EFFECTIVENESS OF REACTIVE OXYGEN SPECIES IN AN OIL-BASED MEDICATION FOR HEALING BURN WOUNDS: A CASE SERIES

EFFICACITÉ DES RADICAUX OXYGÉNÉS RÉACTIFS EN SUSPENSION HUILEUSE SUR LA CICATRISATION DES BRÛLURES : UNE SÉRIE

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SUMMARY. Oxygen is considered a key element in the complex tissue repair process. O_3-Oil antiseptics are obtained from the chemical reaction between ozone and unsaturated fatty acids of vegetable oils. The purpose of this study was to assess the effectiveness of a commercially available O_3-Oil in the management of recalcitrant burn wounds. This study involved 20 patients with mid-deep and deep burn wounds (average TBSA approximately 23%, range 7-35%). Patient average age was 47 years (range 26-74 years), 8 were male. The presence of exudate, pain and delayed re-epithelialization, absence of tissue necrosis and/or sepsis were the inclusion criteria for the topical use of O_3-Oil. In each patient two clinically similar areas were identified and treated every 48 hours with O_3-Oil (Group 1) and conventional medication (Group 2). All the patients had positive bacterial culture results before treatment. 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 4 days on average in Group 1 (range 1-7 days) and 8 days in Group 2 (range 5-11 days). The present study demonstrated that Novox® provides a significant antibacterial effect, while stimulating reparative processes. According to our experience, the use of gel or pad is useful for re-epithelializing lesions, while the impregnated gauzes, which tend to adhere to the wound, are better used on lesions with granulation tissue.

Keywords: burns, ozone, wound healing, epithelialization

RÉSUMÉ. *L'oxygène est un élément- clé du processus complexe de réparation tissulaire. Les antiseptique O_3-huile sont obtenus par réaction chimique entre l'ozone et des acides gras polyinsaturés d'origine végétale. Cette étude avait pour but d'évaluer l'efficacité d'un tel produit sur les brûlures d'évolution torpide. Elle a concerné 20 patients (dont 8 hommes) de 47 ans d'âge moyen (26- 74) ayant des brûlures intermédiaires à profondes sur 23% de SCT (7 à 35). Les indications de mise sous O_3- huile pouvaient être la persistance d'exsudation, la douleur, l'absence d'épithélialisation, l'absence de séparation de l'escarre, l'infection (tous les patients ayant d'ailleurs une bactériologie cutanée positive avant le début du traitement). Chez chaque patient, deux zones similaires recevaient toutes les 48 h soit de l'huile ozonée (groupe 1) soit un traitement conventionnel (groupe 2). La réduction des exsudats, de la douleur et de la charge bactérienne était plus rapide dans le groupe 1, les cultures se négativant à J4 (1 à 7) dans ce groupe contre J8 (5 à 11) dans le groupe 2. Cette étude montre que le Novox® a un effet antibactérien significatif et stimule la cicatrisation. Dans notre expérience, l'utilisation de gel ou de plaques imprégnées sont plus efficaces pour promouvoir l'épithélialisation quand les compresses saturées, qui adhèrent à la plaie, sont préférables sur des tissus hyperbourgeonnants.*

Mots-clés: *brûlure, ozone, cicatrisation, épithélialisation*

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Introduction

Among the already general and local well-known factors that can affect the complex tissue repair process (whose deficiency or absence may slow down or interrupt the sequence of the related progressive phase and make the wound chronic), oxygen can undoubtedly be considered a key element.[1] Indeed, this element not only plays a crucial role in almost any phase of tissue repair, but it has proven to deploy a bacteriostatic and bactericidal action on several species of bacterial and fungal pathogens.[2] In the light of the above premises, a significant aim in skin lesion treatment is to ensure an adequate level of oxygen in the wound bed.

O_3-Oil antiseptics are obtained from the chemical reaction between ozone and unsaturated fatty acids of vegetable oils. A new ozonated virgin olive oil with antiseptic indications (Novox®) has recently demonstrated a positive effect in treating non-healing diabetic and oral ulcers, skin lesions and wound dehiscences.[3-6] The purpose of this study was to assess the effectiveness of a commercially available O_3-Oil in the management of recalcitrant burn wounds.

Patients and methods

This study was carried out between February 2020 and September 2020 and involved 20 patients with mid-deep and deep burn wounds (average TBSA approximately 23%, range 7-35%). Patient average age was 47 years (range 26-74 years), 8 were male. The presence of exudate, pain and delayed re-epithelialization, absence of tissue necrosis and/or sepsis were the criteria for the topical use of O_3-Oil, 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 preliminary preparation of the wound bed by means of an appropriate cleansing with saline solution (*Fig. 1A, Fig. 2A, Fig. 3A*), wound tissue cultures were obtained daily, for 10 consecutive days, and sent to the Microbiological Unit of Pisa University Hospital.

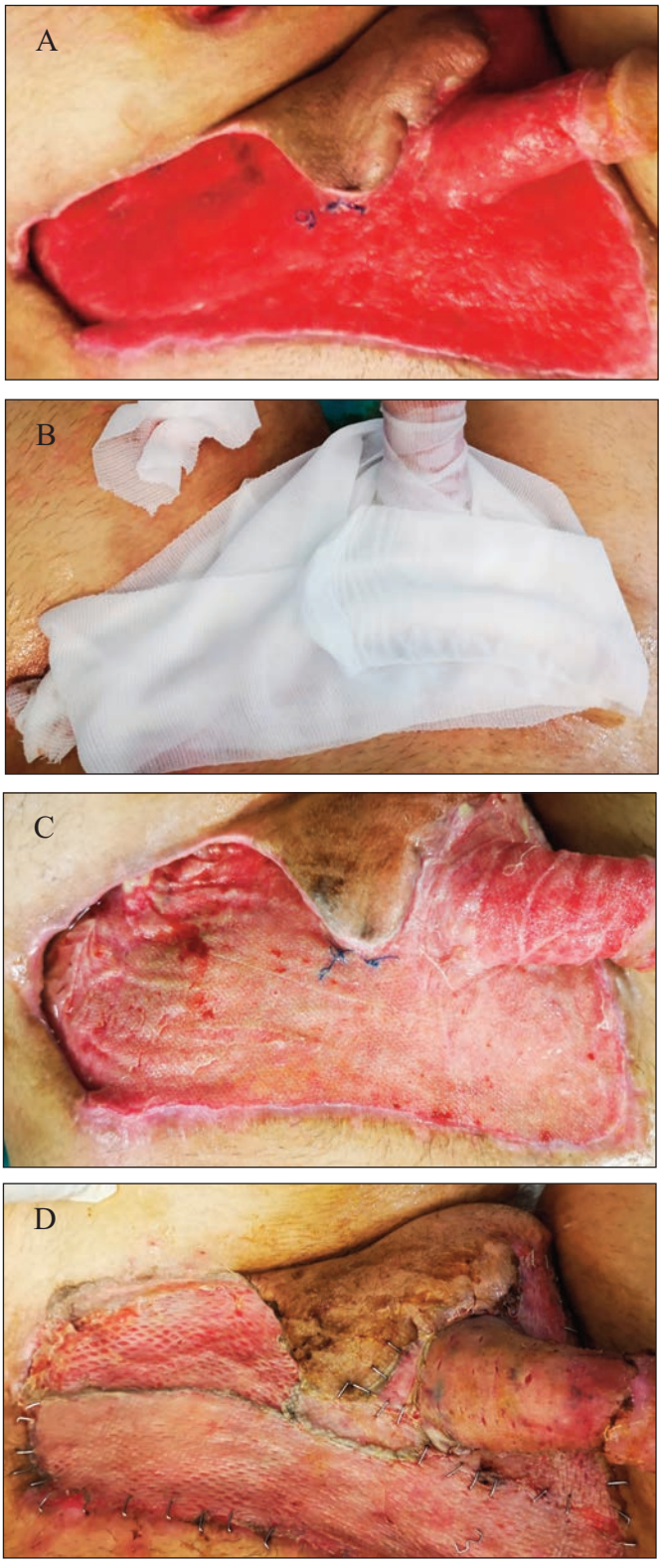

Fig. 1 - After careful removal of all epidermal debris and wound irrigation with saline solution, wound tissue cultures were obtained daily. A) Four-five layers of Novox® impregnated non-adhering gauzes were applied on granulation tissue, then covered with non-woven gauzes; B) Burn wound after 2 days; C) and after 5 days of treatment with Novox®; D) The large wound in the sovrapubic area was covered with a split-thickness graft which completely healed.

In each patient two clinically similar areas were identified and treated every 48 hours as listed below:

Group 1: a uniform layer of O_3-Oil in the form of Novox® gel or pad was applied on the lesions in the process of re-epithelialization and their borders, or inside the fistulas (*Fig. 2B*), then covered with non-adhering dressing and non-woven gauzes to prevent evaporation; 4-5 layers of Novox® impregnated non-adhering gauzes were applied on granulation tissue or undermined chronic wounds, then covered with non-woven gauzes (*Fig. 1B, Fig. 3B*).

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

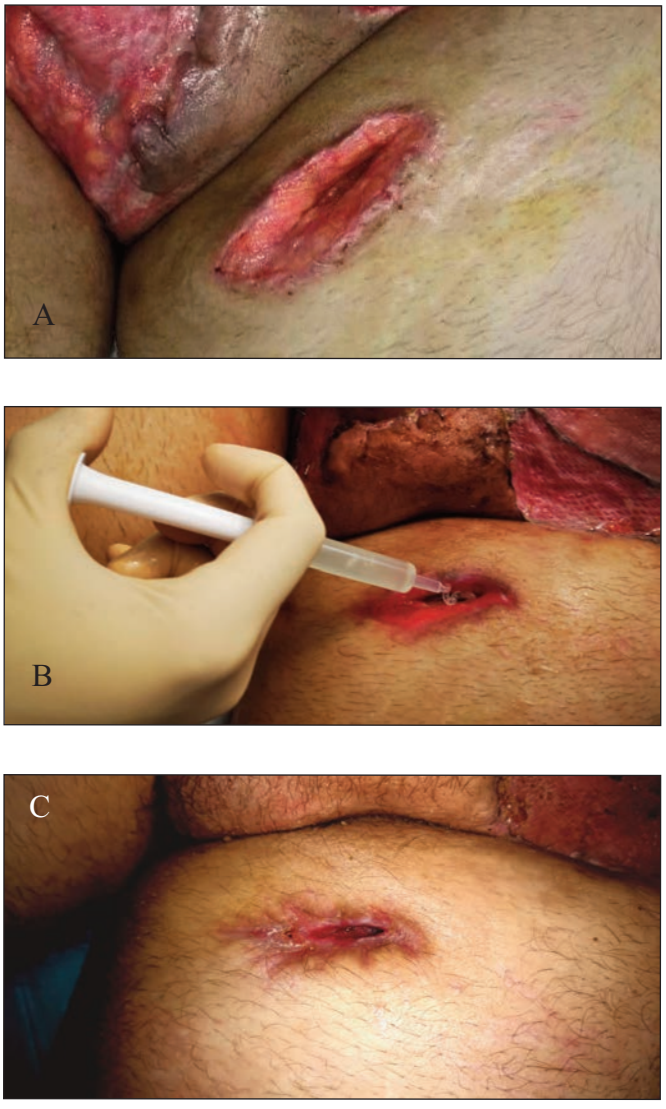

Fig. 2 - A) Burn wound before treatment with O_3-Oil; B) A uniform gel layer of Novox® was applied inside the fistula, then covered with non-adhering dressing and non-woven gauzes to prevent evaporation; C) Burn wound after 2 days of treatment with Novox.®

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

Table I - Group 1 patient data and culture sample results

				Microbiological cultures										
n°	**Age**	**Sex**	**TBSA (%)**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
1	30	F	23	C.p.	C.p.	C.p.	-	-	-	-	-	-	-	-
2	26	M	14	S.h.	S.h.	S.h.	S.h.	-	-	-	-	-	-	-
3	45	M	32	P.a.	P.a.	-	-	-	-	-	-	-	-	-
4	37	F	7	S.e.	-	-	-	-	-	-	-	-	-	-
5	59	F	33	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-	-	-
6	61	M	29	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-	-
7	33	F	18	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-
8	47	F	22	S.c.	-	-	-	-	-	-	-	-	-	-
9	31	M	16	P.m.	P.m.	P.m.	-	-	-	-	-	-	-	-
10	48	F	35	C.p.	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-	-
11	57	F	9	S.h.	S.h.	-	-	-	-	-	-	-	-	-
12	54	M	13	C.a.	C.a.	C.a.	-	-	-	-	-	-	-	-
13	65	F	15	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-
14	27	F	19	P.m.	P.m.	P.m.	P.m.	-	-	-	-	-	-	-
15	35	M	32	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	-	-	-	-	-
16	74	F	28	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-
17	42	M	35	S.e.	S.e.	S.e.	-	-	-	-	-	-	-	-
18	69	M	29	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	-	-	-	-
19	41	F	34	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-	-	-
20	67	F	25	P.m.	-	-	-	-	-	-	-	-	-	-

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

				Microbiological cultures										
n°	**Age**	**Sex**	**TBSA (%)**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
1	30	F	23	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	-
2	26	M	14	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	-	-
3	45	M	32	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-
4	37	F	7	S.e.	S.e.	S.e.	S.e.	S.e.	-	-	-	-	-	-
5	59	F	33	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-
6	61	M	29	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-
7	33	F	18	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.
8	47	F	22	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	S.c.	-	-	-	-
9	31	M	16	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	-	-
10	48	F	35	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	-	-
11	57	F	9	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	-	-
12	54	M	13	C.a.	C.a.	C.a.	C.a.	C.a.	C.a.	-	-	-	-	-
13	65	F	15	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-
14	27	F	19	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	-	-
15	35	M	32	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	C.p.	-	-	-	-
16	74	F	28	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-	-	-	-	-
17	42	M	35	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	S.e.	-	-
18	69	M	29	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.	S.h.
19	41	F	34	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	P.a.	-
20	67	F	25	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	P.m.	-	-	-

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*.

isolated organisms cultured from the wound tissue were *Pseudomonas aeruginosa* (N=5, 25%), *Candida parapsilosis* (N=3, 15%), *Staphylococcus haemolyticus* (N=3, 15%), *Candida albicans* (N=3, 15%), *Proteus mirabilis* (N=3, 15%), *Staphylococcus epidermidis* (N=2, 10%) 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. 4*). Clinically, several macroscopic changes in granulating tissue were observed (*Fig. 1C, Fig. 1D*). Delayed re-epithelialization lesions and fistulas of Group 1 pa-

tients healed faster compared to Group 2 patients (*Fig. 2C*, *Fig. 3C*). Negative cultures were obtained after 4 days on average in Group 1 (range 1-7 days) and 8 days in Group 2 (range 5-11 days).

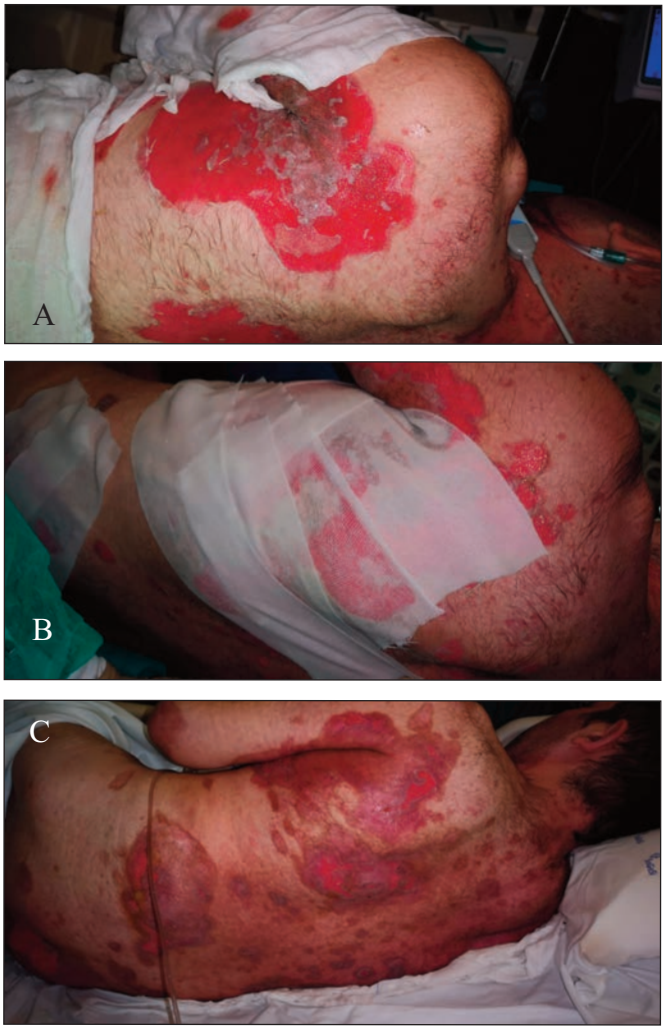

Fig. 3 - A) Burn wound before treatment with O_3-Oil; B) Four-five layers of Novox® impregnated non-adhering gauzes were applied on granulation tissue, then covered with non-woven gauzes; C) Burn wound after 9 days of treatment with Novox.®

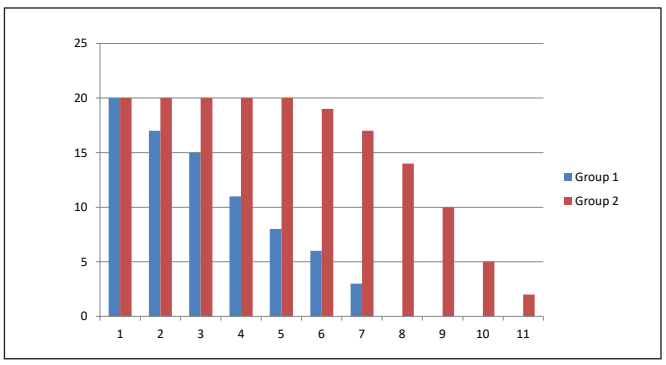

Fig. 4 - A faster bacterial load reduction was observed in Group 1 compared to Group 2.

Discussion

Oxygen has proven to play a fundamental role in almost any stage of wound healing.[1] Cells involved in the inflammatory phase require high oxygen concentration in order to allow cellular respiration and to preserve the related activity each of them is intended for. Additionally, creation of granulation tissue basically depends on oxygen, essential for fibroplasia, angiogenesis and the creation and deposition of new extracellular matrix. Also, in scar remodeling the level of oxygen is fundamental for enzymatic reactions, which determine the realignment of collagen fibers in the extracellular matrix, the deposition of elastic fibers and their retraction, hence favoring a more homogeneous tissue regeneration and better scar results.

Therefore, almost any tissue repair phase is oxygen-dependent, and when this element level decreases, consequences in terms of time and effectiveness of the process are remarkable. Besides, the antimicrobial power of oxygen must not be undervalued, since it deploys a bacteriostatic and bactericidal action, by means of macrophages and thanks to the free radicals which are generated.[2,7] Indeed, ozonated oils have a broad antibacterial spectrum that covers Gram-negative and Gram-positive, and has also proven to be effective against several periodontal pathogens and pathogenic fungal species.[8-10] The possible mechanism by which O_3-Oil act as an antiseptic is the oxidation of microorganisms through a slow release of peroxides.[11,12] The topical application of oxygen-release formulations (e.g. cold creams, ointments) can grant the lesion a correct "extra quantity" of this element, both in those cases where it totally lacks or deficiency is due to the increased local metabolism.

In the present study we used O_3-Oil for treatment of intermediate-severe burns and skin graft donor-sites, even in the presence of wound infection. The over-oxidised oil, in the form of Novox®, is stored in a prefilled plastic syringe in order to allow for safe and easy self-treatment: it was delivered to the wound bed and covered with non-adhering gauzes to ensure painless dressing change. The use of non-adhering pads can be an alternative for re-epithelializing areas, while impregnated gauzes, placed in

several layers, are more useful on granulation tissue or undermined ulcers. This can be performed as frequently as the wound requires, from 2-3 times weekly, to daily with self-treated wounds. The over-oxidised oil has a consistency that allows it to spread homogenously through the wound bed, from the bottom to the top of the wound. The oil forms an oleic matrix that releases a continuous flow of reactive oxygen species (ROS) into the wound bed, as well as having film-forming and protective functions. Moreover, avoiding the accumulation of wound exudates, the oleic matrix should decrease bacterial proliferation.

Wound treatment with O_3-Oil is extremely easy, inexpensive, and can often be performed by the patient himself, leading to a total compliance of the patient and cost reduction. The dressing showed an actual effectiveness, promoting granulation and decreasing bacterial contamination. We believe that our results are valuable and warrant debate, since this is the first study to describe clinical and cultural evidence of bacterial load reduction after topical use of O_3-Oil. However, this is only a preliminary report and further studies will be necessary to confirm our findings.

Conclusion

The present study demonstrated that Novox®, in every available formulation, always provided a significant antibacterial effect, while stimulating reparative processes. According to our experience, the use of gel or pad is useful for re-epithelializing lesions, while the impregnated gauzes, which tend to adhere to the wound, are better used on lesions with granulation tissue. The secondary dressing involved non-woven gauzes to prevent O_3-Oil evaporation. No patient suffered from allergy/intolerance. For these reasons, this product may be useful in different types of burn lesions, especially when they are colonized by multidrug-resistant pathogens.

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