Cellular immunolocalization of gastric and pancreatic lipase in various tissues obtained from dogs

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Objective—To determine cellular immunolocalization of canine gastric lipase (cGL) and canine pancreatic lipase (cPL) in various tissues obtained from clinically healthy dogs.

Sample Population—Samples of 38 tissues collected from 2 clinically healthy dogs.

Procedures—The cGL and cPL were purified from gastric and pancreatic tissue, respectively, obtained from dogs. Antisera against both proteins were developed, using rabbits, and polyclonal antibodies were purified by use of affinity chromatography. Various tissues were collected from 2 healthy dogs. Primary antibodies were used to evaluate histologic specificity. Replicate sections from the collected tissues were immunolabeled for cGL and cPL and examined by use of light microscopy.

Results—Mucous neck cells and mucous pit cells of gastric glands had positive labeling for cGL, whereas other tissues did not immunoreact with cGL. Pancreatic acinar cells had positive labeling for cPL, whereas other tissues did not immunoreact with cPL.

Conclusions and Clinical Relevance—We concluded that cGL and cPL are exclusively expressed in gastric glands and pancreatic acinar cells, respectively. Also, evidence for cross-immunoreactivity with other lipases or related proteins expressed by other tissues was not found for either protein. Analysis of these data suggests that gastric lipase is a specific marker for gastric glands and that pancreatic lipase is a specific marker for pancreatic acinar cells. These markers may have clinical use in the diagnosis of gastric and exocrine pancreatic disorders, respectively. (Am J Vet Res 2002;63:722–727).

Serum lipase activity has been used as a diagnostic tool for pancreatitis in humans and dogs for several decades. However, it is recognized that serum lipase activity has low specificity for pancreatitis and that pancreatic disorders, respectively. (Am J Vet Res 2002;63:722–727).

This is attributable to the fact that many types of cells secrete lipases. Lipases are defined as water-soluble enzymes that hydrolyze lipids into more polar lipolysis products. All lipases share a catalytic triad formed by serine, aspartic acid, and histidine, but overall, they have only limited amino acid sequence homology. Pancreatic lipase (most appropriately referred to as classical pancreatic lipase as a result of the discovery of pancreatic lipase-related proteins 1 and 2) was the first lipase discovered. Many other lipases, such as gastric lipase, hepatic lipase, lipoprotein lipase, hormone-sensitive lipase, and other less prominent lipases, have been described.

We hypothesize that canine gastric lipase (cGL) and canine pancreatic lipase (cPL) can be useful markers for clinical evaluation of gastric and exocrine pancreatic function, respectively. For this to be true, cGL and cPL must be organ-specific; thus, cGL must be synthesized and secreted only by cells of the stomach, and cPL must be synthesized and secreted only by pancreatic acinar cells.

Immunolocalization has commonly been used to establish the cellular localization of many cell products, including collagen, papilloma virus antigen, ATP diphosphohydrolase, hyaluronic acid binding protein, inter-tα-trypsin inhibitor, glutaredoxin, and neuronal apoptosis inhibitory protein. The substance to be evaluated by use of immunolocalization must be antigenic, and antiserum directed against this antigen must be developed in an appropriate host.

Several basic methods exist for immunolocalization. For 1 method, the primary antibody is directly linked with a reporter molecule, such as a fluorescent substance or an enzyme. The tissue that may contain the antigen is incubated with the primary antibody, and excess antibody is removed by washing after incubation. When a fluorescent label is used, the antigen in a cell can be viewed directly by use of fluorescent light during microscopy. When an enzyme is used as a label, a substrate for the enzyme must be provided, and a positive reaction can be identified by a color change of the substrate. Only cells that contain the antigen in question will have a positive reaction. Alternatively, a secondary antibody directed against the primary antibody can be used. This secondary antibody can then be linked with a reporter molecule, as described previously. Finally, the primary or secondary antibody can be linked to a hapten such as biotin or digoxigenin, which can then be detected by reporter molecules bound to avidin or by yet another antibody directed against the hapten. Use of a sec-
ondary antibody or a hapten provides an advantage, because multiple steps allow amplification of the localization reaction, making these latter methods more sensitive.21

The objective of the study reported here was to examine whether cGL was specific for gastric glands and cPL was specific for pancreatic acinar cells. We also wanted to determine cellular immunolocalization of cGL and cPL in various tissue sections obtained from healthy dogs.

Materials and Methods

Purification of cGL—Purification of cGL was performed as described elsewhere. Briefly, gastric lipase was extracted by soaking tissue obtained from the stomach of a dog in 20 mM glycine solution, pH 2.5. The extract was further purified, using cation-exchange chromatography, anion-exchange chromatography, and size-exclusion chromatography. Purity of gastric lipase was determined by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Identity of the purified lipase was confirmed on the basis of lipolytic activity, estimation of molecular mass, and determination of N-terminal amino acid sequence of the first 25 amino acid residues.

Purification of cPL—Purification of cPL is provided in detail elsewhere.2 Briefly, a defatted pancreatic extract was prepared from pancreatic tissue obtained from a dog. The pancreatic extract was further purified, using anion-exchange chromatography, size-exclusion chromatography, and cation-exchange chromatography. Purity of pancreatic lipase was determined by use of SDS-PAGE. Identity of the purified lipase was confirmed on the basis of lipolytic activity, estimation of molecular mass, and determination of N-terminal amino acid sequence of the first 25 amino acid residues.

Production and purification of polyclonal antiserum—Antiserum directed against cGL was obtained. Two New Zealand White rabbits were repeatedly inoculated with purified cGL emulsified in complete Freund adjuvant for the first injection and incomplete Freund adjuvant for all following injections. Antiserum directed against cPL was obtained in a similar manner. Two New Zealand White rabbits were repeatedly inoculated with purified cPL emulsified with a commercial adjuvant for the first 2 injections and with incomplete Freund adjuvant for 9 additional injections. The amount of protein injected varied depending on the titer of the primary antiserum. The first tubes were used to determine total counts (TC), and each received 100 µl of tracer alone. The other 2 tubes were used to determine nonspecific binding (NB), and each received 100 µl of tracer and 200 µl of RIAB. All tubes were thoroughly mixed by use of a vortex and incubated at room temperature (18 to 24 C) for 2 hours. Except for the TC tubes, 100 µl of a solution of carrier serum (1 ml of normal rabbit serum and 99 ml of RIAB) was added to each tube, followed by 1 ml of a commercially available precipitation solution.3 Tubes were mixed and then centrifuged (3,000 X g for 20 minutes at 4 C). Supernatant was carefully decanted, and radioactivity of tubes was counted for 1 minute. Results for each antiserum dilution were expressed as follows:

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\text{Percentage binding} = \left( \frac{\text{mean TC} - \text{mean NB}}{\text{mean count} - \text{mean NB}} \right) \times 100
\]

Polyclonal antisera were purified by use of affinity chromatography. Briefly, affinity chromatography columns were prepared for cGL and cPL, respectively, using the manufacturer’s instructions. After lipoprotein precipitation and change of the buffer solution (75 mM Tris-HCl, 150 mM NaCl, pH 8.0), antiserum was applied to the respective column. After eluent from the column had returned to baseline values, the column was washed with a solution of 100 mM glycine and 500 mM NaCl, pH 3.0. Concentration of the eluent was adjusted to approximately 1 mg/ml; the eluent then was frozen and stored at ~80 C.

Preparation of tissue specimens—Tissue specimens were collected from 2 healthy dogs that were euthanized as part of an unrelated pharmacokinetic study. Tissue specimens were fixed in neutral-buffered 10% formalin for 24 hours. Sections were collected from the striated muscles, skin, eyes, cerebral cortex, cerebellum, medulla, hypophysis, spinal cord, thyroid gland, parathyroid glands, adrenal glands, lymph nodes, spleen, cardiac muscle, liver, gall bladder, pancreas, prostate, and testes. After fixation, sections were embedded in paraffin. Briefly, tissues were placed in a graded series of alcohol solutions (70, 90, 95, and 100% ethanol). Then, the slides were placed in a solution of xylenes or a xylene substitute, which was followed by insertion into 2 paraffin baths at 60 C. Sections were cut at a thickness of 3 µm, placed on silane-coated glass slides, and dried at 18 to 24 C.

Immunohistochemical analysis—Slides were deparaffinized by placing them in 2 solutions of xylene substitute and rehydrated by washing them in a series of ethanol solutions, using the reverse order of concentrations described for the dehydration procedure. In the last rehydration step, slides were washed in water. Each slide then was blocked by addition of a protein solution for 30 minutes at 18 to 24 C. Slides were rinsed with 50 mM Tris-HCl (pH 7.6) and incubated with monospecific anti-cGL antibody or monospecific anti-cPL antibody (approx 1 mg/ml) at dilutions of 1:1,000 to 1:10,000 in primary antibody diluent for 30 minutes at 18 to 24 C. Optimal dilutions of the respective primary antibody for immunohistochemical analysis were determined by use of serial dilutions (1:10 to 1:10,000) applied to deparaffinized sections of the respective antigen source (ie, stomach for cGL and pancreas for cPL). The optimal dilution was determined as that which produced discrete, intense, and localized labeling of individual cells or groups of cells with minimal or no nonspecific background labeling. Nonspecific background labeling was considered to be pale diffuse labeling of tissues within sections not expected to be a source of the associated lipase (eg, interstitial connective tissues). Optimal dilutions ranged from 1:1,000 to 1:10,000 depending on the specific antibody. Incubation with primary antibody was followed by another rinse and incubation with a secondary biotinylated goat anti-rabbit IgG antibody for 30 minutes at 18 to 24 C. After another wash, slides were incubated with streptavidin alkaline phosphatase solution for 30 minutes at 18 to 24 C. Slides were washed again, and then they were incubated (5

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minutes at 18 to 24 C) with a commercially available marker. After another rinse, slides were counterstained with Mayer hematoxylin, rinsed, dehydrated, and covered with a coverslip, using a commercially available product. All slides were examined by at least 1 of the investigators (BRB or JW). Slides were carefully scanned for evidence of red color. A positive label was determined by identification of red stain in any cells on a particular slide.

Results

Purified cGL and cPL had lipolytic activity, as determined by use of a titrimetric assay. Results of SDS-PAGE of cGL and cPL revealed a single band for each protein. Approximate molecular mass of cGL and cPL was 49 and 50.7 kd, respectively. The N-terminal amino acid sequence of the first 25 amino acid residues of cGL was identical to the sequence reported elsewhere. The N-terminal amino acid sequence of the first 25 amino acid residues of cPL had high homology with porcine (88%), equine (88%), and human (84%) pancreatic lipase but lower homology with canine pancreatic lipase-related protein 1.

Both rabbits inoculated with cGL were seropositive for cGL, and both rabbits inoculated with cPL were seropositive for cPL. Rabbit serum was purified by use of affinity chromatography, and it then was adjusted to a final concentration of 1 mg/ml. Radial immunodiffusion revealed a precipitation line between purified anti-cGL and cGL, but a precipitation line was not evident between anti-cGL and cPL. Also, radial immunodiffusion revealed a precipitation line between anti-cPL and cPL, but a precipitation line was not evident between anti-cPL and cGL.

Labeling patterns of all tissue sections for cGL and cPL were determined. Of the 38 types of tissues examined, only gastric secretory cells in the gastric cardia, gastric corpus, and pyloric antrum had positive labeling for cGL (Fig 1-3). Gastric secretory cells, specifically mucous neck cells, mucous pit cells, and more...
faintly labeled superficial parietal cells, had positive labeling for cGL in all 3 tissue specimens. However, pancreatic tissue did not have positive labeling for cGL (Fig 4).

The only tissue that had positive labeling for cPL was the exocrine portion of the pancreas, specifically pancreatic acinar cells and zymogen granules (Fig 5). Positive labeling for cPL was not observed in the gastric cardia, gastric corpus (Fig 6), or pyloric antrum.

Discussion

Purified cGL and cPL each had lipolytic activity, and each appeared as a single band on SDS-PAGE, indicating successful purification of both proteins. Identification of cGL and cPL was achieved by partial characterization of both proteins. Molecular mass and N-terminal amino acid sequence of the first 25 amino acid residues of cGL were identical to those reported elsewhere. Molecular mass of cPL was similar to that for purified pancreatic lipase of other species, and there was a high degree of sequence homology for the first 25 amino acid residues of cPL with that of porcine, equine, and human pancreatic lipase.

Radial immunodiffusion revealed that purified anti-cGL antibody bound to cGL and that purified anti-cPL antibody bound to cPL. It also revealed that neither antibody was immunologically cross-reactive with the other protein.

Several methods can be used to evaluate the cellular origin of an enzyme. The discovery of lipase activity in gastric contents led to the hypothesis that a gastric lipase does exist. However, the mere existence of lipolytic activity in gastric contents can also be explained by lipase being excreted proximal to the stomach and passively reaching the stomach along with a meal. In 1917, other investigators reported the gastric origin of lipase activity in gastric contents of dogs by documenting that lipolytic activity in the stomach of each dog with an occluded pylorus was dependent on the secretory activity of the stomach. However, only the development of immunologic methods such as immunohistochemical analysis allowed conclusive proof of the cellular origin of gastric lipase. Immunolocalization has limitations in that this technique can only be used to suggest evidence of a substance in a cell at a specific time point. It should be...
remembered that a substance detected within a cell was not necessarily synthesized by that cell. In theory, that substance also could have been absorbed by various mechanisms. However, such behavior is unlikely for the cells that were of primary interest in our study (ie, gastric and exocrine pancreatic secretory cells). The only way secondary intracellular protein could be excluded conclusively would have been by use of labeling cells for mRNA that encode the respective polypeptide chains.36-38 However, because of the substantial additional effort and cost that would have been required to perform such mRNA labeling, and because immunolocalization does provide reasonable evidence for the synthesis of a secretory product in a certain cell, it was decided not to pursue this issue further at this time.

Of all the tissue sections examined, only gastric tissue had positive labeling for cGL. All 3 areas of the stomach that were examined (ie, gastric cardia, gastric corpus, and pyloric antrum) had positive labeling for cGL, suggesting that cGL is secreted by cells located throughout the mucosa of the stomach. Most of the cells that had positive labeling for cGL appeared to be mucous-producing cells located in the area of the gastric pits and neck of the gastric glands, mucous pit, and neck cells. This agrees with the location of cGL in another report.34 Fainter positive labeling of some of the superficial parietal cells in all areas also was observed.

The only tissue section that had positive labeling for cPL was the pancreas. Specifically, positive labeling was limited to pancreatic acinar cells.

The study reported here provides evidence that cGL and cPL are organ-specific enzymes. It further suggests that other proteins that may cross-immunoreact with cGL or cPL were not secreted by any of the tissue examined. Thus, cGL and cPL are good candidates for organ-specific markers of the stomach and exocrine portion of the pancreas, respectively, in dogs.

References


