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Tumour Gastrin Expression and Serum Gastrin Concentrations in Dogs with Gastric Carcinoma are Poor Diagnostic Indicators

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Summary

Hypergastrinaemia is observed commonly in human patients with gastric carcinoma and is associated with atrophic gastritis and *Helicobacter pylori* infection, both of which predispose to development of gastric tumours. Increased expression of gastrin is also described as a prognostic indicator for gastric carcinoma in man. Gastric carcinoma is rare in dogs and generally carries a grave prognosis. In this study, the expression of gastrin was investigated immunohistochemically in gastric biopsy samples from 64 dogs with gastric carcinoma. Serum gastrin concentrations were measured in 15 of these dogs and compared with those of seven healthy control dogs. Tumour tissue expressed gastrin in 8% (5/64) of the dogs with gastric carcinoma. There was no significant difference in serum gastrin concentrations between dogs with gastric carcinoma and healthy controls ($P = 0.08$). Expression of gastrin in gastric carcinomas is less common in dogs than in man and may therefore not be relied on as a prognostic marker in this species. Serum gastrin concentration alone is also not a useful biomarker for gastric carcinoma in dogs.

Keywords: dog; gastric carcinoma; gastrin; hypergastrinaemia

Canine gastric carcinomas are usually diagnosed at an advanced stage, resulting in a poor prognosis and limited treatment options. A median survival time of 35 days has been described (Swann and Holt, 2002) and 70–90% of gastric carcinomas have metastasized by the time of diagnosis or humane destruction (Sullivan *et al.*, 1987; Scanziani *et al.*, 1991; Swann and Holt, 2002). Gastric carcinoma is the second leading cause of cancer-related death in man (Jemal *et al.*, 2011) and there is an association between chronic atrophic gastritis and gastric neoplasia (Tatsuta *et al.*, 1993; Vannella *et al.*, 2012). A similar association has also been described in the Norwegian Lundehund (puffin dog) (Qvigstad *et al.*, 2008).

Gastrin is a hormone produced by the endocrine G cells in the gastric antrum (Dockray *et al.*, 2001). After secretion, gastrin binds to cholecystokinin-B receptors on enterochromaffin-like cells and parietal cells (Dockray *et al.*, 2001; Schmitz *et al.*, 2001). Atrophic gastritis causes loss of parietal cells, decreased acid production and thereby interruption of the negative feedback mechanism on the G cells, resulting in hypergastrinaemia (Burkitt *et al.*, 2009). The hypergastrinaemia subsequently stimulates growth of enterochromaffin-like cells and eventually results in neoplastic transformation (Vannella *et al.*, 2012). This transformation is usually a gradual progression from chronic atrophic gastritis to metaplasia, dysplasia and finally neoplasia (Correa, 1988; Burkitt *et al.*, 2009).

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Immunohistochemistry (IHC) has shown that 25–50% of human gastric carcinomas express gastrin (Henwood *et al.*, 2001; Hur *et al.*, 2006; Stephens *et al.*, 2007) and this expression is associated with significantly shorter survival times (Hur *et al.*, 2006; Stephens *et al.*, 2007). Serum gastrin concentrations are significantly higher in patients with gastric cancer compared with healthy controls (Rakic and Milicevic, 1991); however, serum gastrin concentration is not a useful diagnostic biomarker (Lin *et al.*, 1995), nor is it helpful prognostically or in assessing whether a tumour may be resected (Soran *et al.*, 2000). One case of concurrent gastric carcinoma and hypergastrinaemia has been reported in a dog (de Brito Galvao *et al.*, 2009). Hypergastrinaemia and expression of gastrin have been well documented in human gastric carcinomas, but these parameters have not yet been investigated in canine gastric carcinoma.

The primary aim of this study was to investigate the concentration of serum gastrin in dogs suffering from gastric carcinoma and the expression of gastrin by IHC in canine gastric carcinomas. A secondary aim was to evaluate whether serum gastrin concentration could be a useful diagnostic biomarker of gastric carcinoma in dogs. The study protocol was reviewed and approved by the National Committee of Animal Experiments Inspectorate in Denmark and the Ethics and Administrative Committee at the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark.

Gastric biopsy samples from 64 dogs with histologically confirmed canine gastric carcinoma, collected in the period 1979–2013, were obtained from pathology archives at the Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences (NMBU), and the Norwegian Veterinary Institute. The gastric lesions in eight of these dogs, all Norwegian Lundehunds, have been described previously (Qvigstad *et al.*, 2008). The gastric biopsies had been collected by gastroscopy, exploratory laparotomy or at post-mortem examination. Gastric biopsies obtained from seven healthy adult dogs undergoing routine neutering (at the Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark) served as controls. Before participating in the study, each dog's owner gave informed consent. Dogs were excluded as controls if they had experienced gastrointestinal signs in the previous 4 weeks, had a diagnosis of cancer (regardless of location), had chronic kidney disease or had received any medical treatment during the previous 4 weeks. Dogs considered to have an increased risk of complications during anaesthesia were also excluded. After neutering, gastric biopsy samples from the control dogs were obtained endoscopically (gastrointestinal endoscope EC450MP5, Fujinon Cooperation, Saitama, Japan; endoscopic biopsy forceps diameter 2.3 mm, Medwork GmbH, Höchstadt, Germany). All samples were fixed in 4% buffered formaldehyde, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE). All histological slides were re-examined during 2013, and the initial diagnoses of gastric carcinoma or normal gastric mucosa were confirmed.

Sections for IHC (3–4 μm) were subjected to one of the following pre-treatment protocols: (1) heat treated in a microwave oven in 10 mM sodium citrate buffer, pH 6 for 10 min, or (2) for the older specimens, 0.1% trypsin in 0.1 M Tris buffered saline (TBS) containing 1.1% $CaCl_2$ at 37°C for 60 min. Following antigen retrieval, endogenous peroxidase was blocked with H_2O_2 (Thermo Scientific Pierce, Waltham, Massachusetts, USA) for 20 min. The slides were incubated subsequently with TBS containing 5% bovine serum albumin (BSA) for 20 min to reduce further non-specific background staining. The sections were then incubated at 4°C overnight with the polyclonal rabbit anti-human gastrin antibody (Dako, Glostrup, Denmark) diluted 1 in 600 in TBS with 2.5% BSA. Negative controls were performed by omitting the primary antibody from the diluent. After washing three times with TBS, the sections were incubated for 30 min at 37°C with biotinylated goat anti-rabbit IgG (Dako, Glostrup, Denmark) diluted 1 in 500 and with streptavidin–horseradish peroxidase (Vector laboratories, Burlingame, California, USA) diluted 1 in 500. Labelling was 'visualized' using 3,3′-diaminobenzidine (DAB) (Dako) as chromogen. Sections were counterstained with haematoxylin and mounted.

Immunohistochemical labelling was evaluated by EJ and TSW. At least one tissue section was studied for each case, and when several sections were available, a section with both tumour tissue and normal mucosa was chosen. The presence of gastrin in normal mucosa and within the carcinoma cells was noted for each case. A single gastrin-positive cell was sufficient for classification as a positive case. Expression of gastrin in normal pyloric glands was used as a positive control.

Blood samples were collected prospectively from 15 dogs with gastric carcinoma (2010–2013). Biopsy samples from these 15 dogs were included in the pathology material for IHC. As sedation can influence serum gastrin concentration (Nakamura *et al.*, 1997), blood sampling before sedation was preferred, but when the owner chose humane destruction rather than further diagnostic investigations, the samples were collected after sedation ($n = 6$). The blood