

Treatment of a Full-Thickness Skin Wound in Gluteal Region of the Vervet Monkey with Topical Insulin

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Abstract

In a female vervet monkey brought to our hospital with the complaint of anaphylactic shock depending on snake bite, a gangrenous wound was detected in right gluteal region and traditional local cicatrizing and epithelizing pomade application and systemic treatment were performed for 10 days. As no signs of epithelization and shrinkage was observed during this treatment, a new treatment protocol was started with topical insulin pomade. It was determined that epithelization started on the 4th days and completely recovered on the 46th days of the treatment and 30 days after the completion of topical insulin treatment the wound region was became hairy. It was concluded that topical treatment with insulin pomade had positive effects on delayed secondary wound healing in vervet monkeys whom their cutaneous trunci muscle (*M. panniculus carnosus*) responsible for wound contraction was not functional.

Keywords: Insulin treatment, Secondary wound healing, Vervet monkey

Bir Vervet Maymununun Gluteal Bölgesindeki Maddi Kayıplı Deri Yarasının Topikal İnsülin İle Sağaltımı

Öz

Hastanemize yılan ısırığına bağlı anafaktik şok şikayeti ile getirilen dişi vervet maymununda, sağ gluteal bölgede gangrenli deri yarası tespit edildikten sonra 10 gün boyunca geleneksel lokal sikatrizan ve epitelize pomat uygulamaları ve sistemik sağaltım yapıldı. Yarada epitelize ve küçülmenin hiç gözlenmemesi üzerine topikal insülin pomat uygulamasına geçildi. Uygulamanın 4. gününde epitelizeyonun başladığı, 46. günde tamamlandığı, topikal insülin sağaltımının kesilmesinden sonra 30. günde ise epitelizeyon bölgesinin tüylendiği gözlemlendi. Sonuç olarak, yaranın kontraksiyonla küçülmesini sağlayan cutaneous trunci (*M. panniculus carnosus*) kasının non-human primatlarda fonksiyonel olmadığı, topikal insülin pomat uygulamalarının gecikmiş sekonder yara iyileşmesinde pozitif katkıları olduğu gözlemlendi.

Anahtar sözcükler: Sekonder yara iyileşmesi, İnsülin sağaltımı, Vervet maymun

INTRODUCTION

Wound is defined as the breakdown or loss of cellular or anatomical integrity of tissue. Healing of wound is a complex and dynamic process that combines blood vessel and cells, parenchymal cells and mediator functions and extracellular matrix production. Healing stages are classified as inflammation phase, proliferation phase, maturation and remodeling phase. Proliferation phase consists of neovascularization/angiogenesis, fibroplasia-collagen,

epithelization and wound contraction phases^[1]. The key point in the proliferation phase is angiogenesis, which is the formation of new blood vessels in the wound tissue. In cats and dogs, it is widely accepted that subcutaneous panniculus carnosus muscle plays an important role in providing and maintaining the cutaneous circulation, and providing wound contraction^[1,2]. But the existence and development of the panniculus carnosus (cutaneous trunci) muscle in higher primates is variable and is rarely occur in the Old and New World Monkeys and completely



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absent in human [3]. Wounds that caused by excessive tissue loss can be treated with secondary healing. Sometimes secondary healing impaired at one or more points from the stages in the normal healing process, and may lead to a delay in healing beyond the expected time [4]. Insulin is a peptide hormone and regulate blood glucose level. On the other hand, some studies show that insulin application accelerates wound healing, such as facilitates monocytes/macrophages chemotaxis, pinocytosis/phagocytosis, anabolic protein metabolism, local glucose level and secretion of inflammatory and growth mediators [5-7]. In this case report, we aimed to present the positive effects of topical insulin in the treatment of a non-healed skin wound.

CASE HISTORY

A female vervet monkey was brought to Animal Teaching Hospital, Faculty of Veterinary Medicine, Near East University with the complaint of respiratory depression and unconsciousness. In emergency examination, slow breathing, tachycardia, matt voice in the caudal lung lobes and the sternal area was determined, and was given 100% oxygen for respiratory depression and slow infusion IV colloid for 9% dehydration. Aminofilin (Filinsel® 24 mg/10 mL vial, Osel İlaç A.Ş.) was given 6 mg/kg IV. After 15 min, cardiopulmonary resuscitation was performed due to respiratory and cardiac arrest and for this aim adrenaline (Adrenalin® 1 mg/1 mL vial, Osel İlaç A.Ş.) were administered 0.5 mg IV. Based on the clinical findings and considering the development of hemothorax, thoracosynthesis was performed. Patient's respiration and heart rhythms returned to normal after 9 mL of blood was removed in the thoracosynthesis. Cephazolin (Equzolin® 1 g, 4 mL vial, Tüm Ekip İlaç A.Ş.) and enrofloxacin (10% Dufafloxacin® 100 mL vial, Dutch Farm) were administered IV against the risk of sepsis and secondary infections, and lactated Ringer's, 5% dextrose and 6% hydroxyethyl starch solution were given to increase circulation. The patient's consciousness came back after 3 h. Antibiotic use was continued for 5 days. Patient was physiologically return to normal after 1 week, but thereafter, the gangrenous area 5x5 cm in size was thought to be due to snake bite was determined in the right gluteal region (Fig. 1a). Local ethacridine lactate monohydrate (Rivanol®, 1 g, Oro İlaç A.Ş.) wet dressing

afterward 0.12% chlorhexidine gluconate (Klorben® spray, Drogsan San. ve Tic. A.Ş.) was used two times a day for the first 4 days with the aim of demarcation. Procaine penicillin G and potassium penicillin G (Devapen® 800 IM vial, Deva A.Ş.) were given IM for two times per day for one month for the purpose of protection against the infection as recommended by some literatures. On the 4th day of treatment, the necrotic tissues were removed by surgical debridement. After the debridement, in the wound treatment was initially used topical mixture of nitrofurazone pomade (0.2% Furacin® Pomade, Zentiva Sağlık Ürünleri Sanayi ve Tic. A.Ş.), dexpanthenol pomade (Pantenol® 5% cream, Saba İlaç Sanayi ve Ticaret A.Ş.), zinc oxide pomade (20% Oro® Çinko Krem, Oro İlaç A.Ş.) and centella asiatica (1% Madecassol® pomade, Bayer) two times in a day and IM administration of 5% diluted dexpanthenol (Bepanthen® 500 mg/2 mL ampoule, Bayer) once a day for 10 days (Fig. 1b).

In this point, we were not observed any changes in wound size and the epithelialization despite application of the mixture of 4 pomades, therefore the treatment was changed into only topical insulin pomade. The pomade mixture was prepared to contain 1 mL/100 IU of regular insulin (Humilin® R, 100 IU/mL, 3 mL cartridges, Lilly İlaç Tic. Ltd. Şti.) per 10 g of vaseline. The mixture was stored at room temperature as recommended by the insulin manufacturer and was sufficiently applied to the wound twice daily. In 5th day of topical insulin application, epithelization was observed to start. Treatment was terminated with the completion of wound epithelization on the 46th day (Fig. 2). On the 30th day after the end of the treatment, it was seen that the entire region was covered with hair, except for the approximately 0.1 cm² area in the center of the wound (Fig. 3).

DISCUSSION

Panniculus carnosus muscle plays important role in contraction and vascularization phases in wound healing in cats and dogs and some mammals [1,8,9]. But in non-human primates, panniculus carnosus muscle is primarily a shoulder muscle and arises from the superficial fascia of the lateral torso and inserts into the humerus with the mm pectoralis. Contraction of this muscle usually moves the

Fig 1. 5x5 cm in size gangrenous wound in the right gluteal region (a), despite the epithelizing and cicatrizing pomade application for 10 days after debridement, view of atonic wound (b)



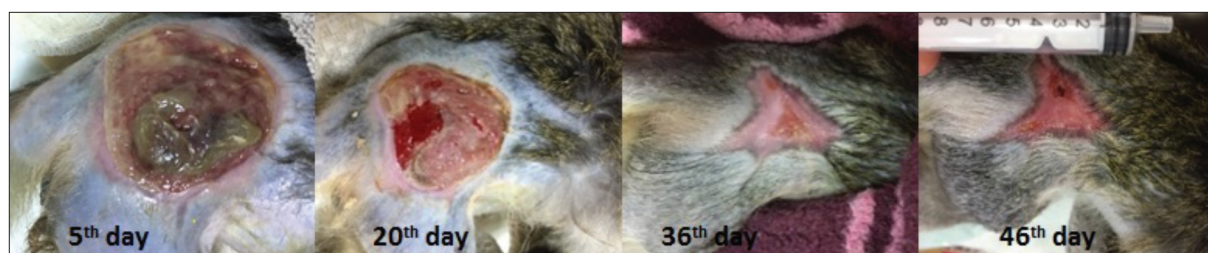


Fig 2. Appearance of the wound on different days of topical insulin treatment

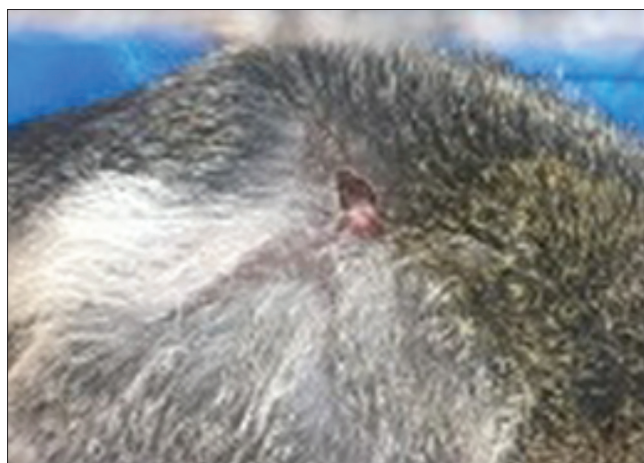


Fig 3. Appearance of the gluteal region on the 30th day following topical insulin therapy was ended

skin of the side [3,10]. In our case, very slow contraction phase was observed in the right gluteal wound after one month of treatment. It was thought that slow contraction phase in the wound may be related to the absence of panniculus carnosus muscle in gluteal area in vervet monkeys.

Wounds that do not heal beyond the expected normal healing process are defined as chronic wounds [4]. Chronic wounds generally originate from an underlying problem, such as vascular failure, diabetic or pressure ulcers, and shows secondary signs such as delayed healing, discolored granulation tissue, pocketing of wound base, and wound breakdown [11]. Biofilm formation in the chronic wounds is one of the causes of delayed wound healing. A planktonic bacteria or part of the biofilm, when bound an appropriate surface, creates a biofilm and has a different structure that is durable to antimicrobials and the host's immune response. Biofilms can affect keratinocytes, fibroblasts maturation, expression of pro-inflammatory cytokines, matrix metalloproteases, and induction of angiogenesis. Furthermore, biofilm bacteria do not cause acute phase inflammation, and may not be detected using conventional sampling techniques. The biofilm treatment is more difficult and different than the normal wound treatment, therefore, it is recommended that a combination of several methods, such as systemic and local antibiotic or antiseptics, or alternative methods be used. Although the effects of systemic and topical antibiotics are limited, they are the

best option for biofilm and normal wound healing [12]. The dexpanthenol is an epithelial agent commonly used in wound treatments that stimulates mRNA synthesis, fibroblastic activity and stimulates cytokines such as IL-6 and IL-8 [13]. Similarly, centella asiatica has been reported to induce angiogenesis through increased monocyte chemoattractant protein-1 (MCP-1) expression and stimulation of Vascular endothelial growth factor expression (VEGF-1) [14]. In our case, although we use a mixture of 4 pomades that it was mentioned their effects, epithelization and wound size changes were not observed on the 10th day after debridement. We thought that the delay in wound healing was due to the suppression of the host's immune response by biofilm formation.

Diabetes mellitus (DM) is a progressive disease characterized by insulin deficiency and insulin resistance or both, and generally the insulin is used in the treatment of DM [15]. The wound healing disorder in diabetes can be attributed to various factors, such as weak blood supply, reduced proliferative potential and low inflammatory changes [16]. Some burn, surgical incision or diabetic wound animal studies were demonstrated that inadequate or non-healing wounds can be treated with topical insulin application [17-21]. In some studies, it has been observed that insulin changes the catabolic metabolism of proteins by providing positive effect on nitrogen uptake, amino acid uptake of cells, protein and DNA synthesis in local wound healing. It was also observed that insulin regulates the use of glucose in cells, reduces exudate, bacterial growth, hypoxia at the wound site, and increases vascularization, proliferation and contraction [5]. Insulin is known to provide wound healing through certain growth factors such as Alpha-smooth muscle actin (α -SMA), Insulin-like growth factor (IGF-1), Transforming Growth Factor- β 1 [5,21]. It has also been reported that insulin provides endothelial cell migration without the use of the VEGF/VEGFR pathway [5]. In our case, despite the use of standard epithelization treatment and noobserved infection, we found that there was not enough epithelization in the wound. It was observed that epithelization started and accelerated after the use of insulin pomade. In our case, we thought that insulin pomade initiates proliferation and epithelialization by regulating glucose level, anabolic protein metabolism and DNA synthesis in the wound, using different growth hormone pathways and removing possible biofilm formation, in

contrast to the effects of other pomades. In conclusion, as in some mammalian studies, we think that topical insulin as an option can be used in non-healing wounds of monkeys.

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