

Human Placenta Extract Therapy for Feline Hepatic Lipidosis

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Abstract: Feline hepatic lipidosis (HL), the most common hepatobiliary disease in cats, is characterized by the accumulation of excessive triglycerides (TGs) in more than 80% of hepatocytes. Forced oral feeding is recommended as the only therapy for this disease but the prognosis is often poor. As human placenta extract (Laennec) has been used to improve hepatic metabolism, we investigated the efficacy of this drug for the treatment of cats with HL. Ten cats diagnosed with HL in this study were treated with Laennec as a therapeutic drug. An immediate improvement in clinical symptoms was observed in all 10 cases. A statistical analysis indicated that the blood level of ALT ($P = 0.029$), AST ($P = 0.029$), ALP ($P = 0.005$), and T-Bil ($P = 0.012$) decreased significantly after treatment. All 7 hospitalized cases were eventually discharged from the hospital with a mean hospitalization duration of 4.8 days. The results of this study suggest that Laennec preparation has potential as an effective medicine for feline HL.

Key words: Feline Hepatic Lipidosis, Human Placenta, Laennec, ALT, ALP

I Introduction

Feline hepatic lipidosis (HL) is characterized by severe triglyceride accumulation in hepatocytes, causing cholestasis and liver disorders¹⁻⁴. The triglyceride content in the liver tissue of cats with lipidosis averaged 43% compared to 10% in that of healthy cats¹⁻⁴. HL is a secondary disorder concurrent with other essential diseases; more than 90% of lipidosis cases also had small intestinal diseases, pancreatitis, neoplasia, kidney disease, or diabetes mellitus¹⁻⁵. Therefore, it is very important to treat the underlying disease. Refeeding syndrome is characterized by severe hypophosphatemia that occurs in patients given enteral or parenteral nutrition⁶. Therefore, even if treatment of the underlying disease was successful and only HL remained,

the treatment period may be extended over the long-term. According to a specific report, in the absence of a diagnosis of a fatal underlying condition, recovery rates of 80%–88% could be expected if enteral feeding was initiated early in the course of the disease and sustained until voluntary intake resumed. However, cats might need tube feeding for several (3–6) weeks, requiring the owner to be an active participant in the recovery of their pet^{7, 8}. Forced oral feeding is of limited benefit in cats that have been anorectic for prolonged periods and can be stressful when performed as long-term hospital care. At present, there are no reports on an effective medical therapy for fatty degeneration of hepatocytes. Human placenta extract (Laennec; JAPAN BIO PRODUCTS Co., Ltd., Tokyo, Japan) contains HGF, a hepatocyte growth factor that has a number of positive effects: (1) promoting liver regeneration, (2) promoting hepatocyte DNA synthesis, (3) preventing liver damage, (4) anti-fat liver, and (5) preventing liver fibroplasia⁹⁻¹¹. In

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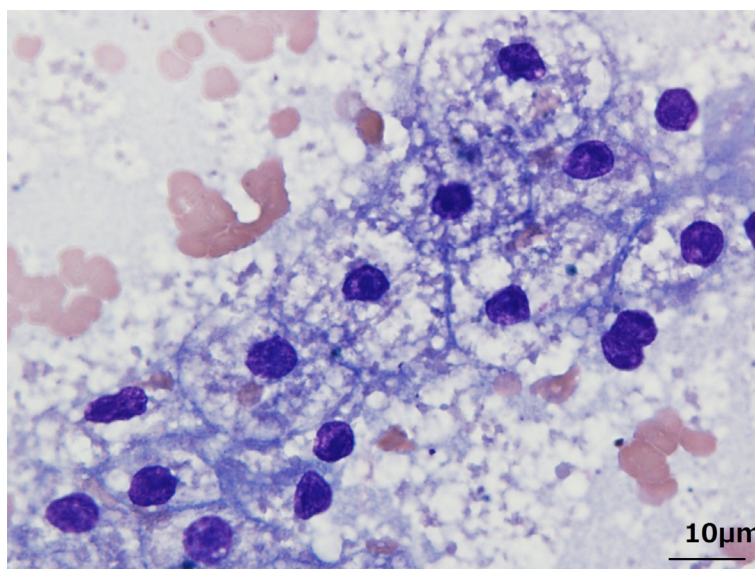


Fig. 1 Cytology findings of hepatic lipidosi.

Hepatocytes showed intense vacuolation. The sample was collected by fine needle biopsy (Case 2; Wright's Giemsa Staining, $\times 1,000$).

this report, Laennec was administrated to 10 cats with HL as an adjunct therapy during the treatment of an underlying disease with conventional nourishment therapy.

II Materials and Methods

A diagnosis of HL was based on a compatible history, clinical symptoms, biochemical and sonographic findings, plus the cytology of a fine needle liver biopsy^{5, 12-18}). The findings of cytological examination showed a vacuolation in the majority of sampled hepatocytes in all 10 cats (Fig. 1). In addition, all 10 cats had underlying diseases; acute pancreatitis (Case 1), inflammatory bowel disease (IBD) (Cases 2 and 10), immune-mediated neutropenia and thrombocytopenia (IMNP/IMTP) (Case 3), diabetic keto-acidosis/acute pancreatitis (Cases 4 and 7), gastrointestinal low-grade lymphoma (Case 5), IBD/acute pancreatitis (Case 6), feline infectious peritonitis (FIP) dry type (Case 8), and FIP wet type (Case 9). These 10 cases were diagnosed with HL at a private veterinary hospital (AKIYOSHI ANIMAL CLINIC [author's] between April 2013 and March 2015.

On the day that HL was diagnosed, Laennec (2 ml/head; Laennec contains a water-soluble agent of the human

placenta extract resolvent 112 mg out of 2 ml of 1 ampoule) was subcutaneously administered to all cats undergoing forced oral feeding and treatment for the underlying disease. All procedures in this study were performed in accordance with the Animal Protection Guidelines of Azabu University (authorization no. 1707019). This study, using client-owned cats, demonstrates a best practice of veterinary therapy and involves informed client consent.

For the seven cases of hospitalized cats, Laennec was administered every day until discharge. For the remaining three ambulatory cases, Laennec was injected from days 3 to 7. Physical examination, the time taken to recover appetite, and a complete blood workup and blood chemistry that included alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), total bilirubin (T-Bil), total cholesterol (T-cho), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), phosphorus (P), glucose (Glu), triglycerides (TG), and electrolytes (Na, K, Cl) were investigated. Table 1 summarizes the information on the concurrent disease, treatment, prognosis and number of Laennec injection in the 10 cats.

Table 1 Information relative to the concurrent disease, treatment, prognosis and number of Laennec injection in 10 cats

	Case1	Case2	Case3	Case4	Case5
Signalment	Age:8y NM Norwegian forest cat	Age:17y NM Domestic short hair	Age:2y NM Scottish fold	Age:10y NM Domestic short hair	Age:15y NM Domestic short hair
History	Anorexia (4days), Vomiting, (firsr opinion) Ran a fever	Anorexia (5days), Vomiting	Anorexia (5days), Vomiting	Anorexia (7days) Weight loss (2months)	Anorexia (7days), Vomiting
Abnormalities on the CBC	Normal	Normal	Normal	Normal	Normal
Abnormalities on the initial biochemical panels	ALT:524U/l, AST:410U/l,ALP:310U/l fPLI:50µg/l<.TBA147.1µmol/l	ALT:907U/l,AST:401U/l ALP:932U/l, T-Bil:4.9mg/dl	ALT:1318U/l,AST:229U/l ALP:657U/l	ALT:428U/l,AST:279U/l, Glu:549mg/dl, Na:137mmol/l, Cl:97mmol/l, fPLI kit:Positive, FIV:Positive	ALT:1132U/l,ALP:215U/l
Concurrent disease	Acute pancreatitis	Inflammatory bowel disease	IMNP IMTP	Diabetic ketoacidosis Acute pancreatitis	Gastrointestinal low-grade lymphoma
Treatment	Enrofloxacin 5mg/kg/sid sc, Maropitant 2mg/kg/sid sc, Ursodeoxycholic 10mg/kg/bid po, Metronidazole 10mg/kg/bid po, Meloxicam 0.2mg/kg/sid sc	Enrofloxacin 5mg/kg/sid sc, Prednisolone 0.5mg/kg/sid sc Maropitant 2mg/kg/sid sc	Enrofloxacin 7mg/kg/sid sc, cyclosporine 7mg/kg sc, Prednisolone 2mg/kg/sid sc, Ursodeoxycholic acid 10mg/kg/bid po	Impipenem-cilastatin 5mg/kg/tid iv, Maropitant 2mg/kg/sid sc Regular insulin iv (0.05U/kg-0.1/ kg/hr)	Maropitant 2mg/kg/sid sc, Ursodeoxycholic acid 10mg/kg/bid po, Mosapride 0.5mg/kg/bid po
Hospitalization management	NA	7days	NA	5days	NA
Number of Laennec injection	1A SC, 1day	1A SC, 7days	1A SC, 3days	1A SC, 5days	1A SC, 3days
Prognosis	Survival >1years	Survival > 1years	Survival > 1years	Survival >6months	Survival >1years
	Case6	Case7	Case8	Case9	Case10
Signalment	Age:9y NM Domestic short hair	Age:8y NM Domestic short hair	Age:16y NM Domestic short hair	Age:2y NM Domestic short hair	Age:14y NM Domestic short hair
History	Anorexia (4days), Vomiting	Anorexia (2days), PU/PD(+)	Anorexia (3days) Weight loss (2months)	Anorexia (4days)	Weight loss
Abnormalities on the CBC	Normal	WBC: 23400/µl, Neu: 14800/µl	WBC: 32800/µl Neu: 18900/µl, Mono: 11100/µl	WBC:27500/µl Neu: 21800/µl, Mono:2200/µl	Normal
Abnormalities on the initial	ALT: 266U/l,AST: 512U/l,ALP: 83U/l,Alb:2.2g/dl, T-Bil: 1.3mg/dl, fPLI kit:Positive	ALT: 352U/l,AST: 493U/l, ALP:208U/l, Glu:584mg/dl, T-Bil: 1.5mg/dl, f-PLI kit:Positive	ALT: 477U/l,AST: 239U/l ALP: 180U/l, T-Bil: 0.8mg/dl TP:10.8g/dl, BUN:39.1mg/dl	ALT: 269U/l,AST: 639U/l ALP: 121U/l, T-Bil: 3.9mg/dl T-bil:2.1mg/dl, Alb 2.0g/dl	ALT: 372U/l,AST: 136U/l ALP: 809U/l, T-bil:2.1mg/dl, Alb 2.0g/dl
Concurrent disease	Acute pancreatitis Inflammatory bowel disease	Diabetic ketoacidosis Acute pancreatitis	FIP/dry type	FIP/wet type	Inflammatory bowel disease
Treatment	Impipenem-cilastatin 5mg/kg/tid iv, Maropitant 2mg/kg/SID sc, Predonizolone 1mg/kg/sid sc	Impipenem-cilastatin 5mg/kg/tid iv, Maropitant 2mg/kg/sid sc, Regular insulin iv (0.05mg-0.1mg/kg/hr)	Impipenem-cilastatin 5mg/kg/tid iv, Predonizolone 2mg/kg/sid sc, IFN 1MU/kg/sid sc	Impipenem-cilastatin 5mg/kg/tid iv, Predonizolone 2mg/kg/sid sc, IFN 1MU/kg/sid sc	Entrofloxacin 5mg/kg/sid sc, Maropitant 2mg/kg/sid sc, Prednisolone 1mg/ks/sid sc
Hospitalization management	3days	3days	4days	5days	7days
Number of Laennec injection	1A SC, 3days	1A SC, 3days	1A SC, 4days	1A SC, 5days	1A SC, 7days
Prognosis	Survival > 1years	Survival > 6months	Survival: 2.5Months (FIP cause)	Survival: 1.5months (FIP cause)	Survival>6months

Reference range: WBC: 4800–17000/µl; Neu: 2500–12500/µl; Mono: 50–1500/µl; ALT: 20–84U/l; AST: 12–45U/l; ALP: 26–160U/l; T-Bil: 0–0.7mg/dl; TP: 5.4–7.8g/dl; Alb: 2.5–3.9g/dl; BUN: 12–32mg/dl; Na: 145–156mmol/L; Cl: 111–125mmol/L; NM = neutered male; PU/PD = polyuria and polydipsia; CBC = complete blood count; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; fPLI = specific feline pancreas lipase; TBA = total bile acids; T-Bil = total bilirubin; Glu = glucose; Na = sodium; Cl = chlorine; FIV = feline immunodeficiency virus; Alb = albumin; WBC = white blood cells; Neu = polymorphonuclear neutrophils; Mono = monocytes; FIP = feline infectious peritonitis; IMNP = immune-mediated neutropenia; IMTP = immune-mediated thrombocytopenia; NA = not applicable; 1A = 1 ampoule.

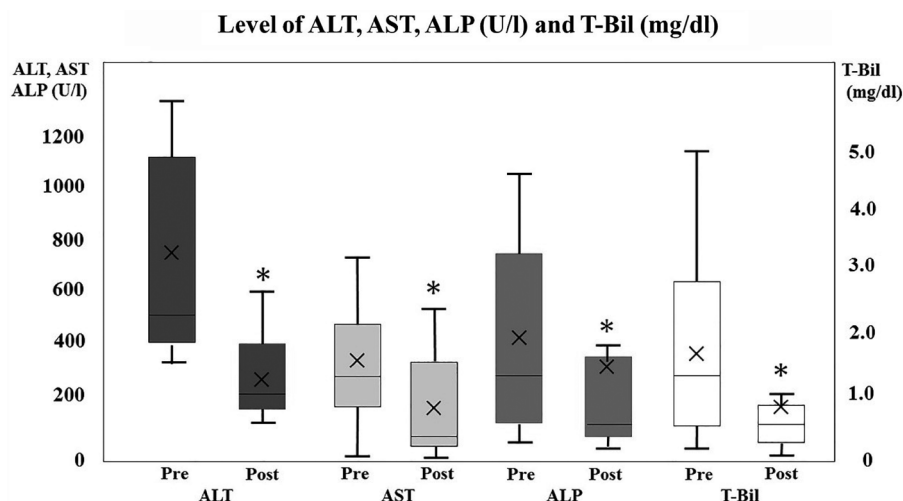


Fig. 2 Changes in blood chemistry.

The boxplot of ALT (dark gray), AST (light gray), ALP (middle gray) and T-Bil (white) levels of 10 cases, pre- and post- Laennec injection. The cross shows the mean and the bar shows the median. The values of ALT, AST, ALP, T-Bil were statistically compared between pre-treatment (pre) and post-treatment (post) values. The asterisk shows a significant difference ($P < 0.05$) in statistics.

Table 2 The transition of blood test results of 10 cases.

Pre shows the value of the blood test before Laennec injection and Post shows the value of the blood test at the time of completion of Laennec injection. NA means that Laennec injection was administrated by going to hospital for treatment

	ALT Pre	ALT Post	AST Pre	AST Post	ALP Pre	ALP Post	T-Bil Pre	T-Bil Post	Hospitalization
Case 1	524 U/l	425 U/l	410 U/l	39 U/l	319 U/l	72 U/l	0.8 mg/dl	0.3 mg/dl	NA
Case 2	907 U/l	545 U/l	401 U/l	258 U/l	932 U/l	1159 U/l	4.9 mg/dl	1.1 mg/dl	7days
Case 3	1318 U/l	159 U/l	229 U/l	32 U/l	657 U/l	253 U/l	0.6 mg/dl	0.4 mg/dl	NA
Case 4	428 U/l	142 U/l	279 U/l	73 U/l	112 U/l	80 U/l	0.5 mg/dl	0.3 mg/dl	5days
Case 5	1132 U/l	95 U/l	NA	NA	215 U/l	172 U/l	0.09 mg/dl	0.1 mg/dl	NA
Case6	266 U/l	123 U/l	512 U/l	41 U/l	83 U/l	68 U/l	1.3 mg/dl	0.6 mg/dl	3days
Case7	352 U/l	273 U/l	208 U/l	275 U/l	49 U/l	90 U/l	1.5 mg/dl	0.6 mg/dl	3days
Case8	477 U/l	176 U/l	239 U/l	64 U/l	180 U/l	58 U/l	0.8 mg/dl	0.2 mg/dl	4days
Case9	269 U/l	141 U/l	639 U/l	514 U/l	121 U/l	90 U/l	3.9 mg/dl	3.3 mg/dl	5days
Case10	372 U/l	132 U/l	136 U/l	129 U/l	809 U/l	350 U/l	2.2 mg/dl	0.6 mg/dl	7days

Reference range: ALT: 20–84U/l, AST: 12–45U/l, ALP: 26–160U/l, T-Bil: 0–0.7mg/dl.

III Results and Discussion

The median pre-treatment ALT, AST, ALP, and T-Bil values were 453 U/l, 279 U/l, 198 U/l, and 1.1 mg/dl, respectively. The median post-treatment ALT, AST, ALP, and T-Bil values were 151 U/l, 73 U/l, 90 U/l, and 0.5 mg/dl, respectively. (Fig. 2) The degree of decreased liver enzyme levels (ALT, AST, and ALP) and T-Bil varied, but a prompt improvement was confirmed by an alleviation of symptoms in all cases. Table 2 shows the transition of blood test results of 10 cases. A statistical analysis included a Wilcoxon

signed-rank test using EXCEL (Microsoft, Washington, U.S.A.) and STATMATE (Nihon 3B Scientific, Nigata, Japan). Statistical analysis indicated that ALT ($P = 0.029$), AST ($P = 0.029$), ALP ($P = 0.005$), and T-Bil ($P = 0.012$) decreased significantly after treatment ($P \text{ value} < 0.05$). All cases were eventually discharged with a mean hospitalization duration of 4.8 days.

This report demonstrated the therapeutic potential of a Laennec preparation as an effective medicine to treat Feline HL. A decrease in ALT levels was seen in all cases and the mean time taken for appetite improvement was 4.8 days,

which was dramatically shorter than that presented in other reports^{7,8}). Furthermore, the ratio of decreased liver enzyme (ALT, AST, ALP) went along with hospitalization days for time to appetite improvement and the average hospitalization days of the non-administrated group was 12.0 days in our hospital. (Akiyoshi, M. and Hisasue, M., unpublished results) A particularly remarkable effect was seen in Case 1. This cat had acute pancreatitis and developed HL several times in the past, always necessitating hospitalization for 10 to 14 days. However, the symptoms and hepatic enzyme abnormalities of HL in this cat improved following a single injection of Laennec with conventional treatment and hospitalization management, and forced feeding was not required. HL and pancreatitis recurred 5 times over the following 2 years, and each time the cat was given Laennec recovery was evident by day 3. Furthermore, in HL cases with IBD or acute pancreatitis (Cases 2, 4, 6, 7, and 10), the length of hospitalization was less than 1 week, and it was suggested that the Laennec injection was more effective than the previously reported treatment in which tube feeding was necessary for 3–6 weeks^{7,8}). In this study, the therapeutic dose of Laennec was extrapolated with reference to the therapeutic dose in humans¹⁹). The determination of a concrete therapeutic dose will be necessary in the future.

In this report, no side effects of a Laennec injection were seen in all cases. The maximum number of Laennec injections given to an individual cat in this study was seven (Cases 2 and 10), and no abnormalities were detected by physical examination. In human clinical trials, side effects were reported in 3.7% of 237 patients. The main side effects were pain at the injection site (2.56%), hypersensitivity (0.37%), and induration of the injection site (0.37%)¹⁹). Laennec was extracted from human placental tissue and is an exogenous material for cats. An increased risk of hypersensitivity and anaphylaxis was thus presumed to occur in cats compared to human patients. As such, careful follow up of the treated cats is required.

There were three problems in this study. The first problem was variability in the underlying disease of the 10 cases. The second problem was that there was no comparison with a control group that was not given Laennec. The third problem was that a definitive diagnosis of HL was not completed. We

were unable to exclude other hepatobiliary diseases such as inflammatory, neoplastic, and blood vessel dysplasia since open wedge liver biopsies were not conducted. Therefore, it will be necessary to solve these problems in the future to prove the effect of a Laennec injection treatment for feline HL. It was suggested that enforcing an evaluation by unifying the underlying diseases, comparing a Laennec injection group with a non-administered group, and a histopathological examination of the liver will be necessary for further confirmation of the efficacy of Laennec.

In this study, clinical symptoms and liver enzyme levels in all cats with HL recovered following a treatment of Laennec injections with a mean hospitalization duration of only 4.8 days. A Laennec injection will be expected to reduce the cost and term of hospitalization and may contribute to improved feline HL outcomes. Further investigations that include a prospective study are necessary to determine the efficacy of Laennec.

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