

Efficacy and Safety of Allogenic Canine Adipose Tissue-derived Mesenchymal Stem Cell Therapy for Insulin Dependent Diabetes Mellitus in Dogs: A pilot study

Sung-Yong Rhew

Seoul National University College of Veterinary Medicine

Woo-Jin Song

Seoul National University College of Veterinary Medicine

Qiang Li

Seoul National University College of Veterinary Medicine

Ju-Hyun An

Seoul National University College of Veterinary Medicine

Hyung-Kyu Chae

Seoul National University College of Veterinary Medicine

So-Young Jeung

Seoul National University College of Veterinary Medicine

Seol-Gi Park

Seoul National University College of Veterinary Medicine

Jeong-Ha Lee

Seoul National University College of Veterinary Medicine

Hee-Seon Cho

Seoul National University College of Veterinary Medicine

Sang-June Sohn

Seoul National University College of Veterinary Medicine

Hwa-Young Youn (✉ hyyoun@snu.ac.kr)

Seoul National University College of Veterinary Medicine <https://orcid.org/0000-0002-0283-1348>

Research article

Keywords: C-peptide, Dog, Diabetes mellitus, Hyperlipidemia, Mesenchymal stem cells

Posted Date: August 6th, 2019

DOI: <https://doi.org/10.21203/rs.2.12448/v1>

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Abstract

Background Since mesenchymal stem cells (MSCs) possess regenerative and immunomodulatory properties, and are capable of controlling the immune dysregulation that leads to β -cell destruction, stem cell transplantation could be used in the management of insulin-dependent diabetes mellitus (IDDM). In this pilot study, we assessed whether canine adipose tissue-derived MSC (cAT-MSC) therapy could be an option for treatment of canine diabetes mellitus. **Results** With the written informed consent of the owners, allogenic cAT-MSCs were infused intravenously in IDDM dogs. C-peptide was elevated by about 5–15% in 3 of 4 cases, and hyperlipidemia was resolved in 2 of 4 cases. Additionally, fructosamine and Hb/A1c levels were improved in 2 of 4 cases. **Conclusions** Considering that C-peptide secretion capacity and lipid metabolism are related to diabetic complications, these results suggest that cAT-MSC therapy in diabetic dogs might help to improve the insulin secretory capacity of dogs with IDDM and prevent diabetic complications.

Background

Canine diabetes mellitus is a common disease, with a prevalence of 13 per 10000 dogs in a cohort study conducted in Sweden [1] and a 0.32% prevalence in one study conducted in the UK [2]. Of 860 dogs diagnosed with diabetes in the Swedish study, the median survival time was 2 years, not including 223 dogs that died at the time of diagnosis. In another study, the 1-year survival rate for canine diabetic cases was 64% [3] and diabetes commonly results in many complications [4, 5] Thus, canine diabetes mellitus is a common disease that affects the dog's lifespan and quality of life.

Although 40% of diabetic dogs die due to ketoacidosis or euthanasia on the day of diagnosis with diabetes mellitus [1], subsequent mortality could be due to diverse complications, including retinopathy, nephropathy, neuropathy, gastrointestinal, and infectious complications. These complications are more likely to occur as the disease duration increases [6, 7] One study reported that diabetic dogs may have a higher prevalence of diabetic microvascular complications when their insulin secretion capacity is reduced; this can be evaluated by measuring fasting serum C-peptide levels [7, 8].

Most cases of diabetes mellitus in dogs are due to insulin-dependent diabetes mellitus (IDDM), which is historically thought to be caused by pancreatitis [9–12] or autoimmune-mediated beta cell destruction [13–16]. One study contradicted the commonly held concepts about autoimmune-mediated beta cell destruction [17], but did not evaluate T cell-mediated autoimmune responses. In another study, although there was no direct evidence of B and T lymphocytic infiltration in the pancreatic tissue of diabetic dogs, except for in those with early diabetes, only a few alpha, beta, PP, and ghrelin cells remained after the extremely selective destruction of beta cells, suggesting a high likelihood of autoimmune-mediated beta cell destruction [16] as a cause of canine IDDM. Given the immunomodulatory effect of mesenchymal stem cells (MSCs) [22–25] and their potency for differentiation into insulin secretory cells [26], administration of MSCs to cases with IDDM is likely to lead to improvement in the insulin secretion ability in diabetic cases [18–21].

Studies on stem cell therapy in diabetes have been conducted in humans and laboratory animals, and it has been reported that treatment with adipose tissue-derived MSCs or bone marrow-derived MSCs is effective and safe [27]. However, there has been no study of the effect of canine adipose tissue-derived MSC (cAT-MSC) therapy on dogs who have been treated for IDDM for more than 1 year. Therefore, this study investigated whether insulin secretion was improved when dogs with IDDM were treated with cAT-MSCs, and whether this treatment prevented or improved diabetic complications.

Results

cAT-MSC differentiation

cAT-MSCs used in this study were successfully differentiated into adipocytes, osteocytes, and chondrocytes. Each cell-type was stained with specific dyes that allowed their identification by microscopic evaluation (Fig. 1).

Monitoring of clinical signs

After stem cell treatment, 3 owners observed improvement of the respective dogs' vitality (Table 1). No tachycardia or hypotension was observed on physical examination in any of the cases. Two of 4 dogs showed improvement in appetite, polyuria and polydipsia (PU/PD), and body weight; the weight of these dogs increased by 6.83% and 8.69%, respectively. The other 2 dogs' body weight decreased by 2.1% and 6% during the treatment period.

Fasting C-peptide monitoring

Fasting serum C-peptide levels were measured on the morning of each dog's visit (Fig. 2). C-peptide secretion was increased in 3 of 4 dogs, while 1 dog showed decreased fasting serum C-peptide levels. Dogs with a positive response to treatment showed improvement in C-peptide secretion after 1 or 2 stem cell treatments and improved 5%–15% compared with levels before stem cell treatment. During the treatment period, C-peptide of case 1 increased from 3.02 pg/ml to 3.47 pg/ml, that of case 2 increased from 3.35 pg/ml to 3.53 pg/ml, that of case 3 increased from 3.46 pg/ml to 3.67 pg/ml, and that of case 4 decreased from 3.55 pg/ml to 3.37 pg/ml.

Blood analysis and treated insulin dose

During the study, fructosamine and glycated hemoglobin (HbA1c) levels decreased in 2 of 4 dogs, while the levels in the other dogs were unchanged or increased (Table 2). In case 1, fructosamine decreased from 516 $\mu\text{mol/L}$ to 354 $\mu\text{mol/L}$ after treatment, and HbA1c decreased from 9.8% to 7.4%. The fructosamine concentration of case 3 decreased from 433 $\mu\text{mol/L}$ to 301 $\mu\text{mol/L}$ and the HbA1c levels

did not change significantly. Insulin doses were reduced in 2 of 4 dogs, unchanged in 1 dog, and increased in 1 dog. Triglyceride (TG) and total cholesterol levels were within the normal range [28] in 3 of 4 dogs. Among these 3 dogs, hyperlipidemia was resolved in 2 dogs; 1 of the 4 dogs showed increased serum TG levels, above the normal range (Fig. 3).

Urine analysis

Two of 4 dogs showed no evidence of proteinuria during this study, 1 showed improvement in proteinuria, and 1 did not have sufficient continuous evaluation data due to the owner's refusal for further examination (Table 3). None of the dogs had bacterial cystitis.

Adverse effects

To evaluate the safety of this treatment, the dogs' vitality, inflammation near the injection site, development of a neoplastic mass, and hypoglycemia were examined by means of history taking, physical examination, and blood tests during the course of treatment. cAT-MSCs were administered 18 times in total. No side effects were observed other than in 1 dog who showed pain at the injection site on the day of treatment and a flare at the injection site lasting until the next day, once, after the 5th injection.

Discussion

A previous study had shown that the insulin secretory capacity remains present in IDDM dogs who have been treated with insulin for a long period of time [29]. Therefore, the therapeutic effects of stem cells [22–26] may involve both transplantation of new beta cells and preservation of the remaining beta cells. It has been reported that insulin secretory capacity was improved in human diabetic patients as well as in laboratory diabetic model animals [27, 30] when AT-MSCs were administered via a venous or splenic route. However, to date, no studies had been performed in dogs suffering from IDDM for more than 1 year. The present study examined whether cAT-MSC therapy in dogs with IDDM could be an option for diabetes treatment by preserving or improving pancreatic beta cell function.

The dogs were monitored throughout treatment, to evaluate the safety of cAT-MSC administration. Only minor side effects were observed in 1 dog, involving pain at the injection site. To evaluate the therapeutic effect, blood tests, including fasting C-peptide concentration [7, 29], serum TG, total cholesterol [4, 31], fructosamine, and Hb1Ac [32] evaluations were performed, and proteinuria and neurological diseases were evaluated as potential complications of diabetes mellitus [5].

Fructosamine and HbA1c are indexes of blood glucose management over the previous 2–3 weeks and previous 10–14 weeks, respectively [32]. Therefore, fructosamine may be effective for the evaluation of the therapeutic effect after 2–3 weeks of stem cell treatment, and Hb/A1c for evaluation after 10–14 weeks of stem cell treatment. However, these values can be affected by adjusting the amount of insulin

treatment, according to the clinical symptoms, as well as by the effect of stem cells [32]. Therefore, it is necessary to evaluate these along with changes in the amount of insulin treatment. In our case 1, fructosamine decreased from 516 $\mu\text{mol/L}$ to 354 $\mu\text{mol/L}$ and HbA1c decreased from 9.8% to 7.4%, while insulin increased by 20% over the same period. Case 2 had a risk of hypoglycemia, because fructosamine was 212 $\mu\text{mol/L}$ in the pre-treatment evaluation. Thus, we reduced insulin from 0.73 U/kg to 0.63 U/kg during the treatment period and the dog's fructosamine increased from 212 $\mu\text{mol/L}$ to 324 $\mu\text{mol/L}$ and HbA1c increased from 4% to 6.5%. In case 3, although we used an equal dose of insulin throughout the treatment period, fructosamine decreased from 433 $\mu\text{mol/L}$ to 301 $\mu\text{mol/L}$ and HbA1c decreased slightly from 7.4% to 7.3%. In case 4, fructosamine increased from 358 $\mu\text{mol/L}$ to 448 $\mu\text{mol/L}$ during the stem cell treatment period. However, we even reduced the insulin dosage due to the risk of hypoglycemia, considering that the blood glucose curve examination results showed a nadir under 80 mg/dL [35]. This dog showed paradoxical results of gradual weight loss, increased fructosamine, and consistent PU/PD occurring with a blood glucose curve indicating a risk of hypoglycemia. In this case, due to a too-low volume of undiluted glargine insulin (around 0.01 ml), it is possible that the owner was not able to administer the appropriate dose at home, or that glargine insulin may have been overdosed while drawing the glucose curve in the hospital.

C-peptide is an indicator of insulin secretion in pancreatic beta cells [8]. The fasting C-peptide concentration increased in 3 of 4 dogs. Elevation of fasting C-peptide levels might suggest that the insulin secretory capacity of these 3 dogs had been preserved or improved. In human studies, fasting plasma C-peptide levels were found to be lower with a longer duration of diabetes, and the lower the fasting C-peptide, the higher the TG level and the more frequently complications due to diabetes were reported [7]. Therefore, a finding of improvement in the lipid metabolism profile along with elevation in C-peptide levels could suggest that cAT-MSCT treatment could help prevent diabetic complications.

Serum TG and total cholesterol were thus measured to assess fat metabolism. Diabetic dogs are more likely to have hyperlipidemia due to aberrant fat metabolism [4, 31]. When diabetes is properly managed, hyperlipidemia improves [33, 34]. Generally, hyperlipidemia could cause pancreatitis, hepatobiliary disease, atherosclerosis, ocular disease, and seizures or other neurologic signs [31, 34]. Therefore, resolving hyperlipemia is an important point in managing the complications of diabetes. Both dogs with improved fructosamine in the present study showed resolution of hyperlipidemia or maintained a normal range of serum TG and cholesterol. One of the 2 dogs without improvement in fructosamine levels showed resolution of severe hyperlipidemia and mild hypercholesterolemia. Case 2, who was treated with a reduced dose of insulin due to the risk of hypoglycemia showed mild hyperlipidemia and hypercholesterolemia. The results suggest that dogs treated with cAT-MSCT may avoid complications due to hyperlipidemia because these dogs, including the case that showed mild hyperlipidemia, did not require medical treatment for hyperlipidemia [34].

The urine test was performed to evaluate diabetic nephropathy. Nephropathy in diabetic dogs is associated with glucose utilization at the cellular level and microvascular complications [5, 36–38]. The test included a dip-stick test and urine sedimentation test. In addition, the urine protein–creatinine (UPC)

ratio was evaluated when proteinuria without cystitis was detected by the dip-stick test. Two of the 4 cases showed evidence of proteinuria on the dip-stick test, which was shown to be improved in the dog who underwent continuous UPC assessment. As 1 dog with proteinuria showed improvement and as no proteinuria occurred in 2 dogs during 6 months, this suggests cAT-MSC treatment could help prevent nephropathy complications due to IDDM.

The study had some limitations. It was conducted on 4 dogs; this small sample size limited assessment of the statistical significance of the treatment effect. In addition, in order to evaluate insulin secretion, continuous C-peptide measurement, using a mixed meal tolerance test or a 90-minute post-dietary test, has been established as a general evaluation method [39]. However, in this study, additional blood sampling was limited due to the owners' refusal. Thus, as fasting C-peptide concentration has been used as an index of the association between diabetic complications and C-peptide levels in humans [7], we used fasting C-peptide concentrations in this study.

Conclusions

In this study, 3 of 4 dogs treated with cAT-MSCs showed improved fasting C-peptide levels, suggesting improved or maintained insulin-secretion capacity, while other results also suggested improvement and prevention of hyperlipidemia and proteinuria with this MSC treatment. Thus, cAT-MSC therapy might be an option for treating diabetes in dogs.

Methods

Case selection

Four dogs (Table 4) that had already been diagnosed with IDDM at Seoul National University Veterinary Medicine Teaching Hospital (SNU VMTH) were enrolled in the study, with their owners' consent. The inclusion criterion was that dogs had been diagnosed with IDDM for more than 1 year. All owners received an explanation of the study in oral and written form, and owners gave written consent for participation before the study commenced. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of SNU (approval number SNU-180321-4).

Study design

This study was a pilot study to evaluate the safety and efficacy of using cAT-MSCs for dogs with type 1 DM that had been treated with insulin at SNU VMTH for more than 1 year. Dogs who met the inclusion criterion were treated with cAT-MSCs and the therapeutic effect was assessed using the following evaluation criteria. The evaluation tests included changes in overall clinical symptoms, laboratory tests (fasting C-peptide, TG, total cholesterol, fructosamine, HbA1c, treated insulin dose, and urine test) and changes in required insulin dosage. The end of the study was 1 month after the completion of stem cell

treatment, and the dogs were evaluated every 1–2 months until that time-point. The DM dogs enrolled in this study had conventional insulin therapy after the end time-point.

Isolation, culture, characterization, and injection of cAT-MSCs

Canine adipose tissue for stem cell isolation was obtained from healthy 4-year-old beagle dogs. The procedure was established with the approval of the IACUC of SNU (approval number SNU–180706–2), and all dogs were negative for major infectious diseases: canine parvo virus, canine coronavirus, and canine distemper virus.

cAT-MSCs were cultured after isolation, according to previously described methods [40]. First, adipose tissue was washed 5 times with a mixture of Dulbecco's phosphate-buffered saline (DPBS, PAN-Biotech, Aidenbach, Germany) and 1% penicillin–streptomycin (PS; PAN-Biotech). Then, the tissue was cut into small pieces with sterile scissors. Physically excised samples were digested with collagenase type IA (0.1%, Gibco/Life Technologies, Carlsbad, CA, USA) for 60 min at 37°C and were then neutralized in a mixture of 10% fetal bovine serum (FBS, PAN-Biotech) and Dulbecco's Modified Eagle's Medium (DMEM, PAN-Biotech). The neutralized adipose tissue mixture was centrifuged at $1,200 \times g$ for 5 minutes and then the undigested fragments were removed using a 70- μm cell filter (Thermo Fisher Scientific, Rockford, IL, USA). The obtained cells were resuspended in a mixture of 10% FBS, 1% PS, and DMEM and then cultured at 37°C and 5% CO_2 at a density of 3000/cm². Cell growth plates were washed with DPBS after 5 days to remove unattached cells and then further cultured in fresh medium. The culture medium was newly replaced every 2–3 days until cell confluency reached 70–80%. Then, the plate was subcultured at a density of 10,000/cm².

The cells were evaluated for stem cell marker expression using flow cytometry prior to the injection experiment. The identified stem cell markers were Clusters of Differentiation (CD) 29-fluorescein isothiocyanate (FITC), CD33-FITC, CD45-FITC, CD34-phycoerythrin (PE), CD73-PE (BD Biosciences, San Diego, CA, USA), and CD90-allopicosin (eBiosciences, San Diego, CA, USA). Cell fluorescence analysis was performed with a FACS Aria II system (BD Biosciences). These cells were evaluated for their differentiation potential using stempro adipogenesis, osteogenesis, and chondrogenesis differentiation kits (Gibco, Grand Island, NY, USA). The differentiated cells were stained with Oil Red O, Alizarin Red, and Alcian Blue, respectively.

Dogs were treated with chlorpheniramine (0.5 mg/kg, intravenously) 15 minutes before stem cell treatment to prevent a hypersensitivity reaction. P4 cAT-MSCs (5×10^6 cells/kg), diluted with 10 mL of 0.9% saline, was injected intravenously over a period of 30 minutes.

Safety tests

History was taken and laboratory evaluations performed to monitor adverse events. This included the incidence of hypersensitivity, inflammation, or other conditions around the injection site, neoplasm, and severe hypoglycemia or hyperglycemia. On the day when the stem cells were administered, the hypersensitivity reaction was monitored through evaluating body temperature, heart rate, blood pressure, and the injection site for 30 minutes after MSC administration. When the dog visited for the next stem cell treatment, we asked the owner about the history of pain at the site after treatment and about side effects suggesting hypoglycemia at home during the previous 1 month. Then, a general physical examination was performed. On the day of treatment, we also measured fasting blood sugar levels to determine whether serious hyperglycemia or hypoglycemia had occurred.

Efficacy tests

Treatment efficacy evaluation included clinical sign monitoring, fasting C-peptide, HbA1c, fructosamine, TG, and total cholesterol levels, treated insulin dose, and urine test evaluations. Monitoring these profiles started after deciding on stem cell therapy.

Clinical sign monitoring. The general clinical symptoms were assessed for changes in body weight, vitality, appetite, and water intake. Vitality was evaluated using a 5-point system: 5. The dog walks lightly, actively responds to external stimuli, including the owner's call, and there is little change in sleeping time. 4. There is no change in response to walking and external stimuli, but the sleeping time is greatly increased. 3. The dog does not move when walking and the response to external stimuli is poor. 2. Moves only for defecation or urination. 1. The dog cannot move and defecates and urinates in the lying position.

The appetite was also evaluated using a 5-point scale: 5. Eats all of the recommended food in 1 sitting. 4. Eats the recommended amount of food, but does not eat it in 1 sitting. 3. Does not eat the recommended amount of food, but eats more than half. 2. Leaves at least half of the recommended amount of food. 1. No appetite. The amount of water intake was evaluated based on the owner's observation using a 120-ml cup. We considered that PU/PD occurred if the dog's water intake was above 100 ml/kg/day [41–43]. In addition, to evaluate the neurological complications caused by diabetes mellitus, we evaluated tachycardia due to increased sympathetic tone or hypotension due to decreased sympathetic tone [4–6].

Fasting C-peptide monitoring. To evaluate the insulin secretion ability of the beta cells [8] and the possibility of complications caused by diabetes mellitus, we measured the dog's fasting C-peptide levels [7, 29]. We collected blood samples when the dogs visited after 12 hours' fasting. The serum samples were centrifuged and stored at –80 °C until the last MSC treatment. The serum samples were then thawed at room temperature and analyzed using a Dog Insulin C-Peptide ELISA kit (LS Bio, Inc, Seattle, WA, USA).

Blood analysis. Fructosamine, HbA1c, TG, and total cholesterol levels were evaluated by blood analysis. Blood samples were collected after 12 hours' fasting. Those results showed the dog's blood glucose

management status during the study and helped to estimate the possibility of diabetes complications [4–7, 31–34].

Urine analysis. A urine test was performed to monitor proteinuria [37] and bacterial cystitis [44], which are common complications in diabetic cases. The urine samples were taken by cystocentesis and was used in a urine dip-stick test (Combur-Test strips, Roche, Basel, Switzerland) and urinary sediment test. When proteinuria was found by the urine dip-stick test, a urinary sediment test was performed to evaluate the presence of cystitis, followed by quantitative analysis of urine protein–creatinine ratio.

Abbreviations

cAT-MSC

Canine adipose tissue-derived mesenchymal stem cell

CD

Clusters of Differentiation

cPBMC

Canine peripheral blood mononuclear cell

FITC

Fluorescein isothiocyanate

IACUC

Institutional Animal Care and Use Committee

IDDM

insulin-dependent diabetes mellitus

PE

Phycoerythrin

PD

polydipsia

PU

polyuria

UPC

urine protein–creatinine

Declarations

Ethics approval and consent to participate

This study protocol and design were approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC) and ethical approval has been granted (SNU–180321–4).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funds were received for this research

Author's contributions

SYR, WJS, and H-YY conceived and designed this study; SYR, WJS, QL, JHA, SYJ, JHL prepared stem cells for dogs; SYR, HKC, SJS, HSC treated stem cell therapy for dogs; SYR, WJS, QL analyzed the data; all authors read and approved the final manuscript.

Acknowledgements

We are very thankful to Research Institute for Veterinary Science and the BK21 PLUS Program for Creative Veterinary Science Research.

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Tables

Table 1. Clinical sign monitoring (Vitality, appetite, PU/PD, BW) results during cAT-MSc treatment

	Treatment	Vitality	Appetite	PU/PD	BW	BP
Case 1	Pre-treatment	5	4	No PU/PD	4.68	140
	Post treatment #1	5	4	PU/PD	4.81	120
	Post treatment #2	5	5	PU/PD	4.89	140
	Post treatment #3	5	5	PU/PD	4.82	140
	Post treatment #4	5	5	PU/PD	4.87	120
	Post treatment #5	5	5	No PU/PD	5	130
Case 2	Pre-treatment	4	5	No PU/PD	2.76	110
	Post treatment #1	4	5	No PU/PD	2.88	130
	Post treatment #2	3	5	No PU/PD	3	100
Case 3	Pre-treatment	4	4	PU/PD	2.40	140
	Post treatment #1	5	5	No PU/PD	2.37	130
	Post treatment #2	5	5	NoPU/PD	2.39	180
	Post treatment #3	5	5	NoPU/PD	2.35	130
	Post treatment #4	5	5	NoPU/PD	2.39	90
	Post treatment #5	5	5	No PU/PD	2.35	110
Case 4	Pre-treatment	4	4	PU/PD	5.43	160
	Post treatment #1	5	5	PU/PD	5.16	150
	Post treatment #2	5	5	PU/PD	5.17	135
	Post treatment #3	5	5	PU/PD	5	110
	Post treatment #4	5	5	PU/PD	5	130
	Post treatment #5	5	5	PU/PD	5.12	120

PU/PD, polyuria/polydipsia; BW, body weight; BP, blood pressure.

Table 2. Blood test results(Fructosamine, Hb/A1c, triglyceride, total cholesterol) and treated insulin dose during cAT-MSc treatment

	Treatment	Fructosamine ($\mu\text{mol/L}$)	Hb/A1c (%)	Insulin dose (U/kg)	TG (mg/dL)	T. chol (mg/dL)
Case 1	Pre-treatment	516	9.8	0.63	97	289
	Post treatment #1	490	9.7	0.63	59	259
	Post treatment #2	464	10.9	0.63	48	243
	Post treatment #3	532	8.9	0.69	46	287
	Post treatment #4	515	8.9	0.69	47	319
	Post treatment #5	354	7.4	0.79	125	320
Case 2	Pre-treatment	212	4.0	0.73	129	387
	Post treatment #1	287	6.6	0.65	97	423
	Post treatment #2	324	6.5	0.63	219	423
Case 3	Pre-treatment	433	7.4	1.1	368	410
	Post treatment #1	382	7.4	1.1	284	219
	Post treatment #2	368	7.8	1.1	-	-
	Post treatment #3	420	7.4	1.1	110	258
	Post treatment #4	375	8.0	1.1	70	324
	Post treatment #5	301	7.3	1.1	131	251
Case 4	Pre-treatment	358	9.1	0.24	1203	355
	Post treatment #1	417	9.1	0.21	54	274
	Post treatment #2	425	-	0.19	-	-
	Post treatment #3	338	9.9	0.20	-	-
	Post treatment #4	492	11.2	0.20	51	263
	Post treatment #5	448	8.7	0.22	-	-

Hb/A1c, hemoglobin A1c; TG, triglyceride; T. chol, total cholesterol.

Table 3. Urinary test results(USG, diptsick, sediment, UPC) during cAT-MSc treatment

	Treatment	USG	Dipstick	Sediment	UPC
Case 1	Pre-treatment	1.037	Glu 4+	No inflammatory evidence	-
	Post treatment #1	1.051	Glu 4+	No inflammatory evidence	-
	Post treatment #2	1.042	Glu 4+	No inflammatory evidence	-
	Post treatment #3	1.039	Glu 4+	No inflammatory evidence	-
	Post treatment #4	1.050	Glu 4+	No inflammatory evidence	-
	Post treatment #5	1.051	Glu 4+	No inflammatory evidence	-
Case 2	Pre-treatment	-	-	-	-
	Post treatment #1	1.043	Glu 4+	No inflammatory evidence	-
	Post treatment #2	-	-	-	-
Case 3	Pre-treatment	1.018	Glu 3+, Pro 2+	No inflammatory evidence	1.64
	Post treatment #1	1.025	Glu 3+, Pro 3+	No inflammatory evidence	3.23
	Post treatment #2	1.010	Glu 2+, Pro trace	No inflammatory evidence	1.89
	Post treatment #3	1.025	Glu 3+, Pro 2+	No inflammatory evidence	1.13
	Post treatment #4	-	-	-	-
	Post treatment #5	1.014	Glu 3+, Pro 2+	No inflammatory evidence	1.2
Case 4	Pre-treatment	1.018	Glu 2+, Pro trace	No inflammatory evidence	-
	Post treatment #1	1.021	Glu 4+, Pro trace	No inflammatory evidence	-
	Post treatment #2	1.020	Glu 4+	No inflammatory evidence	-
	Post treatment #3	1.023	Glu 4+, Pro trace	No inflammatory evidence	-
	Post treatment #4	-	-	-	-
	Post treatment #5	-	-	-	-

USG, urine specific gravity; UPC, urine protein creatinine ratio

Table 4. Enrolled DM case details

	Age	Breed	Sex	BCS	DM duration
Case 1	11 y	Toy poodle	MC	5/9	1 y
Case 2	15 y	Toy poodle	FS	4/9	5 y
Case 3	14 y	Pomeranian	FS	4/9	2 y
Case 4	11 y	Mongrel	MC	5/9	1 y

BCS, body condition score; DM, diabetes mellitus

Figures

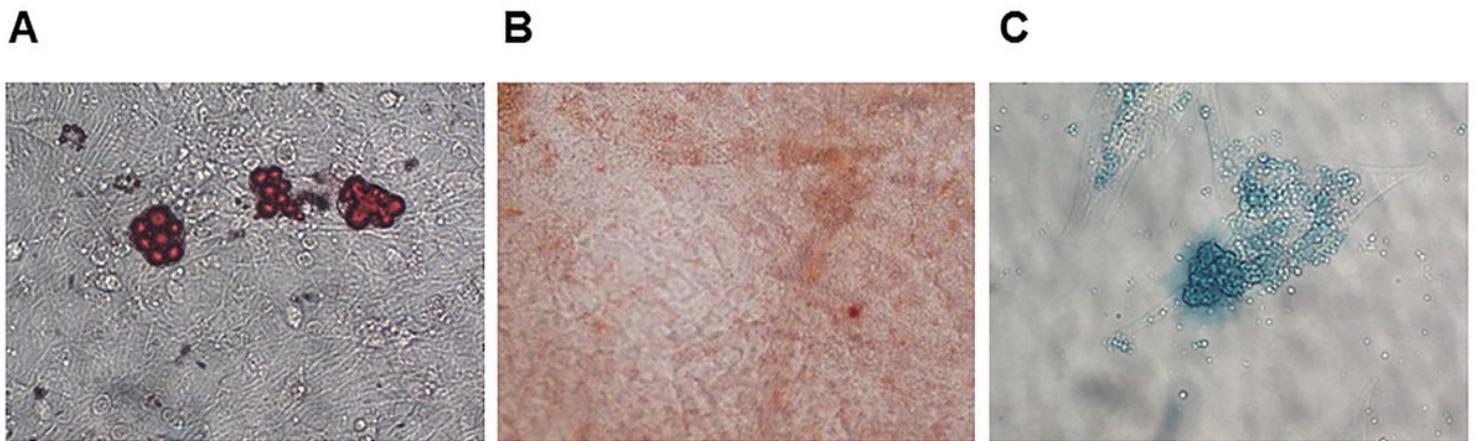


Figure 1

Adipocytes, osteocytes, chondrocytes differentiated from cAT-MSCs (A) Adipocytes stained with Oil Red O (B) Osteocytes stained with Alizarin Red (C) Chondrocytes stained with Alcian Blu

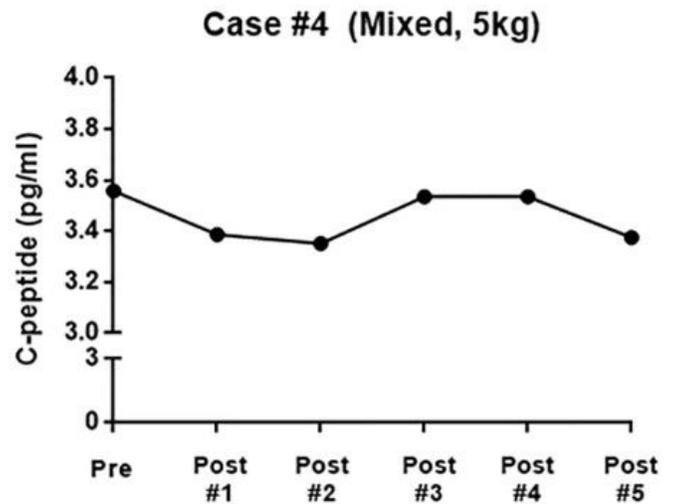
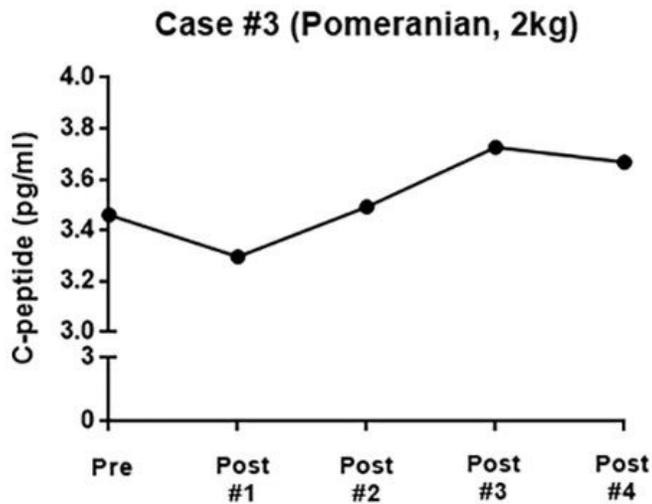
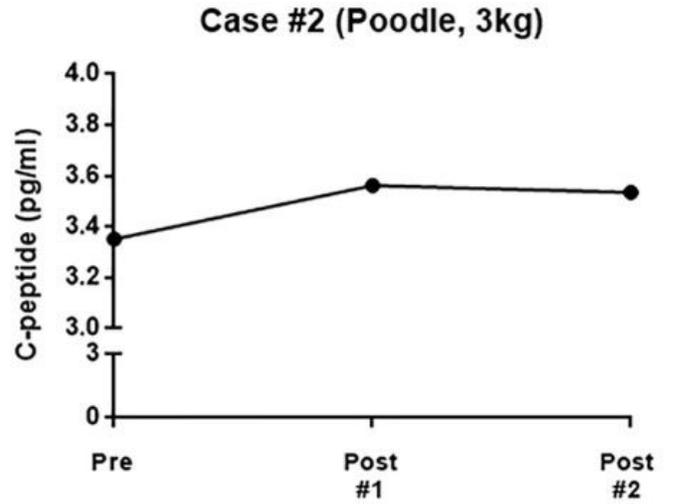
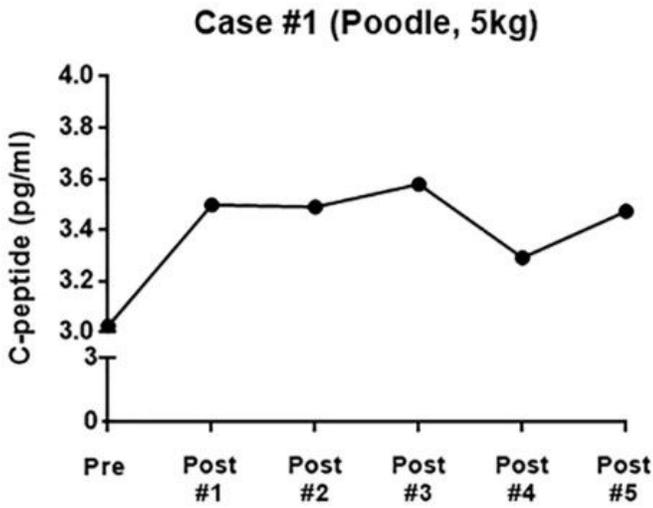


Figure 2

Changes of fasting serum C- peptide in 4 dogs during cAT-MSC treatment

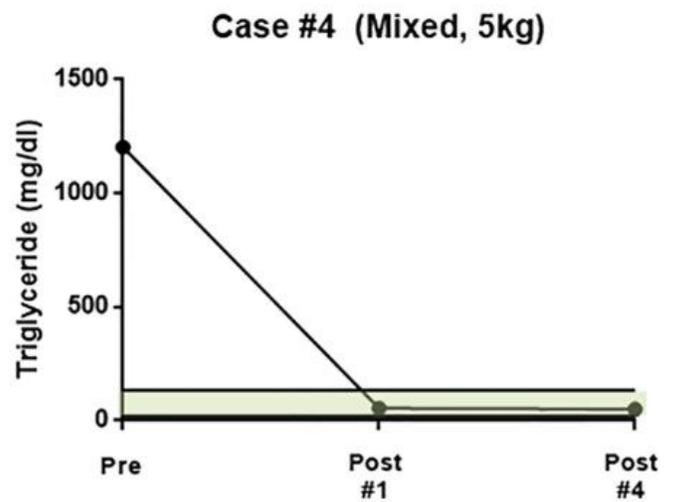
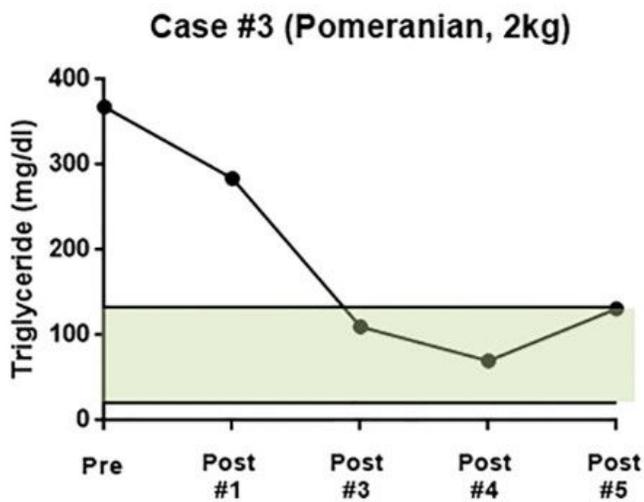
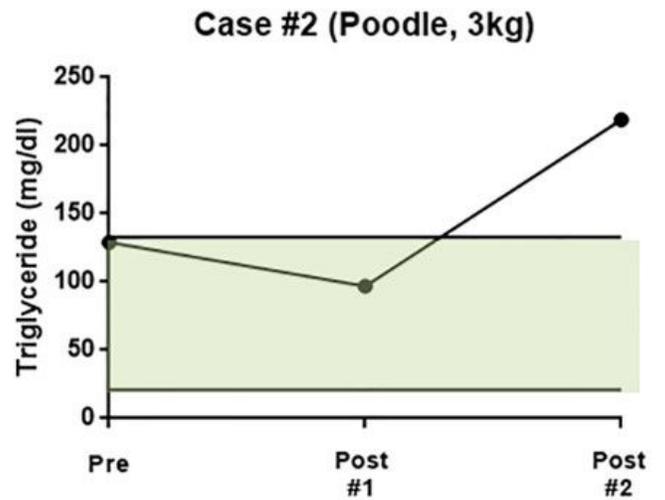
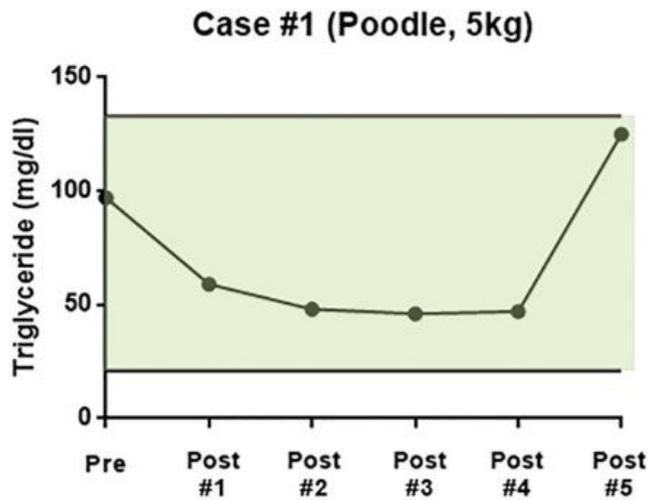


Figure 3

Changes of serum triglyceride in 4 dogs during cAT-MSC treatment Reference range of serum triglyceride: 21-133 mg/dl

Supplementary Files

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