Management of canine insulinomas with toceranib phosphate: 30 cases (2009-2019).

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Abstract

BACKGROUND: Insulinomas are the most common tumour of the endocrine pancreas in dogs. These are malignant tumours with a high metastatic rate and limited efficacious chemotherapeutic options available. Recent literature supports the use of the multi-receptor tyrosine kinase inhibitor sunitinib malate for treatment of metastatic insulinoma in people. Toceranib phosphate is a veterinary targeted therapy that may provide benefit in treatment of canine insulinomas. The primary objectives of this study were to describe the duration of clinical benefit, defined as absence of clinical signs associated with hypoglycemia and/or measurable response according to the response evaluation criteria for solid tumours in dogs (cRECIST), and measures of outcome, namely progression free interval (PFI) and overall survival time (OST) in dogs diagnosed with insulinoma treated with toceranib. A secondary objective was to describe the adverse effects of toceranib in dogs with insulinoma.

RESULTS: A medical record search identified 30 dogs diagnosed with insulinoma and treated with toceranib at five university hospitals and eight veterinary specialty referral hospitals. A majority (66.7%) of dogs with measurable disease experienced either complete response (CR), partial response (PR), or stable disease (SD) with toceranib therapy. The overall median progression free interval (PFI) was 561 days (95% confidence interval: [246, 727 days]). The overall median survival time (OST) was 656 days [310, 1045 days]. Larger dogs were at increased risk for disease progression ($P = 0.0310$) and death ($P = 0.0064$), with every 1 kg increase in body weight resulting in hazard ratios (HRs) of 1.045 [1.003, 1.084] and 1.05 [1.012, 1.090], respectively. In addition, time to disease progression was associated with use of therapies prior to toceranib ($P = 0.0050$) and type of veterinary practice ($P = 0.0025$). The most common adverse events with toceranib therapy were grade 1 or 2 gastrointestinal toxicities.

CONCLUSIONS: Clinical benefit was reported in the majority of dogs diagnosed with insulinoma treated with toceranib, but randomized, prospective studies are needed to assess and quantify the effect of this therapy. The most commonly observed adverse events (AEs) were gastrointestinal AEs.

Background

Insulinomas are insulin-secreting tumours which arise from pancreatic β islet cells. Insulinomas are the most common tumour of the endocrine pancreas in dogs, yet are relatively rare [1-3]. The majority of canine insulinomas are highly malignant tumours. Macroscopic metastatic lesions are present in 45 – 50% of dogs at the time of diagnosis, and it is clinically anticipated that the majority of dogs will develop disease recurrence or metastasis despite attempts at local disease control with surgery [1-4].

The most debilitating clinical signs observed with insulinoma are the result of neuroglycopenia, and include weakness, ataxia, disorientation, behavior changes, and seizures [1, 5]. Initial therapy is aimed at managing these clinical signs, while intermediate to longer-term therapy aims to treat the primary tumour and/or metastasis [1-3].

Surgery with partial pancreatectomy is the mainstay of therapy in dogs [1-3, 6-8]. Dogs that undergo surgical excision of macroscopic disease experience survival times of 10 – 43 months [6-8]. Nevertheless, surgery alone is unlikely to be curative, because most dogs have micrometastatic disease at the time, and in such cases progressive disease invariably leads to recurrent hypoglycemia and secondarily, life-limiting neuroglycopenia [1-3, 6, 9, 10]. Therefore, medical treatments are warranted in attempt to inhibit progression of metastatic disease, ameliorate clinical signs, and extend survival times while maintaining quality of life [1-3, 8-12].

Previously described medical therapies include glucocorticoids, octreotide, diazoxide, alloxan, and streptozocin [8-12]. Potential drawbacks to these options in veterinary medicine include poor response rates, the need for frequent administration, limited availability, the high cost of diazoxide, and significant gastrointestinal, endocrine, and nephrotoxic adverse effects of streptozocin and alloxan [1-3, 8-12]. Consequently, there is a need to investigate alternative, efficacious, and well-tolerated medical interventions to manage insulinomas in dogs.

Sunitinib malate (Sutent®; Pfizer, Inc., New York, NY, USA) is an oral small molecule inhibitor initially approved by the Food and Drug Administration (FDA) for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumour in people, but additional applications have been subsequently identified [13, 14]. Based on the results of a multinational, randomized, double-blind, placebo-controlled phase 3 clinical trial, sunitinib was recently approved by the FDA for treatment of locally advanced or metastatic pancreatic neuroendocrine tumours (pNETs) in people, a classification encompassing all tumours arising from the multipotent stem cells of pancreatic ductal epithelium, including insulinoma [15].

Toceranib phosphate (Palladia®; Zoetis Animal Health, Madison, NJ, USA) is a small molecule inhibitor with similar molecular targets to sunitinib, including the cell surface receptors for vascular endothelial growth factor (VEGF)-1 and -2, platelet-derived growth factor (PDGF)- β , and stem cell factor (SCF) [16,17]. Moreover, in the initial phase I study for toceranib, as well as subsequent off-label investigations of toceranib, clinical responses were observed in dogs with a spectrum of solid tumour types [18-27]. More recently, a case report described a dog diagnosed with metastatic insulinoma experiencing long-term glycemic control with toceranib [28].

The primary objectives of this study were to describe the duration of clinical benefit, defined as absence of clinical signs associated with hypoglycemia and/or measurable response according to the response evaluation criteria for solid tumours in dogs (cRECIST), and measures of outcome, namely progression free interval (PFI) and overall survival time (OST) in dogs diagnosed with insulinoma treated with toceranib [29]. A secondary objective was to describe the adverse effects of toceranib in dogs with insulinoma.

Results

Patient Population

The medical record search, and application of inclusion and exclusion criteria, identified 30 dogs diagnosed with insulinoma and treated with toceranib at five university hospitals and eight veterinary specialty referral hospitals between June 2009 and March 2019. The study population included eight mixed breed dogs, two Boston terriers, two Chihuahuas, two Labrador retrievers, and one of each of the following breeds: Afghan hound, Australian shepherd, chowchow, cocker spaniel, coonhound, dachshund, Doberman pinscher, Irish setter, Jack Russel terrier, papillion, Pekingese, Pomeranian, Scottish terrier, Shar Pei, West Highland white terrier, and Yorkshire terrier. There were 14 male neutered dogs and 16 female spayed dogs. The median age at diagnosis was 9 years (range, 5 – 15 years). The median weight at diagnosis was 14.9 kg (range, 2.9 – 44.4 kg).

Presentation, Diagnosis, and Staging

The majority of the dogs ($n = 25/30$) initially presented with clinical signs associated with neuroglycopenia, including seizures ($n = 13$), collapse ($n = 7$), ataxia ($n = 5$) muscle tremors ($n = 3$), and twitching ($n = 3$). Two dogs presented with lethargy and two dogs presented with vomiting at diagnosis. Four dogs were reported to be asymptomatic for insulinoma at the time of diagnosis. One of these dogs was presented for evaluation of a soft tissue sarcoma, and the other three were presented for routine annual examination. Each of these dogs were further evaluated for possible insulinoma when hypoglycemia was noted on blood work.

Twenty-one dogs underwent partial pancreatectomy with histopathology of the pancreas, confirming the diagnosis of insulinoma. Four dogs had an ultrasound-guided fine needle aspirate biopsy (FNA) with cytology of a pancreatic mass to confirm diagnosis. All of the dogs with either a histologic or cytologic diagnosis also had hypoglycemia reported at the time of diagnosis, additionally, 23 of these dogs had a documented abnormal paired fasting insulin and glucose ratio. Two additional dogs were diagnosed with an abnormal paired fasting insulin and glucose ratio and a pancreatic nodule on CT. Another two dogs were diagnosed with an abnormal paired fasting insulin and glucose ratio and a pancreatic nodule on abdominal ultrasound. The final dog was diagnosed with clinical signs consistent with neuroglycopenia including seizures, weakness, and collapse, and an abnormal paired fasting insulin and glucose ratio, despite no significant abnormalities noted on abdominal ultrasound. In all cases, other causes of hypoglycemia, including sepsis, hepatic failure, adrenocortical insufficiency, and toxin ingestion, were reasonably investigated and eliminated prior to treatment with toceranib.

All 30 dogs had a reported low fasting blood glucose concentration (defined as <3.33 mmol/L) at the time of diagnosis, and 28 dogs were reported to have hypoglycemia in the presence of a concurrent normal or increased fasting insulin measurement, as was reported by each individual reference range. The remaining two dogs did not have baseline insulin levels measured. The median fasting blood glucose concentration was 2.16 mmol/L (range, 1.89 – 3.27 mmol/L), and the median fasting insulin concentration was 101.8 μ IU/mL (range, 14.6 – 647.3 μ IU/mL).

Records of baseline complete blood counts were available for 25 dogs, and serum chemistry profiles were available for 29 dogs at the time of toceranib initiation. The most commonly reported chemistry

abnormality was hypoglycemia ($n = 25$). Concurrent urinalysis was recorded for 14 dogs. Two dogs had hyposthenuria and one dog had isosthenuria. Six dogs had a baseline blood pressure performed, of which three were hypertensive (systolic blood pressure (BP) >140 mmHg).

Staging tests at the time of toceranib initiation included thoracic radiographs ($n = 21$), abdominal ultrasound ($n = 22$), and/or computed tomography (CT) scan of the thorax and abdomen ($n = 12$). The findings of these imaging tests are summarized in Table 1.

Table 1. Summary of Significant Findings from Baseline Imaging

Staging Test	Number of Cases
Thoracic radiographs	21
No significant findings	21
Abdominal ultrasound	22
At least one hypoechoic pancreatic nodule	10
Regional lymphadenopathy	4
Multiple hypoechoic hepatic nodules	5
Thickened intestinal walls	2
Hypoechoic splenic and hepatic nodules	1*
Adrenal mass	1
Cranial abdominal mass	1
No significant findings	0
CT Scan (Thorax and Abdomen)	12
Pancreatic nodules with arterial phase enhancement	11
Regional lymphadenopathy	5
Hypoattenuating hepatic nodules with venous contrast enhancement	2
At least one hypoattenuating splenic nodule	3
Subcutaneous mass	1**
No significant findings	1
Concurrent Abdominal Ultrasound and CT Scan of Abdomen	6
Pancreatic nodule identified on CT scan alone	3

*Determined to be mast cell tumour metastasis upon FNA

****Previously diagnosed as a soft tissue sarcoma**

WHO TNM stage at the time of diagnosis was retrospectively determined for all dogs based upon the data available [30]. There were eight dogs with at least stage I, 13 with at least stage II, and nine with at least stage III disease. Locations of metastasis confirmed by either FNA or tissue biopsy included pancreatic lymph nodes ($n = 13$), liver ($n = 9$), mesenteric lymph nodes ($n = 4$), hepatic lymph nodes ($n = 1$), and spleen ($n = 1$).

Comorbidities documented at the time of toceranib initiation included pancreatitis ($n = 2$), hypertension ($n = 2$), osteoarthritis ($n = 2$), low grade mammary carcinoma ($n = 2$), high grade soft tissue sarcoma ($n = 1$), hepatocellular carcinoma ($n = 1$), metastatic mast cell tumour ($n = 1$), intervertebral disc disease ($n = 1$), meningoencephalitis ($n = 1$), epilepsy ($n = 1$), hyperadrenocorticism ($n = 1$), hypothyroidism ($n = 1$), bilateral keratoconjunctivitis sicca ($n = 1$), tracheal collapse ($n = 1$), and cranial cruciate ligament rupture ($n = 1$).

Treatment

Twenty-one dogs underwent a partial pancreatectomy for treatment of insulinoma prior to initiating toceranib. One dog had a second surgery performed for progressive disease two years after an initial partial pancreatectomy, and then commenced toceranib. One dog also underwent regional lymph node extirpation at the time of partial pancreatectomy. Reasons for toceranib initiation for the dogs that had a prior partial pancreatectomy included regional lymph node metastasis at diagnosis ($n = 8$), hepatic metastasis +/- lymph node metastasis at diagnosis ($n = 7$), inability to excise pancreatic mass ($n = 2$), recurrence of clinical signs associated with hypoglycemia ($n = 3$), recurrent pancreatic nodule 1458 days following partial pancreatectomy ($n = 1$). The median duration of time between initial surgery and toceranib treatment was 93 days (range, 4 – 1458 days).

Four dogs received cytotoxic chemotherapy prior to toceranib initiation. One of these dogs was initiated on toceranib 21 days after it received four doses of doxorubicin (30 mg/m² every 3 weeks) in the adjuvant setting following partial pancreatectomy, one received adjuvant streptozotocin with prednisone following partial pancreatectomy, one received adjuvant vinorelbine with marbofloxacin, and one received cytarabine for meningoencephalitis approximately one year prior to starting toceranib.

Six dogs received prednisone as a sole therapy for insulinoma prior to toceranib initiation. One dog received glucagon with prednisone prior to toceranib initiation, yet clinical signs persisted and glucagon was discontinued only 4 days following diagnosis. All of the dogs that received prednisone as a sole therapy were initiated on toceranib when recurrence of clinical signs associated with hypoglycemia was noted. The median duration between diagnosis and toceranib treatment for the dogs treated with prednisone alone was 50 days (range, 13 – 453 days).

Three dogs did not receive any therapies for insulinoma prior to commencing toceranib. For all three of these dogs, toceranib was offered as a first-line therapy as an alternative to surgical excision. The median duration between initial diagnosis of insulinoma to treatment with toceranib for dogs treated with toceranib as a first-line therapy was 13 days (range, 0 – 22 days).

The median initial toceranib dose administered was 2.67 mg/kg by mouth (range, 2.1 – 3.27 mg/kg). Twenty-four dogs initially received toceranib on a Monday, Wednesday, Friday (MWF) schedule and six dogs on an every other day (EOD) schedule. Fourteen dogs had a dose reduction instituted during treatment with toceranib. All of these dogs had gastrointestinal AEs. All AEs observed during toceranib treatment are listed by grade of toxicity in Table 2.

Table 2. Number of Dogs with Adverse Events During Toceranib Therapy

AE	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Gastrointestinal					
Anorexia	9	1	1	-	-
Diarrhea	7	-	-	-	-
Hematochezia	3	-	-	-	-
Gastric ulceration	-	-	-	-	1*
Vomiting	5	1	-	-	-
Constitutional					
Fever	1	-	-	-	-
Weight Loss	2	-	-	-	-
Lethargy	2	1	-	-	-
Hematologic					
Anemia	5	-	-	-	-
Neutropenia	7	2	-	-	-
Biochemical					
Azotemia	2	-	-	-	-
Increased BUN	2	-	-	-	-
Increased ALP	7	2	3	-	-
Increased ALT	5	4	2	-	-
Increased AST	2	2	-	-	-
Hypoalbuminemia	1	-	-	-	-
Increased Cholesterol	1	-	-	-	-
Hypocalcemia	1	-	-	-	-
Cardiovascular					
Hypertension	2	1	-	-	-
Renal					
Proteinuria	2	1	2	-	-

*Suspected but not confirmed

Five dogs were concurrently administered prednisone at a median dose of 0.5 mg/kg q 24 hr. (range, 0.5 - 0.8 mg/kg q 24 hr). One dog received both prednisone and streptozotocin (dose and frequency not specified) during treatment with toceranib. Two dogs received both prednisone and diazoxide (dose and frequency not specified) during treatment with toceranib.

Additional concurrent therapies and/or supportive medications listed included famotidine ($n = 5$), omeprazole ($n = 4$), maropitant ($n = 4$), enalapril ($n = 4$), metronidazole ($n = 3$), ondansetron ($n = 3$), carprofen ($n = 2$), tramadol ($n = 2$), levetiracetam ($n = 2$), zonisamide ($n = 2$), gabapentin ($n = 2$), phenobarbital ($n = 1$), amlodipine ($n = 2$), mirtazapine ($n = 1$), loperamide ($n = 1$), psyllium fiber ($n = 1$), FortiFlora™ ($n = 1$), amoxicillin/clavulanic acid ($n = 1$), enrofloxacin ($n = 1$), marbofloxacin ($n = 1$), s-adenosylmethionine/silibyn ($n = 1$), ursodiol ($n = 1$), cetirizine ($n = 1$), and cannabinoid oil ($n = 1$).

Response to Toceranib

As a retrospective study, methods of response assessment and intervals between assessments varied between institutions. Response to therapy was defined as an absence of clinical signs associated with hypoglycemia, or in the case of measurable disease, designated according to the criteria described by cRECIST [29].

cRECIST response was reported for 15 dogs [29]. Response to therapy was determined using the absence of clinical signs associated with hypoglycemia ($n = 15$), repeated imaging with abdominal ultrasound ($n = 13$) +/- thoracic radiographs ($n = 6$), or CT-scan ($n = 2$). Intervals between repeated imaging were reported to be either monthly ($n = 4$), bi-monthly ($n = 1$), or tri-monthly ($n = 10$). Of the dogs with cRECIST response reported, 66.7% of dogs experienced a measurable response; six (40%) dogs experienced CR, one (6.7%) dog experienced a PR, three (20%) dogs experienced SD, and five (33.3%) dogs experienced PD. All of the dogs that had either CR, PR, or SD were also reported to be normoglycemic at each response assessment.

Twelve dogs had no new reported clinical signs and repeated blood glucose measurements reported as methods utilized to monitor toceranib response. The median duration of reported normoglycemia was 275 days (range, 12 – 727 days). The remaining three dogs did not have repeated imaging or regular blood glucose measurements reported, but were monitored for recurrence of clinical signs associated with hypoglycemia.

Nineteen dogs ultimately discontinued toceranib. Seventeen of these dogs discontinued toceranib when PD was observed. One dog discontinued toceranib after 883 days due to increased liver enzyme values on monitoring bloodwork. One dog discontinued toceranib after 1261 days due to gastrointestinal adverse events. One dog had splenic metastasis confirmed by FNA after 288 days of toceranib therapy, yet continued to receive toceranib despite PD. The median duration of toceranib therapy for all dogs was 281 days (range, 10 – 727).

One dog that developed progressive disease on toceranib was treated with masitinib following discontinuation of toceranib, but clinical signs worsened acutely, and masitinib was discontinued. Following discontinuation of masitinib, metronomic cyclophosphamide (15 m/m² every 24 hours) was initiated for 6 days before the dog was euthanized for progressive clinical signs.

Time-to-event outcomes: Kaplan-Meier survival curves

Disease progression during toceranib therapy was ultimately observed in 20 dogs (66.6%). In absence of documentation of PD, disease progression was considered not observed for 10 dogs, up until the last veterinary visit, and PFI was right-censored for these cases. The Kaplan-Meier median overall PFI from time of toceranib initiation was 561 days (95% confidence interval (CI): [246, 727 days]) (Figure 1). The probability of progression free by 100, 250, and 500 days was estimated at 0.83 (standard error (SE) ± 0.07), 0.69 (SE ± 0.09), and 0.52 (SE ± 0.10), respectively.

Death attributed to PD was observed for 20 dogs, although details of PD were lacking for five dogs. One dog died of a suspected gastrointestinal perforation 154 days after toceranib initiation, although this was not confirmed by necropsy. In the absence of a death record, OST was right-censored for nine dogs that were alive and still taking toceranib at the time of last assessment. The estimated median OST from diagnosis was 656 days [310, 1045 days] (Figure 2). The probability of survival by 100, 250, 500, and 1000 days was estimated at 0.93 (SE ± 0.05), 0.77 (SE ± 0.08), 0.51 (SE ± 0.10) and 0.34 (SE ± 0.10), respectively.

Time- to event outcomes: Proportional Hazards modeling

Proportional hazards modeling was implemented on each of the two right-censored variables (i.e. PFI and OST). Potential explanatory non-time-dependent covariates (e.g. sex, age, weight, treatment facility, tumour stage, therapies prior to toceranib (e.g. partial pancreatectomy, chemotherapy, and/or prednisone), dosing frequency, and toceranib dose) and all 2-way interactions were considered for inclusion in the models.

For overall PFI, there was evidence of a significant association between time to progression and therapies prior to toceranib ($P = 0.0050$), type of veterinary practice ($P = 0.0025$), and weight ($P = 0.0301$). Specifically, after accounting for type of veterinary practice and weight, dogs that had prior therapies showed an estimated hazard ratio (HR) for disease progression of 8.4 (95% CI: [2.4, 41.4]), relative to dogs that that did not have prior therapy.

Moreover, dogs treated in an academic institution showed an estimated HR for disease progression of 4.6 [1.5, 14.2] relative to dogs treated in private practice, after adjusting for therapies prior to toceranib and weight. And lastly, regardless of therapies prior to toceranib and type of veterinary practice, every 1 kg increase in body weight at diagnosis increased the hazard of disease progression by an estimated multiplier of 1.045 [1.003, 1.084]. There was no evidence of any association between PFI and any other proposed explanatory covariates at a 5% level of significance ($P > 0.17$).

In evaluation of OST, the only relevant explanatory variable was weight at diagnosis, whereby for every 1 kg increase in body weight at diagnosis, there was an increased hazard of death by an estimated multiplier of 1.050 [1.012, 1.090]. There was no evidence of any association between OST and any of the other proposed explanatory covariates at a 5% level of significance ($P > 0.18$).

Discussion

The objectives of this retrospective study were to describe the duration of clinical benefit, defined as absence of clinical signs associated with hypoglycemia and/or measurable response according to cRECIST, and measures of outcome in dogs diagnosed with insulinoma treated with toceranib [29]. The initial clinical signs attributed to hypoglycemia noted at diagnosis were reported to be improved with the addition of toceranib therapy in all cases. The majority (66.7%) of dogs with measurable disease at the time of toceranib initiation were not reported to have recurrent clinical signs, and experienced either a CR, PR, or SD. For all 30 dogs, there was an overall median PFI and OST of 561 days and 656 days, respectively. Overall, toceranib was reported to be well-tolerated; the majority of adverse events reported were grade 1 or 2 gastrointestinal toxicities.

Prior studies that have evaluated OST for dogs treated for insulinoma have found that young age, stage II or III disease, and medical therapy were poor prognostic factors for survival, none of which were found to be significantly associated with either PFI or OST in our study [4, 7, 31]. These inconsistencies are most likely a result of variation in the study population and small numbers, precluding robust statistical analyses. In the present study, the retrospective application of the WHO TNM stage based upon the available case information may have underestimated some case stages. To better elucidate prognostic factors and the influence that disease stage has on outcome, a standardized approach to diagnosis and case management would be ideal.

In the present study, dogs that had therapy for insulinoma prior to toceranib had a greater hazard for disease progression than dogs that received toceranib as a first-line therapy. This could be a result of case selection bias. The majority of the dogs that had prior therapies had residual disease or metastatic disease at the time of toceranib initiation, and dogs that had prior therapies may have also had disease that was inherently or had eventually become resistant to therapy.

Additionally, dogs in our study that were treated at academic institutions had an increased hazard for disease progression, relative to those treated at a private practice. Previous retrospective studies describing therapy for canine insulinoma were performed primarily at academic hospitals [4, 7, 10, 31]. Our study's finding could be explained by biases not specifically evaluated in this study, such as potentially more comprehensive follow-up documentation at academic institutions, or perhaps the enrollment of dogs with more advanced disease. The clinical relevance of this finding is unknown, and comparisons of temporally and demographically disparate and distinct study populations could be misleading. Prospective, randomized or controlled cohort studies would be required to compare these treatment settings and evaluate the clinical implications.

Finally, in the present study, there was also an increased hazard of both disease progression and death for every 1 kg increase in body weight at diagnosis. Canine insulinomas have commonly been reported in medium and large-breed dogs, yet body weight has not been previously described to be associated with prognosis [1, 3, 4, 6, 8, 12].

Similar to previous reports of toceranib administration in dogs, gastrointestinal AEs were the most commonly reported type of AE in the dogs in this study [18,32,33]. The majority of the gastrointestinal AEs were grade 1 or grade 2, but 14 dogs experienced gastrointestinal toxicities that necessitated a dose modification. The dog receiving the highest dose of toceranib in this study did experience a complete clinical response during the treatment period but was presented on an emergency basis 154 days after initiation of toceranib with abdominal pain, abdominal effusion, and suspected gastrointestinal perforation. Although a necropsy was not performed, it is possible that this patient experienced a treatment-associated grade 5 gastrointestinal AE. This case highlights the fact that, while most dogs have only mild to moderate AEs associated with toceranib, it is not without risk [16-27,33]. Given that earlier studies evaluating toceranib doses of 2.5 to 3.5 mg/kg found no differences in biologic activity observed at higher doses, it may be reasonable to administer toceranib at a dose between 2.4 and 3.0 mg/kg to avoid severe gastrointestinal toxicity and frequent drug holidays when treating dogs with insulinoma [32,34].

Additional AEs reported in this study included a combination of grades 1 – 3 constitutional, hematologic, and biochemical AEs. These AEs did not result in overt clinical signs, nor necessitate dosing modifications. Therefore, when comparing medical treatment options for insulinoma, the AE profile of toceranib is seemingly more tolerable than that described for alloxan or streptozotocin [10, 12]. Meleo et al. described the use of alloxan for treatment of five canine insulinomas, and reported complications including renal tubular necrosis, acute renal failure, and persistent hyperglycemia [12]. Development of diabetes mellitus was reported in 42.1% of the dogs treated with streptozotocin in a prospective study by Northrup et al., resulting in euthanasia in the majority of dogs (6/8; 75%) experiencing this AE [10].

In humans, there are reports of diabetic and non-diabetic patients experiencing significant decreases in blood glucose measurements following treatment of various neoplasms with sunitinib [35]. This phenomenon has also been reported in humans treated with other tyrosine kinase inhibitors, including dasatinib, imatinib, and sorafenib [35]. The mechanism by which these tyrosine kinase inhibitors (TKIs) affect blood glucose levels is not well-understood, but one theory hypothesizes that it may be secondary to inhibition of the downstream pathway normally initiated by the binding of stem cell factor (SCF) to its receptor [35]. The gene responsible for encoding the receptor for SCF, c-kit, has been demonstrated to be expressed by insulin-producing pancreatic β -cells in rats [36]. Considering that blood glucose measurements are an integral part of monitoring PFI in patients with insulinoma, use of TKIs in treatment of insulinoma may cause iatrogenic hypoglycemia and impair the ability to monitor treatment response. The authors are unaware of documented hypoglycemia associated with toceranib administration in dogs, but it is important to be aware of this potential issue when selecting toceranib for the management of insulinoma.

Further support for the utilization of toceranib in the management of canine insulinomas could be provided by molecular studies. Human malignant pNET tissues have widespread expression of the receptor tyrosine kinases (RTKs) for platelet-derived growth factor (PDGF)- α/β , and vascular endothelial growth factor (VEGF)-2 and -3, and these tumours are clinically responsive to receptor tyrosine kinase inhibition [15, 37]. Although there have been no veterinary studies verifying the expression of RTKs on endocrine pancreatic tumours such as insulinomas, other neoplastic neuroendocrine histotypes, including thyroid carcinomas and apocrine gland anal sac adenocarcinomas, have been shown to express targets of toceranib [16, 17].

One challenge of this study was accurately defining the extent of disease and objectively measuring response to therapy. As a retrospective study, the diagnostic and staging tests were preexisting and the decisions influenced by owner and clinician preferences. In addition to paired fasting insulin and glucose ratio, the work-up of a dog with suspected insulinoma ideally incorporates both thoracic and abdominal imaging [1, 2, 38]. In the present study, all 30 dogs had either thoracic radiographs and abdominal ultrasound, and/or CT scan of the thorax and abdomen performed for complete staging purposes. In the 22 dogs for which thoracic radiographs were performed, no overt evidence of pulmonary metastatic disease was noted at diagnosis. This finding is consistent with previous reports of dogs with insulinoma in which pulmonary metastasis were not observed at diagnosis [4, 6-10]. Twenty-three dogs had abdominal ultrasound reports available. For these dogs, a pancreatic mass was identified in just 10 dogs (43.5%), which is comparable to previous reports that describe abdominal ultrasound sensitivities ranging from 28% to 75% for detection of insulinoma in dogs [6, 7, 48]. CT scan identified a pancreatic mass in 10/12 (83%) dogs in which it was performed, including three dogs where the mass was not evident on ultrasound. Although CT scan has been shown to identify 71.4% of primary insulinomas in dogs, contrast-enhanced CT has also been reported to inaccurately locate insulinomas in dogs, and intraoperative evaluation of the pancreas and potential metastatic lesions is proposed to be superior for staging purposes [38, 39].

There is also the potential for misdiagnosing metastatic lesions utilizing diagnostic imaging, with one study indicating that 37.5% of suspected intra-abdominal metastases were negative for insulinoma [7]. The original TNM classification for dogs diagnosed with insulinoma recommends that lymph nodes be examined by laparotomy or laparoscopy [30]. Additionally, multiple veterinary studies have observed the advantage of surgical treatment of insulinomas even when intra-abdominal metastatic disease was present [6, 8, 9]. Considering that exploratory laparotomy has both diagnostic and therapeutic value, it should ideally be performed in all cases where insulinoma is suspected.

There were several limitations to this study that are inherent to its retrospective and multi-institutional design. As previously discussed, there was a lack of standardization in the methods used to confirm the diagnosis of insulinoma. Histopathology reports were not available for nine dogs, relying instead on a variable combination of clinical signs attributed to neuroglycopenia, paired blood glucose and insulin measurements, and cytology results, and/or supportive imaging findings. While not ideal, this clinical

approach is consistent with previous publications describing the diagnosis of canine insulinoma [1, 2, 38].

Additionally, our study included one dog that had a previous diagnosis of a hepatocellular carcinoma. Paraneoplastic hypoglycemia and secretion of insulin-like growth factor type-II (IGF-2) has been reported in cases of canine hepatocellular carcinoma [40, 41]. Although this dog met the diagnostic inclusion criteria for our study, the historical diagnosis of hepatocellular carcinoma may have contributed to the hypoglycemia diagnosis and later complicated follow-up blood glucose monitoring. Another dog in our study was previously diagnosed with a metastatic mast cell tumour. Once again, although this dog met the diagnostic inclusion criteria for the present study, monitoring response to therapy in this dog could have been confounded by this prior diagnosis as canine mast cell tumours have been reported to respond to toceranib phosphate [42, 43]. The methods used to monitor treatment response were also not standardized, and therefore the retrospectively applied stage at diagnosis and progression timepoints may have been underestimated. To more accurately evaluate treatment response and measures of outcome, a prospective study with standardized diagnostic and staging criteria are ideal. Finally, the lack of appropriate control populations precluded comparative analyses of endpoints.

The clinical endpoints reported in this study were also potentially confounded by the fact that the clinical setting in which toceranib was initiated was variable. Twenty-one dogs in our study were treated with partial pancreatectomy prior to initiating toceranib therapy. Prior studies report long OSTs ranging from 10 to 43 months with partial pancreatectomy alone [6-8]. Perhaps more importantly, several dogs received concurrent prednisone and other supportive medications, which have the potential to impact both the PFI and OST. Previous retrospective studies have described improved outcomes for dogs with insulinoma receiving adjuvant prednisone therapy alone [6]. Furthermore, owner decisions regarding euthanasia are subjective and influenced by a spectrum of factors, including perceived quality of life, which in turn may be impacted by supportive medications that ameliorate clinical signs.

Conclusions

Dogs with insulinoma treated with toceranib can experience clinical benefit while experiencing minimal adverse events. Although the results of this retrospective study are encouraging, larger scale, prospective, randomized clinical trials in dogs with insulinoma are warranted in order to better evaluate the clinical and survival benefit of toceranib in the management of canine insulinomas.

Methods

The primary objectives of this study were to describe the duration of clinical benefit, defined as absence of clinical signs associated with hypoglycemia and/or measurable response according to cRECIST, and measures of outcome in dogs diagnosed with insulinoma treated with toceranib [29]. A secondary objective was to describe the adverse effects of toceranib in dogs with insulinoma.

Case Selection

The medical record databases of five university teaching hospitals and eight veterinary specialty referral centers were searched for cases in which toceranib phosphate was used to treat dogs diagnosed with neuroendocrine pancreatic neoplasia. Inclusion criteria were: 1) documentation of fasting hyperinsulinemia with paired hypoglycemia, and/or cytologic or histologic diagnosis of a primary pancreatic tumour with neuroendocrine morphology, 2) other causes of hypoglycemia, including sepsis, hepatic failure, adrenocortical insufficiency, and toxin ingestion were reasonably investigated and eliminated 3) details of any prior, concurrent or subsequent treatments, 4) toceranib dosage and schedule, 5) at least one documented follow-up assessment during the toceranib treatment period. Exclusion criteria were: 1) absence of an acceptable clinical diagnosis of insulinoma, 2) insufficient details regarding toceranib treatment, as described in the inclusion criteria, and 3) lack of a documented follow-up assessment.

Information collated from the medical records included signalment, clinical signs at diagnosis, insulin and glucose measurements at diagnosis, the results of any additional clinicopathologic or histologic testing, blood pressure measurement at diagnosis, stage of disease at time of toceranib initiation, dosage and treatment interval of toceranib, reported adverse events (AEs), duration of toceranib therapy, response to therapy, duration of glycemic control while on toceranib, therapies following toceranib if progressive disease was detected, comorbidities, concomitant medications, and the date and reason for death or euthanasia. Stage was retrospectively classified according to the WHO TNM system, with stage I indicating tumour confined to the pancreas, stage II indicating lymph node metastasis, and stage III indicating distant metastasis [30]. AEs were retrospectively graded according to the Veterinary Co-operative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1 [44]. Survival information was obtained from the medical record or telephone conversation with the referring veterinarian and/or owners.

Endpoints were response to therapy, progression-free interval (PFI), and overall survival time (OST). Response to therapy was defined as an absence of clinical signs associated with hypoglycemia and/or measurable response according to cRECIST [29] (Table 3).

Table 3. Criteria for response (cRECIST v1.0) [29].

Response	Criteria
Complete response (CR)	Resolution of all target and non-target lesions, no new lesions
Partial response (PR)	$\geq 30\%$ reduction in the sum of diameters of target lesions, no progression of non-target lesions, and no new lesions
Stable disease (SD)	$< 30\%$ reduction or $< 20\%$ increase in sum of diameters of target lesions, no new lesions
Progressive disease (PD)	Either appearance of one or more new lesions, or $\geq 20\%$ increase in sum of diameters of target lesions, with the sum having an absolute increase of 5 mm

Clinical benefit and response to therapy was evaluated through fasting blood glucose measurements, the presence or absence of clinical signs associated with neuroglycopenia, and/or in the case of measurable disease repeated abdominal ultrasound, computed tomography (CT), or thoracic radiographs.

Duration of toceranib therapy was calculated in days from the time of toceranib initiation to the time of diagnosed disease progression, date of return of clinical signs associated with neuroglycopenia, date of toceranib discontinuation, or to the last date of data submission. PFI was defined as the interval in days from the date of toceranib initiation to the date of reported disease progression or date of toceranib discontinuation. Disease progression was defined as either local progression, the development of metastasis or metastatic disease progression, or the return of clinical signs associated with neuroglycopenia. For cases for which disease progression was not observed, PFI was considered right-censored and defined as interval in days from the date of toceranib initiation to the last date of data submission. OST was defined as the interval in days from the date of diagnosis to the date of death or euthanasia. For cases for which death was not recorded, OST was considered right-censored and defined as the interval from the date of diagnosis to the date of last data submission.

Statistical Analysis

The Kaplan-Meier estimator was used to estimate the survival distribution of each of two right-censored response variables (i.e. PFI and OST). This estimator is a non-parametric statistic that is commonly used in the medical literature to estimate the fraction of patients that have not shown an undesirable event, such as death or disease progression, for certain amount of time. Key to Kaplan Meier curves is that they can take into account right-censored data, which occurs in cases that are lost-to-follow-up or for which the event of interest has not been observed at last follow-up. Censoring criteria were lack of progression diagnosis and alive status for PFI and OST, respectively, at end of the follow-up period. Computations

were conducted using the LIFETEST procedure of SAS (Version 9.4, Cary, NC). Confidence intervals at given time point were calculated based on log-log transformations.

A Cox proportional hazards (PH) model was fitted to each response PFI and OST using the censoring criteria described in the previous paragraph. The Cox PH model is a parametric survival method that further refines the Kaplan Meier approach by enabling covariate adjustments, thus enabling assessment of potential risk factors. The linear predictor considered the following explanatory non-time-dependent covariates, namely sex, age at diagnosis, weight at diagnosis, type of veterinary practice, tumour stage, therapies prior to toceranib, dosing frequency, toceranib dose, veterinary clinic, and all 2-way interactions. The variable of tumour size was excluded from model selection as over 20% of observations were missing. Purposeful selection of covariates into the model was conducted using stepwise selection at a 10% level of significance for entry into the model and 15% level of significance for removal. For each model, the proportional hazard assumption was evaluated. Computations were conducted using the PHREG procedure of SAS (Version 9.4, Cary, NC).

Abbreviations

AE, adverse event; BP, blood pressure; CI, confidence interval; CR, complete response; CT, computed tomography; EOD, every other day; FDA, Food and Drug Administration; FNA, fine needle aspirate; HR, hazard ratio; MWF, Monday, Wednesday, Friday; OST, overall survival time; pNET, pancreatic neuroendocrine tumour; PD, progressive disease; PDGF, platelet-derived growth factor; PFI, progression free interval; PH, proportional hazards; PR, partial response; RTKs, receptor tyrosine kinases; cRECIST, Response Evaluation Criteria in Solid Tumours in Dogs; SCF, stem cell factor; SD, stable disease; SE, standard error; TKIs, tyrosine kinase inhibitors; WHO, World Health Organization; VEGF, vascular endothelial growth factor; VCOG-CTCAE, Veterinary Comparative Oncology Group Common Terminology Criteria for Adverse Events

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE: Not applicable as this was a retrospective records review.

CONSENT FOR PUBLICATION: Not applicable.

AVAILABILITY OF DATA AND MATERIALS: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS: The authors have no competing interests or conflicts of interest to declare.

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AUTHORS' CONTRIBUTIONS: SSO, NB, and RMW designed this retrospective study. SSO, RMW, CJ, SH, BB, TM, MM, BH, and EW supervised case information collection. NB supervised data analysis. SSO, NB, RMW, CJ, SH, and BB assisted in writing and final editing of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Estimated Kaplan-Meier survival curve for median overall progression free interval for 30 dogs with insulinoma treated with toceranib. In absence of documentation of PD, disease progression was considered not observed for 10 dogs, up until the last veterinary visit, and PFI was right-censored for these cases.

Figure 2

Estimated Kaplan-Meier survival curve for median overall survival time for 30 dogs with insulinoma treated with toceranib. In the absence of a death record, OST was right-censored for nine dogs that were alive and still taking toceranib at the time of last assessment.