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Case Report
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Long-Term Management with Adipose Tissue-Derived Mesenchymal Stem Cells and Conventional Treatment in a Dog with Hepatocutaneous Syndrome

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Hepatocutaneous syndrome (HS) is an uncommon skin disorder that occurs in conjunction with liver disease and is diagnosed based on decreased plasma concentrations of amino acids and the histopathology of skin lesions. The survival period generally is <6 months. A 10-year-old castrated male Maltese dog was presented for evaluation of lethargy, polyuria, polydipsia, and skin lesions including alopecia, erythema, and crusts. Based on increased liver enzyme activity, low plasma amino acid concentrations, and findings from liver cytology and skin biopsy, the dog was diagnosed with HS. In addition to administration of antioxidants, hepatoprotective agents, and amino acids IV, allogenic adipose tissue-derived mesenchymal stem cells were infused 46 times over a 30-month period: 8 times directly into the liver parenchyma guided by ultrasonography and the remainder of the times into peripheral veins. After commencing stem cell therapy, the dog's hair re-grew and the skin lesions disappeared or became smaller. During ongoing management, the patient suddenly presented with anorexia and uncontrolled vomiting, and severe azotemia was observed. The dog died despite intensive care. On necropsy, severe liver fibrosis and superficial necrolytic dermatitis were observed. The dog survived for 32 months after diagnosis. A combination of amino acid and stem cell therapy may be beneficial for patients with HS.

Key words: Adipose-derived mesenchymal stem cell; Amino acid; Canine; Superficial necrolytic dermatitis.

A 10-year-old castrated male Maltese dog with a 2-month history of lethargy, polyuria, polydipsia (PU/PD), and alopecia was diagnosed with diabetes mellitus (DM) and allergic dermatitis at a local animal hospital. The dog was unresponsive to treatment including neutral protamine Hagedorn (NPH) insulin and other medications. Consequently, the dog was referred to the Veterinary Medicine Teaching Hospital of Seoul National University (Seoul, Republic of Korea).

According to the dog's owner, no seasonal association had been noticed with skin lesions. On physical examination, the following skin lesions were observed: crusts, erosion, and erythema around the muzzle, perianal region, elbows, and footpads. Regional alopecia was found around the hip, and edema, and pustules were observed on the digits (Fig 1). The dog did not have pruritus but experienced pain and lameness from the lesions on its footpads. A few cocci and inflammatory cells were found using hair plucking and acetate tape tests, but no parasites were observed. Abnormal hematology and serum biochemistry findings included mild anemia (hematocrit, 29.1%; reference range [RR], 35–55%), increased liver enzyme activity (Fig 2), hyperammonemia (115 μg/dL; RR, 16–75 μg/dL), and hyperglycemia (201 mg/dL; RR, 60–120 mg/dL). All other results on blood analysis were within normal RR. Severe glucosuria (300 mg/dL) was observed on urinalysis.

Radiographs were unremarkable except for mild hepatomegaly. Abdominal ultrasound examination identified an enlarged liver with a unique honeycomb pattern (Fig 3). There were no other abnormal findings. Cytologic analysis of the liver using ultrasound-guided fine needle aspiration identified liver fibrosis and some areas of hepatocellular necrosis. Using a 6-mm punch, skin biopsy samples were obtained from 3 lesions, including the interdigital spaces, the skin behind the metacarpal pad, and elbow. Histological abnormalities consistent with hepatocutaneous syndrome (HS) were found,[1] including epidermal thickening attributed to parakeratotic hyperkeratosis, mild intercellular edema, and a hyperplastic change in the basal cells (Fig 4). Plasma amino acid profile, serum glucagon concentration, and serum zinc concentration were measured at the Neodin Vetlab, Seoul, Korea. Glucagonoma was ruled out by a low serum glucagon concentration (36.35 pg/mL; RR,

Abbreviations:

ADSCs	adipose tissue-derived mesenchymal stem cells
DM	diabetes mellitus
HGF	hepatocyte growth factor
HS	hepatocutaneous syndrome
IL	interleukin
MSCs	mesenchymal stem cells
NPH	neutral protamine Hagedorn
PU/PD	polyuria/polydipsia
q12h	twice a day
RR	reference range

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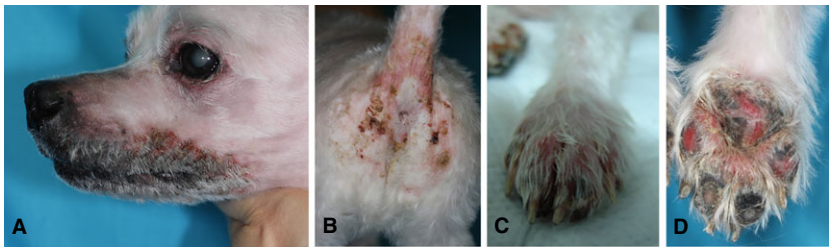

Fig 1. Skin lesions at first presentation. Crusts, erosions, erythema, and hyperpigmentation around the muzzle **(A)**; alopecia, crusting, and erythema in the perianal space **(B)**; edema, erosion, and pustule around the digits **(C)**; and crusting, hyperkeratosis, scale, and ulceration on the footpads and interdigital space **(D)**.

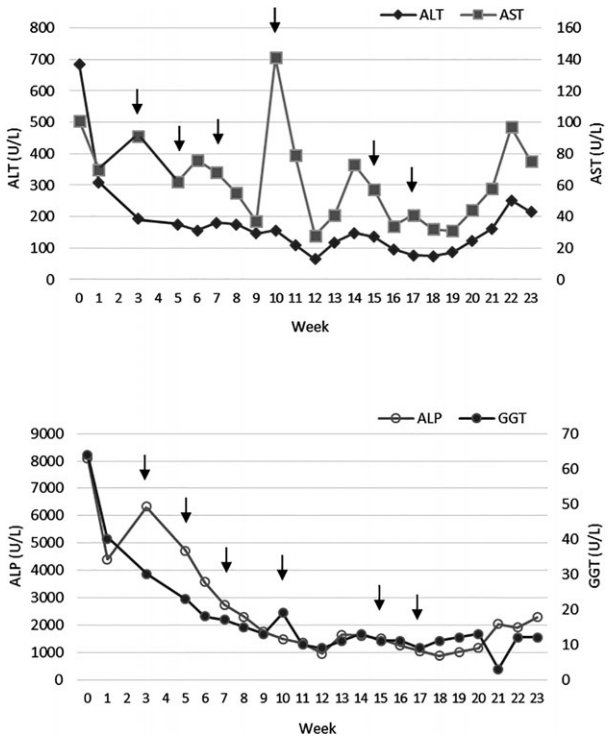

Fig 2. Changes in liver enzyme activity from the day of presentation to 23 weeks. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) **(A)**, alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) **(B)** activities. At first presentation, all liver enzyme activities were markedly increased: ALT 685 U/L (RR, 6–90 U/L), AST 101 U/L (RR, 10–43 U/L), ALP 8099 U/L (RR, 8–100 U/L), and GGT 64 U/L (RR, 0–14 U/L). During stem cell therapy, the activities gradually decreased. Arrows indicate the time of ADSC injection.

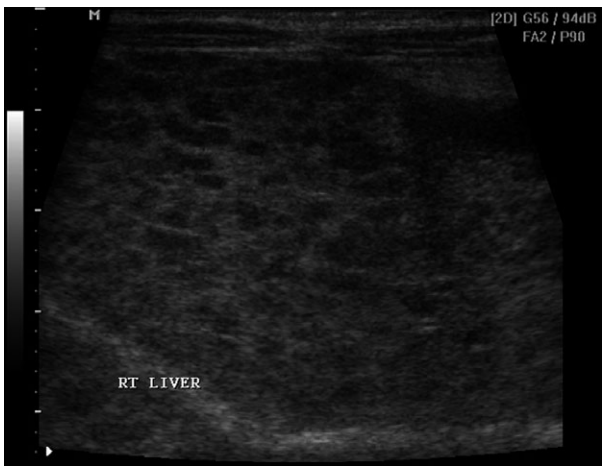

Fig 3. Liver ultrasonograph. Enlarged liver with honeycomb pattern.

50–150 pg/mL). Severe hypoaminoacidemia (Table S1) and normal zinc concentrations (87.8 μg/dL; RR, 70–200 μg/dL) were documented. When ACTH stimulation test was performed, both pre-ACTH, and post-ACTH serum cortisol concentrations were within the normal range (pre-ACTH cortisol 4.58 μg/dL; RR, 1.0–6.0 μg/dL; post-ACTH cortisol 13.1 μg/dL; RR, 5.5–20.0 μg/dL). Based on these test results, the dog was diagnosed with HS.

Initial treatment included hepatoprotective drugs (silymarin 25 mg/kg PO q12h, biphenyl dimethyl dicarboxylate 6.25 mg PO q12h, and S-adenosyl methionine 192 mg PO q24h), antibiotics (cephalexin 30 mg/kg PO q12h and amoxicillin/clavulanic acid 12.5 mg/kg PO q12h), oral supplements (omega-3 liquid, vitamin B, and vitamin E), and topical agents (Malaseb-F medicated shampoo[a] and Aloveen Oatmeal Intensive Conditioner[b]). Branched-chain amino acids[c] were infused via a peripheral vein at a constant rate infusion of 5 mL/kg/h for 6 to 8 hours weekly. DM was managed by SC injections of NPH insulin. Glycemic control was good to excellent. The fructosamine concentration was 313–356 μmol/L, the blood glucose concentration nadir was in the range of 80–160 mg/dL, and the duration of effect of insulin was found to be 8 to 9 hours when the glucose curve was evaluated.

Based on the expectation that the regenerative ability of adipose tissue-derived mesenchymal stem cells (ADSCs) may help improve the patient's liver and skin conditions,[2–4] stem cell therapy was commenced with the owner's consent in the Cell Therapy and Animal Cloning Clinic of Veterinary Medicine Teaching Hospital certified by the College of Veterinary Medicine, Seoul National University (Republic of Korea), 3 weeks after diagnosis. The ADSCs were obtained from the SC fat tissue of young Beagles and were from the same stock of ADSCs previously reported.[5,6] When the stocks

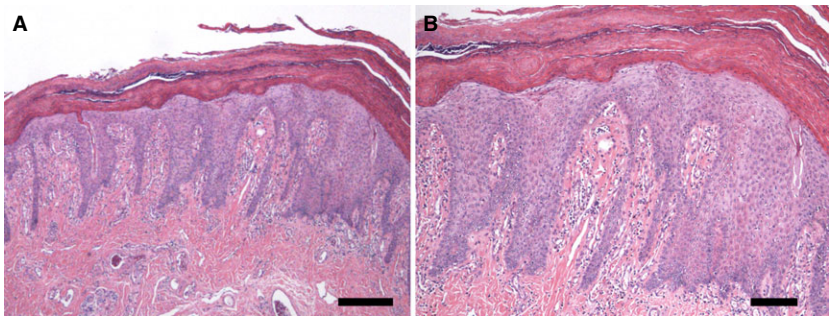

Fig 4. Histopathology of skin lesions. Parakeratotic hyperkeratosis, intercellular edema, and acanthosis. Hematoxylin and eosin stain. Scale bar = 400 μm **(A)** and 200 μm **(B).**

were exhausted, ADSCs were isolated the same way and characterized to have cell surface markers[d] $CD29^+$, $CD73^+$, $CD90^+$, $CD31^-$, $CD34^-$, $CD45^-$, and $CD105^-$[5-7] the latter of which has been shown to have variable expression in canine ADSCs.[8,9] The administration of stem cells was performed 46 times at a dose of 5×10^7 cells; the cells were carefully mixed with normal saline before injection. During the first 4 months, the patient received ADSCs 6 times at intervals of 2 to 5 weeks. Two weeks after the first injection, the increased liver enzyme activity gradually decreased (Fig 2) and the dog's general condition, including vitality and appetite, appeared to improve. Stem cell therapy was discontinued temporarily when the patient's condition reached a plateau. Approximately 6 months later, an additional 40 procedures were performed at intervals of 2 to 3 weeks: 8 times into the liver parenchyma using ultrasound-guided injection and the remainder of the ADSCs by IV infusion. After injection into the liver parenchyma, the skin condition showed remarkable improvement (resembling a normal dog) and the lesions, including crusts, erosions, and erythema, disappeared and the dog's hair re-grew (Fig 5). Moreover, reduction in pain improved the patient's quality of life.

The dog was well managed for 22 months, with IV amino acid administration once per week. However, because of worsening skin problems, the frequency of infusions was increased to 2 or 3 times per week, and the ADSCs sometimes were administered overnight. A branched-chain amino acid supplement[e] (3–8 g/day) administered PO was added.

Approximately 30 months after the initial treatment, the patient gradually lost its appetite and began to experience severe vomiting. Blood analysis identified azotemia (blood urea nitrogen concentration, 66.9 mg/dL; RR, 9.2–29.2 mg/dL; and serum creatinine concentration, 3.2 mg/dL; RR, 0.4–1.4 mg/dL) and anemia (hematocrit, 29.1%; RR, 35–55%). An abdominal ultrasound examination identified decreased gastrointestinal motility. Despite hospitalization and intensive care, including IV crystalloid fluid administration, transfusion of fresh frozen plasma or whole blood, antiemetics, and gastrointestinal protectants, the dog's condition worsened. Although the medical team recommended euthanasia, the patient was discharged according to the owner's wishes and died at home 6 days later.

A postmortem examination showed that the patient was highly cachexic, and had multiple crusts and ulcerations on the body surface including the face, elbows, and footpads. The liver was pale, firmer than normal, and moderately shrunken with indentations on the surface (Fig 6A). Microscopic findings of the skin lesions were similar to those of previous skin biopsies. Severe portal-to-portal bridging fibrosis, biliary hyperplasia, bile stasis, swollen hepatocytes, and hepatocellular vacuolation were observed in the liver (Fig 6B). These findings were consistent with those reported in previous studies.[10–12]

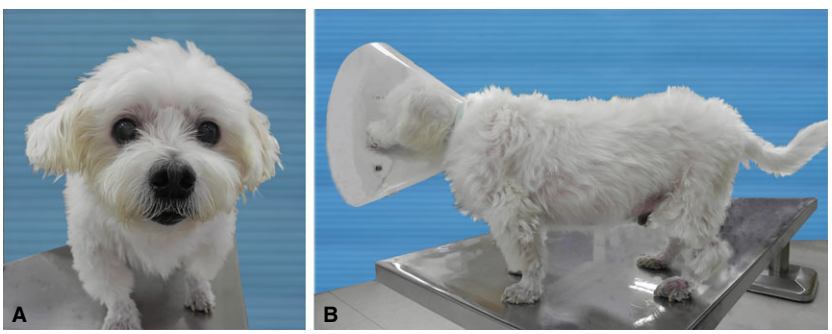

Fig 5. Improvement of skin conditions. Skin lesions such as erythema, crusting, and ulceration substantially improved and new hair grew. Frontal view **(A)** and left lateral view **(B)**.

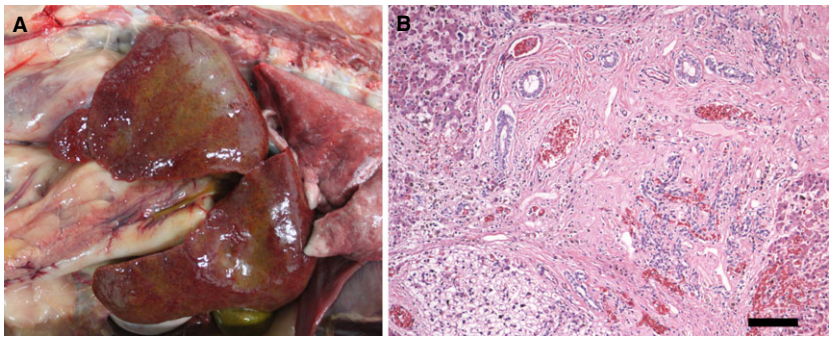

Fig 6. Macroscopic and microscopic liver findings. Decrease in size with irregular surface and yellowish brown color of the liver **(A)** and portal-to-portal bridging fibrosis, biliary hyperplasia, bile stasis, and vacuolar hepatopathy in the liver **(B)**. Hematoxylin and eosin stain, Scale bar = 200 μm.

Hepatocutaneous syndrome, also referred to as superficial necrolytic dermatitis, metabolic epidermal necrosis, or necrolytic migratory erythema, is an ulcerative skin disease associated with liver diseases such as vacuolar hepatopathy or cirrhosis, and long-term phenobarbital administration.[12,13] The disease occasionally occurs with DM.[11,14] Metabolic disturbances of the liver or abnormal glucagon balance caused by underlying disease leads to nutritional deficiencies and decreases in plasma amino acid concentrations, resulting in necrosis of skin cells.[15] Although the definitive pathogenesis of the hypoaminoacidemia is unknown, it may be due to excessive amino acid catabolism.[16] Common skin lesions include erythema, crusting, exudation, ulceration, and alopecia on the footpads, face, perianal regions, and pressure points.[1,11,17] Clinical signs such as lethargy, anorexia, and PU/PD also are present.[10] Severe ulceration of the footpads may result in pain and lameness. A diagnosis of HS is made based on a characteristic honeycomb pattern in the liver on ultrasound examination, and histopathologic evaluation of skin biopsies, including parakeratotic epidermis with striking inter- and intracellular edema, keratinocyte degeneration in the upper epidermis, and hyperplastic basal cells.[1,18] In our patient, hepatic ultrasonography results were consistent with HS, and skin biopsies exhibited typical histopathologic changes of HS. If the results are unclear, measuring plasma amino acid concentrations also is recommended, and plasma amino acid concentrations were low in our patient.

The goal of treating HS is to enhance the quality of life and extend survival time by alleviating dermatopathy and resolving the underlying liver disease. Conventional medications include hepatoprotective agents, antioxidants, zinc, essential fatty acids,[15] and topical agents that are beneficial to the skin barrier. Antibiotics should be considered if secondary skin infections exist. The most effective treatment for HS, which is only palliative, is the IV administration of amino acids.[16,19] One study reported that plasma amino acid concentrations were markedly low in dogs with HS and increased after amino acid infusions.[17] Lesions occasionally are improved by treating the underlying disease, but this outcome is unexpected in cases of serious or irreversible liver disease. The disease will progress, eventually leading to death. Most dogs with HS survive for <6 months but, in some cases, dogs given sufficient protein PO and periodic IV administration of amino acids can live ≥12 months after diagnosis.[17]

Recently, stem cell therapy has been used as part of regenerative medicine in human and veterinary medicine.[2,3,20,21] Mesenchymal stem cells (MSCs) can differentiate into various cell types. Many studies have demonstrated the ability of MSCs to promote dermal fibroblast proliferation and angiogenesis[4,22,23] and to stimulate hepatocyte regeneration, which helps maintain normal liver function in cases of liver fibrosis or after hepatectomy.[24–26] The ADSCs are attractive for research and clinical use because a relatively large number of MSCs can be obtained from SC tissue. Moreover, ethical concerns with harvesting embryos are effectively mitigated, as is the potential for tumorigenesis with embryonic stem cells. One study reported that CD105-positive ADSCs differentiated into hepatocyte-like cells at a high rate and expressed liver-specific markers and hepatic functions such as albumin production, low-density lipoprotein uptake, and ammonia detoxification.[26] In addition, it is known that MSCs normally express liver-specific markers as well as hepatocyte growth factor (HGF).[27,28] Because interleukin (IL)-1 receptor antagonists, IL-6, IL-8, granulocyte-stimulating factor, granulocyte-macrophage colony-stimulating factor, monocyte chemotactic protein 1, nerve growth factor, and HGF are secreted by ADSCs, it is believed that the trophic activity of ADSCs is important for regenerative ability.

Many researchers have investigated the most effective MSC transplantation routes, including direct injection into the liver or spleen, and via the portal or peripheral veins. Stem cell labeling is used to assess the distribution of MSCs,[29,30] whereas histopathologic staining, immunohistochemistry, and biochemical analysis have been used to evaluate therapeutic potential.[31,32] The results, however, have varied. When fetal liver stem (progenitor) cells were transplanted into mice, the intraportal route more effectively targeted the liver compared with the intrasplenic route.[29] Comparing portal, systemic IV, and splenic injections for administration of

MSCs in healthy beagle dogs, MSCs injected by the systemic IV route were trapped in the lungs, whereas both portal and splenic injection led to homogenous high uptake of MSCs by the liver, although the latter method showed mild splenic retention.[30] Another study reported that, of the 3 transplantation routes including tail vein, portal vein, and direct liver, tail vein injection resulted in the most prominent decrease in biochemical parameters (AST, ALT, and ammonia).[31] We selected the peripheral IV route and intrahepatic injection using ultrasound guidance because of accessibility. Injection via the portal vein, which is invasive and requires laparotomy, was excluded, and splenic injection was not necessary because access to the liver parenchyma was possible. Both IV and intrahepatic injection helped improve the patient's disease, but it remains unclear which route was more effective. A limitation of our study was that the number of cells reaching the liver or any changes in the injection area before and after ADSC transplantation was not investigated.

Our patient had severe vacuolar hepatopathy with DM. Despite general treatment including IV amino acid infusions, the skin lesions waxed and waned. It is difficult to conclude that the stem cell treatment alone improved the clinical signs because the patient received amino acids and other conventional treatments. However, considering the distinctive changes before and after injection of ADSCs and the extended survival time, stem cell treatment may have additive effects in treating HS. Further study is necessary to identify whether MSCs directly influence skin renewal or indirectly help by stimulating liver cell development, and to evaluate the most beneficial route and dose for the regeneration of skin and liver cells.

In conclusion, our patient was managed effectively for 32 months after diagnosis of HS using regular IV infusions of amino acids and ADSCs. Stem cell therapy may not only improve skin lesions, but may extend a patient's survival time. Therefore, the results of our case study suggest that ADSCs are a promising alternative in the treatment of patients with HS.

Footnotes

[a] Dermcare, Creek, Australia
[b] Dermcare, Creek, Australia
[c] Hepavia, Daihan Pharm, Gyeonggi, Korea
[d] CD29-FITC, CD31-FITC, CD34-PE, CD73-PE, and CD105-FITC manufactured by BD Biosciences, Franklin Lakes, NJ, USA; CD45-FITC and CD90-APC manufactured by eBiosciences, San Diego, CA
[e] BCAA AMINO & VITAMIN B6, SPOMAX, Gyeonggi, Korea

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Changes in plasma amino acid profiles.