

Clinical systemic lupeol administration for canine oral malignant melanoma

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Abstract. Canine oral malignant melanoma (COMM) is the most aggressive malignant tumor in dogs. Lupeol is a triterpene extracted from various fruits and vegetables that reportedly inhibits melanoma cell proliferation *in vitro* and *in vivo*. In this study, the efficacy of subcutaneous lupeol for spontaneous COMM was evaluated. A total of 11 dogs (3, 5 and 3 dogs diagnosed with clinical stage I, II and III melanoma, respectively) were evaluated. Subcutaneous lupeol (10 mg/kg) was administered postoperatively at various time points to treat these 11 COMM cases. Of the 11 subjects, 7 exhibited no local recurrence 180 days postoperatively and no severe adverse effects were observed in any of the cases. Furthermore, no distant metastasis was observed during the experimental period. Therefore, systemic lupeol may prevent local tumor progression and distant metastasis and may be a novel adjuvant treatment for the treatment of COMM.

Introduction

Canine oral malignant melanoma (COMM) is the most common malignancy in dogs, accounting for 30-40% of all oral tumors (1,2). COMM is characterized by extensive local invasion, as well as distant metastasis (2). Melanoma treatment includes radiation therapy and chemotherapy (CT), either alone, as an adjuvant therapy following surgery, or in combination (2). Although radiation therapy provides effective local tumor control, it is only performed in selected facilities (3-5).

In addition, radiation therapy may be costly and, hence, beyond the financial means of some owners. Several reports have investigated the efficacy of CT in COMM, but there is no definitive evidence regarding its effectiveness (5,6,7-9). Therefore, there is a need for an alternative treatment for COMM.

Lupeol is a triterpene found in fruits such as olives, mangoes, strawberries, grapes and figs, numerous vegetables and several medicinal plants (10). Previous studies reported that lupeol has antitumor properties (11,12) and several reports indicated that lupeol inhibits melanoma cell proliferation *in vitro* and *in vivo* (13,14). Recently, Nitta *et al* (14) reported that systemic lupeol administration inhibited tumor growth in a melanoma-bearing mouse model; however, there is no report evaluating its clinical efficacy in COMM. In this study, we aimed to evaluate the efficacy of subcutaneous lupeol as an adjuvant therapy for spontaneous COMM.

Subjects and methods

Subjects. A total of 11 dogs were included in this study. The characteristics of the subjects are summarized in Table I. The represented breeds included 2 miniature Dachshunds, 2 Beagles, 2 miniature Schnauzers, 1 Golden Retriever, 1 Labrador Retriever, 1 American Cocker Spaniel, 1 Cavalier King Charles Spaniel and 1 mixed-breed dog. The dogs ranged in age between 8 and 17 years. The tumors in all 11 dogs were classified according to the TNM classification (1,2) as follows: 3 dogs had stage I, 5 dogs had stage II and 3 dogs had stage III disease (Table I). Two cases of cancer recurrence were included (C04 and C07). This study was conducted between April, 2010 and March, 2013 in animal hospitals, including the Veterinary Teaching Hospital of Tottori University, Japan. Treatment was administered to dogs whose owners agreed to this study. The owners were informed of the risk of recurrence and other available treatment options, including surgery, radiation therapy and CT, and they all declined radiation therapy. The owners were offered the alternative treatment comprising surgery, subcutaneous lupeol and CT and they were all informed that lupeol was an experimental therapy. All the owners consented to the enrolment of their dogs in this clinical trial.

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Table I. Clinical case characteristics.

Case no.	Breed	Age, years	Gender	Weight, kg	Stage
C01	Golden Retriever	11	M	39	I
C02	Cavalier King Charles Spaniel	17	M	7	I
C03	Labrador Retriever	13	M	28	I
C04	Miniature Dachshund	13	M	5	II
C05	Mixed-breed	15	F	10	II
C06	Miniature Schnauzer	ND	M	6	II
C07	Miniature Dachshund	12	M	5	II
C09	Beagle	8	M	14	II
C10	American Cocker Spaniel	14	F	12	III
C11	Beagle	12	M	8	III
C12	Miniature Schnauzer	14	M	10	III

M, male; F, female; ND, no data.

Table II. Clinical outcomes following melanoma therapy.

Case no.	Stage	Surgical excision	Combined therapy	Local recurrence	Distant metastasis	DFT, days	ST, days	Long-term outcome
C01	I	Partial	-	-	-	210	210	Alive
C02	I	Partial	-	-	-	210	210	Alive
C03	I	Partial	-	Yes	-	110	180	Alive
C04	II	Partial	Melphalan, piroxicam	Yes	-	170	186	Alive
C05	II	Complete	-	-	-	187	187	Alive
C06	II	Partial	-	-	-	180	180	Alive
C07	II	Partial	PHCT	-	-	120	420	Alive
C08	II	Partial	-	-	-	201	201	Alive
C09	III	Partial	-	-	-	248	248	Alive
C10	III	Partial	PHT	-	-	189	189	Alive
C11	III	Partial	-	-	-	39	39	Alive

DFT, disease-free time; ST, survival time; PHCT, photodynamic hyperthermal chemotherapy; PHT, photodynamic hyperthermal therapy.

Surgical excision. Surgery was performed after the tumors were histopathologically confirmed as malignant melanomas. Complete excision was performed in 1 case (C05), in which the tumor could be excised with sufficient surgical margins. Partial excision without complete margins was performed in the remaining 10 cases; these tumors were difficult to excise completely due to their size or location (Table II).

Lupeol therapy. Lupeol solution was prepared as previously described (15). Briefly, lupeol was extracted from Indian lettuce (*Lactuca indica*) and dissolved in olive oil (Wako Pure Chemical Industries Ltd., Osaka, Japan) using heat (37°C) and sonification (3 h). The concentrated lupeol solution was diluted to 5 mg/ml.

Lupeol was administered subcutaneously at 10 mg/kg as an adjuvant therapy at least 1 week postoperatively to prevent

local recurrence or metastasis. Lupeol was initially administered twice a week for 2 weeks (4 administrations in total). Treatment was then decreased to once a week for a total of 4 weeks; alternate weeks for 8 weeks (4 administrations in total); once a month for several months (≥ 2 administrations); and, finally, discontinued. However, this standard protocol was increased or decreased in frequency, depending on the owner's request, travel restrictions, or the physical condition of the subject. Follow-up examinations to detect recurrence and metastasis were performed once monthly during the trial period.

Combination therapy. Photodynamic hyperthermal therapy (PHT) (16,17), photodynamic hyperthermal CT (PHCT) (18,19) and CT were performed, with lupeol administered as an adjuvant therapy. PHT is a local treatment combining photo-

dynamic therapy and hyperthermia that uses indocyanine green as a photosensitizer (16,17). PHCT is an advanced form of PHT, in combination with carboplatin CT (18,19).

Results

Clinical outcome. The clinical outcomes are summarized in Table II. There were no severe adverse effects, such as local pain, diarrhea, or vomiting, in any of the subjects during lupeol treatment. Local tumor recurrence occurred in 1 case with stage I disease (C03) at 110 days and in 1 case with stage II disease (C04), which underwent partial surgical excision initially, at 170 days. Case C03 was immediately restarted on lupeol therapy alone every 2 weeks. After the additional treatment, tumor progression was arrested in case C03; and the disease condition has remained stable in both cases. In case C03, the tumor decreased in size and the owner reported that the dog's quality of life had improved.

Survival. At the end of the experimental period, all the dogs remained alive. Of the 12 dogs, 10 had survived for >180 days after surgery. Moreover, no distant metastasis was observed during the experimental period.

Discussion

COMM is an extremely malignant tumor, with a high propensity for local invasion and metastasis (1,2). The treatment options for COMM include surgery, radiation therapy, CT and immunotherapy comprising xenogeneic DNA vaccination. MacEwen *et al* (20) reported that the median survival time (ST) in dogs with oral malignant melanoma that underwent complete surgical excision was ~17-18 months, 5-6 months and 3 months for stage I, II and III disease, respectively. There may be a potential to prolong ST beyond these reported durations, as all the subjects remained alive during the most recent examination. In stage III cases, the median ST increased in subjects administered adjuvant lupeol treatment (>6 vs. 3 months). The reported median ST for dogs receiving radiotherapy ranges between 5.3 and 11.9 months (3-5,7,9); however, as the radiotherapy equipment, treatment protocols and supplementary therapies differ between studies, it is difficult to directly compare previously reported results. Regardless, the results of previous reports suggest that systemic lupeol administration may help prolong the median ST in COMM cases and may prove efficacious as an adjuvant postoperative treatment.

Distant metastasis was not observed in any of the cases, including the 3 stage III cases, which were at highest risk for metastasis. Furthermore, there was no local recurrence for 180 days postoperatively in 7 of the 11 dogs. The median disease-free time (DFT) of dogs undergoing radiation therapy reportedly ranges between 5 and 7.9 months (5,20). In a study evaluating radiation therapy in 38 dogs with COMM, Theon *et al* (21) reported a median DFT of 11.3 months in stage T1 cases, 6.0 months in stage T2 cases and 6.7 months in stage T3 cases. The results of the present study suggest that surgery combined with lupeol administration achieves local tumor control equivalent to that of radiation therapy. Saleem *et al* (13) reported that lupeol inhibits the growth of

highly aggressive human metastatic melanoma cells *in vitro* and *in vivo* by inducing apoptosis. The results of the present study suggest that, in addition to preventing local progression, systemic postoperative adjuvant lupeol administration may also prevent the development of distant tumor metastasis.

For the local treatment of COMM, aggressive complete surgical excision, such as partial mandibulectomy and maxillectomy is the most effective option (5). Radiation therapy also plays an important role and is an effective treatment for achieving local tumor control; however, radiation therapy is associated with several adverse effects, including dermal desquamation, alopecia and bone necrosis, which may negatively affect the quality of life (7). The subjects must be anesthetized during radiation therapy, which itself is accompanied by serious risks. In addition, therapy is only available at selected facilities and may not be affordable for all owners; therefore, radiation therapy cannot be easily performed in all cases. CT combined with radiation therapy and surgery has been used as an alternative systemic therapy to prevent local recurrence and distant metastasis. Although several reports have evaluated the efficacy of CT in COMM as a sole or adjuvant therapy, there is no clear evidence that CT decreases the risk of local recurrence or metastasis, or that it prolongs median ST (5-9). These limitations highlight the need to develop an effective systemic therapy for COMM.

PHT, PHCT, melphalan and piroxicam were administered in combination, but there is no evidence that these treatments are effective in controlling COMM progression. PHT and PHCT were performed with owner consent after explaining the experimental nature of these treatments. Proulx *et al* (5) reported that melphalan administered for COMM exerted no beneficial effects. There is limited evidence that non-steroidal anti-inflammatory drugs (NSAIDs) may exert a protective effect against malignant melanoma development in humans (22); however, in a study evaluating NSAID therapy in dogs, there was no significant difference in survival attributed to NSAID administration and NSAID therapy did not provide any survival benefit or achieved an improved therapeutic response in the treated subjects (7). Therefore, these adjuvant therapies were unlikely to confer any beneficial treatment effect in this study.

No severe adverse effects, such as local pain, were observed in any of the dogs during lupeol treatment. There are no known reports of adverse effects associated with lupeol administration, which indicates that lupeol is safe when administered at the dosage recommended in the present study. Notably, this study was only a limited clinical trial and evaluated lupeol as a postoperative adjuvant therapy. Prospective controlled studies are required to assess the clinical efficacy of subcutaneous lupeol administration for the treatment of COMM, as well as in combination with other modalities, such as radiotherapy and CT.

In conclusion, to the best of our knowledge, this study is the first clinical trial to evaluate systemic lupeol therapy for COMM. Postoperative lupeol is easily administered, is not associated with severe adverse effects and was shown to prevent local tumor progression and distant metastasis. Therefore, the results of the present study suggest that lupeol is a potential novel adjuvant treatment option and may be used as systemic therapy for COMM.

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