Green Tea with Prednisone or Quinolone Induction for the Treatment of Canine Lymphoma

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The use of green tea to control human lymphoma in the mice* may be adapted for treating canine lymphoma. We offer our experience as an al ternative to euthanasia to the dog lovers.

*Bertolini F, Fusetti L, Rabascio C et al. Inhibition of angiogenesis and induction of endothelial and tumor cell apoptosis by green tea in animal models of human high-grade non-Hodgkin's lymphoma. Leukemia 2000;14:1477-1482.

Abstract

A case of multicentric canine lymphoma with clinical complication of trigeminal neuropathy and unilateral exophthalmos was successfully treated with green tea extract in combination with prednisone induction initially, and ciprofloxacin (Baytril equivalent) induction subsequently. At the 50th week of disease, the patient was totally asymptomatic. A general remission was successfully induced by a combination of green tea and prednisone and maintained by daily tea extract. However, a quinolone in combination with green tea was found to be more effective in inducing remission of the retrobulbar or optic nerve lesions.
The scientific basis of using green tea in combination with quinolone antibiotics as a cancer-suppressing agent was briefly reviewed.

"Doctoring is like cooking: Any cook can follow a cookbook to prepare an edible dish whereas a great chef follows the cooking principle, but is prepared to make calculated deviations from the recipe according to the ingredients available, the season of the year and the taste of the diners to create a culinary masterpiece." -Zen speaks.
This case report was presented and distributed at the 5th Annual A Chef's Delight Food and Wine Tasting Festival organized by the Wa Sung Charity Fund and the American Cancer Society, Oakland, California, October 20, 2002.
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Case Report

A seven-year-old, neutered, 45 lb male Labrador mix initially presented with a mass in the left popliteal fossa. There were no other symptoms and signs of a systemic disease. The body temperature was normal. The complete blood count (CBC) and serum biochemistry profile were normal. Microscopic examination of the stool for ova and parasites was negative. The dog was treated with various antibiotics, including amoxicillin, tetracyclines and Septra with no apparent effects on the mass which increased in size progressively. Over the course of one month, the mass had reached the size of a chicken egg. It was hard on palpation and fixed to the surrounding tissues. At the same time, the right popliteal lymph nodes were found to be enlarged. A fine needle aspirate of the biggest mass in the left popliteal fossa showed that the entire specimen consisted of monotonous immature lymphocytes with large round nuclei and scant cytoplasm. Scattered mitoses were observed. Only very few normal-sized lymphocytes were present (Fig. 1). A multicentric large cell malignant lymphoma was diagnosed. Three weeks after the cytopathological diagnosis, the dog became lethargic and refused food and drink.

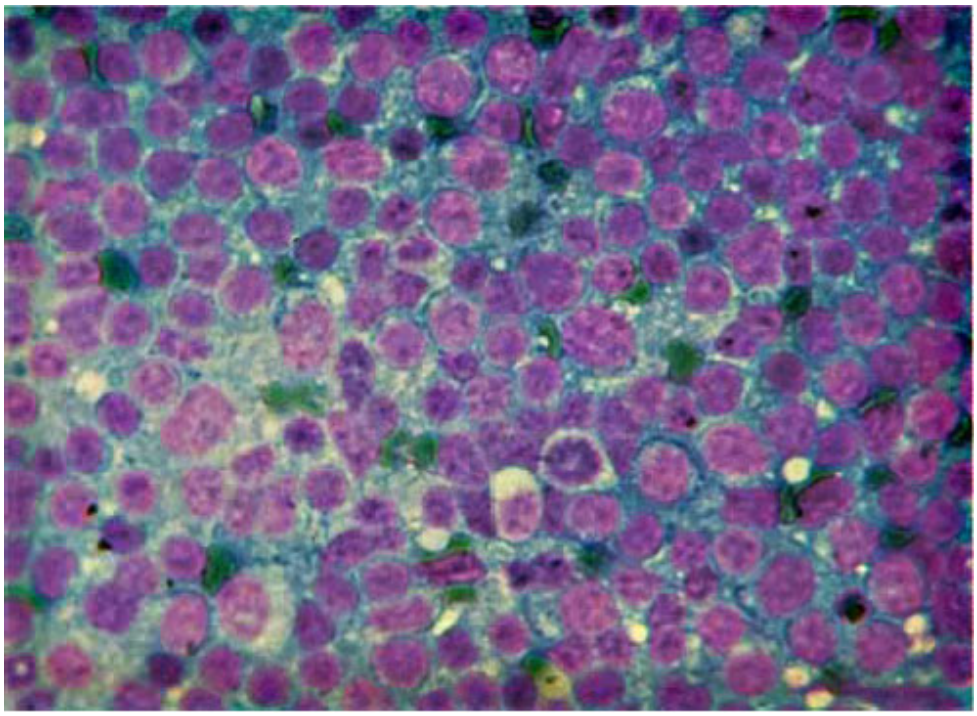

Fig. 1. Fine needle aspirate of an egg-sized mass in the left popliteal fossa of the dog, showing monotonous immature lymphocytes with large nuclei, prominent nucleoli, and scant cytoplasm. One mitotic figure is seen near the center of the microphotograph. A few normal-sized lymphocytes with small darkly stained nuclei are present between the large tumor cells (Air-dried smear. Wright's stain, x430).

Various options of treatment were considered. The owner chose oral administration of green tea extract plus oral prednisone 40 mg per day as the regimen for the treatment. After institution of this regimen, the general condition and appetite of the dog improved rapidly. All enlarged lymph nodes, including the left popliteal, were shrunken to normal-sized in five days. The prednisone was tapered off gradually over a span of three weeks after five days of full dosage while daily administration of green tea extract was continued throughout the entire course of observation. The dog was well enough to make a round flight trip from Connecticut to the Caribbean with the owner.

However, four days after the last tapering-off dose of prednisone, the dog developed bilateral trigeminal neuropathy with an inability to prehend food or drink water. The jaw was dropped at rest with saliva dribbling constantly. The left angle of the mouth opened more widely than the right. There were no voluntary jaw movements. The tongue function was normal. The dog was able to swallow dry food and liquid placed in the back of the mouth. A clinical diagnosis of lymphoma involvement of the trigeminal nerve was made. A full dosage of oral prednisone, 40 mg per day for four days, was reinstituted immediately along with multiple vitamin supplements added to the daily diet. The prednisone was gradually tapered off over the following three weeks as the jaw movements improved to the point that the dog was able to drink liquid and prehend food from the bowl.

Two weeks after tapering off the second course of prednisone, the dog developed malaise, poor appetite and weak legs. Prednisone, 40 mg per day, was administered orally again for five days with rapid improvement of the general conditions, without tapering doses. In fact, the dog remained asymptomatic and behaved like a normal canine of his age thereafter with regained interest in chasing small animals and moving subjects. One month later, i.e. about five months after the first presentation of the left popliteal mass, a course of oral prednisone, 20 mg per day, was given for five days without tapering as a measure to maintain the good "general condition" of the dog.

In the sixth month of the disease, the dog developed progressive unilateral left exophthalmos with tearing and mild hyperemia of the bulbar and palpebral conjunctiva and without purulent discharge. The left eyeball felt hard on palpation, but was non-tender. There was no fever. An ophthalmologic consultation confirmed that there was no chorioretinitis and that there was no loss of vision. All routine laboratory profiles were normal except for a mild anemia. The clinical diagnosis was exophthalmos due to retrobulbar or optic infiltration by a multicentric lymphoma, a diagnosis reached after a group consultation of one veterinarian and three MDs, including a board-certified ophthalmologist. At this stage, the general condition of the dog was excellent and there was no evidence of recurrent lymphadenopathy. Oral prednisone, 40 mg per day, was again reinstituted in an attempt to reduce the exophthalmos. However, no clinical improvement was observed, and the protrusion of the eyeball was worsening while on medication without any symptoms and signs of an infection. The oral prednisone was discontinued after six days without tapering off doses.

An oral quinolone agent, ciprofloxacin (Bayer), 750 mg daily was given to the dog for its potential antineoplastic activities in combination of the daily green tea administration on a trial basis (see Discussion). Surprisingly, the exophthalmos markedly improved after the second day of ciprofloxacin and was no longer clinically noticeable on the fifth day. The dosage of ciprofloxacin was reduced to 500 mg per day after the fifth day for another five days before it was discontinued. The dog remained completely asymptomatic for seven weeks after the left exophthalmos had subsided.

In the eighth month, a slight protrusion of the left eyeball with a mild restriction of free movement of the globe was noticed. Another course of ciprofloxacin, 750 mg per day for five days and 500 mg per day for nine days, was given orally in an attempt to achieve a more sustained remission of the retrobulbar lesion. The left eye returned to normalcy on palpation and observation on the third day of ciprofloxacin administration.

The dog remained completely asymptomatic in the fiftieth (50th) week after first presentation of the disease, and was free of lymphadenopathy continuously for 38 weeks. No corticosteroids were given for the last 21 weeks; in this period the successful maintenance of clinical remission was attributed to the effects of daily administration of green tea and the two quinolone inductions which were used to target the retrobulbar lesions.

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Preparation and Administration of Green Tea

Materials

Loose dry green tea leaves of the tree Camellia sinensis in which the content of total green tea catechins (GTC) was at least 10% and that of (-)epigallocatechin gallate (EGCG) was at least 7% (g/100g dry tea leaves) were used for preparing the liquid tea extract. The GTC and EGCG analysis was kindly performed by Professor Zhen-Yu Chen, Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong, China.

Method of Brewing Tea for the Dog

Three heaping teaspoonfuls (about 12 grams) of dry tea leaves were placed in an empty ceramic tea steeper of about 400 ml in volume capacity. After hot water of boiling temperature was poured into the steeper, filling it all the way to the rim, a lid was placed immediately to cover the tea steeper so that there would be as little atmospheric air trapped between the lid and the hot liquid as possible. The tea was allowed to steep for 20 minutes with the lid on (steeping hot tea in the presence of atmospheric oxygen may result in a loss of 15-20% of its antioxidative activity). After 20 minutes, the temperature of the liquid tea in the steeper usually dropped to about 56°C and the tea leaves sank down to the bottom of the steeper. The liquid tea was poured into about 100 ml crushed ice or ice cubes for an immediate cooling. The cold tea was stored in a capped plastic bottle in a 4°C refrigerator and was to be consumed by the dog within 24 hours.

Tea Dosing

The daily dose of green tea was divided into three portions, about 170 ml each, for the dog to drink in the morning, at noon and in the evening. Various foods that the dog liked had been added to the tea to induce the dog to drink this amount of strong green tea, including evaporated cow's milk and ground boiled pig liver.

Discussion

The standard medication for the treatment of multicentric canine lymphoma includes various combinations of the chemotherapeutic agents, cyclophosphamide, vincristine, methotrexate, L-asparaginase, chlorambucil, doxorubicin and CCNU (Lomustine), often supplemented with prednisone.1-6 Corticosteroids have a direct cytolytic effect on neoplastic lymphocytes. But the corticosteroid-induced remission times are typically brief with early relapses. All chemotherapeutic agents are associated with potentially serious toxic side effects which may be detrimental to the quality of life both for the dog and for the owner. Many dog-loving owners elect euthanasia rather than accepting chemotherapies for their pets. This case report recorded a treatment regimen for the latter group of dog owners and their veterinarian physicians to consider as a potential alternative to euthanasia.

Green tea extract and its components, notably EGCG and related green tea catechins have been shown to inhibit the growth of many types of tumor cells in vitro.7-10 Green tea may inhibit certain types of high-grade human non-Hodgkin's lymphoma in animal models by inhibition of angiogenesis and induction of endothelial and tumor cell apoptosis; it has been reported to be more effective than cyclophosphamide at the maximum tolerable dose in preventing lymphoma recurrence.10 Based on these prior published research data, an experimental regimen of daily green tea extract with prednisone induction was initially designed for the treatment. This treatment achieved complete clinical remission of the lymphadenopathy without the toxic side effects usually associated with the standard chemotherapy.

Multicentric lymphoma with involvement of the central nervous system (CNS) and peripheral nerves, including the trigeminal nerve and optic nerve is a well known, but uncommon clinical presentation in dog. Most dogs suffering from malignant lymphoma do not have the opportunity to live long enough as humans to develop the CNS or peripheral nerve involvement which can be documented by pathological examination. When a dog suffering from canine lymphoma with these complications is treated according to a standard protocol of systemic chemotherapy, such as CCNU (Lomustine), vincristine and prednisone, a clinically complete remission of the generalized lymphadenopathy may be achieved rapidly. But the lymphoma cells in the CNS and in the peripheral nerves within the epineurium, perineurium and the endoneurium are difficult to eradicate.6 In the present case, the combination of green tea and corticosteroids was initially effective in treating the lesions affecting the cranial nerves which presumably contributed to the clinical manifestations of a trigeminal neuropathy. However, when the patient developed a new set of retrobulbar or optic nerve lesions presenting with a unilateral exophthalmos, the tea and prednisone combination was no longer effective as a treatment. Instead, a combination of tea and ciprofloxacin induced a complete clinical remission. This observation indicates that a quinolone compound may be used in combination with green tea extract in controlling the lymphoma when the treatment with the tea and prednisone combination has failed.

The mechanism by which green tea extracts prevent the development and suppress the growth of cancer may be a complex one. Most investigators believe that green tea catechins, especially EGCG, which are known to exhibit antioxidative activity and tumor-suppressing activity, induce cancer cell cycle arrest and cause apoptosis.7-10 Recent studies11,12 have shown that EGCG inhibits DNA topoisomerase I and topoisomerase II which play a key role in controlling the topological state of DNA in all prokaryotic and eukaryotic cells. Other ingredients of the tea leaf extract which may be active in cancer cell biology and in anti-genotoxic effects induced by carcinogens are as yet to be identified.13 For example, a special amino acid in the tea leaves, known as theanine, has been recently shown to enhance the antitumor activity of adriamycin or doxorubicin, a commonly used topoisomerase II inhibitor anticancer drug associated with a high toxicity to the heart and liver. Theanine inhibits the efflux of adriamycin or doxorubicin from the cancer cells selectively, thus raising its intracellular toxic concentration in the malignant cells, but not in the normal tissues. 14-16

DNA topoisomerase II plays an important role in DNA replication and is required for condensation and segregation of chromosomes. It may function as part of a regulatory checkpoint at the entry and progression of mitosis.17 Therefore, DNA topoisomerases have been a subject of intense study in cancer chemotherapy in recent years.11, 18-21 The 4-quinolones, such as nalidixic acid, ciprofloxacin and ofloxacin exert their antibacterial effect by inhibition of the bacterial DNA gyrase, a type II topoisomerase. The eukaryotic DNA topoisomerase II is at least 100-fold less sensitive than its bacterial counterpart to the quinolone antibiotics and therefore usually not significantly affected by the doses of the drugs commonly used for controlling clinical bacterial infections. However, quinolones do have an inhibitory effect on mammalian DNA topoisomerase I, topoisomerase II and DNA polymerase 22, all of which are essential for proper DNA rejoining and structural configuration during cell proliferation. DNA topoisomerases are the target of many anticancer drugs, for example, doxorubicin and actinomycin D among others23. Since these known anticancer drugs and the quinolone antibiotics share the same molecular target, the cytotoxic activity of quinolones against certain cancer cell lines, including those of bladder carcinoma and leukemia, has been investigated.[21,22]

Based on the in vitro studies, ofloxacin and ciprofloxacin have been proposed as a potential adjunct to intravesical chemotherapy for human urinary bladder cancer.21 About 100 µg/ml of ofloxacin or ciprofloxacin is required to inhibit the growth of lymphoblasts and the high-grade bladder cancer cells under in vitro conditions.21,22 Serum concentrations of ciprofloxacin only reach an average level between 3.41 and 4.21 µg/ml after an oral administration of 750 mg of ciprofloxacin to human adult volunteers. Therefore, in general the concentrations of quinolones in the human tissues and body fluids probably cannot reach an expected in vitro anticancer level except in the urine where a concentration of 846 µg/ml can be obtained after an oral administration of the drugs at a tolerable dose.21 However, a low concentration of ciprofloxacin or ofloxacin has been shown to significantly enhance the efficacy of doxorubicin against cancer cells, indicating that there is synergism of two classes of DNA topoisomerase inhibitors in the suppression of cancer cell growth.[21]

Green tea extract and EGCG have been proposed as a nontoxic topoisomerase inhibitor for the treatment of clinical malignancies.11 The case reported here suggests that green tea extracts and a quinolone compound may provide an effective synergistic combination of these two topoisomerase inhibitors for the control of certain types of malignant lymphoma in dogs even when the concentration of the quinolone per se in the animal tissues does not reach an in vitro cytotoxic level for the cancer cells. The presence of other active compounds in the green tea might have enhanced the anticancer activity of these two potentially synergistic topoisomerase inhibitors. For example, theanine might serve as a biochemical modulator and preferentially inhibit the efflux of EGCG and ciprofloxacin from the cancer cells as it does for doxorubicin. It would be of interest to investigate whether theanine indeed functions as a biochemical modulator for topoisomerase inhibitors other than doxorubicin at the molecular level. Nonetheless, since green tea and ciprofloxacin (Baytril on the veterinarian drug list) are of such low toxicity to animal tissues, the combination of green tea and quinolones may serve as an alternative to euthanasia to those dog owners who do not want their pets to go through the standard chemotherapy protocols.

References

1. Price GS, Page RL, Fischer BM et al. Efficacy and toxicity of doxorubicin/cyclophosphamide maintenance therapy in dogs with multicentric lymphosarcoma. J Vet Intern Med 1991; 5:259-62

2. Hahn KA, Richardson RC, Teclaw RF et al. Is maintenance chemotherapy appropriate for the management of canine malignant lymphoma? J Vet Intern Med 1992;6:3-10.

3. Boyce KL, Kitchell BE. Treatment of canine lymphoma with COPLA/LVP. J Am Anim Hosp Assoc 2002;36:395-403.

4. Chun R, Garrett LD, Vail DM. Evaluation of a high-dose chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med 2000; 14:120-4.

5. Mellanby RJ, Herrtage ME, Dobson JM. Treatment of canine lymphoma by veterinarians in first opinion practice in England. J Small Anim Pract 2002;43:198-202.

6. Pfaff AD, March PA, Fishman C. Acute bilateral trigeminal neuropathy associated with nervous system lymphosarcoma in a dog. J Am Anim Hosp Assoc 2000;36:57-61.

7. Ahmad N, Feyes DK, Nieminen AL et al. Green tea constituent epigallocatechin-3-gallate and induction apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997;89: 1881-6.

8. Fujiki H, Suganuma M, Okabe S et al. Cancer inhibition by green tea. Mutat Res 1998; 402:307-10.

9. Li HC, Yashiki S, Sonoda J et al. Green tea polyphenols induce apoptosis in vitro in peripheral blood T lymphocytes of adult T-cell leukemia patients. JPN J Cancer Res 2000;91:34-40.

10. Bertolini F, Fusetti L, Rabascio C et al. Inhibition of angiogenesis and induction of endothelial and tumor cell apoptosis by green tea in animal models of human high-grade non-Hodgkin's lymphoma. Leukemia 2000;14:1477-82.

11. Berger SJ, Gupta S, Belfi CA et al. Green tea constituent (-)epigallo-catechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells. Biochem Biophys Res Commu 2001; 288:101-5.

12. Suzuki K, Yahara S, Hashimoto et al. Inhibitory activities of (-)-epi-gallocatechin-3-O-gallate against topoisomerase I and II. Biol Pharm Bull 2001;24:1088-90.

References

13. Ohe T, Marutani K and Nakase S. Catechins are not major components responsible for anti-genotoxic effects of tea extracts against nitroarenes. Mutat Res 2001;496:75-81.

14. Sadzuka Y, Sugiyama T, Miyagishima A et al. The effects of theanine, as a novel biochemical modulator, on the antitumor activity of adriamycin. Cancer Lett 1996;105:203-9.

15. Sugiyama T, Sadzuka Y. Combination of theanine with doxorubicin inhibits hepatic metastasis of M5076 ovarian sarcoma. Clin Cancer Res 1999;5:413-6.

16. Sadzuka Y, Yamashita Y, Sugiyama T et al. Effect of dihydrokainate on the antitumor activity of doxorubicin. Cancer Lett 2002;179:157-63.

17. Larsen AK, Skladanowski A, Bojanowski K. The roles of DNA topoisomerase II during the cell cycle. Prog Cell Cycle Res 1996;2:229-39.

18. Wang JC. DNA topoisomerases: from a laboratory curiosity to a subject in cancer chemotherapy. NCI Monogr 1987;4:3-6.

19. Lawrence JW, Darkin-Rattray S, Xie F et al. 4-Quinolones cause a selective loss of mitochondrial DNA from mouse L1210 leukemia cells. J Cell Biochem 1993;51:165-74.

20. Chu DT, Hallas R, Clement JJ et al. Synthesis and antitumor activities of quinolone antineoplastic agents. Drug Exp Clin Res. 1992;18:275-82.

21. Kamat AM, DeHaven JI and Lamm DL. Quinolone antibiotics: a potential adjunct to intravesical chemotherapy for bladder cancer. Urology 1999;54:56-61.

22. Hussy P, Maass G, Tummler B et al. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase alpha primase complex, topoisomerase I and II, and growth of mammalian lymphoblasts. Antimicrob Agents Chemother 1986;29:1073-8.

23. Malonne H, Atassi G. DNA topoisomerase targeting drugs: mechanisms of action and perspectives. Anticancer Drugs 1997;8:811-22.