ALA-Porphyrin Science

Adverse effect of repeated treatment with 5-aminoleveuic acid in healthy cats and those with chronic kidney disease

Reo Nishi[a], Mona Uchida[a], Masato Nakahara[b], Shingo Maeda[a], Akio Hayashi[c], Nao Iwatani[d], Toru Tanaka[d], and Tomohiro Yonezawa*[a]

[a] Department of Veterinary Clinical Pathobiology, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan. Fax: +81-3-5841-8012, Tel: +81-3-5841-5420
[b] Mori Animal Hospital, Matsudo city, Chiba 270-2213, Japan
[c] Tissue Engineering Support Holdings, Taito-ku, Tokyo 110-0015, Japan
[d] Neopharma Japan co. Ltd., DS Pharma Animal Health Japan, Chiyoda-ku, Tokyo 102-0071, Japan
** Corresponding author: TY, Email: tomohiro.yonezawa@gmail.com*

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Summary

5-Aminolevulinic acid (5-ALA), an antioxidant, has been reported to exert a protective effect against several types of kidney disease in experimental rodent models. In this study, the appropriate treatment dosage of 5-ALA for cats was investigated. Five clinically healthy cats were used to evaluate the adverse effects of 5-ALA administration. The cats received 5-ALA at doses of 5, 15, or 25 mg/head or cellulose as a placebo orally twice daily for 30 days. Complete blood counts (CBC) and plasma alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (CRE) levels were monitored. These values did not significantly change when 0 or 10 mg/head/day of 5-ALA was administered. ALT levels were significantly elevated when 30 and 50 mg/head/day were administered. Four of five cats had intermittent vomiting with dehydration 2 weeks after the start of the 50 mg/head/day treatment. These symptoms improved after the treatment was stopped. In addition, five cats with chronic kidney disease (CKD) were treated with 5-ALA at a dose of 15 mg/head once daily for 2 months as a pilot trial. ALT levels were temporally but clearly increased in four out of five cats, with no related clinical signs. ALP levels also increased over the reference range in one cat. Notably, CBC, BUN, and CRE levels did not change. These findings suggest that the appropriate dose for repeated treatment of 5-ALA in cats should be less than 10 mg/head/day, and hepatic enzymes and digestive symptoms should be monitored carefully.

Keywords

5-Aminolevulinic acid, Feline, Chronic kidney disease, Alanine aminotransferase

Introduction

5-Aminolevulinic acid (5-ALA) could have protective effects against the progression of kidney diseases. 5-ALA is an endogenous natural amino acid and a mediator of heme synthesis. In mice and rats, it has been reported that 5-ALA with ferrous iron showed protective effects by inducing heme oxygenase-1 (HO-1) expression against renal ischemia-reperfusion injury and cisplatin nephropathy in the kidneys [1; 2]. In addition, the combination treatment of 5-ALA and Fe^{2+} significantly decreased tubular damage and the number of apoptotic cells via heme oxygenase-1 and TNF-alpha, although single treatment with 5-ALA or Fe^{2+} did not improve these [2]. These reports suggest that 5-ALA with ferrous iron could provide protective effects against various types of acute kidney injury (AKI). However, it is still unknown whether 5-ALA with ferrous iron is at least partially effective as a drug for chronic kidney disease (CKD).

CKD is an irreversible disease that progresses to a loss of function in the kidneys [3]. CKD is a common disease associated with morbidity and mortality in cats [4]. Several types of tubulointerstitial inflammation and renal fibrosis have been observed [5-7]. Renal fibrosis is caused by oxidative stress and cell death, followed by multiple factors, such as proteinuria, hypoxia, and oxidative stress [8-10]. As there is no good cause-related therapy for CKD in cats, it is important to develop a new drug targeting these factors. A recent report stated that renal recovery after AKI and progression to CKD could be a sequence of the pathophysiology of mitochondrial dysfunction [11]. Based on these findings of 5-ALA and AKI/CKD, it is possible that 5-ALA could improve kidney dysfunction in CKD, or at least inhibit the progression of CKD. However, the safety of 5-ALA as a repeated treatment for healthy cats and those with CKD has not yet been clearly evaluated.

In this study, the appropriate dosage of repeated 5-ALA treatment was evaluated in healthy cats. In addition, a preliminary trial of 5-ALA treatment with spontaneous CKD cats for 2 months was performed.

Experimental

Exp. 1. Repeated 5-ALA treatment tests in healthy cats

Animals and treatment design

This study was approved by the Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences at the University of Tokyo (P18-085). Clinically healthy cats were included in this study (n = 5). Their health conditions were checked once a month via general physical and blood examinations, including complete blood cell (CBC) count and measuring of the

concentrations of plasma alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (CRE). The subjects were mixed-breed male cats, they were around 6 years of age, and their body weights ranged from 3.4–4.5 kg (average, 4.2 kg). The animals were housed in conventional animal facilities at the University of Tokyo. The cats were kept individually in clean stainless cages (length×width×depth = approximately 1×1×1 m^3) with a water cup, bedding box, and clean pet sheets. The light/dark cycle was loosely maintained at 12L/12D, and the room temperature was maintained at 23 ± 3ºC by an air conditioner. Abundant tap water and an appropriate amount of dry cat food were provided twice daily according to the manufacturer's recommendations (Royal Canin, France). Welfare-related assessments were conducted before, during, and after the experiments. Thirty-day treatment periods and 30-day intervals were designed. A placebo made from cellulose or several doses of 5-ALA (5, 15, and 25 mg/head, Neopharma Japan) with small amounts of cat snack liquid (Chao-Churu, Inaba, Japan) was administered twice daily just before feeding. Sodium ferrous citrate (SFC, Neopharma Japan) at a dose of 25 mg Fe/head was administered at the same time for all treatments. Clinical and laboratory evaluations were scheduled on days 0 and 30 pre- and post-treatment.

Clinical laboratory evaluations

The clinical activity scores (i.e., physical activity, appetite, and dehydration), physical examinations (i.e., heart rate, body temperature, respiration rate, and capillary refilling test), quality of life (QoL) assessments, and treatment impressions were evaluated by the veterinarians according to the 5-level evaluation scales [12]. To evaluate drug safety, CBC, albumin, ALT, ALP, BUN, CRE, calcium, phosphate, and electrolyte levels were measured. CBC was measured using a ProCyte Dx Hematology Analyzer (Idexx Laboratories, USA), and the other blood chemical tests were performed using Fuji Dri-Chem NX500V (Fuji Film VetSystems, Japan).

Exp. 2. Preliminary study of repeated 5-ALA treatment for cats with CKD

Inclusion criteria and treatment design

The preliminary trial was approved by the Animal Ethical Committee of the Veterinary Medical Center, University of Tokyo (VMC16-05). Before enrollment, written informed consent was obtained from all the owners. Cats of all ages, breeds, and sexes with clinically stable CKD were included. A middle-high CRE level, which was designated as 2.0–5.0 mg/dL at the beginning of this test, was used as the inclusion criterion for this test. The diagnosis of CKD was reconfirmed by a sustained

increase in CRE level and a low USG level for at least 3 months before inclusion in the study, based on the International Renal Interest Society (IRIS) guidelines [13]. The endpoints were defined as the QoL assessments by the owners and the treatment impressions determined by the attending veterinarians.

In this study, five cats were enrolled with the owners' approval. They were treated with 5-ALA at a dose of 15 mg/head with SFC (5-ALA:Fe = 1:0.5 as their molecular weight) once daily for the first 2-month period and a placebo tablet for a 2-month period. The owners and attending physicians did not know during which period the cats received 5-ALA or placebo until the end of the trial. To evaluate the effect of 5-ALA, a medical interview determining clinical activity scores, QoL assessment results, and treatment impressions along with physical examinations, blood collection, and urinalysis were performed at months 0, 1, 2, 3, and 4. The interval between the 5-ALA and placebo treatments was only one day. CBC, ALT, ALP, BUN, CRE, calcium, phosphate, and electrolyte levels were also measured.

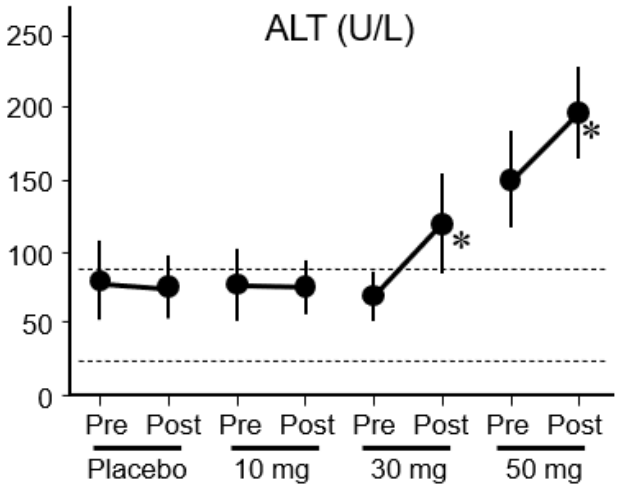
Statistical analyses

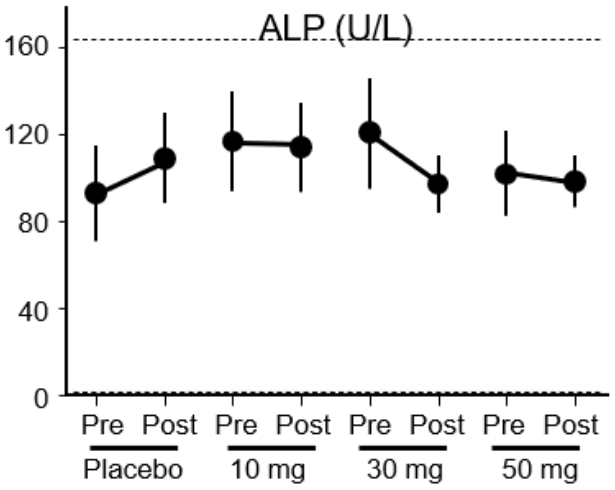
Statistical analyses were performed using XLSTAT Life Science (version 2021.2.2.1141; Addinsoft, Paris, France) as an add-in for MS Excel (Microsoft Corporation, Redmond, Washington, USA). The value changes from the start to the end of the period were evaluated using the Wilcoxon signed-rank test in Exp. 1, and Kruskal–Wallis and Friedman tests were used to compare the time series in Exp. 2. The significance level was set at a p-value of <0.05 for all statistical comparisons.

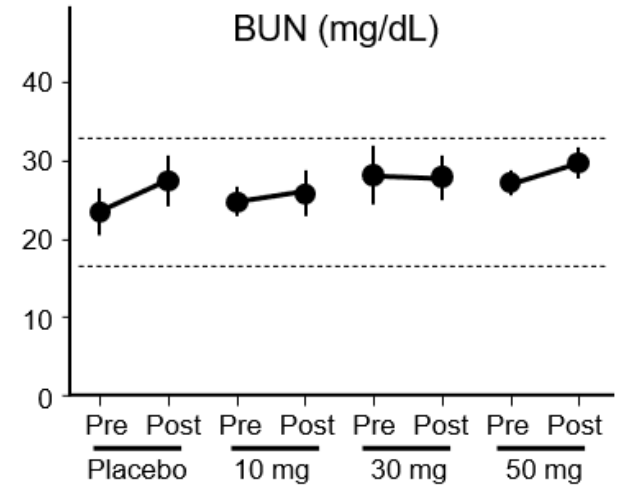
Results

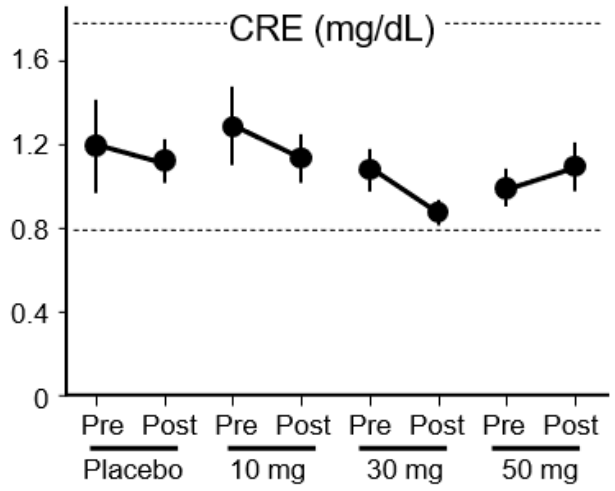
To evaluate the safety of repeated 5-ALA treatment in cats, various doses of 5-ALA were administered for 1 month. The animals were treated with a placebo in the first period and then treated with 5-ALA dosages ranging from 5 mg/head to 25 mg/head twice daily for a month. The experiment was designed with a 1-month interval between treatments. As shown in Fig. 1, ALT, ALP, BUN, and CRE levels were not significantly changed when treated with 0–10 mg/head (range, 2.2–3.0 mg/kg) per day. ALT levels were significantly increased when the animals were treated with 5-ALA at doses of 30 mg/head (range, 6.7–9.1 mg/kg) and 50 mg/head (range, 11.1–15.2 mg/kg) per day. Although there were 1-month intervals between the treatments, ALT levels at the beginning of the 50-mg treatment were still high and did not differ from those at the end of the 30-mg treatment period. Moreover, 2 weeks after the start of the 50-mg treatment, four experimental cats had intermittent

vomiting with dehydration. Their body weight did not change during the placebo, 10-mg and 30-mg treatment periods, but their weight temporally decreased during the 50-mg treatment. These clinical signs spontaneously improved a couple of days after the treatment was stopped. ALP, BUN, and CRE levels did not change during any period of the trial. Other measured factors such as CBC, calcium, phosphate, and electrolyte levels also persisted within the reference range and did not change significantly (data not shown).


ALT (U/L)
250
200
150
100
50
0
*
*
Pre Post Pre Post Pre Post Pre Post
Placebo
10 mg
30 mg
50 mg


ALP (U/L)
160
120
80
40
0
Pre Post Pre Post Pre Post Pre Post
Placebo
10 mg
30 mg
50 mg


BUN (mg/dL)
40
30
20
10
0
Pre Post Pre Post Pre Post Pre Post
Placebo
10 mg
30 mg
50 mg


CRE (mg/dL)
1.6
1.2
0.8
0.4
0
Pre Post Pre Post Pre Post Pre Post
Placebo
10 mg
30 mg
50 mg

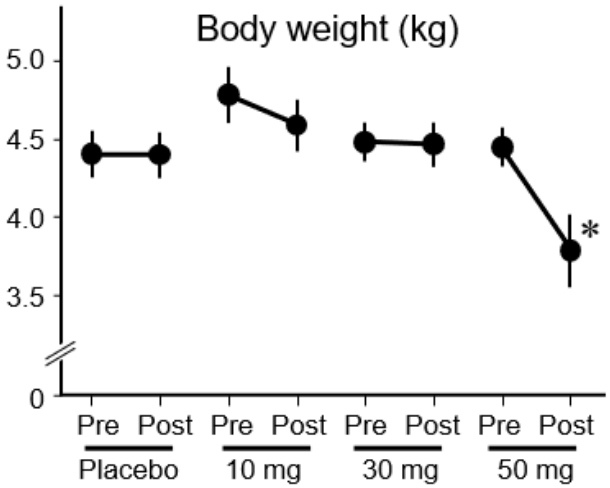

Body weight (kg)
5.0
4.5
4.0
3.5
0
*
Pre Post Pre Post Pre Post Pre Post
Placebo
10 mg
30 mg
50 mg

Figure 1. The effect of repeated treatments with 5-ALA at several doses in healthy cats. Placebo or various doses of 5-ALA were administered twice daily for 1 month. The interval between treatments was 2 months. Data are shown as mean ± SE (n = 5). Asterisks indicate significance ($p < 0.05$, Wilcoxon signed-rank test) when comparing pre- and post-treatment values in the same period. Horizontal dot lines indicate the maximum and minimum values of the reference ranges.

In the next experiment, a pilot trial on cats with CKD was performed. Five cats were enrolled in this trial, with the owners' approval. The animals were already diagnosed with clinically stable CKD at least 2 months before enrollment. The average of their CRE levels at the beginning of the study was 3.08 mg/dL (range, 1.9–4.9 mg/dL). The subjects included two American Shorthairs, two Japanese mixed cats, and one British Shorthair. There were 3 neutered males and 2 neutered females. The mean age of the animals was 12.0 years (range, 11–14 years). The cats had been receiving concomitant treatment for at least 1 month before the study as follows: renal diet therapy in 5 cats; angiotensin-converting enzyme (ACE) inhibitor, pimobendane, cyproheptadine, and sevelamer treatments in 1 cat each; and subcutaneous fluid therapy in 3 of 5 cats. The animals were treated with 5-ALA at a dose of 15 mg/head (mean, 3.96; range, 3.2–4.5 mg/kg) with SFC once daily for the first 2-month period and placebo for the following 2-month period.

As shown in Fig. 2, ALT levels clearly increased in three cats. The level was 967 U/L 1 month after the start of the 5-ALA treatment in one cat, while it was approximately 100 to 200 U/L in two cats. ALT levels were not measured at baseline nor after the first month of 5-ALA treatment in one cat; however, the level was obviously high following the 2nd month of 5-ALA treatment. The ALT levels in one cat did not increase and remained within the reference range. All ALT elevations returned to their original levels 1 month after the end of the 5-ALA treatments. While ALP levels also tended to increase gradually, a level over the reference range was observed in only one cat. These alterations were not statistically different according to the Friedman test (ALT, $p = 0.174$; ALP, $p = 0.105$). Other measurements, such as CBC, BUN, and CRE levels, did not change during the observation period. According to their medical interviews, no general physical conditions related to the treatment event had changed.

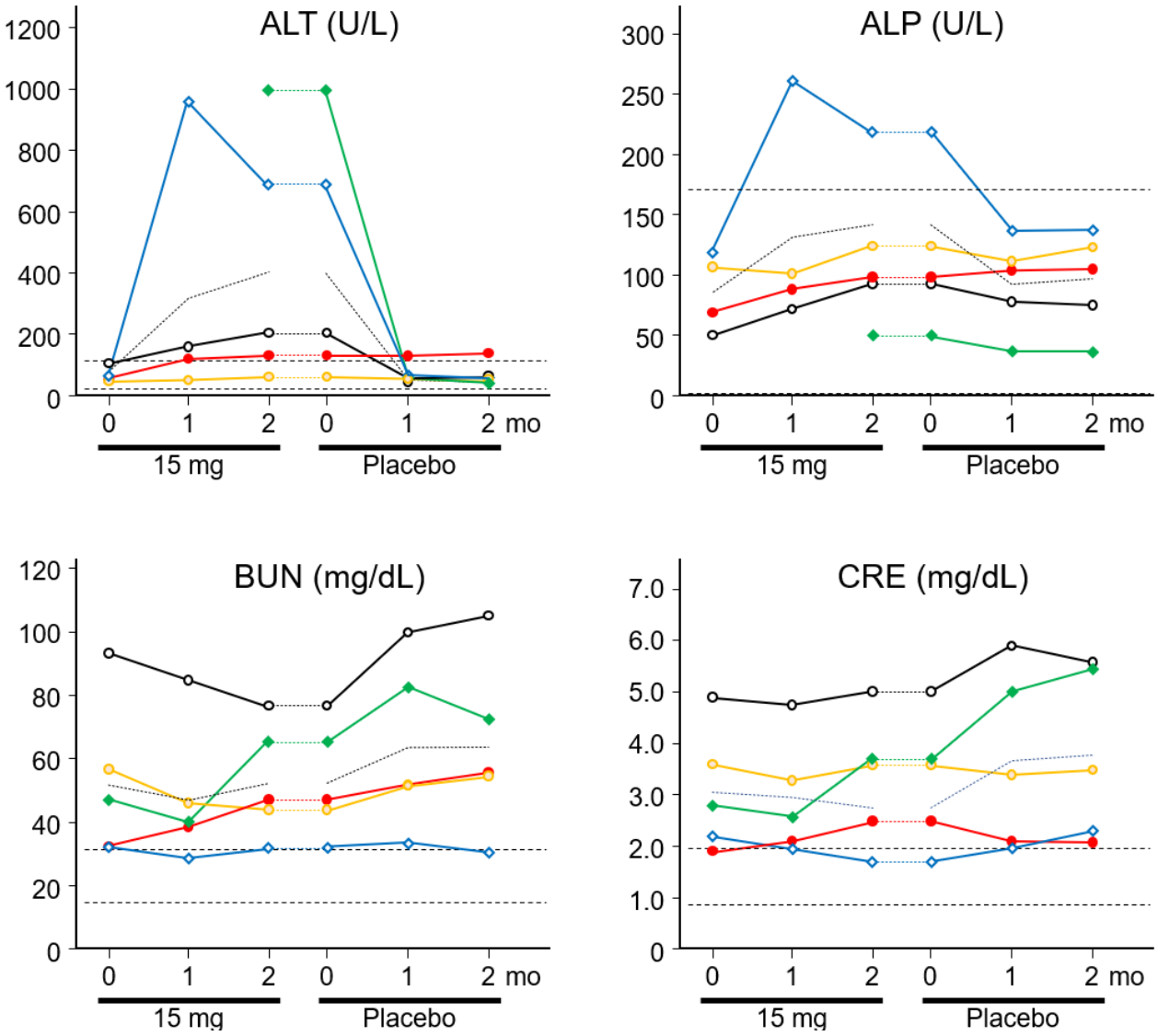


Figure 2. The effect of repeated treatments with 15 mg/head of 5-ALA or placebo in cats with CKD. 5-ALA or placebo was administered once daily for 2 months. Individual data are shown as symbols and solid lines. The mean (n = 4 or 5) is presented as dotted polygonal lines. Horizontal dot lines indicate the maximum and minimum values of the reference intervals.

Discussion

This study aimed to determine the predictive maximum dosage of 5-ALA with SFC in healthy cats and those with CKD using a repeated-dose treatment study. In previous reports, increases in liver enzymes after 5-ALA treatment have been documented in other species [14-20]. For instance, when human brain tumor patients were administered 5-ALA at a dose of 20 mg/kg before surgery, a

postoperative increase in liver enzymes occurred at 45.5%, although most incidents were mild and temporary [14]. In these studies, 5-ALA was used for photodynamic detection (PDD) and photodynamic therapy (PDT) of various tumors. In the veterinary field, PDD and PDT methods for dogs and cats were established by Osaki *et al.* [21-24]. They reported on dogs and cats that were orally treated with 5-ALA-HCl at a dose of 40 mg/kg once [21]. Their findings suggest that a single administration of 5-ALA could be sufficient for dogs and cats. In the present study, ALT and ALP levels were elevated in several cats treated with 30 and 50 mg/head/day. Hence, the no-observed adverse effect level (NOAEL) is suggested to be 10 mg/head, which is approximately 2.2 to 3.0 mg/kg/day. Note that this result was not completely declared, as autopsy or biopsy of various tissues was not performed in this study. Indeed, repeated treatment of rats and dogs was documented in the interview form of 5-ALA hydrochloride (ALAGLIO, Nippon Kayaku, Tokyo, Japan) [25]. In rats, anemia, elevated AST, ALT, and total cholesterol levels, focal necrosis, and bile duct proliferation in the liver were observed after treatment with 183 mg/kg/day for 4 weeks. In dogs, vomiting and mild elevation of AST and ALT levels were observed at a dose of 10 mg/kg/day for 4 weeks. The NOAELs for rats and dogs were 44 and 3 mg/kg/day, respectively, in the interview form. Taken together, it is suggested that the allowable dose of repeated 5-ALA treatment for cats could be lower than that for rats and approximately the same as that for dogs.

In a previous report on humans, treatment-related adverse effects such as vomiting (1%), nausea (1%), and hypertension (1%) were observed following a high-dosage 5-ALA treatment for PDD of urinary bladder carcinoma [26]. Administration of 5-ALA has been reported to cause vomiting in three of four dogs [27]. In this study, intermittent vomiting with dehydration was also observed at high doses in cats. Since 5-ALA is metabolized to protoporphyrin IX (PpIX), a large amount of exogenous 5-ALA can cause PpIX accumulation [28]. Since excessive PpIX is excreted by the biliary system, it can be delivered to the liver and cause temporary inflammation, reduce bile flow, and block the bile canaliculus or bile duct, leading to hepatobiliary injury and increased liver enzymes [28]. The direct cause of vomiting is still unknown, but taken together with these previous reports, a large dosage of 5-ALA administered for a long-time span in cats could cause liver damage. Particular attention should be paid to vomiting and hepatic enzyme levels when 5-ALA is repeatedly administered in cats.

In this study, a preliminary clinical test was performed in five cats with CKD at a dose of 15 mg/head once daily. This trial was designed before Experiment 1, and the dose was determined by extrapolation based on a previous report of other animals and unpublished data from a CKD model

mouse. Fortunately, the health damage and increase in hepatic enzymes in these cats were transient, and irreversible sequelae were unclear in this study. However, the results make us consider with deep remorse that it is essential to conduct a precise safety test using cats in advance rather than inferring by extrapolation when administering to cats.

Renal fibrosis is caused by various factors such as inflammation, hyperphosphatemia, and hypoxia [8]. Since 5-ALA with ferrous iron is an HO-1 inducer and Nrf2-related antioxidant that has protective effects against kidney injury [2; 29], it could also have a protective effect against CKD deterioration. BUN and CRE levels did not change significantly during the 5-ALA treatment. Nevertheless, these levels slightly decreased during the 10 and 30 mg/head treatments in healthy cats and increased during the placebo treatment in cats with CKD. These results implied that 5-ALA could have protective effects against BUN and CRE increases in cats with CKD. However, it is difficult to draw conclusions from these data, and clinical repeated treatment with 5-ALA at an appropriate dose in a larger sample size is needed. Generally, renal filtration abilities and CRE levels are correlated with the severity of renal fibrosis in cats with CKD [5, 7]. Moreover, CRE levels are known to be related to muscle volume in animals. While the body weights of the animals in this study did not change, muscle volumes were not precisely observed. Although the elevation of CRE is a common predictor for evaluating the prognosis of cats with CKD [30], other measurement factors such as symmetric dimethylarginine, urine protein creatinine ratios, and urine albumin creatinine ratios are also useful biomarkers to evaluate kidney function [13]. These items should be monitored during the evaluation of repeated 5-ALA treatment in CKD cats in future large-scale trials.

This study had several limitations. First, the sample size was small. To evaluate adverse effects, morphological evaluation using necropsy or biopsy was not performed in Exp. 1 and 2. Although Exp. 2 was designed as a double-blind crossover test, all patients were treated with 5-ALA in the first period and then with the placebo in the later period. Because the interval between these periods was 1 month in Exp. 1 and one day in Exp. 2, carryover effects were clearly observed.

In conclusion, there is a possibility that repeated treatment with a high dose of 5-ALA could elevate the levels of hepatic enzymes, especially ALT, and occasionally cause intermittent vomiting and dehydration as adverse effects in cats. These findings suggest that an appropriate dose of repeated treatment with 5-ALA for cats could be less than 10 mg/head/day, and that hepatic enzymes and digestive symptoms should be monitored carefully.

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