Clinics of Surgery

Research Article

ISSN 2638-1451 | Volume 5

Role of Topical Amiloride in Acute Wound Healing

Gupta G[1], Sharma B[2], Sharma RK[3*] and Gupta B[4]

[1]Department of surgery, Associate professor, L.L.R.M. Medical college, Meerut

[2]Department of physiology, Associate professor, M.M.C. Muzaffarnagar, Uttar Pradesh

[3]Department of pharmacology, Professor & Head, M.M.C. Muzaffarnagar, Uttar Pradesh

[4]Department of pharmacology, Professor (Retd.), L.L.R.M. Medical college, Meerut

***Corresponding author:**

Rajeev kumar Sharma,
Prof.& Head Department of pharmacology, M.M.C. Muzaffarnagar, Uttar Pradesh,
E-mail: rajeevsharma791@gmail.com

Received: 08 Apr 2021
Accepted: 28 Apr 2021
Published: 05 May 2021

Keywords:

Acute Wound; Amiloride; Urokinase- Type Plasminogen Activator; Wound Repair

Citation:

Sharma RK.et al., Role of Topical Amiloride in Acute Wound Healing. Clin Surg. 2021; 5(8): 1-6

1. Abstract

A wound is defined as a disruption of cellular and anatomic continuity and wound healing is restoration of this continuity.

For clinical study on acute wounds, donor sites for split skin graft were chosen. Fifteen patients (11males and 4 females) age between 5-70 years admitted to the department of surgery and plastic surgery unit L.L.R.M. Medical college, Meerut. Informed written consent was obtained from each patient. An acute wound on the anterior thigh was used as donor site. Grafts were harvested from midline using Watsons modification of humby's knife. Each site was divided into proximal and distal halves and covered with either soft paraffin tulle serving as control or 0.5% amiloride tulle as test site and then dressed conventionally. Healing was evaluated visually on 10th post-operative day. Healing was significantly accelerated by amiloride tulle in terms of days required for complete healing ($P<0.01$), better quality of skin regenerated leading to case of removal of dressing with less of patient's discomfort and hence more acceptability ($P<0.01$). Thus amiloride ointment accelerate acute wound healing.

2. Introduction

A wound is defined as disruption of cellular and anatomic continuity and wound healing is restoration of continuity [1]. Wound healing represents a highly dynamic integrated series of cellular physiological and biochemical events that serve to replace the tissue loss [2].

Discontinuity in the cellular and anatomic structure of a tissue either due to tissue loss or destruction results in acute wound. Although the same events in the same order occur in every healing process regardless of the tissue type or the inciting injury but different type of wounds call for different treatment. So knowledge of wound healing allows the surgeon to manipulate the wound to achieve and optimal response in a rapid period of time. Wound healing is a continuous and not series of steps. It is a summation of a number of processes which follow injury including coagulation, inflammation, matrix synthesis and deposition, angiogenesis, fibroplasias, epithelialization, contraction, remodeling and scar maturation. It is affected by intrinsic and extrinsic factors like amount of damage, foreign body reaction, bacterial contamination, blood supply, age, nutritional status and the systemic disease like arterial disease, venous hypertension etc.

Numerous treatment modalities are currently used to use to control the associated infections and perfusion deficits in an attempt to foster an optimal environment for passive repair of the wound to occur. No such treatment has however been devised which wound stimulate active wound repair, yet remaining within reach of common population. Accelerated healing rate would definitely reduce the suffering pain, morbidity and over all hospital stay thus minimizing loss of man days and cost.

Various factors that have been found to promote active wound healing have limited availability and are very expensive. Various

herbal products have been claimed to be a good healer on the basis of animal studies. The Potassium-Spearing diuretic, amiloride, is a much cheaper and more extensively studied drug [3]. Beside other uses [4], its aqueous solution as well as ointment has been reported to promote to accelerate active healing of the mechanical corneal ulcers in rabbits [5], skin ulcers in albino rats [6].

Previously one study during period from 1999-2000 had been conducted in our department where the efficacy of the topically applied amiloride was tested clinically and experimentally, the results had been encouraging. Hence this study was further continued.

3. Materials and Method

The present study was conducted for a period of one and half year from 2001 to 2002 to investigate the role of amiloride in healing of acute wounds using clinical models.

Fifteen patients (11 males and 4-females aged between 5-70 years) admitted to the department of surgery and plastic surgery unit L.L.R.M. Medical College, Meerut requiring, a split skin graft for variety of conditions were included in this study. The need for split skin graft was due to burn contracture, laceration, necrotizing fasciitis, diabetic foot, of malignant condition in two patients. Only otherwise healthy persons were considered excluding those with uncontrolled diabetes mellitus, pregnancy, collagen vascular diseases, extensive burn of sepsis. Written informed consent was obtained from each patient.

For clinical study on an acute wound, the thigh was used a donor site in each case. After preliminary preparations, painting and draping, grafts were harvested using watson's modification of Humby's Knife which was adjusted for medium thickness, the grafts were lifted by steady to and fro motion of knife on wet skin and then stored in normal saline. The donor site was covered with saline-soaked gauze until the end of surgery.

To assess whether amiloride healed donor sites faster than the traditional dressings, each site was divided into proximal and distal halves and covered with either soft paraffin tulle (Jelonet) serving as control or 0.5 % amiloride tulle as test site. The amiloride ointment was prepared by mixing 0.5 gm of pure amiloride in 100gm of soft paraffin on a tile with spatula. The ointment was impregnated in preliminary preparationto gauze pieces of 10×10 cms and then sterilized in hot air oven at 160 OC for 3 hrs (British pharmacopoeia). In all cases the wound contact layer was then covered with three layers of cotton gauze, cotton wool padding and crepe bandage, secured with adhesive tape & clips,All the patients remained in the hospital throughout the treatment period and received usual antimicrobials and analgesics as required. Healing of donor site was evaluated visually on 10th post-operative day. Dressings were first soaked in normal saline for 30 minutes and then removed leaving only tulle grass at donor site. Every precaution was taken to reduced dressing trauma to the minimum as we know that some wounds may not be healed or at best, be rather fragile. Both sites were assessed before as well as after removal of tulle grass subjectively as well as photographically.

The following parameter were considered for evaluation-

1. Quantity of soaking dressing.
2. Patients discomfort (pain) and ease of removal of dressing.
3. Presence of infection.
4. Status of wound healing.
5. Quality of skin regenerated.
6. Patients acceptability regarding comfort while removal of dressing.

A donor site was classed as healed if the dressing came off without pain to leave a dry-re-epithelialised surface. The quality of new skin at first dressing whether healed or not was assessed by the investigator. The assessment of patients' discomfort with dressing and pain was made jointly by the investigator and the patient.

For statistical evaluation of healing status, each parameter was divided into three categories depending upon the gravity. Scores were then allotted as 5,3 and 1 for best, better and worst healing status respectively. Data obtained was finally analyzed utilizing student- 't'- test.

3.1. The Experimental Study

As the Institution is not registered under the guidelines laid down by "Committee for the purpose of control and supervision of Experiment on Animal" (CPCSEA) till date, the animal experimentation could not be done.

3.2. Observations

The study was conducted on donor site of split skin graft of fifteen patients where half of the harvested site was dressed with 0.5% Amiloride tulle and the other half with standard paraffin tulle(-Jelonet). Since the other half of donor site served as control, this becomes as self-control study where all the inherent biological variables and supportive treatment factors have been eliminated.

It's evident (Table 1 and Figure 1) that there was an acceleration of healing in amiloride treated site.

The most dramatic improvement in patient comfort came with dressing changes. In every patient irrespective of presence or absences of infection, the Amiloride dressing came away either without pain of with for less discomfort than with the traditional dressing.

The rate of healing under amiloride was evidently greater in 86.67% of patients with 5% Amiloride tulle, there was a healed surface at first dressing on 10th post-operative day, whereas only 13.33% conventionally treated area healed by that time.

Although some amiloride treated donor site (13.33%) have not totally healed at the 1st dressing. The Amiloride tulle came off easily

and the regeneration surface was apparently not damaged compared from traditional paraffin tulle dressings which become hard, dry and adherent to the healing skin. Its removal became painful and regenerated epithelium pulled off with the dressing.

A good quality regenerated skin at 1st dressing is defined as an area that no longer required dressing [7]. The healed Amiloride site at first dressing was given only protective dressing with Amiloride tulle till complete healing of the control site.

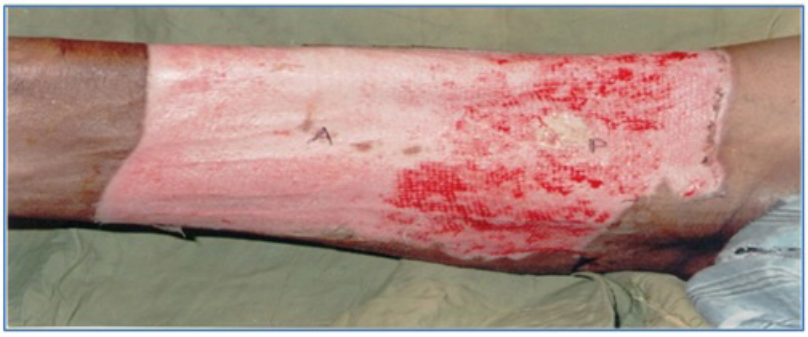

Figure 1: Depiction of comparative Effect of Amiloride (A) and Paratulle (P) on 10th Day

Table 1: The donor area of the graft was arbitrarily divided into two halves and each half was dressed with paratulle and 0.5% Amiloride tulle respectively. On 10th day the results were compared between the two areas by the surgeon and patient separately. Each parameter was divided into three categories depending upon the degree and scores were than allotted as 5, 3 and I for beast, better and worst healing status respectively.

Parameter	Score			
	Best (5)	Better (3)	Worst (1)	Mean ± S.E.
(A) Surgeon's Assessment	F(%)	F(%)	F(%)	
(a)Soakage in Dressing	Nil	Minimal	Present	
-Paratulle site	1(6.67)	13(86.67)	1(6.67)	3.00±0.20
-Amiloridesite	5(33.33)	9(60.00)	1(6.67)	3.53±0.20
(b)Ease of removal of dressing	Easy	Slightly	Difficult	3.27± 0.27
-Paratulle site	3(20.00)	11(46.67)	1(6.67)	4.20± 0.26
-Amiloride site	9(60.00)	6(40.00)	0(0.00)	
(c)Patient's discomfort (pain) while removing the dressing	Mild	Moderate	Severe	4.07 ±0.27
-Paratulle site	8(53.33)	7(46.67)	0(0.00)	4.87 ±0.13*
-Amiloride site	14(93.33)	1(06.67)	0(0.00)	
(d)Infection	Nil	Minimal	Moderate	4.20 ±0.26
-Paratulle site	9(60.00)	6(40.00)	0(0.00)	4.33 ±0.25
-Amiloride site	10(66.67)	5(33.33)	0(0.00)	
(e)Status of wound healing	Complete	Near Complete	Raw	1.27 ±0.18
-Paratulle site	0(0.00)	2(26.67)	13(86.67)	3.13± 0.31**
-Amiloride site	3(20.00)	10(66.67)	2(13.33)	
(f)Quality of skin regenerated	Good	Average	Poor	1.53 ±0.24
-Paratulle site	0(0.00)	4(26.67)	11(73.33)	3.93 ±0.27**
-Amiloride site	7(46.67)	8(53.33)	0(0.00)	
(B) Patient's acceptability of dressing	No Problem	Uncomfortable	Painful	3.80 ±0.33
-Paratulle site	7(46.67)	7(46.67)	1(6.67)	4.73 ±0.18*
-Amiloride site	13(86.67)	2(13.33)	0(0.00)	

The final aspect of healing assessed at the 1st dressing was the surface of wound. It was to be exposed that the paraffin tulle gras treated areas would be rough as the mesh of the tulle gras presses on part of wound surface and islands of regeneration cells grow up in the mesh space [7]. In Amiloride treated areas as smooth cover was noticed. The difference between Amiloride and traditionally treated areas was very much marked and evident in many patients, with Amiloride giving a better and smoother skin.

There were six infections in the study, of them five were in both the sites and one was involving the paraffin tulle site only. There was no difference in dressing slippage between the two groups. No hypertrophic scarring was observed in any case.

4. Discussion

Wounds are Common from child to old age and necessary to surgical entry. Wound (ulcer) means a solution or disruption of the anatomical continuity of any of the tissues of the body and healing is the response to injury. Through the process of healing should be regarded as a continuous stream of interrelated responses. It is conventionally subdivided into three Phases-Inflammations including cell migration, wound closure, Proliferation and post wounding remodeling.

Migration of epithelial cells into the wounded edge occurs quickly and independently of cell division. Extracellular matrix (ECM) and its components may provide a substrate for the epithelium to adhere to and migrate rapidly across and thus augment the first phase of wound healing. The wounding of the connective tissue in the dermis also causes fibroblasts to migrate into the wound. The migration requires the synthesis of proteins and glycoprotein's in extracellular marix(ECM) [8]. High levels of proteases apparently block migration that is first phase of wound healing, by digesting either matrix or cell surface molecules [9]. However, the finding that protease inhibitors slow the migration suggest, that low levels of proteases may be necessary for migration, perhaps by digesting temporary adhesion junctions of their associated matrix [10].

Plasminogen activators (serine proteases) are enzymes which by converting zymogen plasminogen to active proteolytic enzyme plasmin, are implicated in proteolytic degradation [11]. Two types of Plasminogen Activators (PAs) can be distinguished in the intact organism namely tissue-type(t-PA) and urokinase type (u-PA). They seem to take part in different biological processes; t-PA being a key enzyme in thrombolysis whereas u-PA plays a central role in regulating extracellular proteolysis in a variety of normal and pathological processes involving cell migration and tissue destruction [12].

Cell motility is accompanied by an up-regulation of u-PA and a change in the solubility and proteolysis of the u-PA receptor [13]. There are several stages in epidermal migration along or on extra cellular matrix(ECM) where proteinases plasminogen activator and/or plasmin might be involved. Fibronectin fragments, generated through proteinase activity, might be necessary for cell migration Fragments of fibronectin have higher affinity than whole fibronectin for some cell surface receptors [13] and as suggested by Thiery [2], therefore could be required to detach the migrating cell from its fibronectin matrix.

Studies have shown that u-PA is present in the leading edge of migrating corneal epithelium both after abrasion and alkali injuries [14]. The abnormally prolonged presence of enzymatically active u-PA at leading epithelial edge after burn result in persistent corneal epithelial defect [14]. Regulation of u-PA activity at leading edge of epithelium following injury might be expected to be useful therapeutically in the healing of epithelial defects and prevention of ulceration [14, 15].

Later phase of wound healing involves dissolution of fibronectin matrix [16]. Potential therapeutic agents would include compounds that block their degration. During this phase too plasminogen activators and/or plasmin have been suggested to be implicated [16]. Thus protease inhibitors would effect this later phase of wound healing by blocking the digestion of extracellular matrix(ECM) components necessary for cell attachment.

In the present study, amiloride treated half of donor site exhibited better results in terms of lesser number of days required for complete healing and better quality of regenerated skin with less of patient discomfort (Table-1, Figure-1).

One of the cellular events of normal wound repair is angiogenesis, the formation of new blood vessels. Blood vessels are assembled by either vasculogenesis, in which a primitive vascular network is established from mesenchymal progenitor cells during development, or angiogenesis, in which new blood vessels arise from preexisting vessels. Though vasculogenesis is limited to development, angiogenesis occurs both in the embryo and in adults. For angiogenesis to occur, endothelial cells must detach from adjacent endothelia, Proliferate, migrate, and assemble into tubes. Yet the cell signals that regulate and are involved in the process of angiogenesis are only partially known. Tissue injury is immediately followed by coagulation, changing the Extra Cellular Matrix (ECM) environment of endothelial cells, from a collagen and laminin rich matrix to a fibrin and vitronectin rich matrix. These newly deposited extra cellular matrix molecules bind to integrins, a class of cell surface receptors that anchor the cytoskeleton to the surrounding extracellular matrix and provide cellular signals about the matrix environment to the endothelial cells. The changes in the matrix environment along can result in large scale changes in gene expression that favor endothelial cell proliferation and survival, important for blood vessel formation in the wound.

Wound repair angiogenesis requires activation of fibrinolytic enzymes and the plasminogen activator system, which consists of urokinase Plasminogen Activator (u-PA) and tissue Plasminogen Activator (t-PA), balanced by plasminogen activator inhibi-

tor-1(PAI-1) and vitronectin. It has been hypothesized that the dysregulation of the plasminogen activator system can result in abnormal wound healing. Transgenic mice deficient for vitronectin, u-PA, or u-PAR (urokinase plasminogen activator receptor; u-par) genes have been reported to appear phenotypically normal, but with marked disturbances in response to tissue injury. Vitronectin served to limit fibrinolysis following injury and elimination of vitronectin from the provisional wound matrix lead to microvascular hemorrhage and resulted in delayed wound healing. In addition, limited function of u-PA or u-PAR genes disturbed cell migration, result in decreased wound angiogenesis and decreased wound epithelialization. Interestingly, in spite of significant effects on wound microvasculature, deletion of u-PA, or u-PAR genes did not result in delayed healing. The murine wounds with u-PA, or u-PAR deletions compensate with increased wound contraction.

Angiogensis occurs under both physiological and pathological conditions. It is a complex process that probably involves the interplay of a large variety of growth and regulatory factors. Sequence of events in new capillary growth involves degradation of basement membrane, directional migration and alignment, endothelial cell proliferation, tube formation and capillary loop formation. Pathologic angiogenesis can be blocked at several points during the cascade of capillary growth [17] and has led to discovery of angiogenesis inhibitor compounds. A key early step in angiogenesis is the production by endothelial cells of serine proteases (like urokinase plasminogen activator, u-PA) and matrix metalloproteinases that degrade the basement membrane and permit endothelial invasion of the extracellular matrix (ECM) [18] at the leading edge of new vessels [19].

Since plasminogen activators are capable of activating a proteolytic cascade that can digest ECM; u-PA antagonists are likely to prevent angiogenesis. In fact, Antibodies to u-PA have been shown to inhibit tissue invasion and metastasis [20]. Similarly, an inhibitor of u-PA derived from conditioned media of cultured retinal pigmented epithelial cells have been shown to inhibit angiogenesis invitro as well as in rabbit corneal pocket assay [21].

Wound conversion is the term which refers to the dynamic process whereby zone of injury progresses to the zone of tissue necrosis thereby deepening the wound. Environmental hazards can readily lead to conversion of an open wound.

Natural PA inhibitors coexist with pro u-PA in the extracellular milieu and the reaction rate of these inhibitors for u-PA`is several orders of magnitude higher than that of the major plasma protease inhibitors, it is thus likely that they play an important part in controlling u-PA initiated extracellular proteolysis in vivo [22].

Moreover, mechanical injury instigates inflammation which in turn recruit polymorphonuclear cells(PMNs) from the surrounding tissue by migration into the wound area. These PMNs and mononuclear cells can delay the closure of the epithelial wound due to activation of their lysosomal enzymes (acid glycosidases, lysosomal proteases) such that continuing inflammation may foster the persistence of epithelial defect as had been seen in case of corneal ulcers [23]. Activated macrophages that have migrated to inflammatory sites are a good source of u-PA. It seems logical to speculate that abnormally large amounts of u-PA released in the wound area by migrating cells (epithelial cells, macrophages and fibroblasts) becomes unmanageable for natural PA inhibitors leading to much greater proteolysis than is required due to some yet unexplained reason. Probable reason for such disturbance in body's own homeostasis may be either increased inactivation and/or incapability to cope up with their increased demand of naturally occurring PA inhibitors or due to the fact that PA inhibitors can inactivate only the secreted enzymes without affecting the cells associated activity (presumably receptor bound) as has been suggested by chapman et al. [22]. Amiloride and exogenous u-PA inhibitor [24], in such situation might have played a good role by controlling the abnormally prolonged presence of enzymatically active u-PA and thus regulating proteolysis, excess of which is disastrous for wound healing.

The ease of removal of dressing with amiloride tulle on 10th post-operative day in due to earlier healing at amiloride site as compared form paraffin tulle site. The exact mechanism by which amiloride promoted haling is not known with certainty but it may be postulated that it enhanced migration of epithelial cells into the wounded edges by lysing the temporary adhesion junctions and their associated matrix by its urokinase type plasminogen activator inhibitor role providing accelerated healing and better patient acceptability.

Presence of slough and/or plus could not be noticed in any of the ulcer whether treated with soft paraffin or 0.5% amiloride ointment. Since no antimicrobial agent was used topically during the study, it can be stated that intrinsic immunity against infections remained unaltered with the topical use of amiloride. In addition, amiloride did not produce any signs of local irritation.

5. Conclusion

Thus, it is concluded that 0.5 % topical amiloride may prove to be a cheap and better ulcer healing agent (with efficacy similar to 1% amiloride ointment) Particularly in cases of acute mechanical injuries to skin with no apparent side effects.

References

1. Schilling JA. Wound healing. Surg Clib N Amer. 1976; 56: 859-74.
2. Thiery JP. Mechanisms of cell migration in the vertebrate embryo. Cell Differ. 1984; 15: 1-15.
3. Gupta B, Arora P, Singh VS, Bhargava SK. Topical amiloride ointment accelerates healing of mechanical skin ulcers in albino rabbits and patients. Methods Find Exp Clin Pharmacolo. 2006; 28(2): 101-7.

4. Massaeli H, Pierce GN. The protective role of amiloride and its analogs in ischemia/reperfusion challenge. Indian J Pharmacolo. 1993; 25: 68-72.

5. Sood AK, Gupta B, Chung P. Topical Amiloride accelerates healing and delays neovascularization in mechanically produced corneal ulcers in Rabbits. Methods find Exp. ClinPharmacol. 1999; 21(7): 491-7.

6. Gupta B, Singh N, Saxena KK, Srivastava VK. Topical amiloride solution accelerates healing of mechanical skin ulcers in albino rats. Methods and find in Exp. ClinPharmacol. 2000; 22(9): 671-7.

7. Attwood AI. Claciumalginate dressing accelerate split skin graft donor site healing. BRJ Plast Surg. 1989; 42: 373-9.

8. Gipson IK, Kiorpes TC. Epithelial sheet movement: Protein and glycoprotein synthesis. Dev Bio. 1982; 92: 259-62.

9. Frati L, Daniele S, Delogu A, Covelli I. selective binding of the epidermal growth factor and its specific effects on the epithelial cells of the cornea. Exp Eye Res. 1972; 14: 135-41.

10. Zieske JD, Bukusoglu G. Effects of protease inhibitors on corneal epithelial migration. Invest Ophthalmol. Vis Sci. 1991; 32: 2073-8.

11. Quigley JP, RH Gpldfrab C, Scheiner J, O' Donnel-Tormey and TK Yeo. Plasminogen activator and the membrane of transformed cells in tumor cells sufaces and malignancy. R.O. Hynes and C.F. Fox, editor. Alan R. Liss, New York. 1980; 773-796.

12. Paciucci R, Vila MR, Adell T, Kiaz VM, Tora M, Nalaamura T, Real FX. Activation of the urokinase plasminogen activator/urokinase plasminogen activator receptor system and redistribution of E-cadherin are associated with hepatocyte growth factor-induced motility of pancreas tumor cells over expressing met. Am J Pathol. 1998; 1539(1): 201-12.

13. Akiyama SK, Yamada KM. The Interaction of plasma fibronection with fibroblastic cells in suspension. J Biol Chem. 1985; 260: 4492-500.

14. Hayashi K, Berman M, Smith D, El ghatit A, Pease S, Kenyon KR. Pathogenesis of corneal epithelial defects: Role of plasminogen activator. Cur. Eye. Res. 1991; 10: 381-98.

15. Wang HM, Berman M, Law M. Latent and active plasminogen activator in corneal ulceration. Invest Ophthalmol Vis Sci. 1985; 26: 511-24.

16. Clark RAF, Lanigan JM, Della Pelle P, Manseau E Dvorah H, Colvin RB. Fibronecttin and fibrin provide and provisional matrix for epidermal cell migration during wound reepitheliazation. J Invest Dermatol. 1982; 70: 264-9.

17. Folkman J. Editorial comment. Oncology overview on antiangiogenesis: A potential therapantic strategy. Cancer information dissemination and Analysis centre, National centre Institute, U.S. Department of Health and Human Services, November. 1991.

18. Fan TPD, Jagger R, Bicknell R. Controlling the vasculature: Angiogenesis, Anti-angiogenesis and vascular targeting of gene therapy. Trends pharmacol Sci. 1995; 16: 57-66.

19. Ausprunk DH, Folkman J. Migration and Proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc Res. 1997; 14: 53-65.

20. Ossowski L, Reich E. Antibodies to plasminogen activator inhibit human tumor metastasis. Cell. 1983; 35: 611-9.

21. Glaser BM, Adamsons I, Lansing M, Jarden J, Davis JA. Urokinase inhibitors derived from human RPE cells inhibits neovascularization in Vivo. Invest Opthalmol Vis Sci. 1989; 30(Suppl): 395.

22. Chapman JA, Varin Z, Hibbs JB. Macrophage fibrinolytic activity: identification of two pathway of plasmin formation by intact cells and of a plasminogen activator inhibitor. cell. 1982; 28: 653-62.

23. Wagoner MD, et al. Polymorphoncuclear neutrophils delay corneal epithelial wound healing in vitro. Invest. Opthalmol. Vis Sci. 1984; 25:1217-20.

24. Vasali JD, Belin D. Amiloride selectively inhibits the urokinase type plasminogen activator. FEBS Lett. 1987; 214: 187-91.