injection, a series of eight biphasic pulses was applied at 1,200 V/cm (frequency, 1 Hz). Each biphasic pulse was generated using a clinical veterinary electroporator (Onkodisruptor®, Biopulse S.r.l., Naples, Italy) and consisted of two 50- μ sec pulses with a 300- μ sec interval (total treatment time per cm, 3.2 msec) (Fig. 1C and 1D). The procedure was performed under general anesthesia in accordance with current veterinary literature [9]. The treatment did not result in any adverse effect, and the rats were subsequently discharged from the hospital. The patients were checked after one week by performing a CBC. ECT was repeated 2 weeks after the first treatment. The rats were rechecked monthly for 3 months and every 3 months thereafter, with total body radiographs taken for metastatic assessment. After 10 months, the older rat died, and a necropsy showed no evidence of tumor both macroscopically and microscopically (surgical site, lung, kidney, liver and brain). The other rat remained disease free after 14 months.

Mammary carcinomas are rarely reported in male rats and are generally described as ductal carcinomas secondary to the exposure to chemical carcinogens [11]. A recently published study on a large cohort of rats with mammary tumors showed that these neoplasms, independent of the histology type, were more prone to local recurrence than metastasis. Furthermore, the male rats developed malignant tumors rather than benign tumors [16]. Tumor histology results and the observed aggressive biological characteristics are consistent with mammary carcinoma diagnosed for the described cases. The extensive nature of the lesions prevented large excision, resulting in incomplete tumor removal and thereby exposing the patients to risk of recurrence, as reported in literature [16]. Due to the extreme rarity of this malignant neoplasm in male rats and considering scant reports of chemotherapy in pet mice and rats, the two rats were treated by loco-regional injection of cisplatin to the tumor bed. To increase drug uptake by residual tumor cells, multiple series of electric pulses were administered to the surgical bed as per post-surgical electrochemotherapy (ECT) procedure [13]. ECT in exotic animals currently consists of intratumoral injections of chemotherapy agents (usually cisplatin or bleomycin), followed by local delivery of electric pulses to potentiate drug uptake [8,12,13]. In our protocol, we applied local electric currents in the form of bursts of biphasic pulses. The electrical stimulus induces the formation

of pores in the cell membrane and/or clustering of the transmembrane proteins, which facilitates the increase in intracellular drug concentrations. Treatments are delivered every one or two weeks. ECT is generally well tolerated, and side effects are limited to local inflammation, delayed healing of large surgical beds and wound dehiscence [12]. In one cat, ECT was reported to induce a severe radiation recall [12].

ECT shows preferential permeabilization of cancer cells compared to normal cells, exhibits limited side effects, and can potentially activate the immune system response. These advantages are prompting the adoption of ECT as a primary adjuvant treatment among clinicians [12, 13]. In the present report, cisplatin was selected as the treatment drug, because of the higher efficacy of platinum compounds against human mammary cancer compared to bleomycin, the standard drug for ECT [15]. Although cisplatin is less effective, electroporation significantly facilitates increased cisplatin uptake into cancer cells. The cisplatin dose was calculated according to published guidelines for ECT [12, 13]. In the studied cases, adjuvant ECT combined with surgery showed long-term tumor control, which prevented local recurrence and resulted in minimal morbidity. In conclusion, ECT serves as a useful addition to the options currently available to veterinary oncologists for treating neoplasms in exotic animals. Further investigations on larger cohorts of patients are required to further substantiate these observations.

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