Evaluation of the proper dosage of lapatinib and its safety in dogs

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Abstract. Lapatinib is a low-molecular-weight agent targeting the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2). It is one of the antitumor agents used against advanced breast cancer in human. We intended to apply it against canine mammary gland tumors (cMGs). To this end, we evaluated the tolerated dosage and the side effects of lapatinib in healthy dogs. In this study, we conducted a dose-escalation toxicity test starting from 30 mg/kg to determine the maximum tolerated dose. Grade 3 toxicity, which was apparent as weight loss, was observed at the dose of 40 mg/kg/day. We then performed a long-term administration test and found that the dose of 35 mg/kg/day was well-tolerated within 7 weeks but caused grade 3 hepatic toxicity by the eighth week. In conclusion, our findings reveal that the dose of 35 mg/kg/day administered for no more than 8 weeks is fairly safe for use in healthy dogs. This dose is higher than the recommended dose for humans; thus, further studies evaluating the effective dose against canine tumors are needed.

Key words: dog, human epidermal growth factor receptor 2, lapatinib, mammary gland tumor, safety test

Highlights

- Similar to that in human medicine, HER2 is a promising therapeutic target for canine malignant tumors.
- Lapatinib tosylate hydrate is a molecular targeted drug against the HER family including HER2; it has a safe and proven anti-tumor effect in humans.
- A dose-escalation study and long-term administration test of lapatinib were performed in six healthy dogs to determine the MTD and safety of lapatinib in veterinary medicine.
- Oral administration of 35 mg/kg/day lapatinib with meals was found safe for treating cMGs.
- Dose-limiting toxicities of lapatinib were weight loss and ALP elevation.

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor and a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Its overexpression is reported to correlate with the factors influencing the malignancy of human breast cancer, such as proliferation, metastasis, and drug resistance [1, 2]. Human breast cancer is clinically classified into intrinsic subtypes according to the expression status of hormone receptors and HER2 and the proliferation index [3, 4]. The treatment for human breast cancer is selected based on the subtype, and HER2-targeted therapy is administered to patients with HER2-positive cancer. The first-line chemotherapy for these patients is trastuzumab (Herceptin®; Chugai Pharmaceuti-

cal Co., Ltd., Tokyo, Japan), a humanized monoclonal antibody that targets human HER2. Once cancer reaches an advanced stage or is unresponsive to trastuzumab therapy, combination therapy with capecitabine and lapatinib is considered in human breast cancer. Lapatinib is a low-molecular-weight compound targeting epidermal growth factor receptor (EGFR) and HER2 [2] and is reported to be effective against advanced HER2-positive breast cancer [5].

In veterinary medicine, canine mammary gland tumors (cMGs) are among the most prevalent tumors in female dogs, and the first choice of treatment of these tumors in the early clinical stage is surgical excision [6]. For animals with distant metastasis, conventional cytotoxic anticancer agents, such as carboplatin and doxorubicin, are commonly applied. These agents are widely used for neoplasms in dogs and prolong the mean survival time after the surgical operation in cases of advanced cMGs (228 days in cases in which surgery has been performed along with adjuvant

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chemotherapy and 194 days in cases in which surgery alone has been performed) [7]. However, these agents have side effects, such as myelosuppression and cardiotoxicity, which sometimes limit their usage and necessitate the development of a new treatment for cMGTs.

HER2 overexpression has been reported in 18–46% of cMGTs [8]. Therefore, the HER2-targeting agent, lapatinib, might be the new candidate agent against cMGTs. According to the datasheet of lapatinib, two series of safety tests in dogs have been conducted: single- and multiple-dose toxicity tests. In the single-dose toxicity test, doses of 10, 60, and 360 mg/kg were used in healthy dogs and only mild diarrhea was reported as a side effect. On the other hand, in the multiple-dose toxicity test, doses of 10, 40, and 160 mg/kg/day were used for 13 weeks and for 39 weeks, and some side effects including skin inflammation and hepatotoxicity were observed at doses higher than 40 mg/kg/day concluding NOAEL as 10 mg/kg/day in either time period [9]. In our preliminary study, no adverse effect was observed at a dose of 20 mg/kg (unpublished data). On the basis of these findings, we conducted the dose-escalation toxicity test starting from a dose of 30 mg/kg to determine the maximum tolerated dose (MTD) of lapatinib in dogs.

Healthy Beagle dogs were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Three male and three female dogs were included in this study. All the dogs were 18 months old and their mean body weight was 9.2 ± 0.74 kg (range: 7.9–10.1 kg). They were fed with Urban Life Junior dry food (Royal Canin, Dubai, United Arab Emirates), whose quantity was decided according to the animals' body weights, and had *ad libitum* access to fresh water. The room temperature was maintained at around 25°C and the humidity was approximately 60%. All animal experiments were approved by the Animal Use Committee of the Faculty of Agriculture at The University of Tokyo, and we followed the guidelines of The University of Tokyo for the care and use of animals (approval number: P18-048).

Lapatinib tosilate hydrate (Tykerb[®]) was purchased from Novartis (Basel, Switzerland). Lapatinib was encapsulated into capsules (Matsuya, Osaka, Japan) for each dose. The capsules were administered once a day with food, per os. The general physical condition of the animals, including the amount of food intake and the presence of vomiting and diarrhea, was evaluated twice a day. The dogs underwent physical examinations including heart and respiration rate measurement and auscultation once a week, body weight measurement and blood examinations every 2 weeks, and chest X-ray before and after the examination. The blood examination included complete blood count, coagulation test, and biochemistry, including determination of albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total bilirubin (T-BIL), blood urea nitrogen (BUN), creatinine, glucose, and electrolyte levels (DRICHEM 7000V, Fujifilm Corp., Tokyo, Japan). Side effects were evaluated according to the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE) ver. 1.1, and some representative side effects are shown in [Supplementary Table 1](#) [10].

Two studies were planned: a dose-escalation study

and long-term administration test. In the dose-escalation study, starting from an initial dose of 30 mg/kg/day, the dose was increased by 5 mg/kg/day every 2 weeks until a side effect more severe than VCOG-CTCAE grade 3 appeared. The maximum dose that did not result in a grade 3 side effect was considered the MTD and proceeded to the next examination. In the long-term administration test, we administered the MTD for 8 weeks after an interval of 8 weeks from previous study. Similar to many protocols with conventional antitumor drugs, administration for 8 weeks was considered as one term. The side effect was determined as described above, and the appearance of grade 3 side effects was set as the endpoint of the study. All dogs were confirmed to show no clinical abnormalities and normal values in all examinations at the start of both studies.

The dose-escalation study was conducted to determine the proper dose of lapatinib for dogs. [Table 1](#) shows the side effects observed with the administration of 30–40 mg/kg/day lapatinib. One dog developed grade 3 toxicity when lapatinib was administered at a dose of 40 mg/kg/day. The dose-limiting toxicity was a grade 3 adverse effect, which was defined as a weight loss of >15%. In this dog, the percentage weight decreased to 84.4%. At doses of 30 and 35 mg/kg/day, no side effects of severity higher than grade 3 were observed.

Based on the results of the dose-escalation study, the long-term administration test was performed with a dosage of 35 mg/kg/day. The side effects observed during the long-term administration test are shown in [Table 2](#). As the reference range of ALP in dogs is 20 to 156 IU/l, the magnitude of a grade 3 side effect in terms of the ALP level was calculated and defined as 780 to 3120 IU/l in this study ([Supplementary Table 1](#)). After lapatinib administration for 8 weeks, three of the six dogs showed high ALP levels (814, 967, and 1316 IU/l), which were above the lower limit of the range defined. None of the other parameters exceeded the toxicity level beyond grade 3.

Since HER2 expression has been reported in some canine tumors including mammary gland tumors, lapatinib can be considered a useful treatment option [8, 11–13]. Furthermore, HER2 inhibition was reported to be effective in canine mammary cell lines [14]. However, while several safety studies with limited dosages [9] have been performed in dogs, no practical protocols have been established.

In this study, the MTD of lapatinib for dogs was found to be 35 mg/kg/day within 8 weeks. In humans, lapatinib is administered at the dose of 1,250 mg/head/day, which equals to 25 mg/kg for a person weighing 50 kg, with capecitabine. The MTD defined in this study is a clinically applicable dose in dogs, and higher than the extrapolated dose applied in human medicine. A limitation of this study is that it only examined the safety of lapatinib use, and not the effect on canine tumors. Further studies are needed to define a clinically useful protocol for the drug.

In this study, weight loss and a high ALP level were the markers of dose-limiting toxicity. Similar to that in humans, lapatinib can cause hepatobiliary toxicity, although the values reported to be affected differ between dogs and humans: ALT, AST, and T-BIL levels have been reported to increase in humans, but the parameter most affected in this study

was ALP. The reason for weight loss was not clear in this study. Because appetite and diet were unchanged during the study, the causative factor might be an intestinal tract disorder. Determination of the detailed pharmacodynamics of lapatinib and mechanisms of adverse effects in dogs might be necessary for further investigations.

Among antitumor agents many frequently used drugs cause bone marrow suppression. At the same time, molecular targeted agents are not commonly used in veterinary hospitals, possibly because of limited information and costs. However, the results of this study show that lapatinib can be used relatively safely even in dogs, without any adverse myelosuppressive effect. To determine the possibility of

using the drug with conventional antitumor drugs, further safety studies are required.

Drug pharmacokinetics are reported to change depending on the fat intake in humans; a high fat intake is associated with a high area under the concentration-time curve (AUC) and maximum blood concentration [5]. In human medicine, to avoid pharmacokinetic changes, lapatinib is encouraged to be administered between meals. However, in veterinary medicine, administration of oral medication to dogs is often difficult at home, especially without any treats. To establish a possible medication protocol, we decided to administer the drug with meals. At the same time, assuming that various types of diet are given to dogs at home, in this study,

Table 1. Adverse events observed in the dose-escalation study

Adverse event		30 mg/kg/day		35 mg/kg/day		40 mg/kg/day	
		No. of dogs showing adverse events	CTCAE grade	No. of dogs showing adverse events	CTCAE grade	No. of dogs showing adverse events	CTCAE grade
Constitutional clinical sign	Weight loss	4/6	Grade 1	2/6	Grade 1	2/5	Grade 1
		1/6	Grade 2	2/6	Grade 2	1/5	Grade 2
						1/5	Grade 3
Dermatologic/Skin	Erythema	-		-		3/5	Grade 1
Gastrointestinal	Loose stool	1/6	*	1/6	*	1/5	*
Metabolic/Laboratory	ALP	5/6	Grade 1	2/6	Grade 1	5/5	Grade 2
		1/6	Grade 2	4/6	Grade 2		
	ALT	3/6	Grade 1	2/6	Grade 1	3/5	Grade 1
				1/6	Grade 2		
	T-BIL	-		1/6	Grade 1	2/5	Grade 1
						1/5	Grade 2
	GGT	1/6	*	-		2/5	*

*: "loose stool" and "GGT" were not graded because they are not included in the Common Terminology Criteria for Adverse Events (CTCAE). -: no abnormalities or adverse events were observed. ALP, alkaline phosphatase; ALT, alanine aminotransferase; T-BIL, total bilirubin; GGT, γ -glutamyl transpeptidase.

Table 2. Adverse events observed in the long-term administration test

Adverse event		35 mg/kg/day							
		Week 0		Week 2		Week 4		Week 6	
		No. of dogs showing adverse events	CTCAE grade	No. of dogs showing adverse events	CTCAE grade	No. of dogs showing adverse events	CTCAE grade	No. of dogs showing adverse events	CTCAE grade
Dermatologic/Skin	Erythema	-		-		3/6	Grade 1	3/6	Grade 1
Gastrointestinal	Loose stool	-		1/6	*	1/6	*	1/6	*
Metabolic/Laboratory	ALP	-		2/6	Grade 1	6/6	Grade 2	6/6	Grade 2
				4/6	Grade 2			3/6	Grade 3
	ALT	-		2/6	Grade 1	4/6	Grade 1	3/6	Grade 1
				1/6	Grade 2	1/6	Grade 2	1/6	
	T-BIL	-		1/6	Grade 1	1/6	Grade 1	3/6	Grade 1
								2/6	Grade 2
	GGT	-		-		1/6	*	3/6	*

*: "loose stool" and "GGT" were not graded because they are not included in the Common Terminology Criteria for Adverse Events (CTCAE). -: no abnormalities or adverse events were observed. ALP, alkaline phosphatase; ALT, alanine aminotransferase; T-BIL, total bilirubin; GGT, γ -glutamyl transpeptidase.

we provided the dogs with comprehensive nutrition, and the diet chosen had a rather high fat content (less than 16% of total fat). If the drug is administered between meals or with low-fat meals, the MTD might be higher. In conclusion, oral administration of lapatinib at a dose of 35 mg/kg/day with meals within 8 weeks was found to be safe for dogs. This finding might be helpful for a clinical evaluation of its antitumor effect and adverse effects in tumor-bearing dogs.

Conflict of Interest

The authors have no conflict of interest to declare associated with this manuscript.

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