Case Report

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Ex vivo Boosted Immune Cell Therapy for Canine Hepatic Disease

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Abstract A 12-year-old male American Cocker Spaniel was diagnosed with a type of chronic hepatits (CH) called cholangioheaptits. Routine supportive medication was administered to the patient, and ex vivo boosted immune cell (EBI-C) therapy was used for the treatment. A histopathologic examination of the liver 19 months later revealed that the cholangiohepatitis had progressed to cholangiocarcinoma. The medication and immune cell therapy was maintained. Two months after the new diagnosis, the patient's state worsened, and the dog died 635 days after the first visit. EBI-C therapy is a type of immunotherapy, where immune cells are isolated from the patient's peripheral blood mononuclear cells, expanded ex vivo, and then infused into the patient intravenously every two weeks. EBI-Cs (mean: 2.78×10^8 cells) were obtained 38 times and infused every two weeks. Most EBI-C were T-lymphocytes (99.24% of total EBI cells). T-lymphocytes produce large interferon (IFN)-γ, and IFN-γ inhibits liver fibrosis in dogs with CH. Moreover, in bile duct cancer, an increase in T-lymphocytes correlates with decreasing tumor invasion and metastasis. Thus, we propose that EBI-C therapy is applicable as a new supportive therapy for canine liver disease if other treatments like drug medication, surgery, or radiation are unavailable.

Key words EBI-C, immune cell therapy, chronic hepatitis, dog, interferon-gamma.

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Introduction

Chronic hepatitis (CH) is a disease that occurs frequently in dogs (1,8,9,25). Although liver inflammation has been subdivided into several categories, such as chronic active hepatitis, chronic cholestatic hepatitis, and cholangiohepatitis, the World Small Animal Veterinary Association Liver Standardization Group proposed that all conditions are CH (29).

CH is treated as an immune-mediated disease, with oral corticosteroids combined with supportive therapy (6,26). However, if the patient is accompanied by CH and endocrine, such as diabetes mellitus or hyperadrenocorticism, steroid administration is not feasible.

Presentd is a Cocker Spanel case of CH, hyperadrenocorticism, and diabets mellitus, with an eventual progression of the CH to cholangiocarcinoma. Due to the presence of comorbid endocrine disease, corticosteroids for CH were not feasible. Hence, the patient was treated with adjuvant immune cell therapy.

Case Report

A 12-year-old male American Cocker Spaniel, with polyuria, polydipsia, anorexia, and hind limb weakness, was referred to the Small Animal hospital of Veterinary Medicne at Kyungpook National University. On physical examination, there was no specific abnormality. The complete blood count (CBC) and serum biochemistry profile did not reveal abnormalities, except increased alkaline phosphate (ALP, 298 U/I, [RI (reference interval): 47-254 U/I]) and amylase (2215 U/I, [RI: 200-1400 U/I]). Abdominal ultrasound showed a round mass in the splenic body and a snowman-shaped mass in the left adrenal gland. The liver appeared coarse in texture and had an irregular hepatic margin. These abnormalities were also identified in computed tomography imaging.

Abdominal surgery was conducted to excise the masses and obtain a liver tissue sample. The liver's gross appearance was dark red, and various sizes of nodules were observed over the liver surface. The liver sample was obtained from wedge biopsy and fixed in 10% neutral buffered formalin. A splenectomy was performed, and the spleen mass was fixed. No surgical treatment was performed on the left adrenal gland. The tissue samples were subsequently slced and histologically stained with hematoxylin and eosin. In addition to the histopathological examination, and adrenocorticotrophic hormone (ACTH) stimulation test was conducted to confirm hyperadrenocorticism (HAC).

The splenic mass was diagnosed as a benign nodular lymphoid hyerplasia. In the liver tissue, mild to moderate lymphocytic cholangiohepatits with immature portal-portal bridging fbrosis and datrophic heatic lobules were identified. Hepatocytes were swollen with wispy vacuoles, and no neoplasm was observed. The ACTH stimulation test confirmed HAC.

As a daily dose, the patient was treated with ursodeoxycholic acid (10 mg/kg), s-adenosylmethionine (20 mg/kg), trilostane (2.5 mg/kg), and clopidogrel (1 mg/kg) via oral route, concurrent with ex vivo boosted immune cell (EBI-C) therapy. EBI-C therapy is an immunotherapy approach where immune cells are isolated from the patient's peripheral blood mononuclear cells (PBMCs), expanded in ex vivo, and intravenously infused into the patient. Peripheral blood (10 mL) was collected via jugular venipuncture, cultured, and incubated for two weeks. Then, EBI-Cs suspended in 10-20 mL of normal saline was administered to the patient intravenously every two weeks. Two months after the initial hospital visit, the patient was given NPH (0.6-0.7 U/kg) twice a day. The patient responded very well to treatment for HAC and DM. The CVC and serum biochemistry were within the reference ranges. One or more of the serum enzymes, alkaline phosphatase (ALP), alanine transaminase (ALT), creatine kinase, blood urea nitrogen (BUN), and amylase, increased episodically but soon returned to the reference range. The patient's condition was good, except for weight loss. His appetite was normal, but he had lost approximately 2 kg over one year. Histopathological examination of the liver revealed that the cholangiohepatitis had progressed to cholangiocarcinoma 19 months later. The liver samples showed invasive neoplastic cells surrounded by thick fibrous septa. The neoplastic cells had an appearance of biliary epithelium characterized by columnar-cuboidal cells, pale-to-eosinophilic abundant cytoplasm, and prominently round nuclei. However, severe clinical signs of hepatic failure, such as ascites and neurological sings, were not evident. Two months after diagnosis with cholangiocarcinoma, the dog was depressed, with decreased appetite, melena, and diarrhea. Clnical signs had waxed and waned. Even though the insulin dose was adjusted, the blood glucose level was not maintained within the normal range. Serum chemistry showed that the liver enzymes were elevated and did not return within the reference range. At 625 days, the level of BUN, creatinine, amylase, and lipase increased. We treated the patinet for acute renal failure and pancreatitis. THe kidney and pancreatic enzyme activity returned to normal, but lethargy, anorexia, and diarrhea remained. The dog died 635 days following the first visit and 70 days following the diagnosis of cholangiocarcinoma.

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Discussion

CH is frequently reported in dogs (1,8,9,25). American and English Cocker Spaniels were reported to be at risk of developing CH (1). Additionally, the prognosis of these breeds is poorer than other breeds with CH (3,4). Diagnostic confirmation of CH is based on hepatic histopathology (29). Unlike other predisposed breeds, the hepatitis of Cocker Spaniels represented characteristic lesion histopathology (3,13). Both American and English Cocker Spaniels have evidence of increased fibrous connective tissue and predominantly inflammatory lymphocytes rather than plasma cells or neutrophils (3,13). In contrast, English Cocker Spaniels have predominantly shown hepatocyte necrosis and apoptosis, whereas necrotic activity or hepatic apoptosis is low in most American Cocker Spaniels (3,13). CH is one of the causes of cirrhosis, and the prognosis for patients with CH and developed cirrhosis is poor (9,19). In this study, the histopathological finding was similar to previous reports, and we predicted that the liver had progressed to cirrhosis based on gross appearance.

Hence, CH is considered an immune-mediated disease, oral corticosteroids with supportive therapy are the first option for treating CH in dogs (6,26). Corticosteroids have been reported to suppress inflammation and the transforming growth factor (TGF)-β pathway, thus, suppressing fibrosis (5,7). However, in this case, the administration of corticosteroid was not feasible because of HAC and DM. Furthermore, corticosteroid use in patients with hepatic fibrosis is not appropriative because it could cause portal hypertension. Therefore, the patient was treated using adjuvant EBI-C therapy.

EBI-C therapy is a type of immunotherapy, where immune cells are isolated form the patient's PBMCs, expanded ex vivo, and then infused into the patient. Thus, patients receive several activated immune cell transfer can be used to treat various pathological conditions, especially cancer (12,14,24). PBMCs were isolated from a 10 mL peripheral blood sample from the patient. EBI-Cs (0.2-8.4 $\times$ 10^8 cells) was obtained two weeks after the culture and expansion of PBMCs. EBI-C were suspended in 10-20 mL of normal saline and intravenously infused into the patient. EBI-Cs were obtained 38 times in this study, and the mean cell count of injection was 2.78 $\times$ 10^8 cell. Most of the EBI-Cs were $CD3^+CD21^-$ T-lymphocytes (mean: 99.24% of total EBI cells), whereas others in clude Natural killer (NK) cells and regulatory T-cells.

Interferon (IFN)-γ is primarily produced by T-lymphocytes, NK cells and NKT-cells. Thus the number of INF-γ producing cells increased significantly with the culture and stimulation of EBI cells. In several studies, the injection of IFN-γ inhibits liver fibrosis in experimental animals through several mechanisms besides IFN-γ suppression of TGF-β expression (2,21,22,30). Before, we mentioned that CH is an immune-mediated disease and EBI-C therapy is an infusion of numerous immune cells into the body. Thus it was thought that EBI-C therapy may worsen CH. However, the main benefit of EBI-C therapy is to increase the number of INF-γ producing cells in the body, and we expected that EBI cell therapy could have an antifibrotic effect in dogs.

On the second, according to the histopathological liver tissue examination, the patient was diagnosed with cholangiocarcinoma. This biliary tract cancer behaves aggressively, with a high metastasis rate in humans and animals according to clinical studies (10,17). Metastasese occured in 60-88% of the dogs with bile duct carcinoma, and the most common sites of metastasis were the lymph, lungs, and peritoneum (18,28). In human clinics, bile duct carcinoma is treated with radiation therapy, chemotherapy, and aggressive surgery (17). However, in most patients, the cancer is unresponsive or relapses, and the prognosis is poor (17). Therefore, a more therapeutic strategy has been developed to treat bile duct cancer, particularly immunotherapy (11,27). Infiltration of different subsets of immune cells has been demonstrated to inhibit tumor progression and metastatic potential in various types of cancers (12,23,24). In an early study, CD4+ and CD8+ T-cells decreased tumor invasion and metastasis in humans with bile duct cancer (15,16). As mentioned above, EBI-Cs consist primarily of T-lymphocytes. Thus, we expected beneficaial effects on EBI-C therapy on dogs with cholangiocarcinoma. Indeed, we had been conducting diagnostic imaging tests until the day before death. Although a high prevalence of cholangiocarcinoma metastasis has been documented, there was no pulmonary, lymph nodes, and peritoneum metastatic evidence on radiological and ultrasound scans. Un this study, the patient had been teated consistently with EBI-C therapy, except on a few occasions because of the owner's personal reasons.

According to studies, the prognosis in dogs with CH is guarded, with a median survival time ranging from 189 to 913 days (3,19,20). Bexfield et al. (3) noted that 55% of Cocker Spaniels died within three months, and just 17% of dogs survived more than a year following CH diagnosis. Also, several dogs with CH suffered severe clinical symptoms associated with liver function failures, such as ascites and neurological signs (19,20). Presently, the survival time was 635 days from the first hospital visit. During the therapeutic period, the patient's condition had been good.

According to the owner's observation, the dog showed decreased appetite and lethargy when the EBI-C therapy was

discontinued.

To best of our knowledge, this is a novle report on immune cell transfer therapy adjusted for canine CH and bile duct carcinoma. Further studies are needed to confirm the efficacy of EBI-C therapy for canine liver disease. However, we propose that EBI-C therapy is applicable as a new therapy for canine liver disease if other treatments like drug medication, surgery, or radiation are unavailabe.

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Conflicts of Interest

The authors have no conflicting interests.

References

1. Andersson M, Sevelius E. Breed, sex and age distribution in dogs with chronic liver disease: a demographic study. J Small Anim Pract 1991; 32: 1-5.
2. Baroni GS, D'Ambrosio L, Curto P, Casini A, Mancini R, Jezequel AM, et al. Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. Hepatology 1996; 23: 1189-1199.
3. Bexfield NH, Andres-Abdo C, Scase TJ, Constantino-Casas F, Watson PJ. Chronic hepatitis in the English springer spaniel: clinical presentation, histological description and outcome. Vet Rec 2011; 169: 415.
4. Bexfield NH, Buxton RJ, Vicek TJ, Day MJ, Bailey SM, Haugland SP, et al. Breed, age and gender distribution of dogs with chronic hepatitis in the United Kingdom. Vet J 2012; 193: 124-128.
5. Bolkenius U, Hahn D, Gressner AM, Breitkopf K, Dooley S, Wickert L. Glucocorticoids decrease the bioavailability of TGF-beta which leads to a reduced TGF-beta signaling in hepatic stellate cells. Biochem Biophys Res Commun 2004; 325: 1264-1270.
6. Center SA, Warner KL, McCabe J, Foureman P, Hoffmann WE, Erb HN. Evaluation of the influence of S-adenosylmethionine on systemic and hepatic effects of prednisolone in dogs. Am J Vet Res 2005; 66: 330-341.
7. Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. J Hepatol 2004; 40: 646-652.
8. Dill-Macky E. Chronic hepatitis in dogs. Vet Clin North Am Small Anim Pract 1995; 25: 387-398.
9. Favier RP. Idiopathic hepatitis and cirrhosis in dogs. Vet Clin North Am Small Anim Pract 2009; 39: 481-488.
10. Gatto M, Bragazzi MC, Semeraro R, Napoli C, Gentile R, Torrice A, et al. Cholangiocarcinoma: update and future perspectives. Dig Liver Dis 2010; 42: 253-260.
11. Higuchi R, Yamamoto M, Hatori T, Shimizu K, Imai K, Takasaki K. Intrahepatic cholangiocarcinoma with lymph node metastasis successfully treated by immunotherapy with CD3-activated T cells and dendritic cells after surgery: report of a case. Surg Today 2006; 36: 559-562.
12. June CH. Adoptive T cell therapy for cancer in the clinic. J Clin Invest 2007; 117: 1466-1476.
13. Kanemoto H, Sakai M, Sakamoto Y, Spee B, van den Ingh TS, Schotanus BA, et al. American Cocker Spaniel chronic hepatitis in Japan. J Vet Intern Med 2013; 27: 1041-1048.
14. Leen AM, Rooney CM, Foster AE. Improving T cell therapy for cancer. Annu Rev Immunol 2007; 25: 243-265.
15. Nakakubo Y, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, et al. Clinical significance of immune cell infiltration within gallbladder cancer. Br J Cancer 2003; 89: 1736-1742.
16. Oshikiri T, Miyamoto M, Shichinohe T, Suzuoki M, Hiraoka K, Nakakubo Y, et al. Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. J Surg Oncol 2003; 84: 224-228.
17. Patel T. Cholangiocarcinoma. Nat Clin Pract Gastroenterol Hepatol 2006; 3: 33-42.
18. Patnaik AK, Hurvitz AI, Lieberman PH. Canine hepatic neoplasms: a clinicopathologic study. Vet Pathol 1980; 17: 553-564.
19. Poldervaart JH, Favier RP, Penning LC, van den Ingh TS, Rothuizen J. Primary hepatitis in dogs: a retrospective review (2002-2006). J Vet Intern Med 2009; 23: 72-80.
20. Raffan E, McCallum A, Scase TJ, Watson PJ. Ascites is a negative prognostic indicator in chronic hepatitis in dogs. J Vet Intern Med 2009; 23: 63-66.
21. Rockey DC, Chung JJ. Interferon gamma inhibits lipocyte activation and extracellular matrix mRNA expression during experimental liver injury: implications for treatment of hepatic fibrosis. J Investig Med 1994; 42: 660-670.
22. Rockey DC, Maher JJ, Jarnagin WR, Gabbiani G, Friedman SL. Inhibition of rat hepatic lipocyte activation in culture by interferon-gamma. Hepatology 1992; 16: 776-784.
23. Rosenberg SA, Dudley ME. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol 2009; 21: 233-240.
24. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008; 8: 299-308.
25. Strombeck DR, Gribble D. Chronic active hepatitis in the dog. J Am Vet Med Assoc 1978; 173: 380-386.
26. Strombeck DR, Miller LM, Harrold D. Effects of corticosteroid treatment on survival time in dogs with chronic hepatitis: 151 cases (1977-1985). J Am Vet Med Assoc 1988; 193: 1109-1113.

27. Takahashi R, Yoshitomi M, Yutani S, Shirahama T, Noguchi M, Yamada A, et al. Current status of immunotherapy for the treatment of biliary tract cancer. Hum Vaccin Immunother 2013; 9: 1069-1072.
28. Trigo FJ, Thompson H, Breeze RG, Nash AS. The pathology of liver tumours in the dog. J Comp Pathol 1982; 92: 21-39.
29. van den Ingh TS, Van Winkle T, Cullen JM, Charles JA, Desmet VJ. Morphological classification of parenchymal disorders of the canine and feline liver: 2. Hepatocellular death, hepatitis and cirrhosis. In: WSAVA Liver Standardization Group, Rothuizen J, Bunch S, Charles J, Cullen J, Desmet V, editors. WSAVA Standards for clinical and histological diagnosis of canine and feline liver diseases. Amsterdam: Elsevier. 2006:85-101.
30. Weng HL, Cai WM, Liu RH. Animal experiment and clinical study of effect of gamma-interferon on hepatic fibrosis. World J Gastroenterol 2001; 7: 42-48.