

# EFFECTIVENESS OF TOPICAL $\alpha$ -TOCOPHEROL ACETATE IN BURN INFECTION TREATMENT

## EFFICACITÉ DE L'ACÉTATE D' $\alpha$ - TOCOPHÉROL (ATA) DANS LE TRAITEMENT DES INFECTIONS DE ZONE BRÛLÉE

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**SUMMARY.** Infection following burn injury is critical, especially for patients with large total body surface area burns and in skin graft donor sites. Although various aspects of  $\alpha$ -tocopherol acetate ( $\alpha$ -TA) beneficial effects on wound healing have been validated, it appears that no study has specifically addressed its antimicrobial potential. The purpose of this study was to explore the therapeutic efficacy of topical application of  $\alpha$ -TA in terms of bacterial load reduction. Between January 2018 and June 2018, 20 patients with mid-deep and deep burn wounds were included in the present study (average TBSA approximately 42%, range 25-67%). Patient average age was 47.75 years (range 25-72 years), 8 were male. In each patient two clinically similar areas were identified and treated every 24 hours with topical application of  $\alpha$ -TA in the form of Filme Olio® (Group 1) and conventional medication (Group 2). All the patients had positive results for bacterial cultures before treatment. Despite the presence of initial bacterial infection, a quicker reduction of exudates and pain and a progressive and faster bacterial load reduction was observed in Group 1. Negative cultures were obtained after 3 days on average in Group 1 (range 1-6 days) and 8 days in Group 2 (range 5-9 days). This study clearly shows the importance of the therapeutic targeting of infection in the treatment of burns.  $\alpha$ -TA may represent a safe, simple and inexpensive method for improving the healing of difficult wounds with local infection.

**Keywords:** vitamin E,  $\alpha$ -tocopherol acetate, burns, burn infection, burn treatment

**RÉSUMÉ.** Les infections sont une complication majeure des brûlures, en particulier chez les patients largement atteints et au niveau des sites donneurs. Alors que ATA a été évalué, et considéré bénéfique, dans de nombreux aspects de la cicatrisation, aucune étude ne s'est penchée sur son potentiel antimicrobien. Cette étude a exploré l'effet de ATA topique sur la réduction de l'inoculum bactérien. Vingt patients dont 12 femmes, ayant des brûlures intermédiaires et profondes, ont été inclus dans cette étude, qui s'est déroulée entre janvier et juin 2018. Leur âge était en moyenne de 47,75 ans (25- 72), la surface brûlée de 42% (25-67%). Chez tous les patients, deux zones similaires, à la culture bactérienne positive, étaient traitées par ATA (groupe 1) ou traitement conventionnel (groupe 2). La diminution des l'exsudation, de la douleur et de la charge bactérienne était plus rapide dans le groupe 1. Les cultures se négativalent en 3 jours (1- 6) dans le groupe 1 contre 8 (5- 9) dans le groupe 2. Cette étude montre l'intérêt du traitement local des infections de zones brûlées, ATA apparaissant dans ce cadre comme un traitement simple, sûr et peu onéreux.

**Mots-clés :** vitamine E, acétate d' $\alpha$ - tocophérol, infection de zone brûlée, traitement

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## Introduction

Infection following burn injury is the most common complication in burn wound patients, accounting for up to 60-75% of burn-related deaths.<sup>1,2</sup> The majority of superficial burn injuries do not progress to invasive infection, but the problem of wound infection remains critical, especially for patients with large total body surface area (TBSA) burns and in skin graft donor sites. In addition to violating the physical skin barrier, burns disrupt immunological, metabolic and sensory skin functions, leaving a susceptible pathway for bacterial invasion. Burn wounds, classically considered to be sterile immediately upon injury, become colonized and infected within one week after insult, often while the patient is under direct hospital care. Moreover, burn eschar provides a protein-rich, avascular environment that encourages microbial growth, while impeding host immune cells and antimicrobial agent influx. It has also been recently recognized that numerous bacteria (and fungi) exist within burn wounds in the form of biofilms.<sup>3</sup> The combination of virulent and drug-resistant pathogens in a hospitable local milieu, in the setting of functional immune impairment, makes burn wound infection especially difficult to overcome, despite aggressive use of both topical and systemic antibiotics. The modification of medical treatment protocols and the search for new mechanisms involved in the pathogenesis of burns may be helpful for the successful treatment of burn patients.

Vitamin E is a family of essential micronutrients with strong antioxidant activity composed of lipid-soluble tocopherols and tocotrienols, with  $\alpha$ -Tocopherol ( $\alpha$ -TA) the form that is favored by the human cells.  $\alpha$ -TA is involved in immune function and, as shown primarily by *in vitro* studies of cells, cell signaling, regulation of gene expression, and other metabolic processes. Moreover, it inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets and monocytes.  $\alpha$ -TA-replete endothelial cells lining the interior surface of blood vessels are better able to resist blood-cell components adhering to this surface.  $\alpha$ -TA also increases the expression of two enzymes that suppress arachi-

donic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation.<sup>4</sup> In their preliminary report Stanizzi et al.<sup>5</sup> described the positive effects of topical use of  $\alpha$ -TA, such as granulation tissue stimulation, bacterial growth reduction, angiogenesis modulation and epithelialisation improvement. Although various aspects of  $\alpha$ -TA beneficial effects on wound healing have been validated, it appears that no study has specifically addressed its antimicrobial potential.

The purpose of this study was to explore the therapeutic efficacy of topical application of  $\alpha$ -TA in improving the healing of potential infective burn wounds and skin graft donor sites in terms of bacterial load reduction.

## Materials and methods

This study was carried out between January 2018 and June 2018 and involved 20 patients with mid-deep and deep burn wounds (average TBSA approximately 42%, range 25-67%). Patient average age was 47.75 years (range 25-72 years), 8 were male. The presence of exudate, pain and delayed re-epithelialisation, absence of tissue necrosis and/or sepsis were the criteria for the topical use of  $\alpha$ -TA, avoiding conventional treatments such as local antibiotics, polyurethane foams and other occlusive dressings. Exclusion criteria were the presence of severe renal or hepatic failure, positive history of myocardial infarction, ischemic or hemorrhagic stroke, coagulation or psychological disorders.

After careful removal of all epidermal debris and wound irrigation with saline solution, wound tissue cultures were obtained daily (*Fig. 1*), for 10 consecutive days, and sent to the Microbiological Unit of the Pisa University Hospital.

In each patient two clinically similar areas were identified and treated every 24 hours as listed below:

Group 1: topical application of  $\alpha$ -TA in the form of filme Olio® (*Figs. 2a and 2b*);

Group 2: conventional medication (e.g. silver dressings, collagenases, topical antimicrobials).



Fig. 1 - After careful removal of all epidermal debris and wound irrigation with saline solution, wound tissue cultures were obtained daily



Fig. 2b - Topical application of filme Olio® on the infected wound



Fig. 2a - Application of  $\alpha$ -TA in the form of filme Olio® on a tongue depressor

## Results

Patient data and culture sample results are summarized in *Tables I and II*. All the patients had positive bacterial culture results before treatment. The isolated organisms cultured from the wound tissue in Group 1 were *Pseudomonas aeruginosa* (N=7, 35%), *Candida parapsilosis* (N=4, 20%), *C. albicans* (N=3, 15%), *Staphylococcus haemolyticus* (N=2, 10%), *Proteus mirabilis* (N=2, 10%), *Staphylococcus epidermidis* (N=1, 5%) and *Staphylococcus capitis* (N=1, 5%). Despite the presence of initial bacterial infection, a quicker reduction of exudates and pain and a progressive and faster bacterial load reduction was observed in Group 1 (*Fig. 3*). Clinically, several macroscopic changes in granulating tissue were observed (*Figs. 4 and 5*). Negative cultures were obtained after 3 days on average in Group 1 (range 1-6 days) and 8 days in Group 2 (range 5-9 days).

**Table I** - Group 1 patient data and culture sample results

No.	Age	Sex	TBSA (%)	Microbiological cultures											
				Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	
1	49	M	34	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	
2	67	F	46	C.p.	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-	-	
3	64	F	37	S.h.	S.h.	S.h.	S.h.	-	-	-	-	-	-	-	
4	53	F	29	S.h.	-	-	-	-	-	-	-	-	-	-	
5	44	F	67	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-	-	
6	47	M	53	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-	-	
7	49	F	48	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-	-	-	
8	35	M	56	S.e.	S.e.	S.e.	S.e.	-	-	-	-	-	-	-	
9	68	F	43	C.p.	C.p.	-	-	-	-	-	-	-	-	-	
10	51	F	28	P.m.	P.m.	P.m.	P.m.	-	-	-	-	-	-	-	
11	25	M	56	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-	-	
12	72	M	34	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-	-	-	
13	36	F	46	P.a.	P.a.	-	-	-	-	-	-	-	-	-	
14	60	M	25	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-	-	
15	44	F	51	P.m.	P.m.	P.m.	-	-	-	-	-	-	-	-	
16	32	M	35	P.a.	P.a.	P.a.	-	-	-	-	-	-	-	-	
17	58	F	47	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-	-	-	
18	34	F	42	S.c.	S.c.	S.c.	S.c.	S.c.	-	-	-	-	-	-	
19	41	M	27	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-	-	-	
20	26	F	38	C.a.	C.a.	C.a.	-	-	-	-	-	-	-	-	

C.p. *Candida parapsilosis*, C.a. *Candida albicans*, P.m. *Proteus mirabilis*, P.a. *Pseudomonas aeruginosa*, S.c. *Staphylococcus capitis*, S.e. *Staphylococcus epidermidis*, S.h. *Staphylococcus haemolyticus*

**Table II** - Group 2 patient data and culture sample results

No.	Age	Sex	TBSA (%)	Microbiological cultures											
				Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	
1	49	M	34	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	
2	67	F	46	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	
3	64	F	37	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	-	
4	53	F	29	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	-	-	
5	44	F	67	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	
6	47	M	53	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	
7	49	F	48	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	
8	35	M	56	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	-	-	-	-	
9	68	F	43	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	-	
10	51	F	28	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	
11	25	M	56	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	
12	72	M	34	C.p.	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-	-	
13	36	F	46	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	
14	60	M	25	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	
15	44	F	51	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	-	
16	32	M	35	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	
17	58	F	47	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	-	
18	34	F	42	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	-	-	-	
19	41	M	27	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	
20	26	F	38	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	

C.p. *Candida parapsilosis*, C.a. *Candida albicans*, P.m. *Proteus mirabilis*, P.a. *Pseudomonas aeruginosa*, S.c. *Staphylococcus capitis*, S.e. *Staphylococcus epidermidis*, S.h. *Staphylococcus haemolyticus*

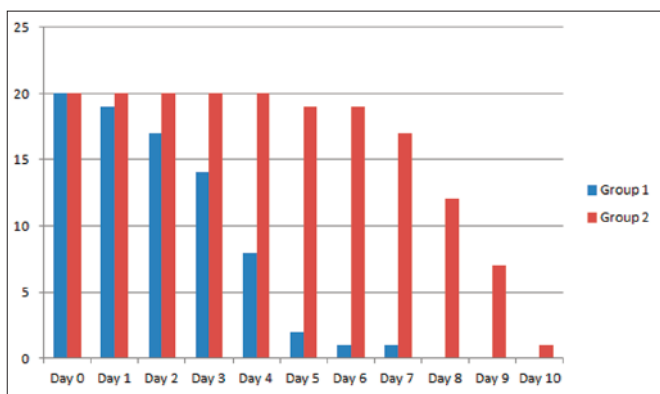


Fig. 3 - A faster bacterial load reduction was observed in Group 1 compared to Group 2



Fig. 4 - Burn wound after 5 days of treatment with filme Olio®



Fig. 5 - Burn wound after 10 days of treatment with filme Olio®

## Discussion

Burns are a major health problem worldwide, with high mortality and morbidity in addition to causing changes in the quality of life of burn patients. The management of burn injuries to limit infection and resurface the wound currently relies on several core strategies: deeper (full-thickness) injuries usually require surgical excision with skin replacement through autografts, allografts, temporary dressings or skin substitutes.

Topical antimicrobials, especially silver-containing compounds such as silver sulfadiazine, have been the mainstay of local burn wound therapy for over 40 years.<sup>6</sup> Progress over this time has been limited and incremental, with most effort devoted to deploying novel carriers for the silver, or extending its use to other types of wounds. However, recent reports have noted multiple drawbacks to the use of topical silver as an antimicrobial: development of resistance (including multi-drug resistance)

by induction of efflux mechanism (including *P. aeruginosa*), local and systemic toxicity to host tissues (e.g. renal toxicity, hepatotoxicity), argyria, and dyschromia of the skin. In this context, there is a pressing need for the development of new therapeutic modalities that can be administered to the infected burn wound and will facilitate wound healing without eliciting harmful side effects.

Locally applied probiotic bacteriotherapy offers an attractive alternative.<sup>7</sup> Probiotic bacteria may be active against a range of pathogens simultaneously, including drug-resistant organisms. They are unlikely to facilitate emerging antibiotic resistance and would be potentially effective even against pathogens in biofilm configuration. Moreover, they are inexpensive and could be easily applied topically to a burn injury site. However, the use of topical probiotics in burn patients is limited to a single report. In addition, the possibility of infective risks of probiotic therapy in functionally immunocompromised patients has not been established.

Utilizing antioxidant therapeutic strategies depending on new mechanisms involved in the pathogenesis of burn-related “oxidative stress” may be considered a promising step in burns management. Vitamin E, first described in 1922 by Evans and Bishop as an essential micronutrient for reproduction in rats,<sup>8</sup> is a fat-soluble vitamin with important functions of maintaining the integrity of the intracellular membrane by protecting its physical stability and providing defense against any tissue damage caused by oxidation.<sup>9</sup> Molecular and cellular effects of vitamin E have been explained by regulating membranes and protein activities by specific reactive oxygen species (ROS) and nitrous oxide systems (NOS) or by interacting and regulating specific enzymes and transcription factors and influencing cellular structures such as membranes and lipid domains.<sup>10</sup> The antioxidant capacity of  $\alpha$ -TA and its effect on reprogramming gene expression allow its topical use in skin diseases where an inflammatory process is activated. Furthermore, it has been demonstrated that epidermal esterases remove from  $\alpha$ -TA the acetic acid that may act against microorganisms simply by lowering the pH and cre-

ating an anhydrous environment unsuitable for their growth and multiplication.<sup>11</sup>

In the present study we used  $\alpha$ -TA for treatment of intermediate-severe burns and skin graft donor sites, even in the presence of wound infection. Topical  $\alpha$ -TA was applied in the form of Filme Olio® on the wound every 24 hours after wound irrigations with saline solution. Topical use of  $\alpha$ -TA is extremely easy, inexpensive, and can often be performed by the patient himself, leading to total compliance of the patient and cost reduction. Despite the presence of initial bacterial infection, a quicker reduction in bacterial load was observed in all the patients, compared to the areas treated with conventional medications. Moreover, clinically, several macroscopic changes in granulation tissue were observed. Initially the unhealthy granulation was a dark red colour, bleeding on contact, characterized by heavy exudates and bumpy irregular overgrowth, and covered with white and yellow shiny fibrinous tissue. After treatment with topical  $\alpha$ -TA, the healthy granulation tissue became light red in colour, without exudates, and was painless. Clinical data appeared to confirm the bacteriostatic effect of  $\alpha$ -TA. In all treated patients no sepsis nor tissue necrosis recurrence was observed until complete healing. Clinical evidence was confirmed by microbiological examination.

We believe that our results are valuable and warrant debate, because this is the first study to describe clinical and cultural evidence of bacterial load reduction after topical use of  $\alpha$ -TA. However, this is only a preliminary report and further studies will be necessary to confirm our findings.

## Conclusion

This study clearly shows the importance of the therapeutic targeting of infection in the treatment of burns. As  $\alpha$ -TA stimulates granulation tissue, it seems to reduce bacterial growth, modulate angiogenesis and improve epithelialization. Filme Olio® may represent a safe, simple and inexpensive method for improving the healing of difficult wounds with local infection.

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