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Cutaneous Epitheliotropic T-Cell Lymphoma in a Dog: Clinical Responses to Lomustine and Gemcitabine

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Abstract : A 5-year-old, spayed female Maltese dog presented with generalized multifocal pruritic erythema and alopecia for a month. Initial skin biopsy suggested cutaneious histiocytosis. The dog had been treated with the immunosuppressive therapy for a month, but multifocal erythematous patches and plaques were newly observed. Direct imprint smear of cutaneous lesions suggested a lymphoma and rebiopsy was performed. Microscopic examination demonstrated a round cell tumor with epitheliotrophism to the epidermis and adnexal structures. The neoplastic round cells were strongly positive for CD3 yet negative for CD79a, indicting the tumor was cutaneous epitheliotropic T-cell lymphoma. After 2 cycles of oral administration of lomustine (70 mg/m^2, once every 2-3 weeks), only partial response was observed. Alternative chemotherapy with gemcitabine (500 mg/m^2, 30-minute IV infusion, once every week) was initiated. A total 3 cycles of gemcitabine failed to control the progression of disease, and the dog was euthanized on Day 69 after the 1st lomustine treatment.

Key words **:** chemotherapy, cutaneous epitheliotropic T-cell lymphoma, gemcitabine, lomustine.

Introduction

Cutaneous epitheliotropic T-cell lymphoma (CETL) is a rare skin malignancy characterized by a clonal T-cell infiltrate primarily involving the epidermis and adnexal structures (5). Cutaneous lymphomas account for only 3-8% of all canine lymphomas, and the prevalence of canine CETL is approximately 0.2-0.7% of canine dermatosis (5,11). The prognosis of canine CETL is poor with a survival time from few months to 2 years (5).

A standard therapy has not been established for canine CETL, because of low response rates (< 50%) and short duration of the existing treatments including synthetic retinoids, topical therapies, radiation therapy, and conventional chemotherapy (15). Recently, two veterinary literatures evaluating lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea (CCNU)) demonstrated an overall response rate of 78-83% in the treatment of canine CETL (13,15). Therefore chemotherapy using lomustine is considered as a reasonable treatment option (5).

In human medicine, cytotoxic chemotherapy is often employed in patients with advanced CETL. Especially, gemcitabine (2', 2'-difluorodeoxycytidine) is widely used in the treatment of aggressive and refractory CETL, with response rates range from 65% to 73% (3,8). One veterinary study showed that gemcitabine can be used safely in canine solid tumor or lymphoma, without evidence for severe toxicity (7).

Previously, the use of prednisolone and isotretinoin to treat a dog with CETL was reported in Korea (2). This report firstly describes clinical responses to lomustine and gemcitabine in a dog with CETL.

Case

A 5-year-old, spayed female Maltese dog was presented due to one month duration of generalized erythema, alopecia, and pruritus. The lesions were described as erythema and focal alopecia of the face, neck, dorsum, legs, and tail. The dog was treated orally with cephalexin (30 mg/kg twice daily for 1 week), itraconazol (10 mg/kg once daily for 1 week), and ivermectin (300 μg/kg once daily for 1 week) without marked improvement. Moreover, erythematous scaly patches were generalized and new crusted lesions developed on the neck. A skin biopsy was performed for histologic analysis, and the findings were suggestive of cutaneous histiocytosis. Skin lesions had been gradually decreased until 2 weeks after the immunosuppressive therapy with prednisone (2 mg/kg twice daily) and azathioprine (2 mg/kg once daily). However, due to relapsing and further worsening of skin lesions,

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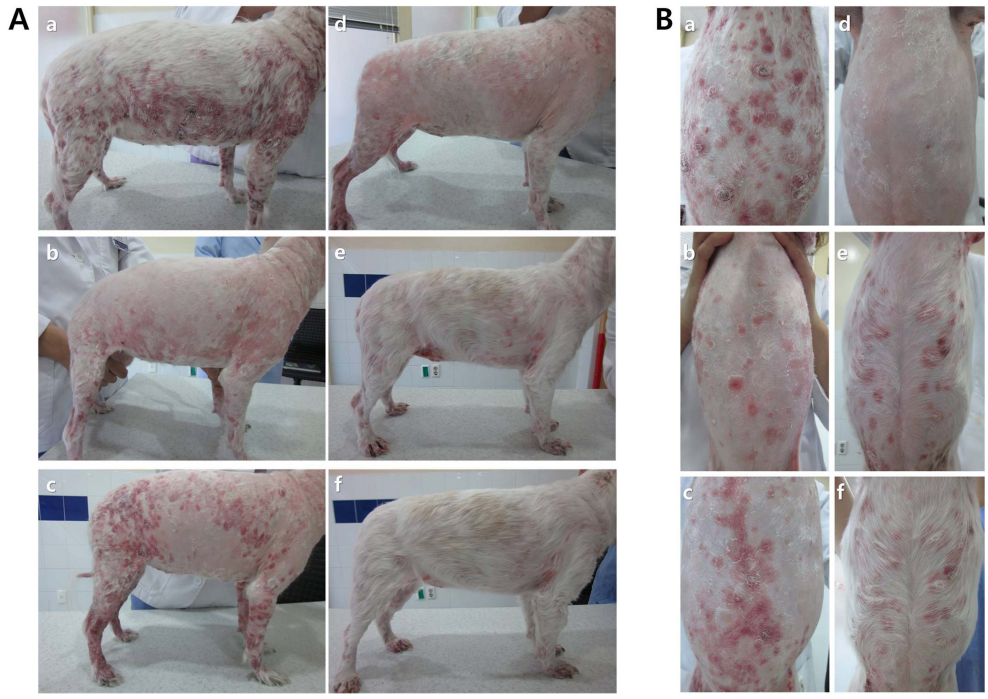

Fig 1. Temporal change of skin lesions observed on right lateral (A) and ventral (B) views following lomustine and gemcitabine therapy. Before chemotherapy, multifocal, asymmetrically distributed erythematous patches and plaques were observed (a). Skin lesions were remarkably improved at 1 week after the 1st lomustine treatment (b), but lomustine was administered again due to relapsing of skin lesions at 16 days after the 1st lomustine treatment (c). The improvement of clinical signs had been maintained until 13 days after the 2nd lomustine treatment (d). At 1 week after the 1st gemcitabine treatment, plaques on the dorsum and ventrum were newly developed (e), and those lesions had been stable until 1 week after the 2nd gemcitabine treatment (f).

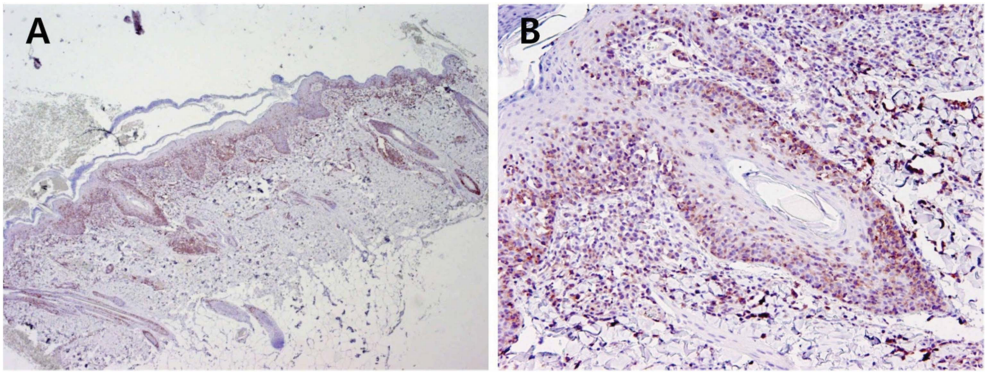

Fig 2. Cutaneous epitheliotrophic T cell lymphoma. Immunohistochemistry for CD3 (T lymphocyte marker). Diffusely neoplastic lymphocytes infiltrated the epidermis, superficial dermis, and adnexal structures, often obscuring dermal-epidermal junction (A; × 20, Romulin AEC, Biocare). Note the intense infiltration of CD3-positive neoplastic lymphocytes along the basal cell layer. (B; × 100, Romulin AEC, Biocare).

the patient was examined 3 weeks later.

The skin examination revealed multifocal, asymmetrically distributed erythematous patches and plaques which were associated with scaling, alopecia, erosion, and crusts (Fig 1a). Submandibular and prescapular lymph nodes were enlarged on palpation. A complete blood count (CBC) with hematology differential showed mild anemia (packed cell volume: 35.5, reference range: 37-55%) and stress leukogram. Severely elevated alanine aminotransferase (ALT: 519, reference range: 21-102 IU/L) was observed on the serum chemistry panel. No abnormalities were identified on abdominal radiographs and sonography. Direct imprint smear of eroded lesions showed medium-large sized lymphocytes with round, cleaved, or folded nuclei. The differential diagnoses included cutaneous lymphoma, plasmacytoma, and malignant histiocytosis.

Skin biopsy was taken from the trunk and submitted for histopathology. Microscopically, a homogenous population of small round cells, resembling lymphocytes, diffusely infil-

Table 1. Assessment of hematologic and hepatic toxicity in a dog with CETL following administration of lomustine and gemcitabine

	Days after the 1st chemotherapy									
	0*	7	13*	23	29	36†	43‡	50‡	57‡	Reference
Neutrophils ($\times10^3/\mu L$)	22.47	1.44(G2)	6.33	7.43	4.09	3.59	3.92	1.42(G2)	2.69	3-11.8
Platelets ($\times10^3/\mu L$)	447	596	672	332	390	290	196	146	170	200-500
AST (IU/L)	58	51	36	40	-	34	-	21	-	23-66
ALT (IU/L)	519(G3)	296(G3)	203(G2)	172(G2)	-	226(G3)	-	60	-	21-102
ALP (IU/L)	53	1973(G4)	> 3000(G4)	2308(G4)	-	1305(G3)	-	497(G3)	-	29-97

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase
*Administration of lomustine (70 mg/m^2, PO)
†Administration of interferon alpha (15,000 IU/m^2, SC, 3 times per week) and isotretinoin (4 mg/kg, PO, once daily)
‡Administration of gemcitabine (500 mg/m^2, 30-minute IV infusion)
G2: grade 2 of neutropenia, 1,000 to 1,499 cells/μL; ALT toxicity criteria, > 1.5 to 2 x the upper limits of the reference range
G3: grade 3 of ALT toxicity criteria, > 2 to 10 x the upper limits of the reference range; ALP toxicity criteria, > 5 to 20 x the upper limits of the reference range
G4: grade 4 of ALP toxicity criteria, > 20 x the upper limits of the reference range

trated in the epidermis and superficial dermis, often obscuring the dermal-epidermal junction. Similar neoplastic lymphocytes infiltrated the hair follicular epithelium, apocrine gland, and sebaceous gland (Fig 2). Immunohistochemistry with antisera against CD3 (Dako, USA) and CD79a (Dako, USA) was performed. The tumor cells exhibited strong immunoreactivity to CD3 yet negative to CD79a, indicating the neoplastic round cells were T lymphocyte in origin. Based on the clinical and histopathological findings, the skin tumor was definitively diagnosed as CETL.

Initial therapy was performed using a lomustine protocol (13,15). A CBC and serum chemistry profile required for assessment of toxicities was performed at 6 to 10 days interval after the 1st chemotherapy (Table 1). The severities of toxicoses were graded from 1 (mild) to 4 (most severe), according to the reported criteria (Table 1) (14). A total 2 cycles of lomustine (Lomustine, medac GmbH, Germany) at 70 mg/m^2 was orally administered to the patient. Generalized erythema, patches, plaques, and lymphadenopathy were remarkably improved at 1 week after the 1st lomustine treatment (Fig 1b). However neutropenia (1,440 cells/μL, grade 2) and hepatotoxicity (ALT: 296 IU/L, grade 3; alkaline phosphatase (ALP): 1973 IU/L, grade 4) were noticed. At 16 days after the 1st lomustine treatment, hepatotoxicity (ALT: 203 IU/L, grade 2; ALP: > 3000 IU/L, grade 4) was not improved, but the 2nd lomustine treatment was performed due to relapsing of skin lesions (Fig 1c). The severity of skin lesions and hepatotoxicity (ALT: 172 IU/L, grade 2; ALP: 2308 IU/L, grade 4) was reduced 1 week later, and then improvement of clinical signs and no myelosuppression had been maintained until 13 days after the 2nd lomustine treatment (Fig 1d). One week later, exfoliative erythrodema, plaques, edema, and erosions were newly observed on the eyelids, ventrum, axilla, paws, and tail. Because of the limitation in purchasing lomustine during treatment period, interferon alpha (15,000 IU/m^2, SC, 3 times per week; Roferon-A, Roche, Switzerland) and isotretinoin (4 mg/kg, PO, once daily; Roaccutane, Roche, Switzerland) were administered. However skin lesions had worsened over 1 week.

Because gemcitabine is widely used in the treatment of refractory CETL in human, alternative chemotherapy was initiated at 43 days after the 1st lomustine treatment, according to a modification of gemcitabine protocol in dogs (7). A total 3 cycles of gemcitabine (500 mg/m^2, 30-minute IV infusion, once every week; Gembine Inj., Hanmi, Korea) was administered. At 1 week after the 1st gemcitabine treatment, erythema and edematous lesions were mildly decreased, but neutropenia (1,440 cells/μL, grade 2) and plaques on the head, dorsum, and ventrum were newly developed (Fig 1e). Although ALP toxicity (497 IU/L, grade 3) was still remained, the 2nd gemcitabine treatment was performed for new lesion development. One week later, skin lesions were stable (Fig 1f) and neutropenia was disappeared, and the 3rd gemcitabine treatment was performed. Because of worsening skin lesions and pruritus, the dog was euthanized at 12 days after the last therapy, according to the request of client.

Discussion

Currently, the most promising treatment options for canine CETL include the use of lomustine (5,13,15). Lomustine is an alkylating agent of the nitrosurea subclass, and its activity for recurrent lymphoma and cutaneous mast cell tumors was reported in dogs (10,12). The recommended dosage for canine CETL was 60-70 mg/m^2 orally once every 3 weeks with a mean of 3-4 treatments (range, 1-12), and the overall median duration of response was 88-94 days (range, 22-282) (13,15). In this case, 2 cycles of lomustine at 70 mg/m^2 were administered to the patient. Although partial response (50-99% reduction in size of measurable tumors) was observed after

the 1st lomustine treatment, skin lesions relapsed 9 days later. Therefore lomustine was administered again at that time, which was 5 days faster than the recommended interval between lomustine treatments. Since the 2nd lomustine treatment, partial response had been maintained for 20 days, thus total duration of response was 36 days.

The main causes of dose reduction or discontinuation in lomustine therapy are toxicoses, which generally include myelosuppression, gastrointestinal signs, and liver enzyme activity increases (13,15). Among them, the most common toxicity is myelosuppression. In one study, incidence of neutropenia was 55-56% at 1 week after initial lomustine therapy (15). Because this toxicity had been gradually resolved, neutropenia was observed in 2-4% of dogs only before the next administration of lomustine. In this case, grade 2 neutropenia appeared at 1 week after the 1st lomustine treatment, but it had not reoccurred until 20 days after the 2nd lomustine treatment.

The true influence of lomustine on liver function could not be determined. However up to 86% of the dogs had increased liver enzyme activity after lomustine chemotherapy, and more than 60% of those dogs received concurrent corticosteroids (15). A retrospective study of 46 dogs with CETL showed that the concurrent use of prednisone provides no detectable improvement in response rate or duration (13). In this case, grade 2-3 ALT toxicity and grade 3-4 ALP toxicity had been noticed during lomustine monotherapy. Although severe ALP toxicity appeared after initial lomustine administration, it had been resolved after the 2nd treatment and did not result in documented hepatic failure. If the dog received concurrent corticosteroids, the severity of hepatotoxicity might be increased. Therefore lomustine monotherapy had a therapeutic activity for the present case without dose reduction. The benefit of lomustine in monotherapy or combined with other agents could be investigated by prospective clinical trials.

Gemcitabine is a pyrimidine antimetabolite that inhibits DNA synthesis through chain termination and ribonucleoside reductase inhibition (6). This drug has demonstrated impressive clinical activity in human patients with advanced-stage and refractory CETL (3). Overall response rates as high as 70% have been reported, but myelosuppression can be problematic (4,9). Previously, the toxicity and efficacy of gemcitabine was evaluated in 19 dogs with aggressive cancer, for which no effective therapy existed or conventional therapy failed (7). Although clinical responses were observed in 2 dogs only, there were minimal hematologic, serum biochemical, and gastrointestinal toxicities. The recommended dosage was 675 mg/m^2 given as a 30-minute IV infusion every 2 weeks.

In this case, a total 3 cycles of gemcitabine at 500 mg/m^2 was intravenously administered to the patient. Although weekly chemotherapy was performed without 1-week rest, only one episode of grade 2 neutropenia and no gastrointestinal toxicity were observed during treatment period. The dog had had stable disease (< 50% reduction or < 10% increase in measurable tumor burden and no new lesion development) during 16 days since initial gemcitabine therapy. However euthinization was performed 12 days later, because of aggravating skin lesions.

Because the clinical sings of CETL is very pleomorphic and can mimic many dermatosis accompanying pruritus and erythema, the final diagnosis is always based on histopathological examination (5). However, there is some similarity among the histological appearances of round cell tumors, thus differentiating multiple cutaneous histiocytomas, cutaneous histiocytosis and cutaneous lymphoma on the basis of light microscopy may be difficult (1). Initially, the present case was misdiagnosed as cutaneous histiocytosis based on histopathology. Therefore the definitive diagnosis of round cell tumors may need detailed immunophemotyping of cumulative cells.

This report firstly describes clinical responses and toxicity of gemcitabine in a dog with CETL. Lomustine monotherapy was reasonably safe and effective for the present case, whereas gemcitabine could not control the progression of disease in contrast to positive responses of human patients. However weekly gemcitabine administration generated minimal toxicities in comparison with lomustine therapy, thus further evaluation of gemcitabine in canine CETL may be needed for patients with dose-limiting toxicity induced by lomustine administration.

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개에서 발생한 피부 상피친화성 T-세포 림프종: Lomustine 및 Gemcitabine에 대한 임상적 반응

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요 약 : 5년령의 중성화된 암컷 말티즈가 1개월 동안 지속된 전신적 발적, 탈모 및 소양감으로 내원하였다. 피부조직구증으로 진단되어 면역억제치료를 1개월 간 지속하였지만 다발성의 발적된 반 및 판 병변들이 새롭게 발생하였다. 피부병변에 대한 압박도말검사에서 림프종을 지시하는 원형세포들이 관찰되었다. 조직학적 검사에서는 종양세포들의 외피 및 부속구조물에 대한 친화성이 나타났다. 면역염색결과 종양세포들은 CD3에 대하여 양성, CD79a에 대해서는 음성반응을 나타내어 피부 상피친화성 T-세포 림프종으로 확진하였다. 70 mg/m^{2}의 lomustine을 2-3주 간격으로 총 2회 투여하였으며 부분적인 치료반응이 관찰되었다. 피부병변의 악화와 lomustine의 이용제한으로 gemcitabine (500 mg/m^2, 1주일당 1회 30분간 혈관주입)을 이용한 화학치료를 시작하였다. 총 3회 치료가 실시되었으나 질병의 진행을 억제하지 못하였으며, 최초 lomustine 치료 69일 후 안락사 되었다.

주요어 : 화학치료, 피부 상피친화성 T-세포 림프종, gemcitabine, lomustine