B-5157
[1-5]

RESEARCH ARTICLE Indian Journal of Animal Research

Ultrastructural Architecture Studies of Pancreas in Guinea pig

S. Rajathi[1], K. Raja[2], V. Ramakrishnan[3], R. Gnanadevi[4], P. Dharani[4], T.A. Kannan[5], Geetha Ramesh[5]

10.18805/IJAR.B-5157

ABSTRACT

Background: The pancreas is an accessory organ of the digestive system and also an important endocrine organ of vertebrates that produce and release substances in the body. The pancreas has both endocrine and exocrine function. Its endocrine function is to regulate blood sugar levels by secretion of hormones like insulin, glucagon, stomatostatin and pancreatic polypeptide and an exocrine function that helps in digestion. The study was performed to document the ultrastructural details of pancreas of guinea pigs by scanning and transmission electron microscopy.

Methods: Six adult healthy guinea pigs of 16-32 weeks of age (Irrespective of sex) were procured from the Department of Laboratory Animal Medicine, TANUVAS as per ethical committee approval. Animals were dissected according to standard operating procedure by using the Carbon dioxide asphyxiations as per CPCSEA norms and pancreatic pieces were utilised for SEM and TEM study.

Result: Pancreas was irregular in shaped and showed splenic, ventricular and intestinal lobes. In SEM, the parenchyma was covered by the dense irregular capsule. Each lobule contained many acini which were connected by a thin, long duct with branched pattern arrangement with increasing wall thickness and diameter. In TEM, the pancreatic tissue consisted of glandular lobules comprised of acini, islets of Langerhans and connective tissue between the lobules. Numerous mitochondria and golgi complexes were also present in the acinar cell cytoplasm along with zymogen granules and rough endoplasmic reticulum. The centroacinar cells were also found. A special type of interstitial cell named telocytes and each was found with many telopodes in the exocrine part of pancreatic parenchyma. Among the four islet cell types, alpha and beta cells could be identified.

Key words: Acini, Alpha cells, Beta cells, Centroacniar cells, Guinea pigs, Pancreas, SEM, Telocytes, TEM.

INTRODUCTION

The guinea pig (*Cavia porcellus*) belongs to the rodent order but differs in many metabolic aspects from rat and mouse and, in particular, exhibits several unique features to humans in its lipid metabolism. Guinea pigs played a major role in the formation of germ theory through the experiments of Louis Pasteur, Emile Roux and Rober Koch (Buchholz and Schoeller, 2004). Guinea pigs had an unusual insulin mutation and the suitable species for the anti-insulin antibodies production (Chan *et al*., 1984). The pancreas is an accessory organ of the digestive system and also an important endocrine organ of vertebrates. Its function is to regulate blood sugar levels by secretion of hormones like insulin, glucagon, stomatostatin and pancreatic polypeptide. As an exocrine gland, it produces pancreatic juice for digestion (Sisson *et al*., 1975). To evaluate the pathologic and clinical reactions, it is essential to understand the normal structure of the organ. Hence, a thorough knowledge on the SEM and TEM of the pancreas is necessary for the proper diagnosis and treatment of ailments. The experimental animals like guinea pigs, mouse, rats, pigs and monkeys play an important role in human medicine, since the anatomical structure of the organs of experimental animals is similar to that of humans (Al-Sharoot, 2014). Studies through the SEM, the smallest acinus observed measured about 8µm in diameter and looked like a bud. The acinar cells were round or oval in shape and measured 30-40µm in diameter in human pancreas. Most acini, were composed of crooked and branching cords of acinar cells (Takahashi, 1984). In guinea pigs, at two days of post-natal life, the acinar cell of the pancreas was found to be small, with the large nucleus, Golgi complex, cisternal Rough Endoplasmic Reticulum (RER), mitochondria and small zymogen granules (De-Assism *et al*., 2003). Fattah (2008) stated that the presence of electron dense granules in beta cells of albino rat. Blood flow pattern in exocrine and islet

[1]Veterinary College and Research Institute, Tirunelveli-627 358, Tamil Nadu, India.
[2]College of Veterinary Sciences, Tirupati-517 502, Andhra Pradesh, India.
[3]Veterinary University Training and Research Centre, Kudrakudi-630 206, Tamil Nadu, India.
[4]Veterinary College and Research Institute, Udumalpet, Namakkal-637 001, Tamil Nadu, India.
[5]Department of Veterinary Anatomy, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India.

Corresponding Author: S. Rajathi, Veterinary College and Research Institute, Tirunelveli-627 358, Tamil Nadu, India.
Email: rajathis9936@gmail.com
ORCID: https://orcid.org/0000-0002-4226-2346.

How to cite this article: Rajathi, S., Raja, K., Ramakrishnan, V., Gnanadevi, R., Dharani, P., Kannan, T.A. and Ramesh, G. (2023). Ultrastructural Architecture Studies of Pancreas in Guinea pig. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-5157.

Submitted: 17-05-2023 **Accepted:** 06-11-2023 **Online:** 16-11-2023

part of pancreas of human was studied by El-Gohary and Gittes (2018). Longnecker (2021) stated that the centroacinar cells in humans can be differentiated from the absence of zymogen granules and less endoplasmic reticulum.

Due to paucity of literature available in guinea pigs, the present basic research on the scanning and transmission electron microscopic studies of pancreas was carried out.

MATERIALS AND METHODS

Sample collection

The present study was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai during the period from 2019 to 2021. Six adult healthy guinea pigs of 16-32 weeks of age (Irrespective of sex) were procured from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, TANUVAS, Chennai-51. After collection of the guinea pigs, they were euthanized as per the standard operating procedure by using the Carbon dioxide asphyxiations as per CPCSEA norms and they were subjected for the dissection. After careful dissection of the animals, pancreas was dissected out.

Scanning electron microscopy

The sections of pancreas of adult guinea pigs were pre-fixed in 2.5 per cent glutaraldehyde-PBS at 4°C for 2 hours. Then samples were washed in PBS and post-fixed in 2 per cent Osmium tetroxide-PBS for 2 hours. Then samples were washed and dehydrated through graded series of ethanol. The samples were mounted on the stubs and coated with gold by a sputter coater as stated by Karahan *et al*. (2007). The SEM images were observed by a Phenom Pro scanning electron microscope at CATERS facility in Central Leather Research Institute, Chennai.

Transmission electron microscopy

Pancreas samples of adult guinea pigs were collected and prefixed at three per cent glutaraldehyde and stored at 4°C. Subsequently, the tissues were washed, three changes (each 30 minutes) in cold sodium cacodylate buffer solution (pH. 7.4) and post fixed in one percent osmium tetroxide for two hours at 4°C. The tissues were then dehydrated in ascending grades of alcohol, propylene oxide: epoxy resin mixture and embedded in Epon-araldite mixture. Semithin (one micron) sections were stained by toluidine blue. Ultrathin sections (600 Å to 900 Å) were prepared on Leica ultracut microtome, mounted on uncoated copper grids and stained with saturated solution of Uranyl acetate and lead citrate. The ultra thin sections were examined under Jeol 1400 Transmission Electron microscope, computer augmented transmission Electron microscope operated at 60-Kilowatt ampere (KV) (Rajathi *et al*., 2022).

Ethics statement

The animal ethical committee of the Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India had approved the collection of laboratory animals and handling as per the Ethical Committee approval (Lr. No. 1467/DFAB/IAEC/2018 dated 13.07.2018). The methods were performed in accordance with the guidelines of the institutional ethical committee of TANUVAS, India. All procedures were performed in accordance with the CPCSEA norms (Periera *et al*., 2004).

RESULTS AND DISCUSSION

The scanning and transmission electron microscopic architecture of pancreas in guinea pigs were similar to other mammals except the following unique findings. Ultrastructurally, guinea pig pancreas showed apparent structural features of digestion and blood sugar maintenance.

Scanning electron microscopy

In the present study, the pancreatic parenchyma was covered by the dense irregular connective tissue capsule with different orientation of connective tissue fibres. The connective tissue fibres were found between the lobules and also found between the individual acini of 16 week-old guinea pig pancreas (Fig 1). It was compound alveolar gland. Each lobule contained many acini which were connected to each other by a thin, long duct with branched pattern arrangement. Takahashi (1984) mentioned about the shape of the acinar cells as round or oval in shape which was similar to the observations of the present study. The acinar cells were connected and drained by intercalated duct which was thin and small. The pancreatic lobule was drained by interlobular duct which was thicker than intercalated duct and the pancreatic lobe was drained by interlobar duct which was larger in diameter than interlobular duct. In between the acini and between the lobules, large fat lobules were also found. Interdigitations of nearby acinar cell membranes were also found. Large blood vessels supplying the lobes and lobules of pancreas were found in the low SEM magnification.

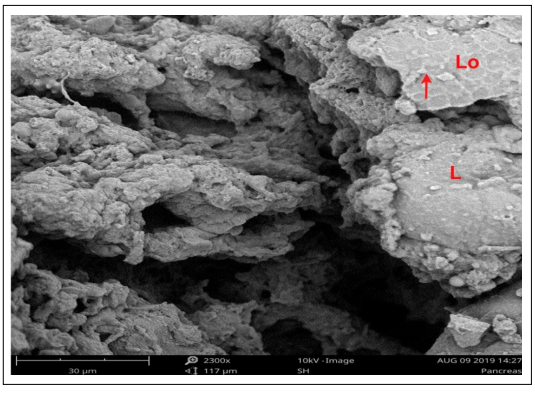

Fig 1: Scanning Electron Micrograph of pancreas of 16 week-old guinea pig pancreas showing lobe (L) and Lobule (Lo) differentiation. Red arrow showing the connective tissue spearting the lobes to lobules. × 2300.

Transmission electron microscopy

The pancreatic tissue consisted of glandular lobules comprised of acini, islets of Langerhans and connective tissue between the lobules. Inside the lobules, lot of small blood vessels and blood capillaries were found surrounding the gland which contained few telocytes. Similar identification of cells was observed in pancreas of aquatic turtles as per (Gandahi *et al.,* 2020). The pancreatic acinar cells were pyramidal in shape and had large irregular shaped euchromatin nucleus with areas of electron dense particles at the periphery. Nucleolus was clear, electron dense and occupied an eccentric position within the nucleus. The cytoplasm was fully packed with cisternae of rough endoplasmic reticulum. The apical portion of the cytoplasm of the acinar cells had numerous homogenous electron dense bodies named zymogen granules. Numerous mitochondria and golgi complexes were also present in the acinar cell cytoplasm along with the zymogen granules and rough endoplasmic reticulum of 20 week-old guinea pig pancreas (Fig 2). The acinar lumen had electron dense particles. Similar observations were also recorded in guinea pigs by De-Assis *et al.* (2003) and in camel by Hafez and (Zaghloul, 2017).

Some acinar cells were electron dense and few acinar cells were electron lucent. The electron dense acinar cells had more proportion of rough endoplasmic reticulum than electron lucent cells. These acinar cells were connected to adjacent acinar cells by junctional complexes. Similar observations were also reported by Beheirya *et al.* (2018) in goose pancreas. The apical portion of the acinar cells showed numerous microvilli of 20 week-old guinea pig (Fig 3). The 5-6 acinar cells were found surrounding the centroacinar cells which was found smaller in size when compared to the acinar cells. The centroacinar cells had many electron dense particles in the cytoplasm. The cytoplasmic and nuclear contents are not clearly distinguishable in the present study. Many microvilli were found surrounding the cell membrane of the centroacinar cell of pancreas of 20 week-old guinea pig (Fig 4). In the proximity to the blood capillary, a special type of interstitial cell named telocytes were found with many telopodias in the exocrine part of pancreatic parenchyma which had association with the acinar cell and blood vessel of pancreas of 20 week-old guinea pig (Fig 5). Nicolescu and Popescu (2012) reported that telocytes acted as important players in intercellular signalling *via* stromal synapses and shed vehicle transfer in human pancreas. Similar observation regarding the presence of telocytes in the stroma of pancreas was also observed in human and turtle pancreas by Nicolescu and Popescu (2012) and Gandahi *et al.* (2020).

Among the four islet cell types, alpha and beta cells could be identified in the present study. Alpha cells had irregular outline and had numerous homogenous small membrane bound granules with moderate to high electron density. Similar observations were also recorded by Lacy and St-Louis (1957) in guinea pig pancreas and by Hafez and Zaghoul (2017) in camel pancreas. Mostly the granules were positioned towards the periphery of the cytoplasm. The alpha cells were observed with ovoid nucleus with indentation, with electron dense condensed chromatin

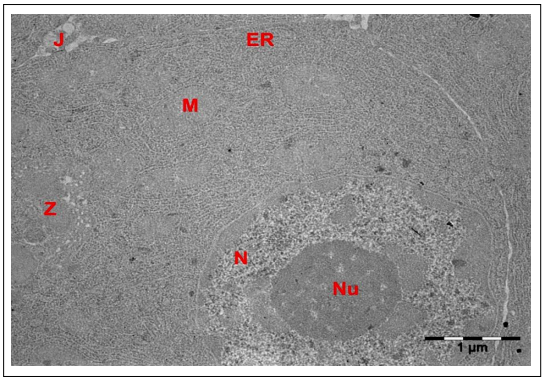

Fig 2: Transmission Electron Micrograph of acinar cells of 20 week-old guinea pig pancreas showing nucleus (N), Nucelolus (Nu), Mitochondria (M), Zymogen granules (Z), Endoplasmic reticulum (ER) ND Junctional Complex (J) Scale bar = 1 µm.

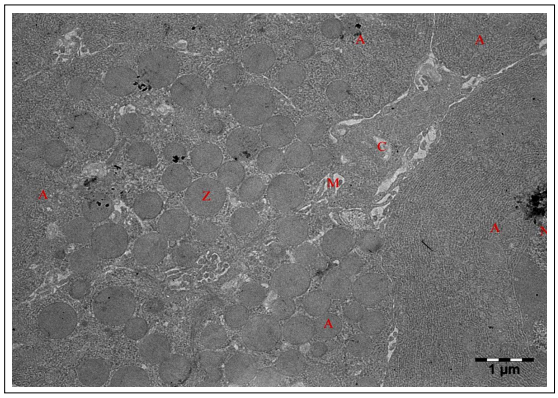

Fig 3: Transmission Electron Micrograph of pancreas of 20 week-old guinea pig showing Centroacinar cells (C) and Acinar cells (A) with nucleus (N) and Zymogen granules (Z) showing microvilli (M). Scale bar = 1 µm.

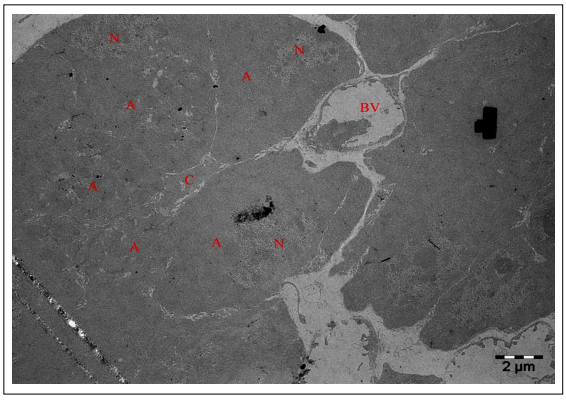

Fig 4: Transmission Electron Micrograph of pancreas of 20 week-old guinea pig showing Centroacinar cells (C) and Acinar cells (A) with nucleus (N) and blood vessel (BV). Scale bar = 2 µm.

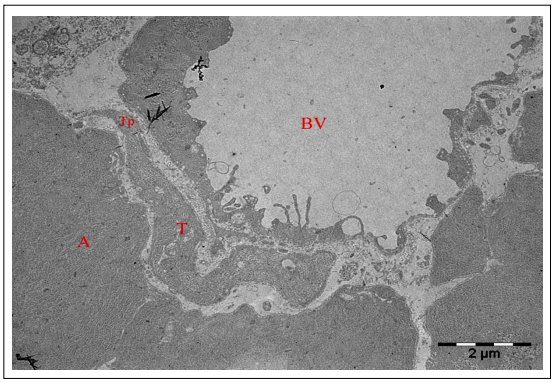

Fig 5: Transmission Electron Micrograph of pancreas of 20 week-old guinea pig showing blood vessel (BV), Telocytes (T) with telopodia (Tp) and Acinar cell (A). Scale bar = 2 µm.

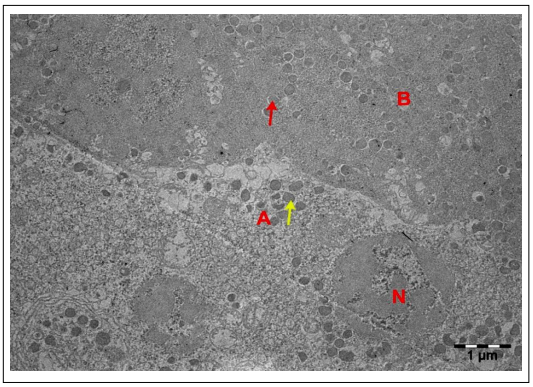

Fig 6: Transmission Electron Micrograph of pancreatic islets of 20 week-old guinea pig showing Alpha cell (A) with its granule (yellow arrow) and Beta cells (B) with its granule (red arrow). N-Nucleus, Scale bar = 1 µm.

particles located at the periphery as stated by Lacy and St-Louis (1957) in guinea pig. The cytoplasm of alpha cells was found with various sized vacuoles and rough endoplasmic reticulum. Smooth and rough endoplasmic reticulum also found in the cytoplasm of alpha cells. Golgi complex also found in the cytoplasm of alpha cells as vesicles of pancreas of 20 week-old guinea pig (Fig 6).

Beta cells were distributed throughout the islet both in the center and periphery. Beta cells were almost similar in size to that of alpha cells. But Hafez and Zaghloul (2017) found large beta cells when compared to alpha cells. The cytoplasmic granules were fewer in number than alpha cells as per the results of Lacy and St-Louis (1957) in the pancreas of dogs, rabbit and guinea pig. The granules were spherical in shape, larger in size and distributed throughout the cytoplasm whereas in rabbits, the granules were rod shaped (Lacy and St-Louis, 1957) and in dogs, the granules were irregular in shape and small, round and indistinct in rabbit pancreas and round in rats (Lacy and St-Louis, 1957). The appearance of electron dense granules was also recorded in albino rat (Fattah, 2008). The beta granules were membrane bound with an electron lucent halo around the granules. Islets were vascularized and endothelial lining of the blood vessels were fenestrated of pancreas of 20 week-old guinea pig (Fig 6).

CONCLUSION

The scanning and transmission electron microscopic architecture of pancreas in guinea pigs were similar to other mammals except the following unique findings. Ultrastructurally, guinea pig liver showed apparent morphological features of carbohydrate, protein and fat digestion and blood sugar maintenance. The pancreatic parenchyma was covered by the dense irregular connective tissue capsule with different orientation of connective tissue fibres. The connective tissue fibres were found between the lobes and lobules and also found between the individual acini. Each lobule contained many acini which were connected to each other by a thin, long duct with branched pattern arrangement. The acinar cells were drained by intercalated duct, interlobular duct, interlobar duct and interlobular duct were found with increasing diameter in the low SEM magnification. The pancreatic acinar cells were pyramidal in shape and had large irregular shaped euchromatin nucleus with areas of electron dense particles at the periphery. Nucleolus was clear, electron dense. The cytoplasm was fully packed with cisternae of rough endoplasmic reticulum. The apical portion had numerous homogenous electron dense zymogen granules. The electron dense acinar cells had more proportion of rough endoplasmic reticulum than electron lucent cells. The apical portion of the acinar cells showed numerous microvilli. Alpha cells had irregular outline and had numerous homogenous small membrane bound granules with moderate to high electron density. Beta cells were almost similar in size to that of alpha cells. The cytoplasmic granules were fewer in number than alpha cells. The granules were spherical in shape, larger in size and distributed throughout the cytoplasm. The beta granules were membrane bound with an electron lucent halo around the granules.

ACKNOWLEDGEMENT

The authors are grateful to Dean, Madras Veterinary College, Chennai for providing facilities for research.

Data availability statement

Data available on request from the corresponding author.

Conflict of interest

The authors declare no potential conflict of interest.

REFERENCES

Al-Sharoot, H.A. (2014). Morphological and histological study of the kidney in guinea pig. Internatioan Journal of Recent Science and Research. 5(11): 1973-76.

Beheirya, R.R., Abdel-Raheema, W.A., Balaha, *et al*. (2018). Morphological, histological and ultrastructural studies on the exocrine pancreas of goose. Beni-Suef University Journal of Basic and Applied Sciences. 7: 353-358.

Buchholz, A.C. and Schoeller, D.A. (2004). Is a calorie a calorie? American Journal of Clinical Nutrition. 79(5): 899-906.

Chan, S.J., Episkopou, V., Zeitlin, S. *et al*. (1984). Guinea pig preproinsulin gene: An evolutionary compromise? Proceeding of the National Academy of Sciences, USA. 81(16): 5046-5050.

De-Assis, G.F., Cestari, T.M., Sesso, A., *et al*. (2003). Post-natal maturation of acinar cells of the guinea pig pancreas: An ultrastructural morphometric study. Anatomy Histology Embryology. 32: 36-41.

El-Gohary, Y. and Gittes, G. (2018). Structure of islets and vascular relationship to the exocrine pancreas. Pancreapedia: Exocrine Pancreas Knowledge Base. DOI: 10.3998/panc.2017.10.

Fattah, E.A. (2008). Histological and immunohistochemical study of changes in the albino rat pancreas during aging. Egypt Journal of Histology. 31(2): 266-277.

Gandahi, N.S., Ding, B. and Shi, Y. (2020). Identification of telocytes in the pancreas of turtles -a role in cellular communication. International Journal of Molecular Sciences. 21: 1-20.

Hafez, S. and Zaghloul, D. (2017). Light and electron microscopy of the pancreas of the Egyptian one-humped camel (*Camelus dromedarius*). European Journal of Anatomy. 21(1): 37-45.

Karahan, S., Yildiz, D. and Bolat, D. (2007). Scanning Electron microscopic features of the ovine interdigital sinus. Acta Veterinaria Hungarica. 55(4): 417-424.

Lacy, P.E. and St-Louis, M.D. (1957). Electron microscopy of the normal islets of langerhans in dog, rabbit, guinea pig and rat. Diabetes. 6: 498-507.

Longnecker, D.S. (2021). Anatomy and histology of the pancreas. Pancreapedia: Exocrine Pancreas Knowledge Base. DOI: 10.3998/panc.2021.01.

Nicolescu, M.I. and Popescu, L.M. (2012). Telocytes in the interstitium of human exocrine pancreas-ultrastructural evidence. Pancreas. 41: 949-956.

Pereira, S., Veeraraghavan, P., Ghosh, S. *et al*. (2004). Animal experimentation and ethics in India: The CPCSEA makes a difference. Alternatives to Laboratory Animals. 32(1): 411-415.

Rajathi, S., Ramesh, G., Kannan, T.A. *et al.* (2022). Liver in guinea pig: Scanning and transmission electron microscopic studies. Microscopy Research and Technique. 1-6. https://doi.org/10.1002/jemt 24194.

Sisson, S., Grossman, J.D. and Getty, R. (1975). Sisson and Grossman's the anatomy of the domestic animals. Philadelphia: Saunders.

Takahashi, H. (1984). Scanning electron microscopy of the rat exocrine pancreas. Arch. Histologica Japonica. 47(4): 387-404.