A comparative immunohistochemical study on amylase localization in the rat and human exocrine pancreas

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ABSTRACT

Objective: To localize amylase enzyme immunohistochemically in the pancreatic acinar cells of rats and humans using polyclonal sheep anti-human amylase antibody, and to compare between the intensities of their amylase-immunostaining.

Methods: Indirect immunofluorescence method was applied on formaldehyde-fixed, and paraffin-embedded pancreatic sections obtained from adult male Wistar rats and autopsied human samples. Primary incubation was performed using sheep anti-amylase antibody followed by secondary incubation with fluorescein isothiocyanate-labeled rabbit anti-sheep IgG serum. Control tests of amylase immunospecificity were also undertaken either by incubation with primary antibodies previously pre-adsorbed with an excess of human pancreatic amylase, or only with secondary antibodies.

Results: The amylase immunofluorescence was positively and homogenously detected in all acinar cells of both rat and human pancreatic stained sections. The immunostaining was clearly demonstrated in the cell apices and peri-nuclear areas, but it was consistently brighter and more intense in the human acinar cells compared with that of the rat pancreas. Control tests of amylase immunofluorescence revealed the specificity of the antibodies applied for amylase localization in rat and human pancreas.

Conclusions: Although many previous immunohisto- and cytochemical reports have successfully localized amylase in the pancreas of different mammalian species, but all of them have used locally prepared anti-amylase antibodies. The present report successfully illustrates immuno-localization of amylase in the pancreatic acinar cells of rats and humans using commercial polyclonal sheep anti-human pancreatic amylase antibodies, and also suggests their useful application in the immunochemical studies on various mammalian species. Additionally, the results indicate a structural similarity between the human and rat pancreatic amylases, a concept required further exploration.

Keywords: Sheep anti-amylase antibody, immunofluorescence, exocrine pancreas, rats, humans.

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The mammalian pancreas, a gastro-intestinal associated gland, has been widely used as a good experimental model for investigating and exploring various morphologic and physio-pathological cellular processes at both light and electron microscopic levels.[1-6] Several immunohistochemical and immunocytochemical reports have successfully illustrated specific amylase immunolocalization in the exocrine pancreatic acinar cells of various mammalian and non-mammalian species.[7-12] All these reports have exclusively implicated locally prepared, species-specific anti-amylase antibody, which have positively and specifically cross-reacted with amylases of other different species. This cross

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reactivity has indicated presence of structural similarity between the amylases of different species. The structure of human alpha-amylase and its active sites has been fully established using x-ray diffraction techniques.[13] Subsequent studies on the molecular structure and activity of amylase family have clearly detected structural homology between mammalian and non-mammalian amylases.[14-16] Since the process of amylase antibody preparation, or their purchase is rather complicated, we have previously succeeded in immunofluorescence localization of amylase in the human exocrine pancreas using commercially available polyclonal sheep anti-human pancreatic amylase.[17,18] The aim of the present work therefore, was first to localize amylase immunohistochemically in the rat and human pancreatic acinar cells using the same commercial anti-amylase antibodies. Secondly, to compare between the intensities of amylase immunostaining in the rat and human acinar cells which gives an indication for cross-reactivity and structure similarity between their amylases.

Methods. The study was conducted on pancreatic tissue samples obtained from normal adult male Wistar rats and from autopsied human pancreas. Five rats with an average body weight of 250g maintained on standard food pellets and water were included in the survey, and the pancreas of each rat was rapidly excised under Nembutal anesthesia, cleared from fat and connective tissue, and was divided into small parts. Multiple human autopsy samples were obtained from the whole pancreas of a 75-year old man who died of chronic renal failure following the ethical consent approved by local human ethics committee. The rat and human pancreatic samples were fixed in a modified Bouin's fluid (formaldehyde: picric acid = 1:3) for 24 hours at room temperature and with continuous shaking. The samples were routinely dehydrated in graded ethanol series and benzene, and were embedded in paraffin. 4-μm thick sections were serially cut and mounted on gelatin-coated clean slides. For routine light microscope histological examination, few rat and human pancreatic sections were processed and stained with hematoxylin and eosin. For indirect immunofluorescence demonstration of amylase, pancreatic sections were deparaffinized in benzene and ethanol series, and were thoroughly rinsed with phosphate buffered saline (PBS). The deparaffinized sections were pretreated with normal swine serum in a dilution of 1:15 to block the endogenous non-specific reaction, and were rinsed in PBS. The human and rat pancreatic sections were incubated with different dilutions of the primary sheep anti-amylase antibody (1:200, 1:100, 1:50, and 1:25; Serotec Ltd., Oxford, England) in a moist chamber. Each dilution was separately tested for 2 hours and for an overnight incubation both at room temperature and at 37ºC. After rinsing with PBS, the sections were further incubated with fluorescein isothiocyanate-labeled rabbit anti-sheep IgG serum in a dilution of 1:50 (Sigma Chemical, Co., St. Louis, Mo., U.S.A.) in a moist chamber for 1 hour at 37ºC in complete darkness. The sections were then thoroughly rinsed in PBS, mounted with a cover slip using 10% glycerin in PBS, and finally were examined with fluorescence microscope with blue excitation (Nikon, Tokyo, Japan). For amylase immunofluorescence specificity, the rat and human pancreatic sections were either incubated with the primary antibodies previously preadsorbed with an excess of pure human pancreatic amylase for at least 24 hours (Athens Research and Technology, Inc., Athens, Ga., U.S.A.), or with the secondary antibodies only (omitting primary antibody incubation).

Results. Histological examination of hematoxylin-eosin stained sections from both rat and human pancreas showed characteristic appearance of both exocrine and endocrine components (Figure 1a and 1b). However, some fibrosis was noticed in the interlobular spaces of human pancreas. In both rat and human pancreatic sections, the exocrine acinar cells were densely packed with zymogen granules, while the endocrine part was represented by the islets of Langerhans, which consisted of many cells separated by spaces and blood vessels. In the preliminary immunofluorescence studies on rats and humans, all pancreatic sections tested at different dilutions of the primary antibodies for various lengths of time and at different temperature conditions showed positive amylase immunostaining. However, the best optimal-reaction was obtained with a dilution of 1:25 for 2 hour at 37ºC, which was exclusively applied in subsequent staining. The amylase immunofluorescence was positively and homogenously detected in all acinar cells of rat and human pancreas (Figure 2a and 2c). The immunostaining was mainly localized in the acinar cell apices and their peri-nuclear areas, which respectively represent the zones of zymogen granules and Golgi complexes (Figure 3a and 3c). Comparing the brightness and intensity of amylase immuno-fluorescence between rat and human acinar cells, it was so clear and obvious that the human pancreatic acinar cells consistently showed a more intense and brighter reaction compared to that detected in rat acinar cells (compare Figure 2c with 2a and Figure. 3c with 3a). The control sections of both rats and human pancreas stained for amylase immuno-specificity consistently showed negative reaction either when the primary incubation was omitted (Figure 2b and 2d), or when the primary antibody was adequately preadsorbed with an excess of

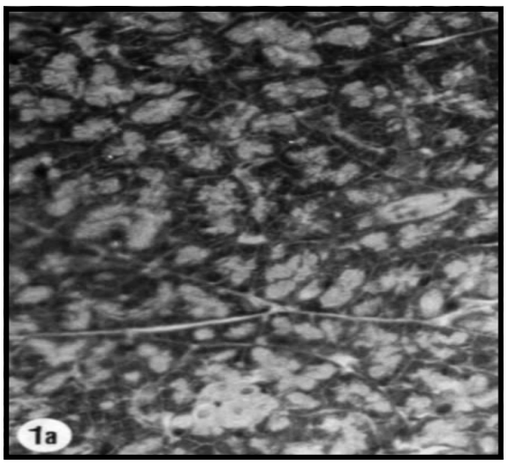

Figure 1a - Hematoxylin-eosin stained section of rat pancreas. (Magnification x 350).

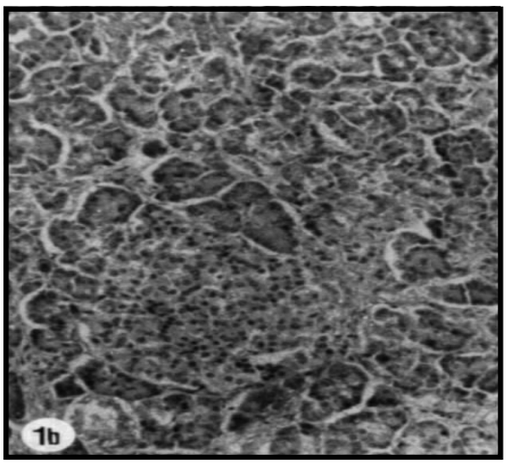

Figure 1b - Hematoxylin-eosin stained section of human pancreas. (Magnification x 350).

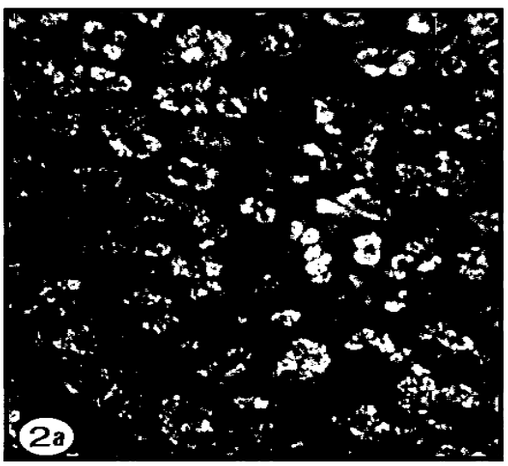

Figure 2a - Low magnification immunofluorescence photograph of rat exocrine pancreas illustrating homogenous positive amylase localization in the acinar cells. (Magnification x 400).

Figure 2b - Immunospecificity control section of rat exocrine pancreas stained with fluorescein isothiocyanate (FITC)-labeled secondary antibody only.

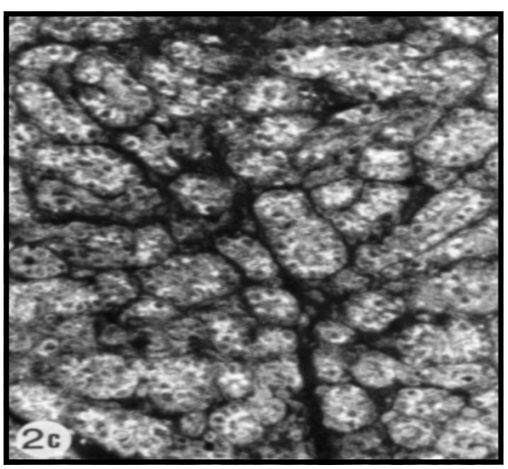

Figure 2c - Low magnification immunofluorescence photograph of human exocrine pancreas. The immunofluorescence in the acinar cells appear brighter and more intense than that of rat acinar cells. (Magnification x 400).

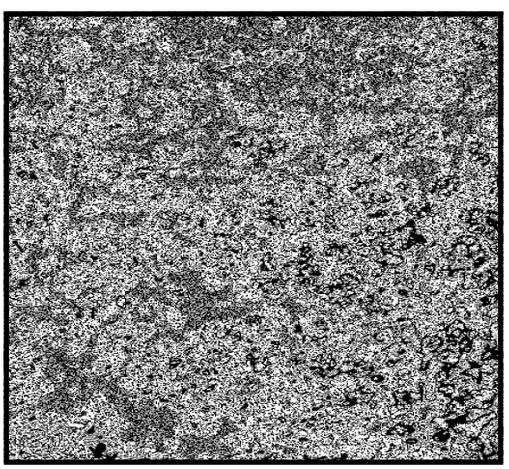

Figure 2d - Immunospecificity control section of human exocrine pancreas stained with fluorescein isothiocyanate (FITC)-labeled secondary antibody only. (Magnification x 400).

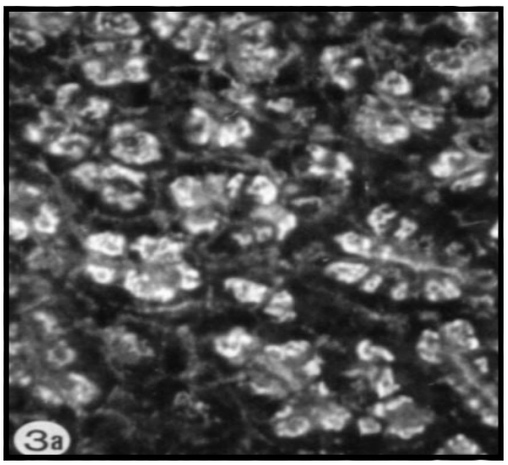

Figure 3a - Higher magnification immunofluorescence photograph of rat exocrine pancreas. The amylase immunostaining is localized in the apices and peri-nuclear areas of acinar cells.

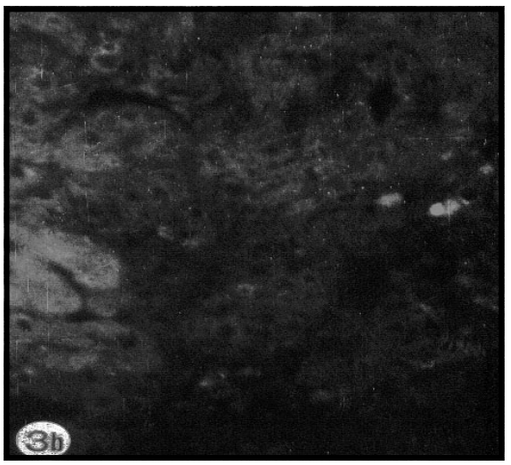

Figure 3b - Immunospecificity control section of rat exocrine pancreas stained with primary antibody previously pre-absorbed with an excess of human pancreatic amylase (magnification x 1100).

amylase (Figure 3b and 3d).

Discussion. The present study successfully illustrates a positive and specific immuno-histochemical localization of amylase in the rat and human pancreatic acinar cells using a commercial, polyclonal sheep anti-human pancreatic amylase antibody. The immunostaining was positively and consistently detected in all acinar cells of rat and human pancreatic sections, and the best optimum reaction was achieved with a primary antibody dilution of 1:25 for 2 hours at 37ºC in a moist chamber. The immunofluorescence was homogenously concentrated in the acinar cell apices and peri-nuclear areas of rat and human exocrine pancreas. It has been well-documented and for a long time that exportable pancreatic enzymes, including amylase, are synthesized on the rough endoplasmic reticulum, transported via small vesicles into the Golgi complex where they are concentrated, conjugated, and delivered into the forming zymogen granules for extracellular discharge.[1,19] Therefore, the detected sites of amylase immunofluorescence in the present study certainly represented the zymogen granule and Golgi complex cellular zones. In our

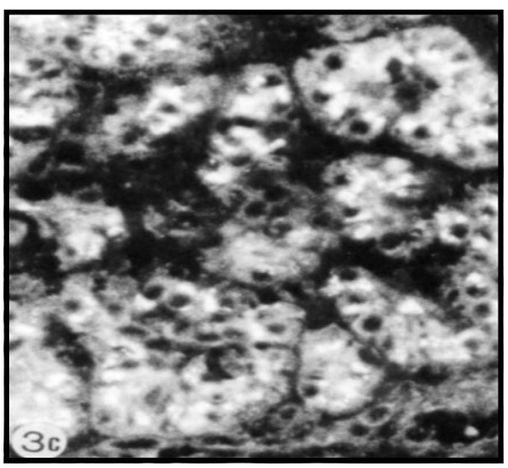

Figure 3c - Higher magnification immunofluorescence photograph of human exocrine pancreas. The amylase immunostaining is also localized in the apices and peri-nuclear areas of acinar cells, but it is brighter and more intense than that of rat acinar cells. (Magnification x 1100).

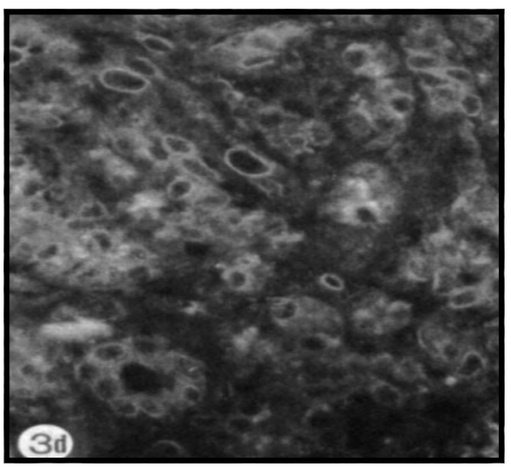

Figure 3d - Immunospecificity control section of human exocrine pancreas stained with primary antibody previously pre-absorbed with an excess of human pancreatic amylase. (Magnification x 1100).

previous studies on the human pancreas,[17,18] we have reported similar immunohistochemical localization of amylase in the acinar cells using similar antibody. Some spots of immunofluorescence were also observed in a few islets and excretory duct cells, which were considered as an indication for presence of pancreatic intermediate islet-amylase, and duct-amylase cells. Although, similar spots of immunofluorescence were also presently detected in the human islet and excretory duct cells, but no attempt in the present study was paid to reconsider them. Similar to our present results, many previous reports have specifically localized amylase in normal and diseased mammalian pancreas by means of immunohisto- and cytochemistry.[20-25] However, in all these reports locally prepared anti-amylase antibodies were exclusively applied. The commercial anti-amylase antibody used in the present study has been raised against human pancreatic amylase, which intensely detected human pancreatic amylase, but less intensely localized rat pancreatic amylase. This cross reactivity between human and rat pancreatic amylases have indicated some similarity in their structure and their binding sites. Similar cross reactivity of rat pancreatic enzymes with bovine and porcine pancreatic enzymes have been demonstrated by Bendayan and Ito.[26] Geuze et al[8] have also reported cross reactivity between rat and guinea pig pancreatic amylases. Moreover, Horri et al[27] have illustrated a close similarity in the structure of human and mouse pancreatic and parotid amylase genes. These data strongly suggest structural identity between pancreatic amylases of different mammalian species.

In conclusion, our present immunohistochemical data clearly illustrates a specific and positive localization of amylase in the rat and human exocrine pancreas using sheep anti-human pancreatic amylase antibodies, and it indirectly points out some structure similarity between rat and human pancreatic amylases. The results also suggest useful application of these antibodies in various immunochemical studies on other mammalian species.

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