The Veterinary Journal 198 (2013) 200–205

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Analytical validation of radioimmunoassays for the quantification of select pancreatic enzymes in jejunal fluid and fecal extracts from dogs

Niels Grützner [a,*], Ingrid Hang [b], Romy M. Heilmann [a], Thomas Spillmann [b], Jan S. Suchodolski [a], Jörg M. Steiner [a]

[a] Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4474, USA
[b] Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki 00014, Finland

ARTICLE INFO

Article history:
Accepted 16 July 2013

Keywords:
Canine
Feces
Jejunal fluid
Trypsin-like immunoreactivity
Pancreatic lipase immunoreactivity

ABSTRACT

Pancreatic enzymes, such as trypsin and lipase, are essential for the digestion of dietary components in the small intestine. Measurement of both enzymes in jejunal fluid and fecal specimens from dogs has not been reported and will be a prelude for further investigations. Therefore, the aim of the study was to validate radioimmunoassays (RIAs) for the measurement of canine trypsin-like immunoreactivity (cTLI) and pancreatic lipase immunoreactivity (cPLI) in jejunal fluid and fecal specimens from dogs. Jejunal fluid and fecal specimens were collected from five healthy Beagles. A commercial ^{125}I-RIA was used for measuring cTLI concentrations and an in-house ^{125}I-RIA was modified for the quantification of cPLI in jejunal fluid and fecal specimens. Both RIAs were analytically validated for canine jejunal fluid and fecal specimens by determining dilutional parallelism, spiking recovery, and intra- and inter-assay variability. For both cTLI and cPLI in jejunal fluid, observed-to-expected ratios for dilutional parallelism and spiking recovery ranged from $\geqslant$77.0% to $\leqslant$115.3% and $\geqslant$79.0% to $\leqslant$120.0%, respectively, and from $\geqslant$87.2% to $\leqslant$118.5% and $\geqslant$74.6% to $\leqslant$116.1%, respectively, for fecal specimens. Intra- and inter-assay coefficients of variation (%CV) for both cTLI and cPLI in jejunal fluid were $\leqslant$7.6% and $\leqslant$10.0%, respectively, and were $\leqslant$10.8% and $\leqslant$9.0%, respectively, for fecal specimens. Both RIAs were demonstrated to be linear, accurate, precise, and reproducible for use with jejunal fluid and fecal specimens from dogs. These results are important for the investigation of pancreatic enzyme concentrations in the gastrointestinal lumen in response to changes in dietary components.

Introduction

The exocrine portion comprises about 90–95% of the entire pancreas and is responsible for the synthesis, storage, and release of digestive zymogens (e.g. trypsinogen) and enzymes (e.g. pancreatic lipase) (Pandol, 2002). Pancreatic enzymes, such as trypsin and lipase, are crucial for the digestion of dietary components in the small intestine, including protein, fat, carbohydrates, DNA, and RNA (James et al., 2009). Over the last decades, the relationship between nutritional substrates and the secretion of pancreatic enzymes (e.g. amylase, lipase, trypsinogen and chymotrypsinogen) has been investigated (Stock-Damge et al., 1984; Chowdhury et al., 2000; Lee et al., 2006). In this context, studies in rats showed that high dietary fat and low/high carbohydrate content alters the synthesis and secretion of pancreatic amylase and pancreatic lipase (Chowdhury et al., 2000; Lee et al., 2006). In dogs, it has been shown that a regular diet supplemented with wheat bran increases secretions of the exocrine pancreas, but did not alter intestinal enzyme activities with regard to carbohydrate and protein absorption (Stock-Damge et al., 1984).

In recent years, canine trypsin-like immunoreactivity (cTLI), an analyte that assesses the concentration of trypsinogen, trypsin and some trypsin molecules bound to protease inhibitors, and canine pancreatic lipase immunoreactivity (cPLI), an analyte that assesses lipase specifically originating from pancreatic acinar cells, have been investigated in serum from dogs using species-specific assays (Williams and Batt, 1988; Steiner et al., 2006). The analyte measured as cTLI in serum originates exclusively from the exocrine pancreas; it reflects the amount of functional pancreatic tissue and leads to traces of cTLI that can be measured in healthy and diseased dogs (Fig. 1). For instance, it has been shown that dogs with exocrine pancreatic insufficiency (EPI) have significantly decreased serum cTLI concentrations (Wiberg et al., 1999; Battersby et al., 2005). However, a study showed that serum cTLI concentrations were unaffected in healthy dogs fed a variety of different diets

* Corresponding author. Tel.: +1 979 4582293.
E-mail address: ngruetzner@cvm.tamu.edu (N. Grützner).

1090-0233/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2013.07.022

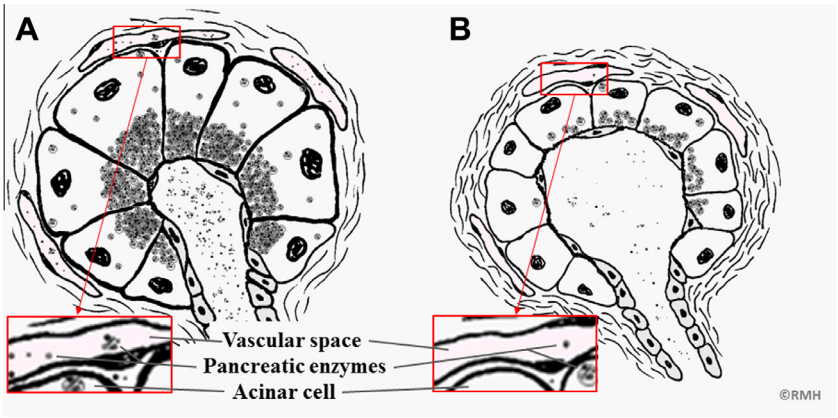

Fig. 1. Canine trypsin-like immunoreactivity (cTLI) in healthy dogs and dogs with exocrine pancreatic insufficiency (EPI). Immunoassays for serum cTLI measure cationic trypsinogen, trypsin and trypsin molecules bound to proteinase inhibitor molecules in the circulation. Trypsinogen and trypsin are specific for pancreatic acinar cells and enter the vascular and/or lymphatic spaces from pancreatic acinar cells. Because the analyte measured as cTLI in serum originates exclusively from the exocrine pancreas, it reflects the amount of functional pancreatic tissue present, leading to (A) traces of cTLI that can be measured in healthy individuals. (B) In dogs with EPI the concentration of cTLI in the serum is markedly decreased.

(James et al., 2009), indicating that serum cTLI lacks sensitivity to detect changes in exocrine pancreatic secretion due to diets of different composition. The serum cPLI concentration is specific for exocrine pancreatic function and an increased serum concentration is specific for pancreatitis (Steiner et al., 2008; Neilson-Carley et al., 2011), but also has been reported to be unaltered in response to different diets in healthy dogs (James et al., 2009).

In healthy dogs, only traces of pancreatic enzymes, such as pancreatic lipase, enter the vascular space, whereas increased amounts of pancreatic enzymes escape into the systemic circulation from the inflamed pancreas and lead to increases in serum cPLI concentrations (Fig. 2). In this context, measurement of the pancreatic secretion of cTLI and/or cPLI in small intestinal jejunal fluid or feces, which has not been investigated previously, may be more sensitive and thus may be better suited for evaluating the effect of dietary composition on exocrine pancreatic enzyme secretion.

To date, only an assay for the measurement of elastase-1, another exocrine pancreatic enzyme, has been established for use in dogs to investigate pancreatic function using fecal samples. Dogs with exocrine pancreatic insufficiency have been shown to have decreased fecal elastase-1 concentrations. However, it has also been reported that healthy dogs and dogs with chronic enteropathies can show a large degree of variation in fecal elastase-1 concentrations (Spillmann et al., 2000, 2001; Wiberg et al., 2000; Battersby et al., 2005). Thus, the aim of the present study was to analytically validate two radioimmunoassays (RIAs) for the quantification of cTLI and cPLI in jejunal fluid and fecal specimens as a prelude to further investigate cTLI and cPLI concentrations in small intestinal jejunal fluid or fecal samples from healthy and diseased dogs in response to feeding diets of various compositions.

Materials and methods

Sample population, collection and processing of specimens

Five jejunal fluid and five fecal specimens were collected from each of five healthy Beagle dogs with a permanent jejunal fistula (approximately 60 cm distal to the pylorus) for an unrelated study. All Beagle dogs were 5-year-old intact males. Health was assumed based on defecation history (no history of diarrhea for at least 4 weeks), unremarkable physical examination and normal results of standard laboratory test in blood (hematology, clinical chemistry) and feces (parasitology). The dogs were fed twice daily with different diets, as described previously (Hang et al., 2012). They were housed individually in a research colony at the Faculty of Veterinary Medicine, University of Helsinki, Finland, and were regularly vaccinated and dewormed. The protocol for collection had been reviewed and approved by the Finnish Ethics Committee (license number ESLH-2008-04002/Ym-23).

Jejunal fluid was collected through permanent jejunal nipple valve fistulas 2 h after feeding, as described previously (Harmoinen et al., 2001). Fecal samples were obtained after the morning feeding time. Fecal samples were collected immediately

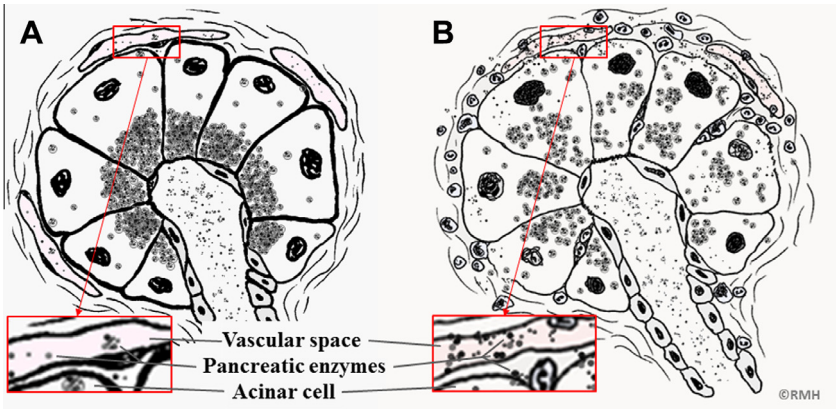

Fig. 2. Canine pancreatic lipase immunoreactivity (cPLI) in healthy dogs and dogs with pancreatitis. The immunoassay for serum cPLI measures lipase that is exclusively synthesized by pancreatic acinar cells after its release or leakage from pancreatic acinar cells into the vascular and/or lymphatic space of the pancreas. (A) While only traces of pancreatic enzymes, such as pancreatic lipase, enter the vascular space in healthy dogs, (B) an increased amount of pancreatic enzymes escape into the systemic circulation from the inflamed pancreas and lead to increases in serum cPLI concentrations.