

Morphological, Cytochemical and Ultrastructural Studies of Blood Cells in Irrawaddy River Dolphin (*Orcaella brevirostris*): A Case Study

Somporn Techangamsuwan¹ Supradit Wangnaithum¹ Jariya Sutanonpaiboon²
Nantarika Chansue² Achariya Sailasuta^{1*}

Abstract

The characteristic of erythrocytes, leukocytes and thrombocytes of a captive Irrawaddy river dolphin (*Orcaella brevirostris*) was described using blood sample collected from caudal vein. By morphological and ultrastructural studies, erythrocytes showed similar size as in humans and contained cytoplasmic electron dense hemoglobin without nucleus. Multilobulated granulocytes composed of cytoplasmic round-to-elongated granule-containing neutrophils and round-to-oval shaped granule-bearing eosinophils while basophil was very scanty. Monocytes displayed azulophilic granules and vacuolated cytoplasm with pseudopodia. Lymphocytes showed equal amounts of heterochromatin and euchromatin with numerous mitochondria in their cytoplasm. Thrombocytes, the smallest circulating blood cells, presented small pseudopodia with many cytoplasmic microtubular structures. By cytochemical staining, lymphocytes were specifically reactive to the acid phosphatase. Neutrophils, eosinophils and monocytes were positive with myeloperoxidase and Sudan black B. The advantage of this study will help standardize on the health monitoring of marine mammal species especially Irrawaddy river dolphin.

Keywords: Blood cell morphology, cytochemistry, *Orcaella brevirostris*, ultrastructure

¹Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Road, Pathumwan, Bangkok 10330 Thailand

²Aquatic Animal Medical Center, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Road, Pathumwan, Bangkok 10330 Thailand

Corresponding author E-mail: achariya.sa@chula.ac.th

บทคัดย่อ

การศึกษาลักษณะเม็ดเลือดของปลาโลมาอิรวดีทางจุลพยาธิวิทยา ทางจุลทรรศน์อิเล็กตรอน และการติดสีชีวเคมี: กรณีศึกษา

สมพร เตชะงามสุวรรณ¹ สุประดิษฐ์ หวังในธรรม¹ จริญญา สุตานนท์ไพบูลย์²

นันทริกา ชันซื่อ² อัจฉริยา ไสละสุต^{1*}

ทำการศึกษาคุณลักษณะของเม็ดเลือดแดง เม็ดเลือดขาว และเกล็ดเลือดของปลาโลมาอิรวดีที่เลี้ยงในบ่อ โดยดูจากลักษณะรูปร่างทางจุลพยาธิวิทยา จุลทรรศน์อิเล็กตรอน และการติดสีทางชีวเคมี ทำการเก็บตัวอย่างเลือดจากเส้นเลือดดำที่หาง ผลการศึกษาพบว่าเม็ดเลือดของปลาโลมาอิรวดีมีขนาด รูปร่าง และการติดสีใกล้เคียงกับในสัตว์เลี้ยงลูกด้วยนม เช่น มนุษย์ เม็ดเลือดแดงมีลักษณะว่า ไม่มีนิวเคลียส และพบถุงบรรจุกีโมโกลบิน เม็ดเลือดขาวแบบนิวเคลียสหลายพูประกอบด้วย นิวโทรฟิล อีโอสิโนฟิล และเบโซฟิล ซึ่งภายในมีแกรนูลบรรจุอยู่ในไซโตพลาสซึม เม็ดเลือดขาวแบบนิวเคลียสพู่เดี่ยว ประกอบด้วย โมโนไซต์ และลิมโฟไซต์ ที่ภายในมีแกรนูลแบบไม่จำเพาะบรรจุอยู่ในไซโตพลาสซึม เกล็ดเลือดเป็นเม็ดเลือดที่มีขนาดเล็กที่สุด มีขายื่นเล็กๆ ลักษณะเหมือนเท้าเหิม ผลการศึกษาการติดสีทางชีวเคมีพบว่า ลิมโฟไซต์จะติดสีจำเพาะต่อ Acid phosphatase ในขณะที่นิวโทรฟิล อีโอสิโนฟิล และโมโนไซต์จะให้ผลบวกต่อการย้อมด้วย Myeloperoxidase และ Sudan black B ข้อมูลจากการศึกษาในครั้งนี้ สามารถนำไปใช้ประโยชน์ในด้านการตรวจสอบสุขภาพและเฝ้าระวังการเกิดโรคในสัตว์น้ำที่เป็นสัตว์เลี้ยงลูกด้วยนม โดยเฉพาะปลาโลมาอิรวดีได้ต่อไปในอนาคต

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¹ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

²ศูนย์วิจัยโรคสัตว์น้ำ ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

*ผู้รับผิดชอบบทความ E-mail: achariya.sa@chula.ac.th

Introduction

Irrawaddy river dolphin (*Orcaella brevirostris*) is a species of dolphin found near coasts in parts of south-east Asia. It is belonging to Kingdom Animalia, Phylum Chordata, Class Mammalia, Order Cetacea, Family Delphinidae, Genus *Orcaella* (Stacey and Peter, 1999). There are estimated to be less than 1,000 Irrawaddy river dolphins left in the world (Baird et al., 1994). As an endangered species, they are legally protected from hunting as in the list A of the Convention on International Trade in Endangered Species (CITES) in 2004 (Fisher and Reeves, 2005). The anatomical features of Irrawaddy river dolphin have a small, blunt, rounded triangular dorsal fin and large flippers with narrow, pointed, peg-like teeth about 1 cm in length in both the upper and lower jaw (Stacey and Peter, 1999). The blood cells can be classified into 3 main categories: erythrocytes, leukocytes and thrombocytes. Basically, the leukocytes comprise neutrophils, eosinophils, basophils, lymphocytes and monocytes. The nuclear structure together with the cytoplasmic granules of leukocytes can be grouped as multilobulated granulocytes (neutrophils, eosinophils, basophils) and mononuclear agranulocytes (lymphocytes, monocytes) (Jain, 1993).

The morphology, cytochemistry and ultrastructure of blood cells have been reported in various teleosts including big head carp (*Aristichthys nobilis*), oscar (*Astronotus ocellatus*), traia (*Hoplias*

malabaricus), lambari (*Astyanax bimaculatus*) (Tavares-Dias, 2006), piracanjuba (*Brycon orbignyanus*) (Tavares-Dias and Moraes, 2006), channel catfish (*Ictalurus punctatus* Raf) (Tavares-Dias and Moraes, 2007). For the hematologic data of an endangered dolphin, the vast majority of studies are investigated in a particular bottlenose dolphins (*Tursiops truncatus*) (Medway and Geraci, 1964; Hall et al., 2007; Venn-Watson et al., 2007; Schwacke et al., 2009). Therefore, the purpose of this observation was to determine the morphology, cytochemical reactions and ultrastructural features of Irrawaddy river dolphin blood cells.

Materials and Methods

The caudal venipuncture peripheral blood sample was collected from a captive-healthy male Irrawaddy river dolphin and was collected in ethylenediamine tetraacetic acid (EDTA) anticoagulant tubes. The air-dried thin blood smears were routinely performed and stained by Wright's-Giemsa stain. The cytochemical staining procedures of the leukocytes for myeloperoxidase (PER), Sudan black B (SBB), leukocyte alkali phosphatase (LAP) and acid phosphatase (AcP) were performed using commercial kits (Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. The peripheral leukocytes from a healthy dog were used

as reaction's positive controls. The cytochemically positive dolphin's blood cells were identified for the granular characteristics and intracytoplasmic distribution. Transmission electron microscopy (TEM) was performed on glutaraldehyde-fixed EDTA-anticoagulated peripheral blood buffy coat samples. Identification of blood cells on TEM was based on the nuclear appearance, cytoplasmic granular distribution, size and shape of blood cells.

Results and Discussion

All blood cell types of Irrawaddy river dolphin displayed the morphological, ultrastructural and cytochemical appearances similar to other mammals (Figure 1) (Raskin and Valenciano, 2000; Sakulwira et al., 2008). Erythrocytes were round, biconcave shape with 6-7 μm in diameter and enucleated. The cytoplasm was filled with electron dense hemoglobin without any organelles (Figure 1A). The thrombocytes were the smallest blood cell with 1-2 μm in diameter. Ultrastructurally, they presented anucleation and a few number of small pseudopodia projecting from the cell membrane. Their cytoplasm contained a variety of organelles composing microtubules, glycogens, mitochondria and varying size of granules (Figure 1B). Occasionally, the giant thrombocyte was presented (Figure 1B inset, arrow).

Neutrophils were about 10-12 μm in diameter. The nuclei were bi-to trilobed surrounded

by pale amphophilic cytoplasm. Electron micrographs displayed a moderately condensed chromatin in their nucleus. Cytoplasm had numerous round to elongated specific granules with homogeneous moderately to intensely electron dense and mitochondria (Figure 1C, arrow). Lymphocytes had 8-10 μm in diameter. Their morphology displayed a round deep-basophilic nucleus with scanty bluish cytoplasm at the rim of cell implying a high nucleus:cytoplasm (N:C) ratio. Under the electron microscope, nucleus demonstrated almost equal amounts of heterochromatin and euchromatin. Their cytoplasm contained many mitochondria, some azurophilic granules and glycogen (Figure 1C, arrowhead). Eosinophils had 8-10 μm in diameter. Their nuclei divided into 2-3 lobes and the cytoplasm contained numerous eosinophilic specific granules. Ultrastructurally, they showed a moderately condensed chromatin with plenty of round-to-oval shaped granules, a few mitochondria and golgi vesicles (Figure 1D, arrow). Monocytes were ranging from 14-16 μm in diameter and contained a large bean-shaped nucleus with transparent vacuolated cytoplasm. The electron micrograph of monocytic nuclei revealed a peripheral rim and central aggregates of heterochromatin localized among euchromatin. Their cytoplasm contained a few translucent, round vacuoles and some azulophilic granules (Figure 1E). Pseudopodia usually were presented on the surface of the plasma membrane (Figure 1E, inset, arrow).

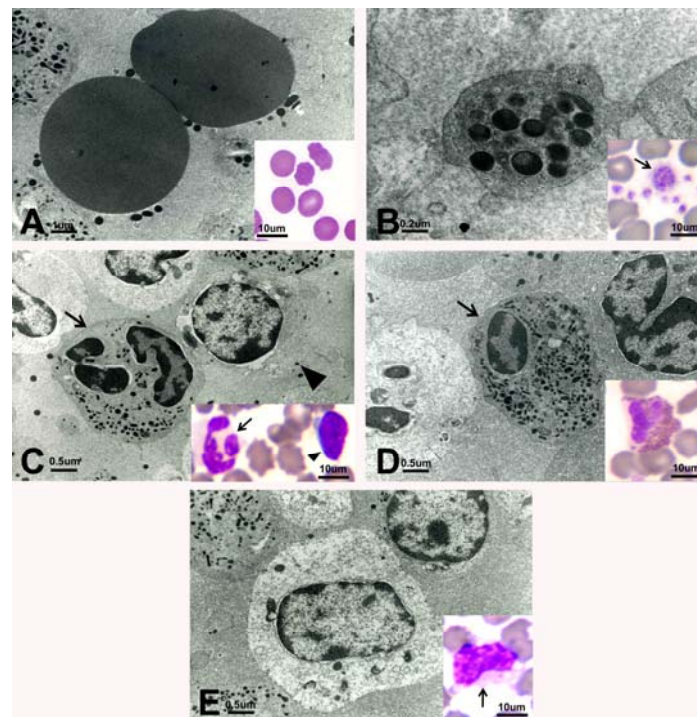


Figure 1 Electron micrographs of Irrawaddy river dolphin blood cells with their light micrographs showing in the right bottom inset. (A) Erythrocytes show anucleated round disk filled with electron dense hemoglobin. (Inset) Erythrocytes are biconcave shape with pale basophilic cytoplasm. (B) Thrombocyte is non-nucleated with a few small pseudopodia. (Inset, arrow) A giant platelet is noted. (C) Neutrophil (arrow) shows tri-lobulated nuclei with numerous cytoplasmic granules while lymphocyte (arrowhead) is mononuclear cell with many cytoplasmic organelles. (Inset) Cytoplasm of neutrophil is eosinophilic and more abundant than lymphocyte. (D) Eosinophil (arrow) contains numerous varying-sized cytoplasmic granules. (Inset) Many eosinophilic granules obscure some parts of nucleus. (E) Monocyte reveals a large oval-shaped nucleus with some cytoplasmic granules. (Inset) Monocyte shows a bean-shaped nucleus and transparent vacuolated cytoplasm. Pseudopod is frequently observed (arrow).

The cytochemical technique commonly uses for detection of the chemical reaction in various cell types and investigates for intrinsic cellular properties. In this observation, leukocyte subtypes of Irrawaddy river dolphin were cytochemically stained with leukocyte alkaline phosphatase (LAP), Sudan black B (SBB), myeloperoxidase (PER) and acid phosphatase (AcP) (Table 1, Figure 2). The canine leukocytes were used as a positive control (Figures 2C, F, I). All leukocytic subtypes were uniformly reactive with LAP displaying a faint light brown chromogen (Figure 2G, arrow). The strong PER (dark red-brown chromogen) and SBB (brown-black chromogen) activity was observed in Irrawaddy river dolphin

neutrophilic, eosinophilic and monocytic granules (Figures. 2A, B, D, E) as it has been described previously for mammals and some other fishes (Zinkl et al., 1991; Tavares-Dias and Moraes, 2006; Shigdar et al., 2009). Therefore, the presence of peroxidase and SBB activities can be used to identify feature of leukocytes in Irrawaddy river dolphin which indicated an effective phagocytic function and a high affinity for fats, respectively. This observations are in agreement with a recent report in Murray cod (*Maccullochella peelii peelii*, Mitchell) on the basis of the parallel staining pattern between PER and SBB reactions (Shigdar et al., 2009).

Table 1 Cytochemical staining reactions of the Irrawaddy river dolphin (*Orcaella brevirostris*)

	Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte
LAP	+/-	+/-	ND	+/-	+/-
SBB	+	+	ND	-	-
PER	+	+	ND	-	+
AcP	-	-	ND	+	-

+: positive, -: negative, ND: not determine

LAP: Leukocyte alkali phosphatase, SBB: Sudan black B, PER: Myeloperoxidase, AcP: Acid phosphatase

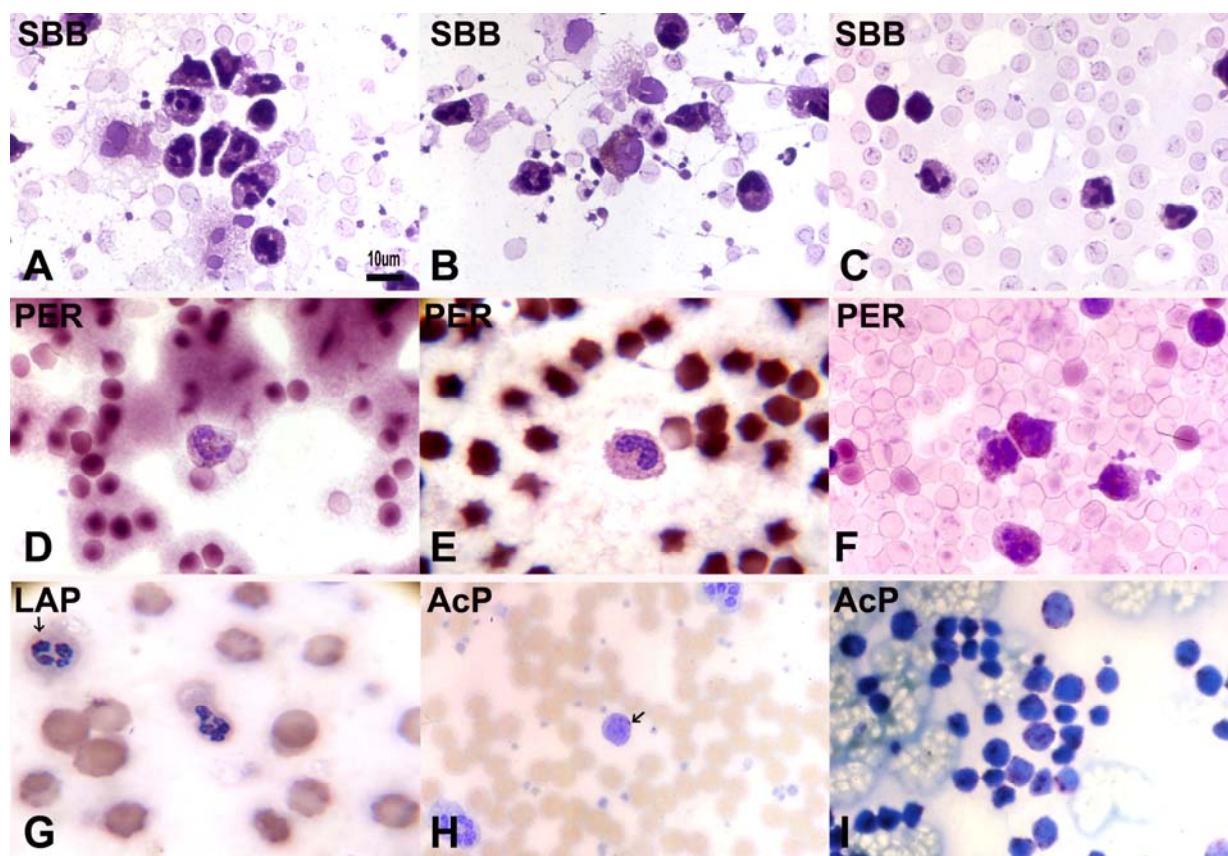


Figure 2 Cytochemical properties of Irrawaddy river dolphin leukocytes. Neutrophils (A) and eosinophil (B) are positive for sudan black B (SBB) showing brown-black staining. Neutrophil (D) and monocyte (E) stain dark red-brown with myeloperoxidase (PER). Leukocyte alkali phosphatase (LAP) stains weakly positive to some neutrophils (G, arrow). Acid phosphatase (AcP) are specifically positive to only lymphocyte showing focal magenta staining (H, arrow). Canine leukocytes are used as positive control (C, F, I) for SBB, PER and AcP reactions, respectively.

Unlike other fishes, Irrawaddy river dolphin lymphocytes were specifically positive to AcP (focal magenta staining, Figure 2H) whereas neutrophils, eosinophils and monocytes were negative. Unspecifically, leukocytes including neutrophils, monocyte and lymphocytes from many aquatic fishes

showed positivity to AcP (Zinkl et al., 1991). Taking this advantage, Irrawaddy river dolphin lymphocytes could be classified confidently by cytochemistry technique using AcP.

We found only a few basophil from thin blood smear and they were mostly negative for all

cytochemical stains, except PAS reactivity (data not shown). This is in agreement with previous findings. The low incidence of circulating basophils was well known in various cartilaginous and marine teleosts as well as freshwater fishes (Saunders, 1966; Ranzani-Paiva et al., 2000). The absence of basophils from blood smear probably resulted from inadequate staining procedure. In the future study, to retrieve more numbers of Irrawaddy river dolphin basophil, a major hematopoietic tissue should be collected (Barber and Westermann, 1975; Lopez-Ruiz et al., 1992).

Taken together, the results of this study considered to be the available information on the hematology of an endangered marine mammal, Irrawaddy river dolphin, regarding their morphology, cytochemistry and ultrastructure of erythrocyte, leukocyte and thrombocytes. Moreover, these results provide baseline data for use in the health monitoring and hematologic diagnosis of healthy and sick marine mammal species.

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