Intestinal Histology and Infiltration of Intestinal Eosinophils of the Golden Tree Snake *Chrysopelea ornata* (Shaw, 1802)

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บทคัดย่อ

การปรากฏของอีโอซิโนฟิลแกรนูโลไซต์ในกระเพาะอาหารและลำไส้ในสัตว์มีกระดูกสันหลังเกี่ยวกับการตอบสนองทางสรีรวิทยาสะท้อนถึงการอักเสบแบบเรื้อรัง แต่ข้อมูลเหล่านี้ยังไม่ได้รายงานในงูเขียวพระอินทร์ *Chrysopelea ornata* (Shaw, 1802) ซึ่งพบได้ทั่วไปในประเทศไทย วัตถุประสงค์ของการศึกษาเพื่อบรรยายถึงมิญชวิทยาของลำไส้และการแทรกตัวของอีโอซิโนฟิลแกรนูโลไซต์ในงูเขียวพระอินทร์ด้วยเทคนิคการย้อมพิเศษ Periodic acid-Schiff (PAS) ตัวอย่างงูเขียวพระอินทร์จำนวน 3 ตัว มีขนาดความยาวลำตัวเฉลี่ยเท่ากับ 61.02±0.83 เซนติเมตร จากบริเวณเขาคอหงส์ จังหวัดสงขลา ประเทศไทย หลังจากนั้นเก็บลำไส้และนำไปผ่านกระบวนมาตรฐานทางมิญชวิทยา ผลการศึกษาพบว่างูชนิดนี้มีโครงสร้างลำไส้มีการยกตัวขึ้นและมีการจัดเรียงท่อด้วย 4 ชั้นหลัก คือ 1. ชั้นมิวโคซา ประกอบด้วยชั้นเยื่อบุผิว และลามินา โพรเพลีย 2. ชั้นซับมิวโคซา 3. ชั้นมัสคิวลาริสกับชั้นย่อยของกล้ามเนื้อเรียบ และ 4. ชั้นซีโรซา ส่วนความชุกชมและการแทรกตัวของอีโอซิโนฟิลแกรนูโลไซต์พบทั้งชั้นมิวโคซาและซับมิวโคซา โดยความชุกชุมของเซลล์ชนิดนี้พบในเยื่อบุผิวมากกว่าลามินา โพรเพลีย แต่ละเซลล์มีรูปร่างทรงกลม นิวเคลียสอยู่ทางด้านข้าง และประกอบด้วยแกรนูลที่มีปฏิกิริยาเชิงบวกกับ PAS จากผลการศึกษาครั้งนี้ทำให้เข้าใจถึงโครงสร้างลำไส้พื้นฐานของงูเขียวพระอินทร์ที่เชื่อมโยงกับการอักเสบแบบเรื้อรัง

คำสำคัญ: อีโอซิโนฟิลแกรนูโลไซต์ งูเขียวพระอินทร์ มิญชวิทยา ลำไส้ ประเทศไทย

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The appearance of eosinophilic granulocytes in the gastrointestinal tract of vertebrates often occurs as a physiologic response to chronic inflammation, but such information of *Chrysopelea ornata* (Shaw, 1802), as a common wild-snake in Thailand, probably remained unanswered. The current study aimed to describe the intestinal histology (PAS) staining method. All snakes [n = 3 individual snakes with 61.02±0.83 cm of the average snout-vent length (SVL)] were collected from Khohong Hill, Songkhla, Thailand. The intestines were kept and processed by using the standard histological techniques. This snake's intestinal histology revealed a variety of intestinal fold distinctions. Its wall clearly is composed of four distinct layers including 1) mucosa with lining two sub-layers (simple columnar epithelium and lamina propria), 2) submucosal connective tissue, 3) muscularis with patterning smooth muscle, and 4) serosa. The abundance and infiltration of EC were identified in both mucosa and submucosa. It should be noted that the mucosal layer was more abundant in the intestinal epithelium than in the lamina propria. Each EC had an oval shape, an eccentrically situated oval-nucleus, and contained PAS-positive granules. Our understanding of the basic biology of the snake intestine suggested that it could be also associated with chronic intestinal inflammation.

Keywords: Eosinophilic granulocyte, Golden tree snake, Histology, Intestine, Thailand

Introduction

The characterization of reptilian eosinophils has been documented via the blood smear slides (Young & Meadows, 2012). It is clearly identified that the nucleus of an eosinophil has a single or round to oval shape and is located eccentrically due to the special accumulation of the cytoplasmic granules (Martínez-Silvestre, Marco, Rodriguez-Dominguez, Lavín, & Cuenca, 2005; Salakij et al., 2002; Raskin, 2012). Throughout a modulating inflammatory response, eosinophils serve as an infectious pathogen and bactericidal activity (Martinez-Silvestre et al., 2005; Salakij et al., 2002). However, the remarkable morphological diversity of eosinophils is exhibited among reptilian species with systematic information (Stacy & Raskin, 2015). For most of the exclusive works on the snake group, this cell has been frequently displayed in peripheral blood films for example grass snake (*Natrix natrix*) (Jan, 1991), monocled cobra (*Naja kaouthia*), Indochinese spitting cobra (*N. siamensis*) and Sumatran cobra (*N. sumatrana*) (Salakij et al., 2002), Indian cobra (*Naja naja*) (Dissanayake et al., 2017) and northwestern garter snake (*Thamnophis ordinoides*) (Katie, 2014) but the

appearance of this cell is also migrated to liver tissue of the plains garter snake (*Thamnophis radix*). This situation could be related to the pathologic process and caused by the biliary cystadenoma, or bile duct adenoma (Knotek, Grabensteiner, Knotková, Kübber-Heiss, & Benyr, 2012); however, the presence of intestinal eosinophils in snakes is extremely rare in the literature.

The golden tree snake *Chrysopelea ornata* (Shaw, 1802), belonging to the family Colubridae, is commonly observed in several habitats in Southeast Asia, and especially Thailand (Nurngsomsri, 2015). Various ways including venom toxins (Weinstein, Warrell, White, & Keyler, 2011), reproductive mode (Vitt & Caldwell, 2014), the distribution and taxonomy (Nurngsomsri, 2015) of *C. ornata* were reported. In this present study, the intestinal histology and its eosinophil existence of the Golden tree snake *C. ornata*, as a commonly important wild snake was described using the histological techniques.

Materials and methods

The fixed-three healthy samples of Chrysopelea ornata (about 61.3 ±0.89 cm of the average snout-vent length (SVL)) were obtained from January to June 2018 from Khohong Hill, Songkhla province (7° 0' 25.5" N100° 29' 54.5" E) in Thailand. This voucher specimen was approved by the Animal Care and Use Committee of the Faculty of Science, Chulalongkorn University (Protocol Review No. 1723001). The intestinal regions were dissected and then processed using the standard histological techniques (Presnell & Schreibman, 2013; Suvarna, Layton, & Bancroft, 2013). The paraffin blocks were cut into 4-µm-thickness and were histochemically stained with PAS (Periodic Acid Schiff) protocol (Presnell & Schreibman, 2013; Suvarna, Layton, & Bancroft, 2013). Finally, the intestinal histology and its eosinophil distribution from the routine H&E staining slides were investigated and photomicrographed under a Leica DM750 light microscope. The size of the eosinophil phenotype was measured (n = 10/samples) and determined as mean±SD. The abundance of this cell was counted using three longitudinal folds at higher magnification (magnification 40x). All measurements were made using the Leica LAS imaging software version 4.5.

Results and Discussion

Intestinal histology of *C. ornata* showed that its wall is completely composed of

mucosa, submucosa, muscularis and serosa (Figure 1A). It is indicated that this is the small intestine, as similarly observed in rainbow water snakes (*Enhydris enhydris*) (El-Mansi, Al-Kahtani, Abumandour, & Ahmed, 2020) and hissing sand snakes (*Psammophis sibilans*) (El-Mansi et al., 2020). Observation of the intestinal fold was triangular-shaped or elongated and formed with the mucosa and submucosa (Figure 1B). As a result, the mucosal layer was covered with a simple columnar epithelium, as namely designed “an absorptive enterocyte”, on the basement membrane, but the position of their nuclei was varied (Figures 1C-1E). The apical surface of the epithelium was strongly stained with the PAS method (Figure 1C). The intestinal goblet cells (or mucus-secreting goblet cells) were found among the epithelium, which had an empty and unstained cell (Figure 1E). These structures were similarly reported in the intestinal mucosa of rainbow mabuya (*Mabuya quinquetaeniata*) (Anwar & Mahmoud, 1975), Roughtail rock Agama (*Stellagama stelilo*) (Amer & Ismail, 1976), and rock semaphore gecko (*Pristurus rupestris*) (Dehlawi & Zaher, 1985). Also, the intraepithelial lymphocytes were identified and infiltrated among the mucosal epithelium (Figure 1E). Each cell had a small round shape, but its cell edge was hardly identified at higher magnification (Figure 1E). The function of lymphocytes is associated with a critical role in the specific defense mechanisms in the digestive tract (Diaz, Garcia, & Figuero, 2008). At times, the lamina propria was a loose connective tissue with a relatively high proportion of fibroblast and capillary (Figures 1D-1E). A thick layer of loose connective tissue and numerous blood vessels was found in the substance of the submucosa (Figure 2A). The muscularis was an involuntary non-striated muscle with diving into an inner ternal circular and outer longitudinal muscles, respectively (Figure 2B). The serosa was finally formed by connective tissue and the mesothelial cells (Figure 2B). Not surprisingly, these structures above were similar to those described in the intestine of other snakes such as hissing sand snake (*P. sibilans*) (Jegede, Sonfada, & Salami, 2015), Brazilian arrowhead viper (*Bothrops jararaca*), and rattlesnake (*Crotalus durissus*) (Gogone et al., 2017). This is in agreement with the findings of rainbow water snake (*E. enhydris*) (Masyitha et al., 2020) and hissing sand snake (*P. sibilans*) (El-Mansi et al., 2020).

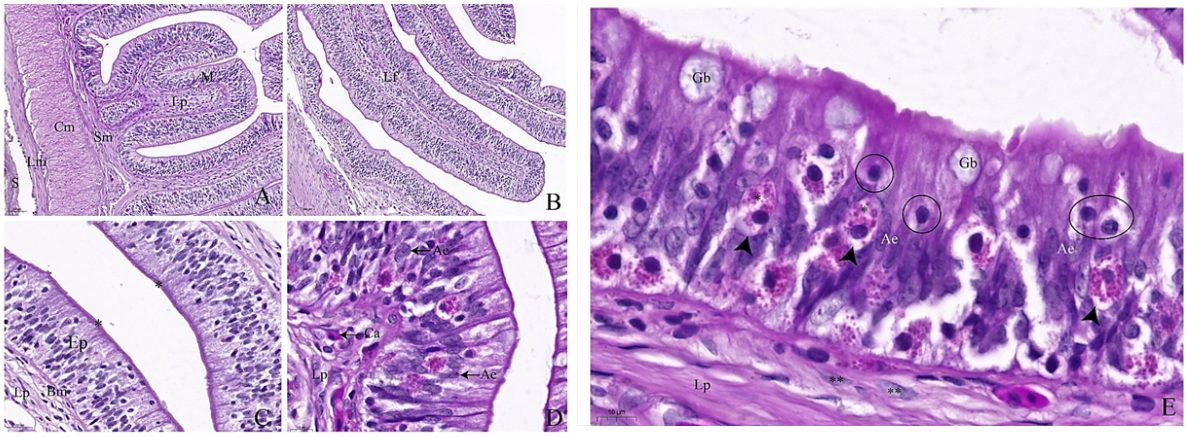

Figure 1 Light microscopy showing the intestinal histology of the Golden tree snake *Chrysopelea ornata*. A-B: Longitudinal section reveals that the intestinal wall consists of mucosa (M), submucosa (Sm), muscularis having two muscular layers (circular (Cm) and longitudinal (Lm) smooth muscles) and serosa (S). The intestinal fold (or longitudinal fold (Lf) is clearly identified. C: High magnification shows that the mucosa contains epithelium (Ep) (or an absorptive enterocyte), and lamina propria (Lp). The apical surface of epithelium is positively reacted (asterisks). D-E: High magnification reveals that the mucosal epithelium contains several cell compositions including an absorptive enterocyte (Ae), goblet cell (Gb) and lymphocyte infiltration (circles). The presence of eosinophils (arrow heads) and secretory granules (asterisks) is only found in the epithelium.

Abbreviations: Bm = basement membrane, Ca = capillary, Lp = lamina propria, double asterisks.

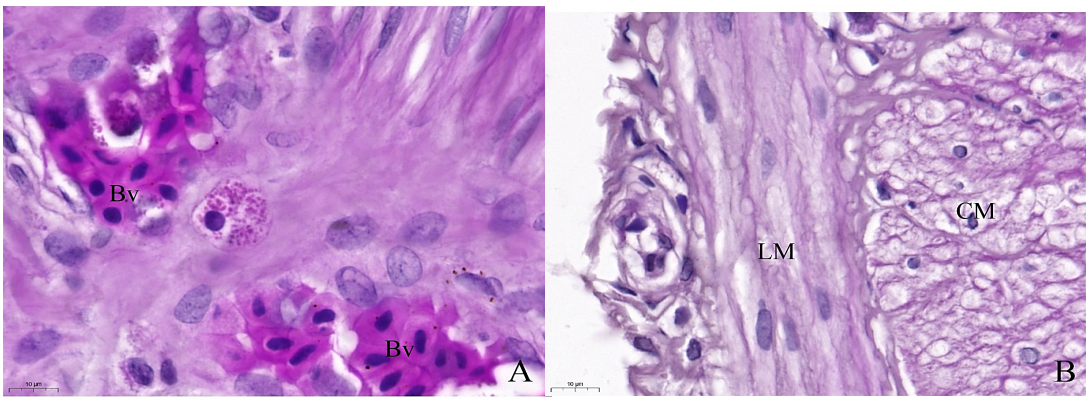

Figure 2 Light microscopy showing the submucosa and muscularis of the Golden tree snake *Chrysopelea ornata*. The loose connective tissue and blood vessel (Bv) in the submucosa are identified (A). Some eosinophils are still exhibited (B).

When present, the abundance and infiltration of eosinophils (EC) were only exhibited in the mucosa, and submucosa (Figures 1D, 1E-2A). The highest abundance of EC was found in the intestinal epithelium (8.46±1.10 individual cell) than in the lamina propria (1.86±0.73 individual cell) (Figure 1E-2A). However, the existence of eosinophils may be anatomically varied in the digestive regions throughout the globe (Pascal, Gramlich, Parker, & Gansler, 1997; Spergel et al., 2011). An oval shape for each EC was 12.04±0.96 μm in diameter. It had eccentrically situated oval-nucleus, and contained with finely granular PAS-positive method (Figure 1E and Figure 3). This is a granular protein containing major basic protein [MBP], eosinophil peroxidase [EPO], eosinophil cationic protein [ECP], and eosinophil-derived neurotoxin [EDN) (Blanchard & Rothenberg, 2009; Kanda et al., 2020). It is proposed that these secretions in the current study also are essential to maintain homeostasis in the steady state (Shah, Ignacio, McCoy, & Harris, 2020). Some studies suggest that eosinophilia at any site in the GI tract is associated with eosinophilic gastroenteritis (EGE) and may influence multiple biological activities such as parasitic helminth, allergic disease, and fungal infections during chronic inflammation (Blanchard & Rothenberg, 2009; Yantiss, 2015; Kanda et al., 2020). Although there remained an abundance of eosinophils in the snake intestine, we proposed that the presence of eosinophils in the *C. ornata*.

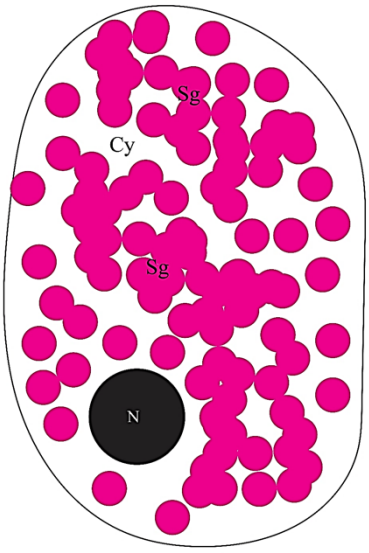

Figure 3 Schematic diagram of eosinophil of the Golden tree snake *Chrysopelea ornata*. Eccentrically observed nucleus (N) and the prominent secretory granules (Sg) in the cytoplasm are noted.

Conclusion

It is concluded that our understanding revealed the intestinal biology of *C. ornata* for the first time, as commonly seen in other reptiles like lizards, snakes and skinks. Also, the abundance of eosinophil in the intestine in *C. ornata* might be associated with chronic intestinal inflammation. Results from our study will be applied to intestinal physiology and histology of this snake.

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