Radioimmunoassay of Serum Pepsinogen in Relation to Gastric (pars oesophagae) Ulceration in Swine Herds

Germain Nappert, André Vrins, Michel Beauregard, Luc Vermette and Normand Larivière

ABSTRACT

A radioimmunoassay for the detection of serum swine pepsinogens is described. The sensitivity and reproducibility of the assay were satisfactory for its clinical use. In normal pigs, the serum pepsinogen level was 1.51 ± 0.56 ng/mL. Cases with parakeratosis, erosions and ulcerations of the pars oesophagae had elevated pepsinogen levels (5.15 ± 1.98 ng/mL).

INTRODUCTION

Gastric ulceration is being recognized increasingly in intensive swine-raising operations (1-3). The nonglandular pars oesophagae is found to be the site most frequently involved in the ulcerative process (4-7). The disease is reported from many countries in swine of all ages, but the highest incidence appears to be in rapidly growing animals (5,7). The pathogenesis has not yet been fully characterized, but it is likely that multiple factors are involved (5,7). The fineness of grinding of the feed has been the only documented cause of gastric ulceration in swine (8-11). Antemortem diagnosis is difficult because clinical signs are not always evident and may be masked by other problems (5). Recent studies indicate that the determination of levels of different types of serum pepsinogen by radioimmunoassay might be useful in humans with gastric or duodenal ulcers (12-14). No correlation has yet been found between the presence of gastric ulcers and pepsinogen values in swine (15-17). However, swine pepsinogen concentrations have only been determined by the spectrophotometric method. This study evaluates the sensitivity and precision of the determination of serum swine pepsinogen level by radioimmunoassay. In this paper, the term pepsinogen is used to cover all the isoenzymes of pepsinogen.

RESUME

Les auteurs decrivent une methode pour la determination du pepsinogene sereique porcin par dosage radioimmunologique. La sensibilite et la reproductibilite des resultats sont satisfaissantes pour une utilisation clinique du test. Les porcs normaux presententaient une concentration en pepsinogene sereique de 1.51 ± 0.56 ng/mL. Les sujets des troupeaux ou l'on observait des lesions d'hyperkeratoese, d'erision et d'ulceration de la pars oesophagae de l'estomac presententaient une concentration elevee en pepsinogene sereique (5.15 ± 1.98 ng/mL).

MATERIALS AND METHODS

New Zealand white rabbits were immunized with purified pepsinogen from porcine stomach: 3700 IU/mg dry weight (No. P-4656, Sigma, St-Louis, Missouri). The immunizing dose, 500 μg, was given by the intracutaneous route and dissolved in 0.01 M phosphate buffered saline, pH 7.4, and emulsified with an equal volume of complete Freund's adjuvant (Gibco Laboratories, Grand Island, New York). Booster injections of 100-500 μg in incomplete Freund's adjuvant were given by the intrasubcutaneous route at two week intervals over a period of three months. The selected antibody was stored at -70°C in a 1:100 dilution in phosphate buffer 0.1 M, pH 7.4 containing 0.1% sodium azide, 2% normal rabbit serum and ethylenediamine tetraacetic acid 0.05 M.

The tracer was labelled with 125I (Amersham, Oakville, Ontario) by the lactoperoxidase and glucose oxidase technique (Enzymobead reagent, Bio-Rad Laboratories, Mississauga, Ontario) (18). The quantities of the components used in the reaction mixture were as follows: 60 μg of purified swine pepsinogen; 10 μL of Na125I (about 1 mCi); 50 μL of phosphate buffer 0.2 M, pH 7.2; and 25 μL of beta-D-glucose 1%. The reaction was performed at room temperature for 20 min followed by centrifugation at 1000 x g for 10 min. The supernatant was mixed with 1 mL of phosphate buffer 0.5 M, pH 7.5. Separation of the free 125I from the 125I labelled pepsinogen was done on a 1 x 30 cm column (Sephadex G-25, Pharmacia Canada Ltd, Dorval, Quebec) which was previously washed with 2 mL of 1% bovine serum albumin (Albumin:Fraktion V, Boehringer Mannheim Canada, Dorval, Quebec) and equilibrated with phosphate buffer 0.15 M, pH 7.5. Eluate aliquots of 0.5 mL were collected into tubes containing 50 μL of 1% bovine

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serum albumin and aprotinin (Trasyol, Boehringer Mannheim Canada, Dorval, Quebec). The free $^{125}$I and $^{131}$I labeled pepsinogen were separated after radioactive determination of the fractions with an automatic gamma spectrometer (Biogamma, Beckman Instruments, Mississauga, Ontario).

The buffer in the radioimmunoassay (RIA buffer) consisted of 0.05 M phosphate buffer pH 7.5 with 0.15 M sodium chloride, 2.5 g/L of bovine serum albumin and 33 mg/L of aprotinin. The standard curve ranged from 0.1 to 100 ng/mL of purified swine pepsinogen. The standard reaction mixture contained 100 $\mu$L of tracer (approximately 20,000 cpm), 100 $\mu$L of standard, 100 $\mu$L of antiserum (diluted 1:3000) and 300 $\mu$L of RIA buffer. The swine serum sample mixture contained 100 $\mu$L of tracer, 10 $\mu$L of serum sample, 100 $\mu$L of antiserum and 390 $\mu$L of RIA buffer. Total radioactivity was determined with four samples of 400 $\mu$L of RIA buffer, 100 $\mu$L of antiserum and 100 $\mu$L of tracer. Nonspecific binding was determined with four samples of 500 $\mu$L of RIA buffer and 100 $\mu$L of tracer. All serum samples and standards were analyzed in triplicate.

The three day incubation period was carried out in 55 x 10 mm disposable vials (Catalogue No. 566353, Beckman Instruments, Mississauga, Ontario). At the completion of incubation 400 $\mu$L of goat antirabbit immunoglobulin immunobead reagent (Catalogue No. 170-5602, Bio-Rad Laboratories, Mississauga, Ontario) was added to each vial and allowed to react at 37°C for 2 h. The vials were centrifuged at 1600 x g for 10 min, the supernatant decanted and the precipitate counted in an automatic gamma spectrometer.

Sera were obtained at weekly intervals from 136 six month old pigs slaughtered by electrocution. Blood samples were collected during exsanguination. The stomachs were collected and examined within a few hours of slaughter. The stomachs were opened along the greater curvature, from the cardia to the pylorus. The contents were removed and the gastric mucosa was lightly rinsed with tap water. The total gastric and ulcerated area of the pars oesophages were measured with a fiber glass board ruled in cm². Representative tissue sections were taken from the pars oesophagae and fixed in 10% buffered formalin. The tissue blocks were embedded in paraffin, sectioned and stained with hematoxylin, phloxin and safran (HPS). The final classification of swine gastric lesions to be presented was based on gross and histopathological findings (19). The correlation between the pepsinogen levels and the area of gastric ulceration was evaluated with the Pearson correlation test.

RESULTS

Figure 1 demonstrates a typical elution diagram showing the complete separation of the free $^{125}$I and $^{131}$I labelled pepsinogen. Figure 2 illustrates the standard curve in which the concentration of pepsinogen ranged from 0.1 to 100 ng per mL of incubation mixture. The curve is essentially linear between 2.5 and 25 ng per mL. The interassay reproducibility was assessed by analyzing 15 plasma samples in three monthly consecutive assays. The coefficient of variation was calculated and varied from 15.7 to 35.7% (mean 24.8%). The intra-assay precision was evaluated by repeated tests of one sample. The coefficient of variation was 7.7% (n = 20).

The pathological changes observed in the stomachs are summarized in Fig. 3. The sex effect was not considered in this study. The pars oesophagae was noted to be normal in 30 cases (22%), but showed initial parakeratosis in 53 cases (39%), parakeratosis and erosions in 46 cases (34%), and ulcers in seven cases (5%). The serum pepsinogen levels, and the total and ulcerated area of the pars oesophagae were measured on all samples (Table I). High levels of pepsinogen were associated with high incidences of lesions of the pars oesophagae in the first two groups. The lowest levels of pepsinogen were observed in the fifth group in which the pars oesophagae of each stomach appeared normal. There was no correlation between the pepsinogen levels and the gastric area ulcerated ($r = 0.408; p = 0.4957$).

DISCUSSION

Like other enzymes secreted into the lumen of the digestive tract, swine pepsinogen can be identified in the blood in small, but measurable quantities. In studies on experimentally induced gastric ulcers in swine, there has been no correlation between the presence of ulcers and pepsinogen values (17). However, the pepsinogen and corticosteroid concentrations...
were found to be significantly higher during cold weather, suggesting that the pepsinogen level was a potential indicator of stress. Considering the specificity and the sensitivity of the radioimmunoassay, the technique described in this study has advantages over the previous spectrophotometric methods.

The typical elution diagram presented in Fig. 1 is comparable to the radiodination of human pepsinogen with $^{125}$I as described elsewhere (20). Reportedly, human pepsinogens have only been labelled with $^{125}$I by a chloramine-T method (12,13,20). In this study, the lactoperoxidase technique was preferred, because this technique causes less protein denaturation than the chloramine-T method (18). However, both techniques were successfully used for the labelling of swine pepsinogen with $^{125}$I.

As shown in Fig. 2, the antibody dilution of 1:3000 gave 88% binding at the 0.1 ng/mL standard, which is better than some standard curves presented for human pepsinogen (12,13). The elevated coefficient of variation of the interassay reproducibility (mean 24.8%) was probably influenced by protein denaturation during the three month interval assay. The storage of human serum could be extended for many years at -20°C without risk of pepsinogen degradation (20). In this study, the swine serum samples were kept at -70°C between each test. The three consecutive freezing and thawing periods were probably responsible for the protein denaturation. The coefficient of variation of the intra-assay precision (7.7%) was comparable to the results obtained elsewhere with human pepsinogens (12,13,20).

All the pigs examined in the first and second groups were from the same herd and the gastric lesions observed were mostly characterized by parakeratosis, erosions and ulcerations of the *pars oesophagea*. In accordance with the literature, they presented a slower growth rate than those of other herds on the same farm (21). In the third and fourth groups, the gastric lesions were less severe, but still characterized by parakeratosis of the *pars oesophagea*. In the fifth group, all stomachs appeared normal. The pattern of swine serum pepsinogen concentrations obtained in this study paralleled the gastric lesions observed. However, there was not always a correlation between the pepsinogen levels and the gastric area ulcerated. The serum pepsinogen concentration probably depended more on the depth of the gastric mucosa damage than on the extent of gastric lesions.

The findings in this study suggested that the determination of serum pepsinogen level by radioimmunoassay may have diagnostic value for gastric erosions or ulcerations in swine. However, the serum pepsinogen concentration can be influenced by several factors including the rate of its elimination by the kidneys and the age.
of the pigs (12,17,22). Further studies will be needed to determine the diagnostic value of the technique.

ACKNOWLEDGMENTS

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REFERENCES


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<th>Groups</th>
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<th>Serum pepsinogen levels [ng/mL]</th>
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<th>Total ulcerated area</th>
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<tr>
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<td>4</td>
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*Means within a column with different superscripts differ significantly (p < 0.05) |
**Analysis of variance between groups (p = 0.0001): F-test x = 37.89 |
F-test x = 71.47    F-test x = 21.99    F-test x = 26.87