Therapeutic Trial of Granulocyte-Colony Stimulating Factor for Dilated Cardiomyopathy in Three Dogs

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NOTE Internal Medicine

Idiopathic dilated cardiomyopathy (DCM) is a cardiac muscle disease of unknown origin characterized by enlargement of the cardiac chambers and severe systolic dysfunction [13]. Typical findings include increased left ventricular end-systolic dimension (ESD) and end-diastolic dimension (EDD), decreased fractional shortening (FS), and increased E-point septal separation (EPSS). Usually, M-mode and B-mode echocardiographic parameters are the most reliable methods of diagnosing DCM [2, 8, 16]. There are no specific therapeutic recommendations for treatment of DCM other than administration of angiotensin converting enzyme (ACE) inhibitors, diuretics, positive inotropic agents, antiarrhythmic agents, and nutritional support. Recently, pimobendan, a phosphodiesterase (PDE) III inhibitor and calcium sensitizer, has provided a revolutionary approach to DCM treatment. However, in the case of no response to the medications mentioned above, there may be no choice available for therapy.

Human medical research [3, 4, 7, 15, 17] has shown that granulocyte-colony stimulating factor (G-CSF) improves cardiac function after myocardial infarction (MI) through bone marrow cell mobilization and protection of cardiomyocytes from apoptotic cell death. Specifically, the importance of the number of mobilized stem cells for clinical effects was supported by the facts that a therapeutic effect was only seen in patients with increased circulating mobilized CD34+ stem cells [16]. The purpose of G-CSF therapy for medically refractory DCM in this case report was focused on enhancement of cardiac function. This case report describes therapeutic trial of G-CSF (10 µg/kg, subcutaneously) and improvement of cardiac function by G-CSF therapy for medically unresponsive DCM in three dogs.

Case No. 1: A 6-year-old, intact, male Rottweiler weighing 32.0 kg was presented to us with a 3-day history of anorexia, dyspnea, and abdominal distension. Electrocardiography (Cardiofax GEM ECG, Nihon Kohden, Tokyo, Japan) revealed that the dog had irregular ventricular tachycardia (VT) and atrial fibrillation (AF: showing no P waves, F waves, and irregular R-R intervals) with a lack of distinct P waves (280–300 beats per minute, bpm) (Fig. 1). Abdominal radiography and ultrasonography showed mild ascites. Blood pressure examination (Cardell® #9402, CAS Medical Systems, Brantford, CT, U.A.) revealed mild hypotension. The animal’s mean systolic blood pressure was 100.8 mmHg (reference range: 120–140 mmHg).

Echocardiographic examination (Logiq 400 Pro Series, GE Healthcare, Stamford, CT, U.A.) revealed marked dilation of the left atrium (LA) and ventricle (LV) with abnormal wall movement and an intact mitral valve structure. Poor FS (8.7%, reference range: 33–45%; Fig. 3A) and increased EPSS (23.4 mm, reference range: 3.27 ± 1.29 mm) were observed via M-mode echocardiography. The dog’s plasma cardiac troponin I (cTnI) level was mildly elevated (0.16 ng/mL) compared with the reference range (median cTnI was 0.03 ng/mL with a range of 0.01 to 0.15 ng/mL) [11].

Case No. 2: A 9-month-old, intact, female Miniature Pinscher weighing 1.97 kg was presented to us because of a 5-
month history of dyspnea and periodic syncopal episodes. Radiographic examination revealed severe generalized cardiomegaly and an increased vertebral heart score (VHS=14.5; Fig. 2). An ECG recording revealed sinus rhythms (normal to tachycardia) with an average heart rate of 170 bpm and tall QRS complexes with deep Q waves indicating left ventricular enlargement. The animal’s blood pressure was mildly decreased compared with the normal range (mean systolic blood pressure was 100.8 mmHg; reference range: 120–140 mmHg). M-mode echocardiography revealed marked dilation of the LA and LV as indicated by decreased FS (22.5%, reference range: 33–45%) and increased EPSS (6.3 mm, reference range: 3.27 ± 1.29 mm). Mild transmitral blood turbulence with an intact mitral valve leaflet was detected by color flow imaging of echocardiography. The dog’s plasma cTnI concentration was mildly increased (0.22 ng/ml, median cTnI was 0.03 ng/ml with a range of 0.01 to 0.15 ng/ml).

Case No. 3: An 8-year-old, spayed, female Miniature Poodle weighing 2.8 kg was evaluated due to chronic hacking cough and exercise intolerance. Serum biochemistry revealed mild hyponatremia and hyperkalemia. Radiographic findings revealed tracheal collapse and elevation in addition to mild cardiomegaly (VHS=11.7). Ventricular tachycardia (240–260 bpm) with paroxysmal rhythm and occasional ventricular premature complex were observed in an ECG examination. Lack of distinct P waves and AF were evident. The animal’s blood pressure results indicated mild hypotension (the mean systolic blood pressure was 106.5 mmHg; reference range: 120–140 mmHg). Echocardiographic findings (Acuson128, Acuson, Mountain View, CA, U.S.A.) revealed decreased FS (12.0%, reference range: 33–45%; Fig. 3C) and a severe increase in EPSS (20 mm, reference range: 3.27 ± 1.29 mm). The mitral valve was mildly thickened without prolapse. The dog’s plasma cTnI concentration was mildly increased (0.27 ng/ml, median cTnI was 0.03 ng/ml with a range of 0.01 to 0.15 ng/ml).

The three dogs were initially treated with furosemide (Lasix®, Handok Phama Co., Chungbuk, Korea, 2 mg/kg, PO, BID), pimobendan (Vetmedin®, Boehringer Ingelheim GmbH, Ingelheim, Germany, 0.3 mg/kg, PO, BID), benazepril (Cibacen 10®, Novartis Pharma AG, Switzerland, 0.3 mg/kg, PO, BID), diltiazem (Diltiazem, Youyoung
Pharm, Chungbuk, Korea, 1.0 mg/kg, PO, TID), L-carnitine (L-carnitine®, Rexall Inc., U.S.A., 50 mg/kg, PO, BID), and digoxin (Digoxin®, Hanil Pharm. Ind. Co., Ltd. Hwasung, Korea, 0.0075 mg/kg, PO, BID). ECG and echocardiographic evaluations were performed regularly during the therapy. However, VT with AF and echocardiographic parameters did not improve over the course of 2 months of cardiac medication.

Thus, we added G-CSF as a therapeutic trial as we had previously performed an experimental study and investigated the effects of G-CSF (10 µg/kg, subcutaneously) in dogs of the experimentally induced myocardial infarction model [12]. After the first treatment of G-CSF (Leukokain®, CJ Corp., Korea) at a normal dose (5 µg/kg, subcutaneously), we found a significant increase in leukocytes in the peripheral blood on day 2. G-CSF (10 µg/kg) was administered subcutaneously daily to the three dogs for 5 consecutive days to three dogs. Based on complete blood count, the number of white blood cells (WBC) peaked on the 5th day after the first injection of G-CSF (Fig. 5A). There was no evidence of abnormal renal or hepatic function in the serum biochemical results. The plasma cTnI concentrations of the dogs were mildly improved to 0.06 ng/ml, 0.12 ng/ml, and 0.07 ng/ml, respectively, after injection of G-CSF. Blood pressure, ECG, and echocardiographic examinations were performed on days 2, 7, and 14. Each dog experienced a mild increase in blood pressure within the normal range after G-CSF injection (Fig. 5B). FS and EPSS were significantly improved by therapeutic trial of G-CSF. FS of the three cases increased to 20.0% (Fig. 3B), 35.4%, and 25.2% (Fig. 3D) respectively. In addition, EPSS decreased to 13.4 mm, 5.7 mm, and 12.0 mm respectively. There was also some small improvement in the abnormal ventricular wall movement of initial examination. Although VT was slightly corrected, AF was still present in case No. 1 and case No. 3. We also confirmed increased amounts of CD34 positive cells in the peripheral circulation of the patient dogs after G-CSF injection using flow cytometry (FACScan, Becton Dickinson, San Jose, CA, U.S.A.; Fig. 4). Mouse anti-canine CD34:FITC (MCA2411F, AbD Serotec, Oxford, UK) was used for fluorescence-activated cell sorting (FACS) analysis. The dogs are now quite stable without clinical problems.

This case report demonstrates the therapeutic effects of G-CSF in medically refractory DCM dogs. In human medicine and experimental animal medicine, stem cell therapy is a rare scientific subject that causes controversy not only among scientists but also among the general public. However, currently, there are numerous reports that support therapeutic approaches of stem cell therapy that show convincing effects on myocardial damage unresponsive to conventional therapy. A previous study showed that mononuclear bone marrow cells injected into the reopened coronary artery within a few days after acute myocardial infarction resulted in significant improvement of stroke volume index, left ventricular end-systolic volume and contractility, and myocardial perfusion of the infarct region [14]. Zeiher recently conducted a similar pilot study using bone marrow cells and progenitor cells isolated from peripheral blood [1]. Theoretically, G-CSF may increase the delivery
Fig. 4. Flow cytometry of peripheral blood samples from case No. 1 was performed before (A) and after (B) the first therapeutic trial of G-CSF. The results indicated that CD34-positive cells in the peripheral bloodstream increased after bone marrow stimulation by G-CSF (refer to the underlined values). *UL: Upper left. UR: Upper right. LL: Lower left. LR: Lower right.

Fig. 5. Changes in the number of WBCs and the systolic blood pressure with time in the three dogs after G-CSF injection. The number of WBCs of the three dogs peaked on the 5th day after G-CSF therapy (A) and their systolic blood pressures were mildly increased after G-CSF therapy (B).
of stem cells to the impaired heart by mobilizing numerous bone marrow cells that are attributed to myocardial regeneration. Numerous studies have reported that G-CSF mobilized bone marrow-derived stem cells induce neovascularization (CD34- and CD31-positive cells) and differentiate into endothelial cells and cardiomyocytes (Sca-
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lized bone marrow-derived stem cells induce
eration (flow) to left ventricle is much larger than that to the
erventricular wall motion after G-CSF therapy. However,
terventricular wall contraction was also mildly improved based on M-mode echocardiographic examination of the three dogs. Although we do not know the reason for this, we believe that the left ventricular wall was more improved than the interventricular wall because blood supply (flow) to left ventricle is much larger than that to the interventricular wall. In relation to veterinary concepts, it is thought that cardiac remodeling secondary to DCM is the highest priority for ultimate stem cell therapy induced by G-CSF application. Histologically, there are 2 distinct forms of canine DCM, an attenuated wavy fiber type with the thinner myocardial fibers than normal and a fatty infiltration-degenerative type. Therefore, we believe that activation of mononuclear cells by G-CSF may have a beneficial effect on the myocardial regeneration of DCM dogs resulting from improvement of cardiac function. G-CSF therapy was applied to the present three cases of medically non-responsive canine DCM and all showed clear improvement of cardiac function in terms of improvement of cardiac parameters in echocardiographic and ECG examinations. Finally, experimental study and large planned randomized clinical trials are considered necessary to determine the effectiveness of G-CSF treatment in DCM dogs.

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