Case Report

Normal Pregnancy and Lactation in a Cat after Treatment of Mammary Gland Tumor When Using Photothermal Therapy with Gold Nanorods: A Case Report


1Department of Animal Reproduction, Veterinary Research Division National Research Center (NRC), Egypt
2Spectroscopy Department, Physics Division (NRC), Egypt
3Environmental Research Division (NRC), Egypt
4National Cancer Institute, Cairo University, Egypt
5Pathology Department, Medical Research Division (NRC), Egypt
6Biological Anthropology Department, Medical Research Division (NRC), Egypt
7Laser Dynamics Lab, Chemistry and Biochemistry Department, Georgia Institute of Technology, USA

Abstract

**Background:** Breast cancer therapy, which includes surgery, radiotherapy, chemotherapy and/or systemic therapy can have a profound impact on reproductive functions, leading to loss of fertility. To our knowledge, this is the first report on cancer photothermal therapy using gold nanorods on the mammary gland treatment of a cat, and the safe effect of the treatment on the reproductive function after tumor remission.

**Case presentation:** A seven years old Shirazi cat (Toatoa) was evaluated because of a 2-week history of progressive dyspnea, signs of depression, and loss of appetite. The cat has a large tumor mass at the left caudal mammary gland. The diameter of the tumor mass was measured using caliper with the dimensions of 14 × 12 × 10.5 cm for length, width and depth, respectively. This was confirmed with ultrasonography. Biopsy samples were taken and fixed in 10% formalin for histopathological investigation, and it was diagnosed as mammary gland adenocarcinoma Grade II. Toatoa was injected intratumoral (IT) with 75 µg gold nanorods (GNRs)/kg body weight followed by exposure to 808 nm laser light for 10 min. GNRs were injected twice, with 15 days apart. After 15 days from the first GNRs injection, there was 60% ablation of the tumor size, while, after 17 days from the second GNRs dose, there was a complete tumor remission. Ultrasound scanning revealed complete ablation of the tumor mass. Complete blood picture (CBC), liver and kidney function analyses showed no changes in any of the tested parameters and indicated that GNRs photothermal treatment is safe and have no immediate toxic effects. After complete remission of the mammary gland tumor, the cat restores all the biological activities including the reproductive function. After 2 months from complete tumor remission, the cat was pregnant after mating with a fertile male, and 62 days later she delivered 3 kittens of normal morphology and growth rate. Breast feeding was found to be normal from all the nipples including the previously affected nipple.

**Conclusion:** Photothermal therapy with gold nanorods can be used for the treatment of mammary gland tumor with apparently no impairments of reproductive functions in cats.

Keywords: Cat; Mammary gland tumor; Photothermal therapy; Gold nanorods; Pregnancy; Lactation

Introduction

Breast cancer (BC) is the most common malignancy, and its prevalence increases with age in women worldwide. In the developed countries, BC represent more than 40% of all cancers patients of <40 years [1]. In Egypt, BC is estimated to be the most common cancer among females accounting for 32.0% of their total [2]. In addition, dogs and cats have a high prevalence of mammary tumors (MTs), and tumors are more aggressive in cats [3]. Feline MTs (FMTs) comprise approximately 11% of feline neoplasms, are more commonly malignant than benign, highly aggressive, carry a poor prognosis attributable to a high probability of local recurrence and metastasis, and mainly hormone receptor-negative cancer [4-6]. Furthermore, feline and canine mammary tumors have epidemiological, clinical, morphologic and prognostic features similar to those of human breast carcinoma, and it is considered as an excellent model for hormone-independent human breast cancer [7]. Therefore, it is necessary to identify new approaches that can be used for treatment of mammary tumor in pets. According to our knowledge, this is the first report on the application of photothermal therapy with gold nanorods in feline mammary gland tumor.

Conventional treatments of breast cancer are surgical removal and/or chemotherapy [8,9]. Alternative treatments are hormone therapy and/or direct control function of estrogen receptors [10,11]. The adverse effects of chemical substances and postsurgical metastasis usually result in a shorter survival time.

The application of nanotechnology in cancer therapy research

*Corresponding author: El Sayed MA, Laser Dynamics Lab, Chemistry and Biochemistry Department, Georgia Institute of Technology, USA, Tel: 404-894-0292; E-mail: melsayed@gatech.edu

Abdoon AS, DVM, Ph.D., Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, 13221 Dokki, Cairo, Egypt, Tel: +201221941292; Fax: +20233370931; E-mail: assabdoon@yahoo.com

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started with the use of gold silica core-shell nanoparticles by Halas’s group [12] and the use of gold nanorods by El-Sayed group [13]. In both of these studies, the nanoparticles absorb near infra-red light and convert it into thermal energy that ablates the cancer cells. Due to the ease of its synthesis, and the purity of the nanoparticles, the gold nanorods have been used successfully on the small animals (mice and rats) [14]. Intratumoral injection of gold nanoparticles followed by near infrared laser treatment resulted in tumor ablation in treated mice [15-18].

The present study is the first report on the application of photothermal therapy using gold nanorods on large animals. It work reports on the photothermal therapy using the gold nanorods to treat the mammary gland tumor of a cat.

Current therapies, while improving the overall survival rate with mammary tumors, have not eliminated this disease as an important cause of morbidity and mortality. In addition to the high risk of cancer relapse [19], BC therapy could have a profound impact on the reproductive function, and may leads to loss of fertility. In women, BC survivors have the lowest chance for future pregnancy [20]. Only 4-7% of patients with conventional BC therapy actually conceive [21]. The side effects of chemotherapy include damage to ovarian follicles [22], leading to temporary or premature menopause and infertility [23]. Another concerns about pregnancy outcomes after BC therapy, is the presence of inherent risks on the babies born after treatment of BC. To our knowledge, the present case represents only the first case to be reported on the pregnancy and lactation outcomes after remission of feline mammary gland treated using photothermal therapy using gold nanorods.

We here discuss for the first time a report on the treatment of feline mammary gland tumor using photothermal therapy using gold nanorods, and the possibility for the normal pregnancy and lactation after tumor remission, and to evaluate the possible toxic effect of GNRs on blood profile, liver and kidney functions.

Case Presentation

A seven years old Shirazi cat (Toatoa,) sexually intact with previous pregnancy came to a private Vet. Clinic, with 2-week history of progressive dyspnea, signs of depression and loss of appetite, as she was suffering from a large mammary gland tumor at the left caudal nipple.

The tumor was covering the whole abdomen (14.0 cm length, 12.0 cm width x 10.5 cm depth, Figure 1A and 1B). The cat was examined for the history of mammary gland tumor two months earlier. The cat was checked for physically examined for body weight, temperature, heart and respiratory rates. Also, the tumor size (length, width and depth) was measured by using a caliper followed by ultrasonography examination (Figure 1C). The cat was injected with 0.25 ml Ketamine and 0.25 ml Zylaject for sedation. Biopsy samples were taken from the tumor mass using true-cut needle for histopathological examination. Tissues were fixed in 10% formalin, processed by embedding in paraffin blocks, and then sliced into 5 µm in thickness. Hematoxylin-eosin staining was performed. Images were acquired using Olympus microscope equipped with color CCD camera. According to the case history and histopathological examination the cat was suffering from mammary gland adenocarcinoma Grade II (Figure 1D). Gold nanorods (GNRs) were prepared at the Spectroscopy Department, NRC, according to the method developed by Nikoobakht and El-Sayed [24]. The dose used in the present study was chosen according to previous literature reports [14]. The cat was subjected for two sessions of treatment. In the first session, 75 µg/kg body weight was diluted in 20 ml physiological saline solution, then injected intratumoral (Figure 2A). Ten minutes after GNRs injection, the site of injection was exposed to continuous laser beam (λ=808 nm) from the top at a power density of 50 mW/cm² for 10 min (Figure 2B). After two weeks the same dose and method of injection was repeated. After administration of GNRs, the cat was examined day after day for survival and evidence of behavioral or motor impairments. Also, changes in tumor shape and size were recorded.

After treating Toatoa with 2 sessions of GNRs injection followed by laser exposure (2 weeks apart), a complete remission of the tumor mass was achieved after 31 days from the first injection (Figure 3A). Ultrasound examination revealed complete ablation of the tumor in response to treatment (Figure 3B), and the cat restored her vital activity.
including the reproductive one. The cat was mated with a fertile male 70 days after complete recovery, and delivered after 62 days of pregnancy 3 kittens with normal morphological and health conditions (Figure 4A), as well as normal growth rate. Breastfeeding of the kittens was normal including the previously affected teat (Figure 4B).

Blood samples were collected before and one month after treatment for evaluating liver and kidney functions as well as hematological profile. Two blood samples were collected by direct vein puncture, the first was collected in vacutainer tube containing EDTA for CBC, and the second one was used for serum separation. Whole blood was centrifuged twice at 3000 rpm for 10 min in order to separate serum. Using autoanalyzer (Moduler Analyzer Series, Roche Diagnostics, USA), serum biochemical analysis was carried out to determine the serum level of total bilirubin (TBIL), direct bilirubin, albumin, alkaline phosphatase (ALKP) and serum glutamic-pyruvic transaminase (SGPT) as a measure of hepatic and biliary functions. Nephrotoxicity was assessed by determination of the levels of uric acid (URIC), urea nitrogen (UREA), and creatinine (CREA).

Hematological autoanalyzer (Exigo EOS Vet, SE-126 13 Stockholm, Sweden) was used to determine hematological parameters such as red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets counts. Analyses of these metabolites in serum of the cat treated with GNRs before and after treatment indicated no difference was observed in any of the tested parameters. Liver and kidney functions were the same before and after treatment (Table 1). Also, values of blood profile were the same before and after treatment (Table 2).

## Discussion

There is some controversy about the number of women that became a pregnant after BC diagnosis. Personal fears or lack of appropriate and rapid fertility counselling probably contribute to this observation. Positive association between abortion and breast cancer was frequently reported from case-control studies [25,26].

After 2 weeks of IT injection of 75 µg GNRs/kg body weight, there was 60% reduction in tumor size. A second dose of IT injection of GNRs was given, and there was a complete remission of the tumor after 31 days from the start of treatment. Similar results were previously reported in rats and mice [14]. Previous results showed that the AuNRs can generate defects in the cell membrane and induce apoptosis of cancer cells [27]. Moreover, thermal therapy with NIR laser and gold nanoparticles has proven effective in breast cancer cell lines in vitro [28,29] and in mice [14].

Interestingly, the cat restored her biological activities after tumor remission, and it became pregnant after mating with a fertile male (60 days after complete remission). She delivered three kittens in a good health and normal morphology. The best available retrospective evidence suggested that pregnancy after BC does not increase the risk of disease recurrence [30-32]. Recently, among risk factors for breast cancer, a history of full-term pregnancy was inversely associated with the risk of second primary cancer [33]. Also, breastfeeding was normal, particularly for the previously affected nipple. Similarly in women, there is no evidence that breastfeeding increases the risk of breast cancer recurrence or a second breast cancer development [34,35]. According to our knowledge this is the first report discussing the effect of pregnancy after mammary gland tumor remission in pet animals.
The present results revealed that IT injection of GNRs has no toxic effects on blood, liver or kidney functions in the treated cat. RBCs, WBCs, and PLT counts were within normal ranges. The liver and kidney function tests, including ALT, AST, ALP, and LDH, were all within normal limits. Furthermore, the urinary and fecal analyses did not show any abnormalities.

Declaration of Interest

No potential conflict of interest was reported by any of the authors.

References


