RESEARCH ARTICLE

Microanatomical Studies on the Lungs in Guinea Pigs

S. Rajathi[1]*, K. Raja[2], Geetha Ramesh[3], T.A. Kannan[4], S. Ushakumary[5]

Abstract

The present work was undertaken with the aim to study the microanatomy of the lungs of six adult healthy guinea pigs. Animals were dissected as per CPCSEA norms and lung pieces were taken. The lungs were cut into small pieces and routine histological tissue processing was done and images were recorded. Histologically lung was covered with visceral pleura and comprised of conductive and respiratory portions. The parenchymatous portions showed bronchi within the lung, tertiary bronchiole and respiratory bronchioles, alveolar duct, and sac which possessed alveoli on its walls. The bronchi were lined with pseudostratified ciliated columnar epithelium. With the bronchi divided from primary to tertiary, the epithelium was changed from respiratory to ciliated simple columnar epithelium with goblet cells. The respiratory bronchioles were lined by cuboidal epithelium without cilia and goblet cells. The alveolar duct, sac and alveoli were lined by simple squamous epithelium. The alveoli contained two types of cells namely type I pneumocytes and type II pneumocytes. Alveolar macrophages were found.

Key words: Alveoli, Guinea pigs, Histology, Lungs.

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Introduction

The function of the respiratory system is gas exchange between oxygen from the inhaled air and excretion of carbon dioxide formed within the mammalian body. Using respiratory system, the animal maintains homeostasis with normal functioning of other organ systems (Dellmann and Eurell, 1998). The importance of veterinary medicine is to prevent the occurrence and spread of diseases which requires the macroscopic and microscopic functional anatomy of the organ (Voloshyn *et al.* (2020). As per Gunthel *et al.* (2018), prevention, diagnosis, surgery and treatment of the pathologies of respiratory system are impossible without the knowledge of functional anatomy of these organs. The respiratory system of domestic mammals extends from nasal cavity to the alveoli within the lung parenchyma. The gases are exchanged between air and blood through the alveolar wall and capillary wall (Cadiz and Jonz, 2020). Visceral pleura covered the right and left lung parenchyma, and septa from this pleura entered the parenchyma of the lung and divides it into lobes and lobules. The literature for the histological study in the lung of the guinea pigs is scanty. So, this work was undertaken with the aim to study the microanatomical features of lungs in guinea pigs.

Materials and Methods

Six adult healthy guinea pigs of Dunken-Hartley breed, aged 16-32 weeks (irrespective of sex), were procured from the Department of Laboratory Animal Medicine, TANUVAS, Chennai (India) as per the approval of animal ethical committee (Lr. No. 1467/ DFAB/IAEC/2018 dated 13.07.2018). Animals were brought to Department of Veterinary Anatomy, Madras Veterinary College, Chennai and were dissected as per CPCSEA norms and lung pieces were utilised for microanatomical study. The lungs were cut into small pieces, fixed in neutral buffered formalin and Bouin's fluid and routine histological processing was done and paraffin blocks were obtained. Paraffin sections of 4 to 5 µ thickness were taken from the blocks using manual rotary microtome and stained using Haematoxylin and Eosin (Luna, 1968). Images were recorded using image size recording system and digiscope imaging system.

Results and Discussion

The microanatomy of the lung of the guinea pig was composed of conductive parts and respiratory parts as in other mammals (Dellmann and Eurell, 1998). The lining epithelium

[1]Department of Veterinary Anatomy, Veterinary College and Research Institute, Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Tirunelveli, 627 358, TN, India

[2]Department of Veterinary Anatomy, College of Veterinary Sciences, SVVU, Tirupati-517 502, Andhra Pradesh, India

[3]Department of Veterinary Anatomy, Madras Veterinary College, Chennai-600 007, TANUVAS, Tamil Nadu, India

[4]Education Cell, Madras Veterinary College, Chennai-600 007, TANUVAS, Tamil Nadu, India

[5]Veterinary College and Research Institute, Salem-636 112, TANUVAS, Tamil Nadu, India

Corresponding Author: Dr. S. Rajathi, Department of Veterinary Anatomy, Veterinary College & Research Institute, Tirunelveli-627 358, TANUVAS, TN, India. e-mail: rajathis9936@gmail.com

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of conductive pathways was pseudostratified columnar epithelium or respiratory epithelium to columnar epithelium with cilia and presence of goblet cells, but the respiratory pathways were lined by ciliated to non-ciliated cuboidal to simple squamous epithelium. The airway conductive pathways started with trachea and divided into two principal bronchi which in turn divided into secondary and terminal bronchioles within the lung parenchyma. The bronchi were divided into smaller and smaller bronchi and bronchioles. The smallest component was the terminal bronchioles (Fig. 1).

Microscopic anatomy of the lung of guinea pigs showed the presence of bronchi with small lumen, tertiary bronchiole and respiratory bronchioles, alveolar duct, alveolar sac and alveoli, which possessed alveoli on its walls, except terminal bronchioles and bronchi (Fig. 1) and formed the parenchyma of lung. The non-parenchymatous portions of lungs included visceral pleura, interlobar and interlobular septa, which were derived from pleura and the connective tissue elements, arteries, veins, capillaries, nerves and lymphatic vessels. The connective tissue elements were found more in proportion in the present study (Fig. 1), but Koptyev *et al.* (2014) in rats showed the presence of less proportion of connective tissue in the interlobes and interlobular septa and between the alveoli which may be due to species difference.

The microscopic anatomy of the bronchi found within the lung of the present study was of four layers from inside to outwards, namely, tunica mucosa, tunica submucosa, fibro-cartilaginous and serosa. This finding was in line with the observations of Koptyev *et al.* (2014) in rats. The lining epithelium of tunica mucosa was found to be respiratory or pseudostratified ciliated columnar epithelium surrounded by a thin layer of loose connective tissue lamina propria, thin discontinuous band of muscularis mucosa with smooth muscle layer and submucosa with connective tissue layer without glands, tunica muscularis layer with a band of smooth muscle and plates of hyaline cartilage and thin layer of loose connective tissue serosa. Similar observations were recorded by Khaleel and Baker (2018) in indigenous guinea pigs, but they observed few submucosal glands, which were not observed in the present study, which may be due to breed variation. Similar results were also observed in rabbits by Al-Hussan *et al.* (2022) but they observed layer of adipose tissue in the serosa which were not observed in the present study might be due to species difference. When the bronchi divided from primary to tertiary bronchi, the epithelium was changed from respiratory to ciliated simple columnar epithelium with goblet cells, the proportion of hyaline cartilage layer decreased and the proportion of smooth muscle layer increased and the wall thickness of the bronchi and diameter was also reduced as stated by Kennedy *et al.* (1978) in hamsters, Koptyev *et al.* (2014) in rats, Khaleel and Baker (2018) in guinea pigs and Bosch *et al.* (2021) in humans. Along with the ciliated simple columnar epithelium, goblet cells and non-ciliated clara cells were also observed as noted by Koptyev *et al.* (2014) in rats and Khaleel and Baker (2018) in indigenous guinea pigs. The pseudostratified epithelium possessed with tall columnar cells with oval shaped nucleus located in the basal part of the cell and small angular basal cells with round shaped centrally located nucleus, and all rested on the basement membrane. Basal cells function as a reserve cells for the epithelial replication and also for the attachment of columnar cells to the basement membrane in humans (Corkmack and Ham, 1989). Brush cells were found in association with pseudostratified epithelium in human bronchi too (Bosch *et al.,* 2021). But brush cells were not found in the present study, which may be due to species difference.

The terminal bronchioles were the terminal conductive part of the lung. The lining epithelium of terminal bronchioles was columnar to cuboidal cells without goblet cells which were surrounded by a comparatively thick layer loose connective tissue lamina propria, muscularis mucosa was not observed, hyaline cartilage was not found and smooth muscle was found as a thin continuous to discontinuous band in tunica muscularis (Fig. 2), whereas in red fox, epithelial cells

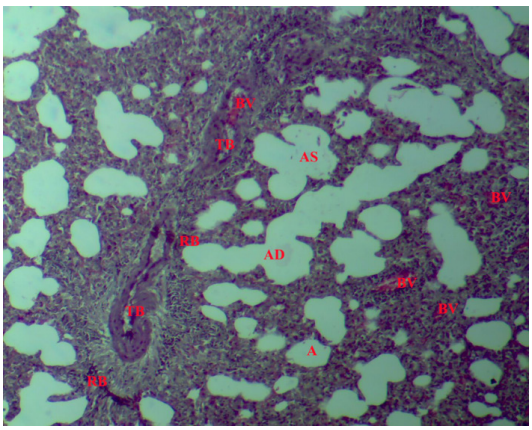

Fig. 1: Photomicrograph of lung of guinea pig showing TB - Terminal bronchioles, RB - Respiratory bronchiole, BV - Blood vessel, AD - Alveolar duct and A - alveoli, H & E x 40

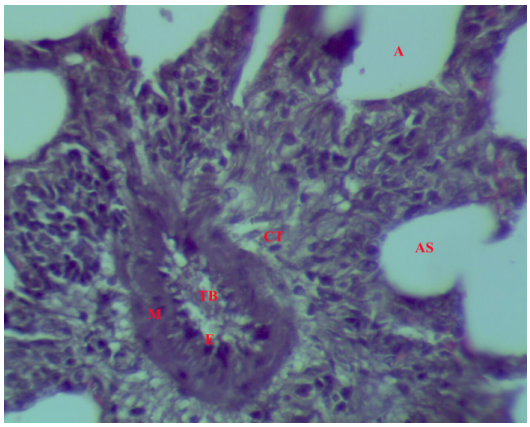

Fig. 2: Photomicrograph of lung of guinea pig showing TB - Terminal bronchioles, E - Epithelium M -Muscularis, CT - Connective tissue Serosa, AS - Alveolar sac and A – alveoli, H & E x 100

were simple columnar non-ciliated to cuboidal and possessed some goblet cells which may be a species difference (Moussa and Hassan, 2015). Similar results regarding lack of muscularis mucosa layer was found by Kalita (2014) in pigs. But in dogs, the lining epithelium of poorly developed terminal bronchioles were surrounded by a very thin layer of lamina propria and discontinuous band of smooth muscle (Horalskyi *et al.,* 2023), but in the present study it was well-developed with all the tunics (Fig. 2). Fraser (2005) stated the bronchiolar epithelial cells in human possessed ciliated columnar cells. Moreover, the human bronchioles contained exocrine bronchiolar cells which secrete surfactant and regenerate the bronchiolar epithelium were however not found in the present study in guinea pigs.

Respiratory parts consisted of respiratory bronchioles, alveolar duct, alveolar sac and alveoli (Fig. 3) as reported by Koptyev *et al.* (2014) in rats, Al-Hussan *et al.* (2022) in rabbits and Horalskyi *et al.* (2023) in dogs, which perform both respiratory and conductive functions. Horalskyi *et al.* (2023) found that terminal bronchioles in dogs were surrounded by many respiratory bronchioles which were not observed in the present study but branching of terminal bronchioles into few respiratory bronchioles were observed (Fig. 3). Ibe *et al.* (2011) in rabbit and gerbils, and Khaleel and Baker (2018) in guinea pigs did not observe respiratory bronchioles which may be due to environmental, species and breed variation. The respiratory bronchioles were lined by simple columnar epithelium without cilia (Fig. 3) similar to that in rats (Koptyev *et al.,* 2014) and in guinea pigs (Khaleel and Baker, 2018), whereas in dogs Horalskyi *et al.* (2023) found it lined by simple prismatic epithelium. The alveolar duct, alveolar sac and alveoli were lined by simple squamous epithelium (Fig. 4) as stated by Horalskyi *et al.* (2023) in sexually mature dogs and Khaleel and Baker (2018) in guinea pigs. The gaseous exchange takes place between the alveoli and the surrounding capillaries.

The walls of the alveolar ducts, sacs and alveoli were made of alveoli. The respiratory bronchioles open into the alveolar ducts and alveolar sac, which in turn contain alveoli (Fig. 3). The structures of alveolar region including alveolar duct, sac and alveoli were lined by simple squamous epithelium surrounded by a thin decreasing layer of connective tissue fibres from alveolar duct to sacs. The alveolar region was found in contact with capillaries which is essential for gas exchange. The epithelium of alveoli was located on the basement membrane and connected with the basement membrane of the capillary endothelial cells (Fig. 4) in some parts as observed by Koptyev *et al.* (2014) in rats. In other parts the alveolar epithelial basement membrane was found surrounded by loose connective tissue fibres as found by Horalskyi *et al.* (2023) in sexually mature dogs.

The lining epithelium of alveoli was simple squamous epithelium which contained two types of cells namely type 1 pneumocytes and type 2 secretory pneumocytes (Fig. 4, 5). Similar observations were also recorded by Koptyev *et al.* (2014) in rats, Moussa and Hassan (2015) in red fox, Khaleel and Baker (2018) in guinea pigs and Horalskyi *et al.* (2023) in dogs. Koptyev *et al.* (2014) in rats also identified type II brush pneumocytes, which were not observed in the present study, may be due to species differences. The type 1 pneumocytes were simple squamous cells with fusiform shape and had a flat centrally placed nucleus (Fig. 5) as stated by Koptyev *et al.* (2014) in rats and were responsible for gaseous exchange. The type 2 pneumocytes were bigger, fewer cells, cuboidal in shape with centrally placed round nucleus and were found projecting into the lumen (Fig. 4) between the type 1 pneumocytes, which are involved in the production of surfactant and also function in repair of alveolar epithelium

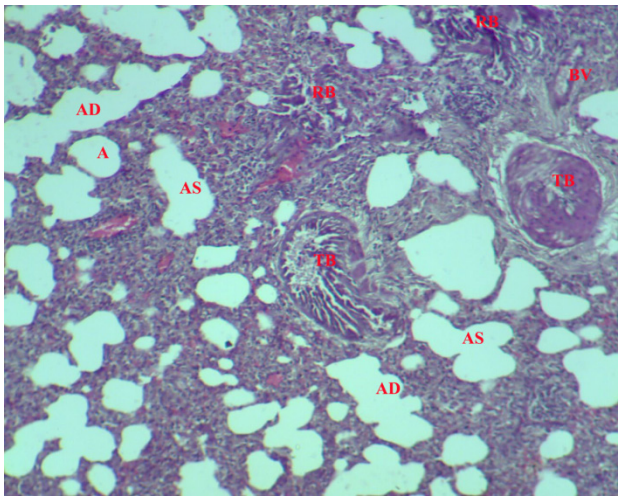

Fig. 3: Photomicrograph of lung of guinea pig showing TB - Terminal bronchioles, RB - Respiratory bronchiole, BV - Blood vessel, AD - Alveolar duct, AS - Alveolar Sac, A - Alveoli, Red dot - Epithelium and Blue line - smooth muscle, H & E x 100

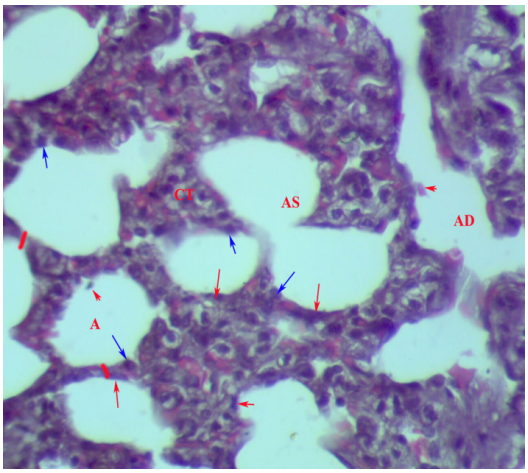

Fig. 4: Photomicrograph of lung of guinea pig showing A - Alveoli, AS - Alveolar Sac, AD- alveolar duct, Red arrow head - Alveolar macrophages Blue arrow- Pneumocyte II, Red Arrow - Pneumocyte I, CT - Connective tissue, Red line - Alveolar basement membrane in connection with capillary basement membrane, H & E x 400

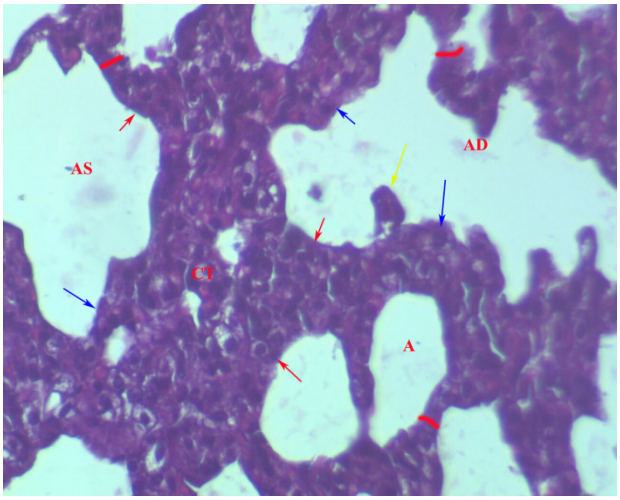

Fig. 5: Photomicrograph of lung of guinea pig showing A - Alveoli, AS - Alveolar Sac, AD - alveolar duct, Yellow arrow -Alveolar macrophages Blue arrow - Pneumocyte II, Red Arrow - Pneumocyte I, CT - connective tissue, Red line- Alveolar basement membrane in connection with capillary basement membrane, H & E x 400

after the destruction. Similar results were also observed by Koptyev *et al.* (2014) in rats regarding the shape of type II pneumocytes, but they also found microvilli and secretory granules in the cytoplasm of type II penumocytes, which were not observed in the present study. Within the lumen of alveoli, alveolar macrophages were also found projecting from the wall of the alveoli (Fig. 4) which plays an important role in immune system. The alveolar macrophages possessed many cytoplasmic processes and were basophilic and contained many vesicles. Nuclei of alveolar macrophages were small and irregular in shape and centrally placed. Similar findings were also recorded by Koptyev *et al.* (2014) in rats.

Conclusion

The histological study of lungs of guinea pigs described in detail in the present study will form the basis for understanding the treatment, surgery and disease mechanism involved in the lower respiratory tract like chronic obstructive pulmonary disease, asthma etc in guinea pigs. The histological study of lung of guinea pigs was similar to other laboratory animals like rats, hamsters and gerbils with few differences regarding the occurrence of respiratory bronchioles and differences in the epithelial cells. Further study is also needed in laboratory animal research field regarding the identification, quantification and study of cells involved in the experimentation study regarding function of respiration. This study can be used on full scale with immunohistochemistry and electron microscopy study.

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