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Improvement Effect of 5-Aminolevulinic Acid on Hyperlipidemia in Miniature Schnauzer Dogs: An Open Study in 5 Cases of One Pedigree

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ABSTRACT

This is the first study to examine the long-term effect of 5-aminolevulinic acid mainly on serum lipoproteins in dogs with hyperlipidemia. We studied 5 Miniature Schnauzer cases whose fasting serum total triglyceride and very-low-density lipoprotein of triglyceride levels were extremely high (635 ± 116 and 520 ± 92 mg/dL, respectively). Although the total cholesterol values were normal, the very-low-density lipoprotein of cholesterol level was high (49 ± 7 mg/dL). Each dog received a 5-aminolevulinic acid supplement (5 mg/day) orally for 6 months. The mean values of total triglyceride, very-low-density lipoprotein of both triglyceride and cholesterol decreased significantly after the treatment period (319 ± 29, 245 ± 18, and 27 ± 2 mg/dL, respectively, $P < 0.05$). Our present results may present evidence that 5-ALA administration contributes to improvement of hyperlipidemia in Miniature Schnauzer.

Key words 5-aminolevulinic acid; dogs; hyperlipidemia

Hyperlipidemia in Miniature Schnauzers (MSs) is a breed-related lipid disorder,[1, 2] which is characterized primarily by increased serum concentration of triglyceride (TG) and occasionally cholesterol (Chol), accompanied by the accumulation of either very-low-density lipoprotein (VLDL) or a combination of VLDL and chylomicron (CM).[2] Recent studies raised the possibility that hyperlipidemia in MS can cause several complications such as pancreatitis,[3] hepatobiliary disorder,[4] glomerular injury,[5] ocular disease,[6] and neurological symptoms.[7] Unfortunately, the cause of primary hyperlipidemia in this breed has not yet been fully elucidated, although the deficiency or decreased activity of lipoprotein lipase were formerly considered to be one factor of this disease.[2] As for the treatment of canine hyperlipidemia, feeding the animals a low-fat diet is initially recommended.[2] In addition, administration of lipid-lowering drugs such as omega-3 fatty acids,[8] fibrates,[9] niacin,[10] and statins[11] is required in patients that do not sufficiently respond to dietary management. Notably, a recent study[9] demonstrated the efficacy of bezafibrate, a fibrate, in treatment hyperlipidemia in dogs including MS. However, these agents are originally marketed as human drugs, and thereby veterinary drugs for the treatment of canine hyperlipidemia remains to be developed.

5-Aminolevulinic acid (5-ALA) is a natural δ-amino acid, and acts as a precursor to heme,[12] which has an energy-generating function in the mitochondria.[13] Recent studies report that the administration of 5-ALA upregulates aerobic energy metabolism by increasing the activity and protein expression in the mitochondria of mice.[12, 14] Hence, the administration of 5-ALA may result in an improvement in lipid and glucose metabolisms, and a reduction in obesity in obese mice.[12, 15, 16] In Japan, 5-ALA supplement has been recently developed for dogs.[17] The previous study confirmed that the effect of 3-month administration of 5-ALA on serum TG concentration in MS dogs[17]; however, the effects of longer-term administration on the lipoprotein concentration in the dogs have yet to be investigated. In this study, we assessed the 6-month effect of a commercially-available supplement of 5-ALA on serum lipoproteins in 5 closely related MS with hyperlipidemia.

SUBJECTS AND METHODS

The 5 dogs were brought to Anchor Trust Hospital (Tokyo, Japan) for medical checkup and were apparently healthy but overweight [bodyweight, 8.3–9.4 kg (Table 1); body condition score, all 4/5]. They were privately owned and were closely related: case 1 was the mother of case 2, and case 2 was the mother of cases 3–5, which had been born at the same time. The mating partners of cases 1 and 2 were unrelated to all of the cases.

Complete blood cell counts and biochemical blood tests were carried out under fasting conditions (i.e., 12 h or more after meals). Blood cell counts showed

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Abbreviations: 5-ALA, 5-aminolevulinic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; Chol, cholesterol; CM, chylomicron; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MS, Miniature Schnauzer; TG, triglyceride; VLDL, very-low-density lipoprotein

Table 1. Details of 5 cases of Miniature Schnauzer dogs on the date of the first medical examination

Basic Information	Case 1	Case 2	Case 3	Case 4	Case 5	Reference range*
Background						
Sex	Female (Spayed)	Female (Spayed)	Female (Spayed)	Female (Spayed)	Female (Spayed)	
Age (yr)	13	12	6	6	6	
Bodyweight (Body condition score)	9.4 kg (4)	9.2 kg (4)	9.4 kg (4)	8.9 kg (4)	8.3 kg (4)	
Biochemical test						
Total protein (g/dL)	**7.9**	**8.0**	7.1	**8.0**	**8.5**	5.4–7.1
Albumin (g/dL)	3.0	**3.5**	**3.7**	**3.6**	**2.3**	2.6–3.3
ALT (U/L)	43	**163**	96	40	56	21–102
AST (U/L)	41	39	34	26	36	23–66
ALP (U/L)	**512**	**1008**	**235**	**183**	130	20–156
γ-GTP (U/L)	**7.0**	**11.0**	5.0	3.0	3.0	1.2–6.4
Total Bilirubin (mg/dL)	0.4	0.2	0.2	0.2	0.2	0.1–0.5
BUN (mg/dL)	21.8	21.3	20	11.8	16.1	10–28
Creatinine (mg/dL)	1.0	1.2	1.1	0.7	1.1	0.4–1.8
Glucose (mg/dL)	109	91	**60**	75	**56**	65–118
Na (mEq/L)	150	148	149	147	149	141–152
K (mEq/L)	5.3	**5.5**	4.4	**4.3**	4.4	4.4–5.4
Cl (mEq/L)	107	**103**	109	110	112	105–115
Lipase (U/L)	**269**	106	144	61	21	10–160
Endocrinology test						
T4 (μg/dL)	1.0	1.4	1.9	1.4	1.8	1.0–4.0
FT4 (pmol/L)	**5.7**	7.7	18.5	11.5	15.2	7.7–47.6
c-TSH (ng/mL)	**1.99**	0.84	0.61	**1.23**	0.4	0.05–0.42
Basic cortisol (μg/dL)	4.8	2.8	**1.5**	**1.9**	**1.1**	2.0–6.0
TG-related lipoprotein						
Total TG (mg/dL)	**1094**	**515**	**529**	**461**	**576**	0–107
CM-TG (mg/dL)	**148**	**42**	**96**	**23**	**28**	0–10
VLDL-TG (mg/dL)	**879**	**420**	**384**	**410**	**508**	0–67
LDL-TG (mg/dL)	**39**	18	31	19	24	3–38
HDL-TG (mg/dL)	**28**	**34**	**18**	9	**15**	0–10
Chol-related lipoprotein						
Total Chol (mg/dL)	274	255	192	177	180	91–293
CM-Chol (mg/dL)	10	3	7	2	2	0–10
VLDL-Chol (mg/dL)	**76**	**38**	**40**	**39**	**50**	0–10
LDL-Chol (mg/dL)	33	29	17	15	12	0–57
HDL-Chol (mg/dL)	155	185	129	123	115	90–241

*Reference ranges of biochemical test were derived from the Textbook of Veterinary Internal Medicine,[13] whereas those of endocrinology test and lipoprotein analysis were indicated by IDEXX Laboratories, Inc. (Tokyo, Japan), and Spectrum Lab Japan Inc. (Tokyo, Japan), respectively. †Bold values mean the values outside each reference range. AST, aspartate transaminase; BUN, blood urea nitrogen; c-TSH, canine thyroid stimulating hormone; FT4, free thyroxine; γ-GTP, gamma-glutamyltranspeptidase; T4, thyroxine; yr, years.

normal or insignificant abnormal values in all cases (data not shown). Blood biochemistry tests revealed that in some cases, higher values than reference range[18] were observed in total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase, and lipase activity (Table 1).

The measurements of the serum concentration of hormones (T4, FT4, c-TSH, and basic cortisol) and TG- or Chol-related lipoproteins [CM, VLDL, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)] were outsourced. High c-TSH concentrations were observed in two cases, low FT4 concentration in one case, and a low concentration of basic cortisol in 3 cases. In all cases, fasting serum total triglyceride (T-TG), TG-related lipoprotein of CM (CM-TG), VLDL (VLDL-TG), and HDL (HDL-TG) were much higher than normal. Fasting serum total cholesterol (T-Chol) fell within the reference range, whereas VLDL of Chol (VLDL-Chol) was relatively high. Based on the blood test results (i.e. high concentration of T-TG and several TG-related lipoproteins), we diagnosed all 5 cases as hyperlipidemia, and enrolled them in this open clinical trial.

This clinical trial was carried out for a total of 7 months, consisting of 6-month treatment and 1-month wash-out periods. In the treatment period, 5-ALA was orally administered as a 5-mg supplement tablet (EneALA®, Neopharma Japan Co., Ltd, Tokyo, Japan) to each dog once per day, as recommended by the manufacturer. All of the cases have been given commercial low-fat food (SELECT BALANCE SLIM, Vet's Choice Japan Co., Ltd., Gifu, Japan) and no other treatment before and during the study period. Blood tests were conducted as described above at 3, 6, and 7 months. The statistical analysis was carried out by using the commercially available computer soft (BellCurve for Excel®, Social Survey Research Information Co., Ltd., Tokyo, Japan). Temporal changes in test results were statistically analyzed by one-way repeated-measures analysis of variance with Bonferroni correction. Statistical significance was set as $P < 0.05$.

Informed consent and consent for the use of EneALA® were obtained from the owner, who also granted permission for the publishing of this case report. The clinical study in this study was conducted under an ethics committee-approved protocol in accordance with the Tottori University Animal Clinical Research Ethics Committee (approval number: H31-001).

RESULTS AND DISCUSSION

In this study, 5-ALA (5 mg/head) was administered once a day for 6 months, and no adverse effects were observed. Temporal changes in the mean serum concentrations of TG- and Chol-related lipoproteins in 5 cases over 7 months are shown in Figs. 1(a) and 1(b), respectively. As for TG-lipoproteins, the values of T-TG, CM-TG, and VLDL-TG in all 5 cases remained to be higher than the reference range after 6-month treatment (range, 209-372 mg/dL), but the mean values of T-TG and VLDL-TG (mean ± SE) were significantly lower at 3 and 6 months than before treatment, respectively ($P < 0.05$). LDL-TG levels were slightly but significantly lower at 3 months than initial values ($P < 0.05$). However, the concentrations of T-TG, VLDL-TG, and LDL-TG increased again one month after the treatment ended, indicating that the effects of 5-ALA are likely to be limited to the administration period only. These findings indicate that 5-ALA treatment cannot fully normalize but can decrease T-TG in MS with hypertriglyceridemia, mainly through a decrease of VLDL-TG. Unfortunately, the efficacy of 5-ALA supplement in this study was considered to be weaker than that of bezafibrate, which can normalize serum T-TG concentration in most dogs with primary hypertriglyceridemia.[9] In order to promote the effective use of the 5-ALA supplement, further study to verify the combined therapeutic effect with lipid-lowering drugs, including bezafibrate, and/or therapeutic diets is greatly anticipated.

In Chol-lipoproteins, a slight but significant decrease in the mean VLDL-Chol was observed during the 5-ALA treatment period compared with before treatment ($P < 0.05$). However, no significant difference in T-Chol before and during the 5-ALA treatment period was found. This implies that 5-ALA has little effect in reducing Chol-related lipoproteins. By contrast, Koganei et al.[16] found that the administration of 5-ALA resulted in a decrease of T-Chol in diet-induced obese mice. Further studies are needed to clarify the effect of 5-ALA on hypercholesteremia in dogs.

Lipid disorders may be due to primary or secondary factors in dogs,[2] although in this study, it is likely that primary causes played a great role in hyperlipidemia as the dogs were extremely closely related, middle or old-aged MS, and all had elevated T-TG and VLDL-TG.[1, 2, 11] Such familial (genetic) hyperlipidemia is very common in humans,[19] as well as dogs. Incidentally, sib-pair linkage analysis has been conducted to identify the responsible genes of various familial diseases in human medicine,[20] but demands an enormous amount of time. In contrast, the intergenerational studies in companion animals, such as this study, can be finished in a shorter time because of their short life-span, and thus would contribute to advance understanding of the similar diseases in humans.

As for secondary causes of hyperlipidemia, all

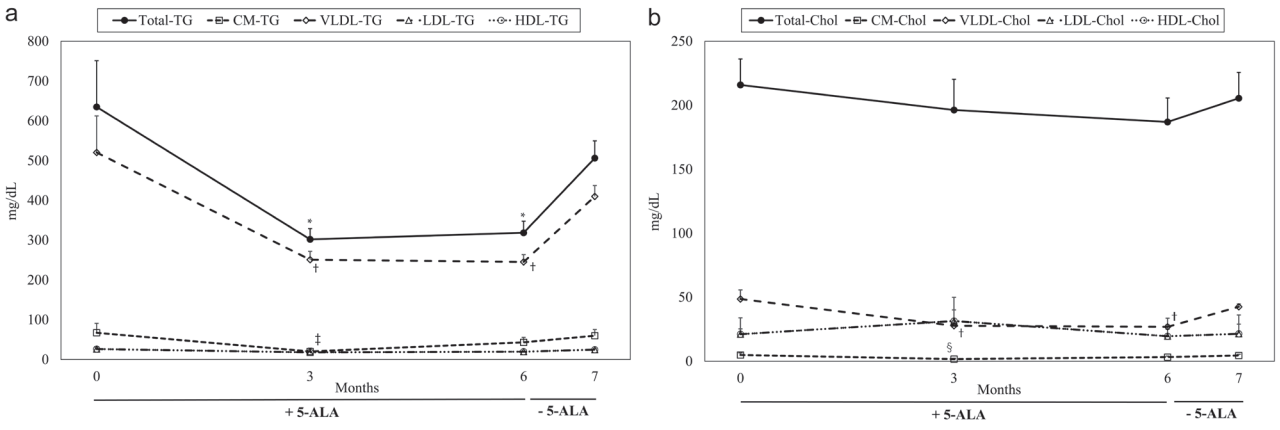

Fig. 1. The temporal changes of the blood concentrations (mean ± SE) of TG-related lipoproteins (**a**) and Chol-related lipoproteins (**b**) in 5 cases of Miniature Schnauzer dogs during the observation period. $P < 0.05$ vs. 0 month in T-TG (*), VLDL (†), LDL (‡), and CM (§). The results on T-TG concentrations at 0 and 3 months were previously reported.[17]

cases were overweight, which can affect serum lipoprotein concentrations.[21, 22] Blood tests revealed that all cases had normal fasting serum glucose levels, essentially ruling out diabetes mellitus. Case 1 had low FT4 levels, and cases 1 and 4 showed normal T4 but high TSH concentrations, indicating potential hypothyroidism or occasional thyroid axis alteration.[23] Pancreatitis might also be associated with hypertriglyceridemia in case 1,[3] because of the elevated lipase activity.

The bodyweight of all cases decreased with 5-ALA treatment, and the mean (± SE) bodyweight (kg) was significantly lower after 3 (8.38 ± 0.17), 6 (7.82 ± 0.16), and 7 (7.38 ± 0.17) months, compared with that prior to treatment (9.04 ± 0.21, $P < 0.01$). Thus, 5-ALA treatment may contribute to weight loss in overweight dogs, which is supported by the findings of the obesity model in mice.[12, 15, 16] The biochemical blood tests after 6 months of 5-ALA treatment indicated a decrease in ALP in cases 1, 2, 3, and 4 to 361, 539, 161, and 101 U/L, respectively. ALT in case 2 decreased to 79 U/L, and lipase activity in case 1 decreased to 163 U/L. Thus, 5-ALA administration might contribute to subclinical hepatobiliary disorder and/or pancreatitis possibly because of the improvement of hypertriglyceridemia in these cases. On the other hand, the mean T4 levels (μg/dL) were kept nearly constant during the clinical trial period (1.52 ± 0.19, 1.64 ± 0.16, and 1.44 ± 0.12 at 3, 6, and 7 months, respectively), and thus 5-ALA treatment is unlikely to affect T4 concentration in dogs. In addition, the mean concentrations of heme-related products, i.e., hemoglobin (g/dL) and total bilirubin (mg/dL) at 3 (15.5 ± 0.73 and 0.26 ± 0.02), 6 (16.5 ± 0.86 and 0.26 ± 0.04), and 7 (16.9 ± 0.33 and 0.24 ± 0.05), respectively, did not differ from those before treatment, although the previous study reported that in mildly hyperglycemic human subjects, blood concentrations of these products increased by 5-ALA treatment.[24] Thus, the effect of 5-ALA administration on heme-related products might be different between dogs and humans.

This study has several limitations. Firstly, we focused on a small number of MS dogs that had very close blood relationships. Secondly, it remains unknown whether 5-ALA has any efficacy in dogs with severe hyperlipidemia-related complications. Thirdly, the start timing of effectiveness of 5-ALA and the enhancement of the efficacy by an increased dose were not fully clarified in this study, and thus should be verified by further studies with modified test protocol.

In conclusion, to our knowledge, this is the first study to report on the improvement effects of long-term administration of 5-ALA on VLDL-TG and LDL-TG, in addition to T-TG, in MS dogs. There may be several possible explanations for the effects of 5-ALA. As shown in this study, 5-ALA treatment can help the elimination of obesity, which can increase the risk of high concentrations of T-TG and VLDL-TG,[25] possibly because of increase in activity of mitochondrial cytochrome *c* oxidase and production of ATP.[14] Additionally, 5-ALA may induce a reduction in white adipose tissue volume and expression of uncoupling protein-1 in brown adipose tissue.[26] Furthermore, 5-ALA can increase expression of lipoprotein lipase and suppress expression of fatty acid synthase, and thereby may improve liver lipid metabolism.[16] Our results raise expectations that the 5-ALA supplement can be available as a treatment option of canine hyperlipidemia. Further, large-scale clinical studies are needed to fully demonstrate the efficacy of 5-ALA for hyperlipidemia in dogs.

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