

Efficacy of Autologous Serum as An Adjunct Treatment for A Melting Corneal Ulcer in A Captive Asian Elephant

Thittaya Janyamethakul¹ Prasit Moleechat² Rinku Gohain² Chalermchat Somgird^{1,3}

Pornsawan Pongsopavijit^{3*} Boontarikaan Wititkornkul⁴

Abstract

This is the first case report on the use of autologous serum as an adjunct to successfully treat a melting corneal ulcer in a captive Asian elephant (*Elephas maximus*). A 40-year-old female Asian elephant was presented with epiphora of the right eye. It had already received treatment for 3 weeks, but had not responded to conventional treatment with antibiotics and analgesic eye drops. Upon examination, a melting corneal ulcer was found. The elephant received a combined treatment that included autologous serum twice a day for 79 days in addition to antibiotic, antifungal and analgesic drugs administered topically. After just 19 days of treatment, neovascularization of the ulcer site occurred and the ulcer reduced in size, which indicated that the elephant responded well to the treatment. The autologous serum, antibiotic and antifungal eye drops were continued for an additional 2 months to prevent secondary infection during the corneal healing. Throughout the treatment, the elephant lived and worked as usual. This study demonstrated the benefits of using autologous serum as a combined treatment for a melting corneal ulcer in a captive Asian elephant. Autologous serum has tear properties to bathe the eye and is also rich in growth factors and anti-collagenase, which can promote wound healing.

Keywords: Asian elephant, autologous serum, corneal melting, corneal ulcer

¹Excellent Center of Elephant Research and Education, Chiang Mai University, Chiang Mai 50100, Thailand

²Save the Elephant Foundation, Chiang Mai 50100, Thailand

³Elephant and Wildlife Clinic, Department of Companion Animals and Wildlife, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

⁴Large Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

*Correspondence: pornsawan.p@gmail.com

Introduction

Autologous serum eye drops have been used in human medicine for at least 30 years, primarily in patients suffering from ocular surface disorders such as kerato-conjunctivitis sicca and persistent epithelial defects (Geering et al., 2004). Autologous serum is a suitable tear substitute because it has the same pH and osmolality as normal tears. Because of its high concentration of vitamin A, fibronectin, and various growth factors, autologous serum can promote epithelial wound healing. In the field of veterinary medicine, the use of autologous serum has been reported in equines (Brooks, 2002). In canines, autologous serum is the main protease inhibitor for clinical use especially for keratomycosis (Ben-Shlomo et al., 2010) and keratitis (Gelatt, 2008). This is the first report to describe the use of autologous serum as an adjunct to treat a melting corneal ulcer in a captive Asian elephant.

Ocular problems commonly observed in Asian elephants (*Elephas maximus*) include conjunctivitis, corneal opacity, corneal ulcers, corneal edema, hypopyon, cataracts, and lens luxation (Fowler and Mikota, 2006; Angkawanish et al., 2009). During 2005-2008, 196 elephants presented ocular disorders, which represented 14.1% of the referral cases at the elephant hospital at the National Elephant Institute in Lampang, Thailand (Angkawanish et al., 2009). Because they live in dense forests near natural water sources, elephants' eyes are exposed to dust, sand, and strong sunlight. Moreover, elephants normally use their trunks to blow dust on themselves and rub their eyes in response to irritation or trauma. This can lead to development of secondary bacterial or fungal infections in the eye that can rapidly progress if mahouts lack concern and fail to seek prompt treatment.

Materials and Methods

Clinical signs: A 40-year-old female captive Asian elephant presented prolonged lacrimation of the right eye despite having been treated with Gentamicin Sulfate 0.3% eye drops (GENTA-OPH®) every 4 hours for 3 weeks (Fig 1). Physical examination revealed blepharospasm, epiphora and photophobia. A corneal ulcer was observed with fluorescein staining. Palpebral reflex, menace reflex, and dazzle reflex of both eyes were normal. Although the pupillary light reflex was normal on the left eye, it could not be observed on the right eye due to opacity. Tetracaine hydrochloride 0.5% drops (ALCAINE®) were applied to the affected eye to facilitate further examination and treatment. After 15-20 minutes, the elephant was still able to blink her eye and cover the cornea with the third eyelid. Because information about nerve anatomy in elephants is limited, particularly in regard to auriculopalpebral nerve blocks that are used for ocular examination in equines (Wiedner et al., 2006), a more detailed ophthalmic examination was abandoned. A bacterial culture from a corneal swab of the infected eye was identified as *Neisseria* spp. Subsequent fungal identification from the same eye showed no fungal infection.



Figure 1 Initial appearance of the right eye before treatment that included autologous serum

Treatment: Upon examination, medical treatment was initiated with topical Diclofenac sodium 1% (VOLTA-OPH®), Amphotericin B eye drops (AMPHOTERICIN-B®), and autologous serum twice daily in combination and topical Gentamicin was applied every 4 hours. In addition, Atropine 0.5% eye drops (ISOTO-ATROPINE®) were administered once daily only in the evening.

Preparation of elephant autologous serum: Adapted from Liu et al. (2005), the elephant autologous serum was prepared in the laboratory of Veterinary Medicine at Chiang Mai University. Five 10 ml. syringes were used to collect a total of 50 ml. of blood from an ear vein using either a 21g butterfly needle or a IV catheter. Then, the blood was allowed to clot for 2 hours at room temperature before being centrifuged at 3,000 rpm for 15 minutes. The separated serum was collected (about 20-25 ml.) into a sterile container to which 1 mg. of gentamicin was added. The autologous serum was then aliquoted into sterile tubes, each containing 3 ml. Additionally, the serum was stored at 4°C and used within 7 days.

Results & Discussion

Seven days after the initial examination and subsequent treatment (Fig 2), the progression of the melting ulcer stopped completely. A decrease in blepharospasm was noted with epiphora and photophobia still present. By the 19th day (Fig 3), recovery signs included absence of both epiphora and photophobia, presence of neovascularization at the cornea, and smoothing of the corneal surface. Because of the marked improvement, Diclofenac eye drops were discontinued while the remaining treatments were continued for another 2 months.

This elephant was diagnosed with a melting corneal ulcer. The laboratory diagnosis of *Neisseria* spp. identified from the eye swab was outside the study of normal ocular flora previously found in Asian elephants (Tuntivanich et al., 2002). *Neisseria* spp. has been found on normal conjunctival membranes of sheep (Langford, 1971) and on mucosal surfaces in bovine eyes (Wilcox, 1970). Additionally, it has been found in both normal and ulcerative keratitis in dogs

(Wang et al., 2008) and conjunctivitis in humans (Bharathi et al., 2010). Therefore, *Neisseria* spp. may represent a pathogenic bacteria for Asian elephants.

In this case, although there were concerns about potential side effects of 2 eye drop medications, they were selected because of their ready availability. Diclofenac is an anti-inflammatory drug that has been frequently used topically after ocular surgery in

humans. According to Lin *et al.* (2005), complications should be closely monitored in corneal pathophysiology for animal use. Additionally, side effects of Atropine in horses can include gut motility decrease and predisposition to colic (Gilger and Deeg, 2011). However, in this case, those side effects were not found.



Figure 2 The right ocular on Day 7 of treatment. progression of the melting ulcer stopped. The eye showed reduced blepharospasm with epiphora and photophobia still present.



Figure 3 The right ocular on Day 19 of treatment. Neovascularization was present, the corneal surface became smoother and epiphora was absent.

Autologous serum is more suitable than artificial tear lubricants because the latter do not provide nutrients to the eye (Poon et al., 2014). Not only is autologous serum biologically nontoxic to the eye because of its similar osmolality and pH to natural tears, it is also rich in vitamin A, which is an essential factor for epithelial cell growth (Liu et al., 2005) and contains TGF- β 1, lysozyme, and alpha-2 macroglobulin, which have anti-proteinase properties (Brooks, 2002). Epidermal growth factor (EGF) has been found to promote wound healing due to anti-apoptotic properties. Typically, veterinarians have used various concentrations for the treatment of eye disorders.

Kodikara *et al.* (1999) estimated the average recovery time for corneal ulceration in Asian elephant as approximately 2 months (without autologous serum). However, in this case, we observed the neovascularization, absence of epiphora and smoothing of corneal surface within nineteen days after beginning our treatment. Because the adjunct serum significantly reduced recovery time, we therefore recommend that it can be used in conjunction with traditional treatment to melt corneal ulcers in captive Asian elephants.

Before using autologous serum, the following considerations should be evaluated: the severity of the ulcer, the efficacy of drug administration on-site, and the co-operation of both elephant and mahout. Another significant factor includes the facility where the autologous serum is prepared and stored.

In conclusion, this is the first report on the use of autologous serum to enhance current protocols to treat a melting corneal ulcer in a captive Asian elephant. The successful result of this case indicates that the autologous serum, which promotes epithelial healing, may significantly reduce the recovery time of corneal ulcers in Asian elephants.

Acknowledgements

We would like to thank the veterinarians, mahouts, veterinary students and volunteers of the Save the Elephant Foundation who continued the treatment procedure until the elephant recovered. We are grateful to the Laboratory of Veterinary Medicine, Chiang Mai University for the use of their equipment. We thank Assistant Professor Dr. Chatchote Thitaram, Dr. Janine L. Brown, Assistant Professor Dr. Anucha Sirimalaisuwan, and Dr. Weerapongse Tangjitjaroen for their advice and recommendations.

References

- Angkawanish T, Boonprasert K, Homkong P, Sombutputorn P, Mahasawangkul S, Jansittiwate S, Keratimanochaya T, and Clausen B 2009. Elephant health status in Thailand: the role of mobile elephant clinic and elephant hospital. *Gajah*. (31):15-20.

- Ben-Shlomo G, Plummer C, Barrie K, and Brooks D 2010. Curvularia keratomycosis in a dog. Vet Ophthalmol. 13(2):126-130.
- Bharathi MJ, Ramakrishnan R, Shivakumar C, Meenakshi R and Lionalraj D 2010. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. Indian J Ophthalmol. 58(6):497-507.
- Brooks DE 2002. Ophthalmology for the Equine Practitioner. Wyoming: Teton NewMedia.
- Fowler ME and Mikota SK 2006. Biology, Medicine, and Surgery of Elephants. United Kingdom: Blackwell.
- Geerling G, MacLennan S, and Hartwig D 2004. Autologous serum eye drops for ocular surface disorders. Br J Ophthalmol. (11):1467-1474.
- Gelatt KN 2008. Essentials of Veterinary Ophthalmology. 2nd ed. Iowa: Wiley-Blackwell.
- Gilger BC and Deeg C 2011. Equine Recurrent Uveitis. In: Equine Ophthalmology. 2nd ed. Saint Louis: W.B. Saunders. 317-349.
- Kodikara DS, De Silva N, Makuloluwa CAB, De Silva N, and Gunatilake M 1999. Bacterial and fungal pathogens isolated from corneal ulcerations in domesticated elephants (*Elephas maximus maximus*) in Sri Lanka. Vet Ophthalmol. (3): 191-192.
- Langford EV. 1971. Mycoplasma and associated bacteria isolated from ovine pink-eye. Can J Comp Med Vet Sci. 35(1):18-21.
- Lin JC, Rapuano CJ, Laibson PR, Eagle Jr RC and Cohen EJ 2000. Corneal Melting Associated With Use of Topical Nonsteroidal Anti-inflammatory Drugs After Ocular Surgery. Arch Ophthalmol. 118(8):1129-1132
- Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, and Geerling G 2005. An optimised protocol for the production of autologous serum eyedrops. Graefes Arch Clin Exp Ophthalmol. (7):706-714.
- Poon AC, Geerling G, Dart JKG, Fraenkel GE, and Daniels JT. 2001. Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. Br J Ophthalmol. (10):1188-1197.
- Tuntivanich P, Soontornvipart K, Tuntivanich N, Wongaumnaykul S, and Briksawan P 2002. Conjunctival Microflora in Clinically Normal Asian Elephants in Thailand. Vet Res Comm. (4): 251-254.
- Wang L, Pan Q, Zhang L, Xue Q, Cui J and Qi C 2008. Investigation of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Beijing, China. Vet Ophthalmol. 11(3):145-149
- Wiedner EB, Isaza R, Galle LE, Barrie K, and Lindsay WA 2006. Medical Management of a Corneal Stromal Abscess in a female Asian elephant (*Elephas Maximus*). J Zoo Wildl Med. (3): 397-400.

บทคัดย่อ

การใช้ซีรัมร่วมรักษากระจกตาอักเสบ (Melting Corneal Ulcer) ในช้างเลี้ยงเอเชีย

ทิฏฐยา จรรยาเมธากุล¹ ประสิทธิ์ โมฬีชาติ² Rinku Gohain² เฉลิมชาติ สมเกิด^{1,3}
พรสวรรค์ พงษ์โสภณวิจิตร^{3*} บุญพริกานต์ วิทิตกรกุล⁴

ช้างเลี้ยงเอเชีย เพศเมีย อายุ 40 ปี ตาขวามีอาการน้ำตาไหลมากผิดปกติ ได้รับการรักษาเบื้องต้นด้วยการหยอดยาปฏิชีวนะและยาลดอักเสบเป็นเวลา 3 สัปดาห์ แต่อาการไม่ดีขึ้น นอกจากนี้ยังพบแผลหลุมลึกที่กระจกตา (melting corneal ulcer) อย่างไรก็ตาม ผลการเพาะเชื้อแบคทีเรียและเชื้อราไม่พบเชื้อก่อโรค จึงได้ทำการร่วมรักษาด้วยการเสริมซีรัมของตัวช้างเอง (autologous serum) โดยให้ทางการหยอดตาวันละ 2 ครั้งๆละ 1.5 มิลลิลิตร ร่วมกับการให้ยาปฏิชีวนะ ยาต้านเชื้อรา และยาลดอักเสบ ตลอดจนการให้ยาทั้งหมด 79 วันพบว่า การดำเนินไปของความผิดปกติลดลง ทั้งนี้พบการตอบสนองต่อการรักษาในส่วนของน้ำตาไหลที่ลดลงและมีการเกิดการสร้างเส้นเลือดฝอยมาเลี้ยงในส่วนของกระจกตาโดยเฉพาะตำแหน่งของแผลหลุมลึก ในช่วงท้ายของการรักษาพบว่า ไม่มีอาการน้ำตาไหล เส้นเลือดฝอยที่มาเลี้ยงในส่วนของกระจกตาที่เกิดขึ้นเริ่มมีการหดเล็กลงและกระจกตาทกลับมาสภาพที่เกือบจะเป็นปกติ ช้างสามารถดำรงชีวิตได้ตามปกติ ยังคงมีการให้ซีรัมของตัวช้างเอง ยาปฏิชีวนะ และยาต้านเชื้อราจนกระทั่งครบ 2 เดือนเพื่อป้องกันการติดเชื้อระหว่างการหายของแผล จากกรณีศึกษาพบว่า ช้างมีการตอบสนองที่ดีต่อการรักษาโดยใช้ซีรัมของตัวช้างเองร่วมการรักษาภาวะกระจกตาอักเสบ ซึ่งซีรัมมีคุณสมบัติคล้ายกับน้ำตา และมี growth factor ที่ส่งเสริมการหายของแผลที่กระจกตา และป้องกันคอลลาเจนถูกทำลายซึ่งสามารถใช้เป็นอีกทางเลือกในการรักษาความผิดปกติในระบบการมองเห็นของช้างเลี้ยงได้

คำสำคัญ: ช้างเลี้ยงเอเชีย ซีรัมของตัวสัตว์เอง กระจกตาหลอมเหลว แผลหลุมที่กระจกตา

¹ศูนย์ความเป็นเลิศด้านการศึกษาและวิจัยช้าง มหาวิทยาลัยเชียงใหม่ จ.เชียงใหม่ 50100

²มูลนิธิอนุรักษ์ช้างและสิ่งแวดล้อม จ.เชียงใหม่ 50100

³คลินิกช้างและสัตว์ป่าภาควิชาคลินิกสัตว์เลี้ยงและสัตว์ป่า คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ จ.เชียงใหม่ 50100

⁴โรงพยาบาลสัตว์ใหญ่ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ จ.เชียงใหม่ 50100

*ผู้รับผิดชอบบทความ E-mail: pornsawan58@gmail.com