Ascorbic Acid-Induced Necrosis in Canine Mammary Adenocarcinoma

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Abstract *This report highlights the result of treatment with large doses of ascorbic acid (AA, vitamin C) in the terminal stage of a dog's mammary tumour with pulmonary metastasis following surgery. An 11-year-old female, golden retriever had been histopathologically diagnosed as having a simple tubular adenocarcinoma. The dog had been given AA at 250 mg/kg/day intravenously for 2 weeks, followed by 4,000 mg/day orally and fed with an organic homemade diet. Partial remission of metastasized lymph nodes and clinical improvement were observed during 35 days of treatment. The dog was then taken home and given ordinary food and dessert, in addition to 4,000 mg/day of oral AA. After one week, the dog's clinical status worsened and the lymph nodes returned to their pre-AA size. Three weeks later the dog died and necropsy was performed. This case report suggests that AA, in conjunction with an organic homemade diet, might forestall death in dogs in the terminal stages of cancer.*

Introduction

Canine mammary tumours (CMTs) are the most common neoplasms in intact female dogs.[1] Fifty percent of the CMTs are malignant.[2,3] Most of the malignant CMTs in dogs are carcinomas, and less than five percent are sarcomas.[3] In general, the occurrence and severity depends on the extent and location of the metastases.[3-5] Although carcinomas metastasize mainly via the lymphatics to the regional lymph nodes and lungs, haematogenous metastasis may also occur. Other less commonly reported metastatic sites include the liver, bone, brain, spleen, kidney, skin, eye, adrenal glands, uterus, heart, muscle and pancreas.[3,4,6]

Approximately 48% of dogs with mammary carcinoma die or are euthanized within one year after surgical removal of the primary tumour or recognition of metastases.[7] Conventional treatment options are limited, although CMTs remain an important disease entity in veterinary oncology.[1] Surgery remains the treatment of choice. Therapies used to treat human breast cancer have been adopted, such as several chemotherapeutic agents and their combinations, hormonal therapy, anti-cyclooxygenase-2, desmopressin and radiotherapy.[5, 8-10] Since the results from these treatments have been limited, and are also associated with significant toxicities and certain complications, an effective treatment strategy continues to be warranted.

In human alternative medicine, large dos-

es of ascorbic acid (AA, vitamin C) have been applied to treat and prevent various types of cancer, such as breast cancer and lymphoma.[11] Thirty years ago, an important review by Pauling, Cameron, and Leibovitz documented the scientific evidence in support of using AA as a therapeutic agent in the treatment of cancer.[12,13] The results obtained from a small number of clinical studies suggest that intravenous AA can increase well-being, and in some cases can produce outstanding anti-cancer effects, and that adverse effects are minor in properly screened patients.[13,14] A recent Phase I clinical trial indicated that the addition of intravenous AA to conventional chemotherapy produced a highly favorable disease-stabilizing effect in advanced cancer of the pancreas; a cancer that is unrelentingly progressive in the great majority of patients who received conventional therapy.[15-17] In addition, Yoem et al studied the effect of intravenous AA on the quality of life of terminal cancer patients. Their research reports have led to a call for greater attention to the therapeutic potential of intravenous AA in cancer therapy for patients in the terminal stages of their disease.[11] There remains an urgent need to objectively document and ultimately determine the biological basis and clinical efficacy for the anti-cancer effects of AA.

The use of large doses of AA for cancer treatment in dogs has not been studied. Subsequent accumulating scientific evidence, including *in vitro* and *in vivo* studies in experimental animals and the clinical use of intravenous AA in human patients with terminal cancers, strongly supports the possibility of an important role for intravenous and oral AA in cancer therapy. We applied large doses of AA as an alternative treatment in the terminal stage of dog's mammary tumour, which already had metastasized to the lungs, and had not responded to prior conventional chemotherapy.

Case history

An 11-year-old female, golden retriever, weighing 25 kg, was referred to the oncology clinic, located at the animal teaching hospital, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The dog had been histopathologically diagnosed as having a simple tubular adenocarcinoma of the mammary gland (**Figure 1**, p. 65). The mass had been removed 2 times. The first surgical removal was done 2 weeks after biopsy, and the second surgical removal was performed 4 weeks later where normal tissue (i.e., the skin margin) was removed with surgical excision of the tumour. Seven months after the second surgical removal, the dog had right axillary (size: 7x3x3 cm), right prescapular (size: 12x4x3 cm) and left prescapular lymph node (size: 6x3x2 cm) enlargement. The cytology result was mammary adenocarcinoma with metastases to the lymph nodes (**Figure 2,** p. 65). Radiography showed multiple tumour masses, varying in sizes, invading all lung lobes (**Figure 3**, p. 66), and mild splenomegaly.

Conventional chemotherapy with a single intravenous infusion of doxorubicin (A.D. mycin; Boryung Pharma, Korea) 30 mg/m^2 body surface area every three weeks for 2 cycles was the initial treatment. Two weeks following the infusion, the dog developed cancer cachexia and mild anaemia caused by bone marrow toxicity. The notable findings from the CBC were: RBC 6.1 (reference range: 5.5-8.5 x 10^6 RBCs /µl), hemoglobin 11.7 (reference range: 12-18 g/dl), and hematocrit 31 (reference range: 37-55%). The dog's palpable lymph nodes also remained unreduced in size.

As a result, conventional treatment with doxorubicin was discontinued and replaced by large doses of AA and an organic homemade diet. On the basis of intravenous doses used in humans,[14] we speculated that the dog would require lower intravenous doses of AA. Regarding the life-threatening effect of tumour haemorrhage and necrosis that can result from intravenous AA,[18] we used an intravenous dose that we believed was safe, followed by daily oral doses of AA. The dog was administered 250 mg/kg/day of AA (Vitamin C Injection; T.P. Drug Laboratories, Thailand) over 60 minutes in a 100 mL normal saline bag intravenously for 14 days. This was followed by 4,000 mg/day orally of AA (Nat-C; MEGA Life Sciences, Thai-

land) for 30 days.

The organic homemade diet was given to the dog twice daily and included brown rice, marine fish, organic vegetables and sometimes eggs, cheese and a brewer's yeast supplement. The dog was kenneled in the private hospital for the duration of its treatment.

One week after beginning the AA and organic homemade diet, the dog developed symptoms of depression, an intermittent fever, and a productive cough. Blood work done during this period showed moderate anaemia, thrombocytopaenia, and mild neutrophilic leukocytosis. The results were as follows: RBC 4.2 (reference range: 5.5-8.5 x 10^6 RBCs/µL), hemoglobin 8.3 g/dL (reference range: 12-18 g/dL), hematocrit 25% (reference range: 37-55%), platelets 1.74 (reference range: 2-5 x 10^5/µL), neutrophils 14,090/µL (reference range: 3,000-11,500/µl), and leukocytes 17,400/µL (reference range: 7,000-17,000/µL). Liver and kidney function tests were within normal limits.

By day 16 (i.e., 2 days after finishing the course of IV AA), another CBC was done. Hematologically the anaemia was improving, even though the neutrophilic leukocytosis persisted. The results were as follows: RBC 5.66 (reference range: 5.5-8.5 x 10^6 RBCs / µL), hemoglobin 11 (reference range: 12-18 g/dL), hematocrit 34 (reference range: 37-55 %), platelet 2 (reference range: 2-5 x10^5/µL), neutrophils 16,852 (reference range: 3000-11,500/µL), and WBC 19,150 (reference range: 7,000-17,000/µL). The antibiotic, Marbofloxacin (Marbocyl®; Vétoquinol), was prescribed at a dose of 2.75 mg/kg orally for two weeks to prevent bacterial infection.

By day 35, the dog looked better and displayed the "typical" features of a normal dog. The dog's weight had increased by 3 kg (i.e., 25 kg before treatment to 28 kg). All the mentioned lymph nodes gained partial remission (defined as a greater than 50% reduction in tumour volume). Remarkably, the right axillary lymph node decreased to 2x1x1 cm in size compared to its enlarged size before treatment (7x3x3 cm). The owner took the dog home from the private hospital and fed the dog with ordinary food and dessert. After a week at home the dog's health worsened, as evidenced by weakness, lethargy, anorexia, tachypnoea, weight loss, and increased size and induration of all the smaller lymph nodes. The dog was readmitted to the hospital. The CBC showed severe anaemia and neutrophilic leukocytosis. The results were: RBC 2.7, neutrophils 17,745/µL, and WBC 19,500/µL. Pain was elicited on abdominal palpation. An abdominal ultrasound revealed a large hepatic mass measuring 3x5 cm. No other laboratory investigations were undertaken since the owner was unable to afford more testing. Nevertheless, the dog continued to receive 4,000 mg/day orally of AA. The dog died 3 weeks later and necropsy was performed.

Necropsy results

Gross evaluation demonstrated right axillary (**Figure 4,** p.66), and right and left pre-scapular lymph node enlargement. The thoracic cavity was filled with approximately 1,000 mL sero-sanguineous transudate. Multiple white firm nodules, varying in size, appeared in all lung lobes (**Figure 5,** p.67). A cyst-like mass at the base of the heart, measuring 8x6 cm, contained clotted blood and a red colour transudate. A white nodule, size 3x5 cm in diameter, was found in the liver. Histopathology of the mass sections showed extensive necrosis; pyknosis of tumour cells and suppurative areas with intralesional haemorrhage **(Figures 6,7,** p.67, 68**).** The tumour cells were loosely arranged, having an island and glandular structure, with few inflammatory cell infiltrations. Fibrosis was found in some areas of the masses. The lymph nodes and lungs showed massive necrosis of metastasized tumour areas. The cause of death was respiratory failure due to hydrothorax. Hydrothorax developed from anaemia and hypoproteinaemia, as a result of anorexia and cancer cachexia.

Discussion

Generally, in the advanced terminal stages of tumours in dogs, the disease will rapidly progress and result in death. Before

AA treatment, this dog showed the same pattern of disease as other dogs having cancer. However, dramatic results were observed during five weeks of AA treatment, starting with large intravenous doses for 14 days followed by large oral doses for 30 days. The lymph node mass diminished in size and the dog's quality of life and behaviour normalized, indicating a therapeutic effect from AA. The results we obtained from AA were comparable to results reported in humans decades ago.[19,20]

The dog was also prescribed an organic homemade diet that possibly contributed to its improved condition. When the owner brought the dog back home and the diet was changed to ordinary food and dessert, the dog's health declined in a week, and the smaller lymph nodes became as big as they were at the onset of treatment. The relapse of the disease may have been caused by the foods given to the dog when it was brought home. The desserts given to the dog likely contained high amounts of sugar (i.e., glucose and fructose); glucose is known to be the main energy source required by tumour cells.[21] Other foods given to the dog might have also contributed to the relapse. A case-control study by Alenza et al showed that the development of mammary gland dysplasia and CMTs in dogs was associated with a high intake of red meat, especially beef and pork, and a low intake of chicken.[22]

We speculate that AA caused tumour haemorrhage, which might have resulted in the decrease we observed in the RBCs and platelets during therapy. The neutrophilic leukocytosis may have been the result of tumour necrosis and/or the enhancement of the immune system by AA. Nevertheless, these effects were not deemed clinically detrimental while the dog was treated in the hospital since there was regression of lymph node size and the dog's quality of life and behaviour normalized.

The important actions of AA include both antioxidant and pro-oxidant activities. The former action reduces reactive oxygen species, which is an important promoter of carcinogenesis. The latter action is selectively cytotoxic to tumour cells when: (1) the concentration of antioxidant enzymes (e.g., catalase) are low; (2) more intracellular transition metals (i.e., iron and copper) are available; and (3) glucose transport of cells are over-expressed.[23] These lead to an increase in hydrogen peroxide and hydroxyl radical, causing cancer cells to die. Furthermore, AA is involved in the apoptotic process when cells have damaged DNA.[24] We believe that the pro-oxidant activities of AA likely caused the massive destruction of neoplastic cells and the accompanying necrosis and haemorrhage. Since some of the necrotic areas showed evidence of repair (i.e., they were being replaced by fibrosis), it is also possible that AA was preventing further tumour dissemination as a result of its function in collagen synthesis.[23,25]

This is the first report illustrating the efficacy of AA as a novel chemotherapeutic agent for the terminal stages of CMTs in a dog. This dog had survived for 17 months following the first surgery, which is longer than what has been previously reported.[7] We recommend that the veterinary oncology field pursue more research evaluating the efficacy of AA as treatment for CMTs and other types of canine cancers.

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Competing Interests

The authors declare that they have no competing interests.

Statement of Animal Rights

The deceased dog was donated, for the purposes of necropsy, to the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn, University, Bangkok, Thailand. The institutional guide was

adhered to and respected during all phases of the necropsy.

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Figure 1. Histopathology of tumour mass revealed solid nests and tubular adenocarcinoma (H&E staining, bar=25 µm).

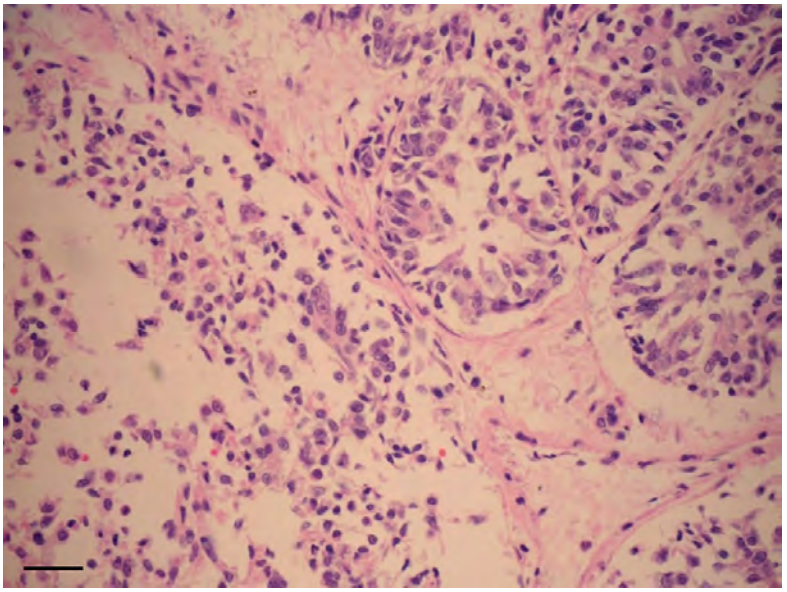

Figure 2. Cytological sample aspirated from the right axillary lymph node revealed cluster of large cuboidal cells, pleomorphic round to ovoid nuclei with prominent nucleoli; diagnosed as adenocarcinoma (Giemsa staining, bar=15 µm).

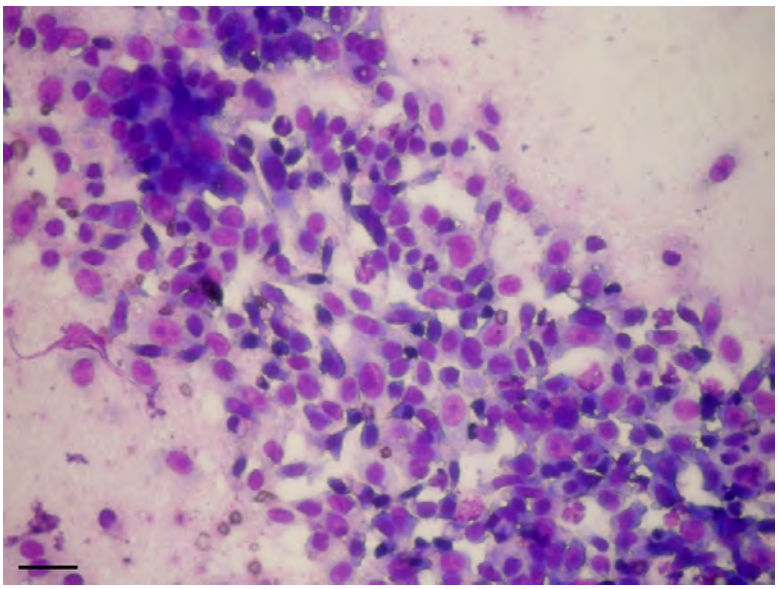

Figure 3. Radiography revealed diffuse multiple masses occupied in pulmonary parenchyma, lateral view.

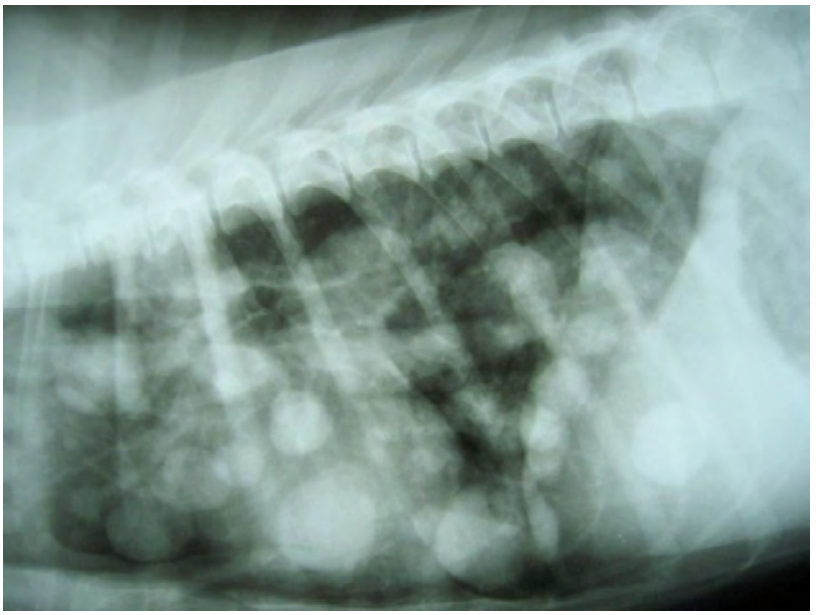

Figure 4. Metastatic solid mammary tumour mass of the axillary lymph node with mutifocal necrotic areas.

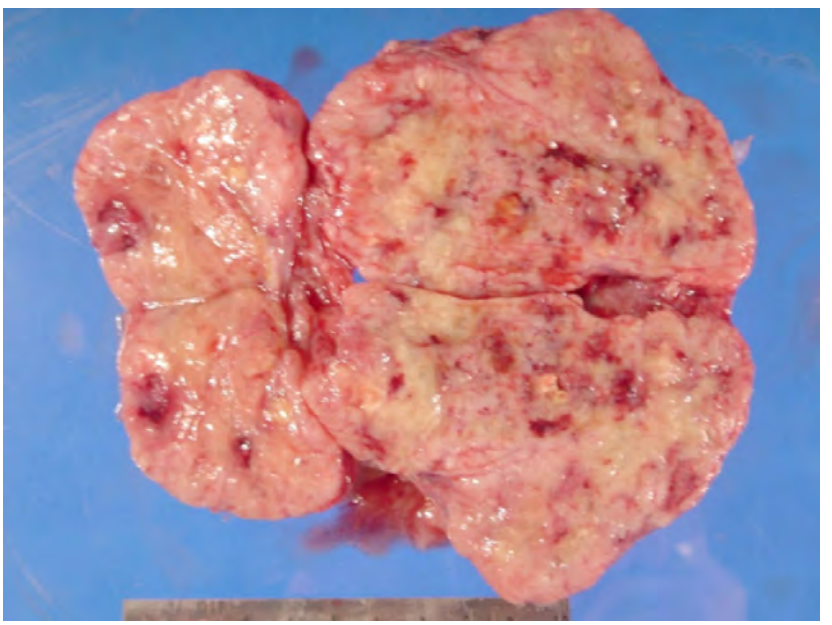

Figure 5. Gross specimen of the lung revealed multiple tumours of various sizes invading all lobes.

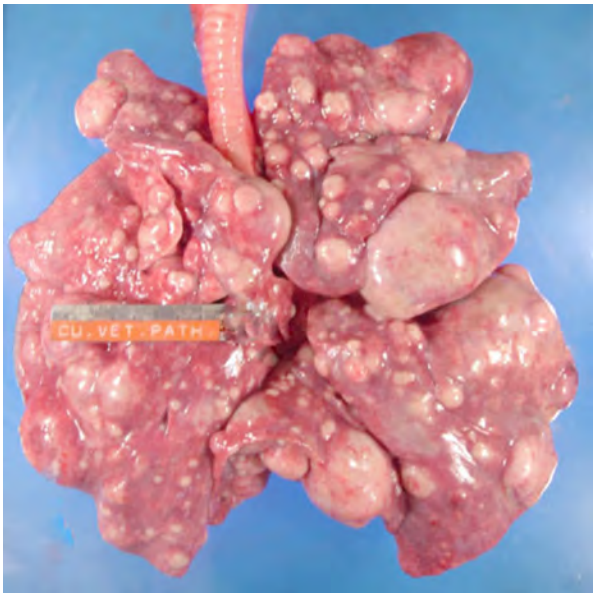

Figure 6. Histopathology of tumour mass revealed massive necro-haemorrhagic areas after treatment (H&E staining, bar=100 µm).

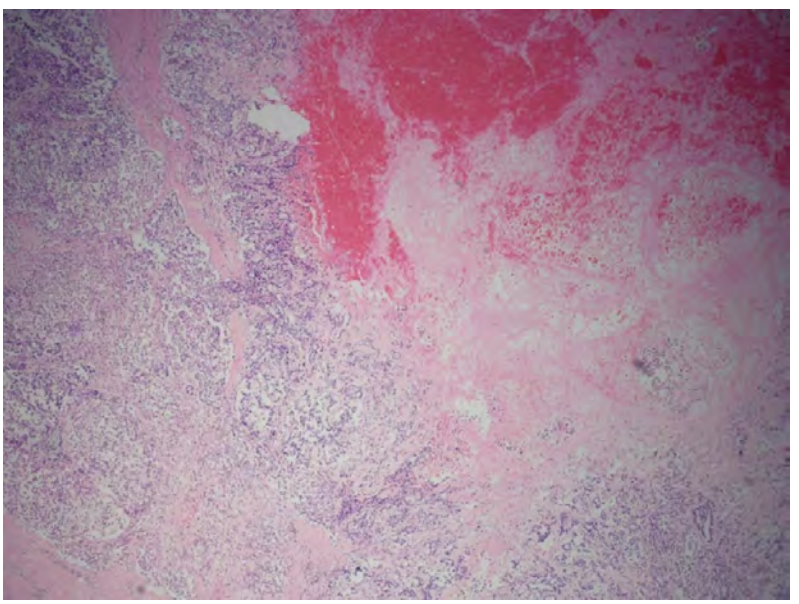

Figure 7. Histopathology of tumour mass at necrotic area revealed scattered necrotic cells which are characterized by pyknotic, karyorrhexis and karyolytic nuclei (H&E staining, bar=25 µm).