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“ENHANCING INDUSTRIAL COMPETITIVENESS THROUGH BIOTECHNOLOGY INNOVATION”
Surakarta, 6-7 September 2016

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STRUCTURE AND MUCOPOLYSACCARIDE TYPE OF MAJOR SALIVARY GLANDS OF THE SUNDA PORCUPINES (HYSTRIX JAVANICA)

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Abstract

Sunda porcupines is one of the rodent species endemic to Indonesia. Although the conservation status of Sunda porcupine is the least concern, their populations in the wild tend to dramatically decrease due to high interest of human consumption. Moreover, information related to the anatomical structure of their organ system is still limited. The purpose of this study is to identify the topography, anatomical structures and types of mucopolysaccharides produced by the major salivary glands of Sunda porcupine. The study used four female Sunda porcupines. Tissue samples of major salivary glands which include parotid, submandibular and sublingual glands were processed for paraffin method and analyzed using macroscopic observation, Hematoxylin-Eosin (HE), Alcian Blue-Periodic Acid Schiff (AB-PAS) and lectin histochemistry for 	extit{saphora japonica agglutinin} (SJA) and 	extit{wheat germ agglutinin} (WGA). The parotid gland was found in the preauricular region and along the posterior surface of the mandible, while the submandibular and sublingual glands were located on the floor of the mouth posterior to each mandibular canine. The parotid gland was divided into two lobules, each composed by different types of acini in a separate lobulation. HE staining showed that parotid gland looks unique because in the anterior lobe, the acini are dominated by serous cell-type, while the acini of posterior lobe are composed by mixed of serous and mucous cell-types. Submandibular gland acini consist of serous cells-type and sublingual gland acini are covered by mucous cell-type. All of three major salivary glands have complete duct system comprising intercalated, striated and excretory ducts. The acini of parotid gland contains acid and neutral mucopolysaccharides, the submandibular gland contain neutral mucopolysaccharides and sublingual glands contain acid mucopolysaccharides according to the AB-PAS staining method. Lectin staining using SJA and WGA indicates that acini in salivary glands of sunda porcupine contain sugar residue of N-acetylgalactosamine and N-acetylglucosamine which is a derivative of galactose and glucose by the order of intensity from weak to strong in the parotid, sublingual and submandibular glands. The present results provide the first time data on the anatomical structure and mucopolysaccharides type produced by major salivary glands of Sunda porcupines.

Keywords: Sunda porcupine, major salivary gland, anatomical structure, mucopolysaccharides

1. Introduction

The Sunda porcupine (\textit{Hystric javanica}) is one of the rodent species endemic to Indonesia. Although the conservation status of Sunda porcupine is the least concern, their populations in the wild tend to dramatically decrease due to high interest of human consumption. Moreover, there is lack information on the anatomical structure of their organ systems. The Sunda porcupine has a distinct gastrointestinal system. Even the histological structure of pancreatic tissues of Sunda porcupine similar to the other mammalian species \cite{1}, its contains four types of major pancreatic endocrine cells with approximately similar distribution patterns to the other rodents, except for abundant glucagon cells in the peripheral area of the islets of Langerhans \cite{2}.
Histochemistry can be defined as the chemistry of tissue components and its relation to tissue morphology. In histochemical study, lectins have extensively been used as probes in studying the cell surface interaction and carbohydrate composition in many tissues because lectins, naturally polypeptides, can bind specifically to carbohydrate residues in terms of glycoconjugates [3]. Many authors have focused on the importance of glycoconjugates in salivary glands of mammalian species and correlated them with body functions such as, transporting of macromolecules for digestive efficiency, preventing proteolytic damage on epithelia, and defending against bacteria [4, 5].

Anatomical structures and types of mucopolysaccharides produced by the major salivary glands of Sunda porcupine, however, are not available. Therefore, the study was conducted with conventional staining for histological analysis and lectin histochemical methods for detecting sugar residues in the glycoconjugates of major salivary gland.

2. Methods

Four major salivary glands of adult Sunda porcupines, Hystric javanica, about 67 cm in length, were purchased from a merchant in Tawangmangu, Central Java, Indonesia were used as samples. Salivary gland tissues of *Hystriz javanica* were fixed for 24 hours in Bouin’s solution, dehydrated in ethanol, cleared in xylene, and embedded in paraffin.

Tissue samples of major salivary glands (parotid, submandibular and sublingual glands) were processed for paraffin method, cut serially in 4–5 μm thicknesses and stained by hematoxylin and eosin (HE) for conventional histological evaluation. Alcian Blue-Periodic Acid Schiff (AB-PAS) and lectin histochemistry for *saphora japonica* agglutinin (SJA) and wheat germ agglutinin (WGA) were applied for further analysis of sugar residues in the glycoconjugates of major salivary gland. Sections were examined with a conventional light microscope, and photomicrographs were taken with Optilab digital camera.

3. Results and Discussion

The parotid gland was found in the preauricular region and along the posterior surface of the mandible, while the submandibular and sublingual glands were located on the floor of the mouth posterior to each mandibular canine. The parotid gland is divided into two lobules, each composed by different types of acini in separated lobes.
Figure 1. Alcian Blue-Periodic Acid Schiff (AB-PAS) staining reaction in the submandibular, sublingual and parotid glands of Sunda porcupines (520x). The submandibular (A) and anterior lobe of parotid glands (B) positive with PAS. The sublingual (C) and posterior lobe of parotid gland (D) positive with AB. Stars and arrows indicated the acini and ducts of the glands.
Figure 1. Lectin histochemistry for *saphora japonica* agglutinin (SJA) and *wheat germ agglutinin* (WGA) in the major salivary glands of Sunda porcupine (520x). Lectin histochemistry method showed that SJA (A, B, C) and WGA (D, E, F) were detected in all major salivary glands of Sunda porcupine by the order of intensity from strong, medium and weak in the submandibular, sublingual and parotid glands, respectively. Stars and arrows indicated the acini and ducts of the glands.
Routine histologic examination of hematoxylin and eosin stained sections revealed that the submandibular and sublingual glands were located in close proximity, separated by thin fibrous connective tissue. HE staining showed that submandibular gland acini consist of serous cell-type and sublingual gland acini are covered by mucousmucous cell-type. Parotid gland looks unique because in the anterior lobe, the acini are dominated by serous cell-type, while the acini of posterior lobe are composed by mixed of serous and mucous cell-types. As a major salivary gland of the body, a submandibular, sublingual and parotid gland of different rodents shows a variety in the lobes and form of its secretory endpieces. In agreement with our finding in Sunda porcupine, the submandibular and sublingual secretory endpieces of rat [6] and European hamster [7] showed serous and seromucous cell-types, respectively. However, in contrast with other rodents which consist of 1 lobe with serous cell-type acini [6, 7], the parotid gland of Sunda porcupine consist of 2 lobes with serous cell-type in the anterior lobe and seromucous cell-type in the posterior lobe.

In the present study, the three major salivary glands of Sunda porcupine have complete duct system comprising intercalated, striated and excretory ducts. Similar results were found in the rodents [6, 7]. The acini of submandibular gland contain neutral mucopolysaccharides, sublingual glands contain acid mucopolysaccharides and the parotid gland contains acid and neutral mucopolysaccharides according to the AB-PAS staining method. The acidic mucopolysaccharides are thought to contain terminal sialic acid residues [8] and neutral mucopolysaccharides compose of free aldehyde groups within the monosaccharide units [9]. Predominant glycoconjugates with terminal sialic acid in serous cells may coat the mucosal surface so as to provide an environment designed to preserve hydration [10] and to protect the cell from pathogenic organisms [11, 12].

The identification of sugar residues was improved by using lectin histochemistry in comparison with conventional histochemistry. Lectin histochemistry method showed that SIA and WGA were detected in all major salivary glands of Sunda porcupine by the order of intensity from weak to strong in the parotid, sublingual and submandibular glands. Lectin staining using SIA indicates that acini in salivary glands of Sunda porcupine contain sugar residue of N-acetylgalactosamine [13]. Lectin staining using WGA indicates that acini in salivary glands of Sunda porcupine contain sugar residue of N-acetylgalactosamine which is a derivative of glucose [14].

4. Conclusion

The present results provide the first time data on the anatomical structure and mucopolysaccharides type produced by major salivary glands of Sunda porcupines.

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