A clinical evaluation of the efficacy and safety of singlet oxygen in cleansing and disinfecting stagnating wounds

• **Objective:** This cohort study evaluated the clinical efficacy of singlet oxygen, ActiMaris (AM) a hypertonic (3%) ionised (pH 9.8) sea water solution. It was assumed that when used for wound cleansing, disinfection and the reduction of inflammation, AM would be safe and effective.

• **Method:** Between May 2008 and May 2009, ambulant patients presenting at one of four wound healing centres were included in the study. Patients had critically colonised and/or infected, malodorous wounds, covered with slough/fibrin or wounds showing inflammation of the periwound skin. Wounds were assessed in terms of percentage changes in fibrin, slough and granulation tissue, they were assessed clinically and high resolution digital photographs were scored by a physician who was blinded to treatment allocation. Results were compared at baseline (week 0) and following 42 days of AM treatment (week 6).

• **Results:** Seventy-three patients were included in the analysis. Dressing changes were at 2-day intervals on average, and the median treatment period was 46.04 days (range: 3–197). At 42 days, 33% (n=24) of included wounds had healed, 57% (n=42) had improved and 10% (n=7) remained stagnant. Cleansing and wound disinfection with AM was effective. In 31 patients (42%) wounds had showed clinical signs and symptoms of critical colonisation and/or infection at day 0, whereas at day 42 the infection was completely eradicated. Inflammation was reduced in 60% (n=44) of cases and patients did not report pain or discomfort when using AM.

• **Conclusion:** The use of singlet oxygen was shown to be safe and the results of this study indicate AM to be useful for wound cleansing, disinfection, reducing inflammation and promoting wound healing.

• **Conflict of interest:** The centres were supplied with the study product by the sponsor. The authors have no financial interest in writing this article.

singlet oxygen; redox system; stagnating wounds; wound cleansing; wound disinfection

Chronic and stagnating wounds often provide an ideal habitat for microbial colonisation, which together with the lack of a host response, can impair healing.[1,2] Furthermore, the environment within such wounds can be ideal for bacterial proliferation, especially in the presence of necrotic or sloughy tissue.[2,3]

The removal of devitalised tissue is generally accepted as a necessary precondition for the formation of new tissue.[4,5] Devitalised tissue can mask infection, act as a physical barrier to healing and may impede normal matrix formation, angiogenesis or the development of granulation tissue.[6] Devitalised tissue contributes to the production of inflammatory cytokines, which in turn leads to the overproduction of matrix metalloproteinases (MMPs).[6,7]

The value of chronic wound cleansing, including debridement, is a basic principle in the modern approach to wound management.[7,8] It is a part of wound bed preparation — it gently and continuously removes debris and exudate, preparing the wound bed for wound closure.[1,2,5]

Topical antimicrobial substances such as silver, povidone iodine or polihexanide are increasingly used to treat multi-resistant wound infections.[7–10] Antiseptics have a lower potential to induce bacterial resistance compared with antibiotics, although overuse of these products may reduce their efficacy.[7,8]

In recent years, there has been debate over the appropriateness and efficacy of various different methods of wound cleansing and disinfection. In Europe we differentiate between debridement (removal of dead tissue) and cleansing (removal of senescent cells and exudate). However, this distinction is not made everywhere. There are grey areas. For instance, in the management of stagnating wounds, the removal of an excess of MMPs may be done by absorbent dressings and not just by sharp debridement.[4,5]

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For wound cleansing, a variety of strategies are currently applied, such as short rinsing, or leaving a dressing impregnated with an antimicrobial in place for approximately 20 minutes (the so-called 'wet-to-dry' phase) before applying the usual dressing regime.[5,9] Antimicrobials have a time to onset, so a better effect can be expected when applied for 20 minutes, rather than a quick rinse. We previously published this method in *JWC* and it has since been widely practiced in Continental Europe and is currently gaining favour in the UK.

To use these tissue friendly solutions in a moist wound healing dressing, of course, gives an even better effect. However, for this study we used AM the same way as polyhexamethylene biguanide (PHMB) or other antiseptic solutions would be used, applying best practice — the wet-to-dry phase.

The wet-to-dry phase is a multi-phase concept, which starts with an active cleansing phase, the 'wet' phase, in which a cleansing fluid is applied to the wound for 20 minutes to one hour, followed by a short resting phase, the 'dry' phase. During the dry phase, the wound is covered with a gauze dressing and peri-wound skin integrity is restored. Cleansing fluid evaporates during both phases, resulting in the release of wound debris, exudate and pathogens, which saturate the gauze dressing during the dry phase. Next, a moist wound healing dressing, usually an alginate, foam or Hydrofiber, is applied. If local infection is present, then an antiseptic may be used as the cleansing agent during the wet phrase and an antiseptic dressing might be used afterwards, instead of an absorbent dressing. The aim of the wet-to-dry phase is not to create an optimal healing environment or temperature (although excessive cooling off is to be prevented), but rather to cleanse the wound and reduce itching and inflammation.[5]

There are currently no conclusive data to show which strategy, out of continuous treatment with an antimicrobial combined with a dressing, or a short cleansing phase using an antimicrobial before dressing application is the most effective strategy for wound disinfection.[9]

However, it has been suggested that the antimicrobial carrier used and the time during which the antimicrobial can become activated can influence the results obtained with the treatment.[5,9]

Singlet oxygen

Singlet oxygen, a form of molecular oxygen (O_2) which is less stable than the normal triplet oxygen, is a reactive oxygen species (ROS). ActiMaris (AM) (QuantumMedis Est, Vaduz, Liechtenstein) is an ionised (pH 9.8) solution of sea water with active singlet oxygen, that has been used as an antimicrobial for wound disinfection in Austria, Switzerland and Germany.[10,11] It is hypertonic (3%) and thus draws water out of cells by osmosis.[11-13]

ROS are implicated in cellular activity to a variety of inflammatory responses.[12-14] Effects of ROS on cell metabolism have been well documented for a variety of species.[14-16] These include not only roles in apoptosis (programmed cell death) but also in other mechanisms such as the induction of host defences.[12] This can be explained with the redox system. ROS generated within cells or, more generally, in a tissue environment, can damage cells and tissues. Aerobic organisms can carefully control the generation of ROS and other oxidative stress-related radical and non-radical reactive intermediates (that is, aerobic organisms can maintain redox homeostasis), and 'make use' of these molecules under physiological conditions, to modulate signal transduction, gene expression and cellular functional responses ('redox signalling').

When AM is in contact with the wound bed, singlet oxygen is released slowly, as during the Krebs cycle in mitochondria.[10,11,14] At a high pH (9.8), AM's singlet oxygen interacts with hydroxide groups as a redox system,[11,13,14] which occurs through a series of complex electron transfers.[15] Redox signalling can have positive effects, such as the induction of host defence.[11,12,14-16] AM's clinical activity is based on these mechanisms reducing inflammatory reactions, promoting neovascularisation, granulation and epithelialisation in stagnating wounds.[10,11]

In AM, active oxygen is bound and stabilised between sodium and chloride ions (NaOCl) in water or gel.[10,11] Bacteria and viruses do not have an efficient defence against singlet oxygen.[11] Singlet oxygen, has been shown to have microbicidal activity against *Staphylococcus aureus* and *Escherichia coli in vitro*, and also when applied to chronic wounds *in vivo*.[10,11] When in contact with skin and/or wounds, active oxygen induces a reaction by binding electrons from other cells or substances.[10,11] This destroys the sulphate groups of bacterial membranes in seconds, and the bacteria are soon engulfed.

The fast onset of activity makes AM especially suitable for wound rinsing.[10,11] AM is available as a solution, a forte solution and a gel. The solution and the gel are indicated for cleansing contaminated wounds and those at risk of infection. The forte solution is indicated for critically colonised and clinically infected wounds. AM has a low concentration of natrium oxichlorit (0,2%) and both the solution and gel are alkaline (pH 9); the forte solution and gel are hypertonic (3,0%).

The product may be combined with various dressings, such as alginates, hydrofibres and foams, for continuous application.[4,5,10,11] Due to excellent tissue compatibility and an absence of irritation, ubiquitous application is possible on the skin, mucous membranes, cavities, the middle ear and cartilage and beneath semi-occlusive and occlusive dressings.[10,11] AM may be used for acute wounds, chronic

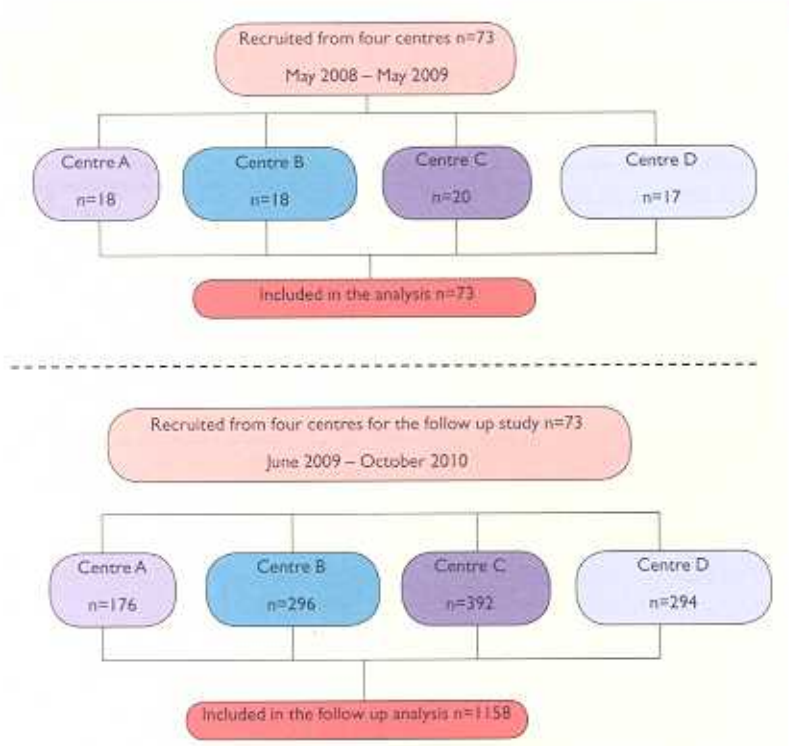

Fig 1. Patient flow chart

ulcers and burns.[10,11] The active oxygen product may bleach dark clothes and it is contraindicated in individuals who are allergic to sea salt.

This study is the first clinical evaluation of AM. A clinical cohort study was designed using case ascertainment to evaluate the use of singlet oxygen for cleansing and disinfecting stagnating wounds of various aetiologies, which had previously been treated with polihexanide at the clinics that participated in the study. Patients had all been treated with PHMB during dressing changes (wet-to-dry phase for 20 minutes) for at least 14 days, and did not respond sufficiently, in terms of their clinical signs and symptoms. High colonisation persisted and wounds remained stagnant.

We designed a multi-centre cohort study to evaluate the efficacy of AM for the treatment of patients with stagnating wounds of various aetiologies.

Materials and method

This multi-centre cohort study evaluated AM on the following:

Primary outcomes

- The percentage of slough in the wound bed and inflammation of the peri-wound skin, comparing day 0 with day 42
- The percentage of malodorous wounds that demonstrated a reduction in odour during the course of the study period
- A shift in wound bed tissue types, to determine the stimulation of granulation and epithelialisation, comparing the wound bed condition at day 0 and day 42.

Secondary outcome

- Ease of use, safety and suitability in deep wounds.

Patients

Between May 2008 and May 2009 ambulant patients aged over 18 years with various wound types were recruited from four complex wound healing clinics, two in Austria (centres A and B), one in Germany (centre C) and one in Switzerland (centre D) (Fig 1). The treatment protocol and level of expertise is comparable across these centres.

Local ethics committee approval was obtained and patients gave written informed consent before entry into the study. The study included patients that had presented at the centres with non-healing wounds of different aetiologies. Stagnation was confirmed before entry into the study, by demonstrating a lack of improvement despite two weeks' treatment with appropriate standard treatment. Patients had critically colonised and/or locally infected[2] malodorous wounds, covered with slough/fibrin and wounds showing symptoms and signs of inflammation of the peri-wound skin. In wounds with signs of infection, swabs were taken for bacterial analysis.[2] Patients with systemic and spreading wound infections and those with critical ischaemia were excluded.

Demographic and clinical data

At day 0, patients' general condition, nutritional status and intake, mobility status, age and risk factors were assessed, together with social status and specific factors that can delay wound healing, such as alcohol use, circulation disorders, diabetes mellitus, illicit drug use, medications and smoking.

Wound healing, reduction of wound area and wound bed condition

Wound area reduction and wound bed condition were assessed at dressing changes on days 0, 7, 14, 28 and 42. Baseline (week 0) versus day 42 (week 6)

AM treatment results were compared, looking at the percentage changes in fibrin, slough and granulation tissue. Both clinical assessment and high resolution digital photographs were used, the photos scored by two physicians, who were blinded to treatment. Photographs were analysed using a digital tool, which was applied to assess wound size and evolution of the wound bed.[17]

A computer program, ZWM WDSI (www.wfi.ch) was used to calculate the wound area from these digital images.[17] This program includes an adapted version of the Dutch Wound Care Society (DWCS) colour classification, which was used to calculate

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Table 1. Clinical characteristics of the consenting participants

Clinical Characteristics		Consenting participants (n= 73)
		Mean ± SD
Age (years)		68.8 ± 7.97
		Frequency (%)
Presence of comorbidities	Lymphoedema	8 (10.96)
	Diabetes Mellitus	8 (10.96)
	Polyneuropathy	3 (4.10)
	Peripheral arterial disease	14 (19.18)
	Hemiplegia	2 (2.74)
	Paraplegia	1 (1.37)
	Critical ischemia	1 (1.37)
	Dementia	2 (2.74)
	CVI	22 (30.14)
	Other	12 (8.76)
Wound duration (months)		0.5–221
Wound types	Mixed leg ulcer	13 (18.0)
	Arterial leg ulcer	1 (2.0)
	DFU	8 (11.0)
	Post infect.	4 (5.0)
	Trauma	5 (7.0)
	Surgical	10 (14.0)
	PU	4 (5.0)
	VLU	25 (34.0)
	Other	3 (4.0)
		Percentage
Wound location	Upper leg	3
	Sacrum	1
	Trunk	3
	Trochanter	3
	Abdomen	4
	Toes	7
	Foot	4
	Foot sole	7
	Heel	3
	Malleolus	22
	Lower leg	43

CVI=chronic venous insufficiency; DFU=diabetic foot ulcer; PU=pressure ulcer
VLU=venous leg ulcer

the percentages of slough, necrotic, granulation and epithelial tissues present.[17]

Clinical assessment of wound healing was performed during dressing changes, by specialist clinicians, a scale with the following categories was used:[17]

- Wounds were defined as stagnating when the status of the wound bed did not change between baseline and end of the study
- Wounds had deteriorated when the clinical signs and symptoms of inflammation and/or infection increased, or when sloughy tissue and/or necrosis had increased, or when granulation and epithelialisation did not progress
- Wounds were defined as improved when the clinical signs and symptoms of infection/inflammation[2,5] had reduced and/or when granulation/epithelialisation had progressed.
- Wounds were defined as closed when epithelialisation was complete.

Peri-wound skin condition

The condition of peri-wound skin was assessed at dressing changes on days 0, 7, 14, 28 and 42. Baseline (week 0) results, versus those at 6 weeks' treatment were compared using a four-point scale, based on a modified physician global assessment scale (PGA).[17,18] The presence of inflammation was evaluated using the following scores: 1 = absent, 2 = minimal, 3 = moderate, 4 = severe. Evaluation considered both the degree of redness and the area of peri-wound skin involved.

Odour assessment

Odour was assessed subjectively at dressing changes, asking both clinicians and patients to score it on a five-point scale (1=no offensive odour; 2=slight offensive odour; 3=moderate offensive odour; 4=much offensive odour; 5=severe offensive odour). Patients and clinicians were both asked if they observed any changes when comparing the odour to that smelled at the previous dressing change. Finally, patients were asked if wound odour had influenced their daily living. A five-point scale measuring quality of life in relation to wound odour was used for this (1=very good; 2=good; 3=moderate; 4=poor; 5=very poor).

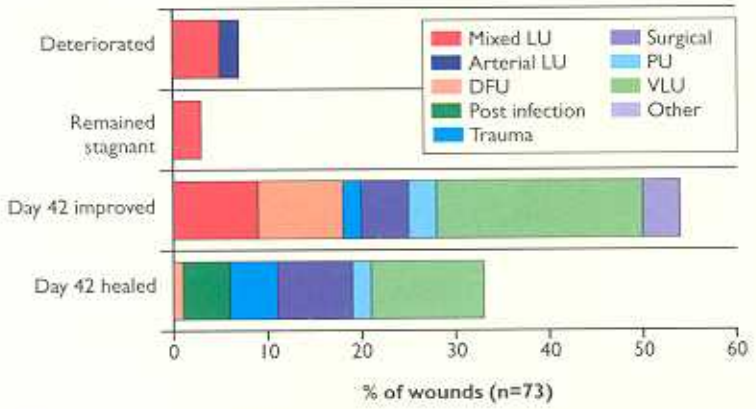
Dressing regime

Using AM as the cleansing agent, the treatment protocol used the wet-to-dry phrase. The choice of moist wound healing dressing (alginate, foam or Hydrofiber) used after the wet-to-dry phase was at the clinician's discretion. Both wound cleanser and dressing were applied in accordance with the manufacturer's instructions by experienced clinicians — the nurses and doctors at all five centres are certified wound care specialists, having completed a two-year course. The treatment period lasted a maximum of 6 weeks.

The study protocol stipulated that clinicians should treat the underlying aetiology — for instance, venous leg ulcers should be treated with compression and diabetic foot ulcers should be managed with off-loading and callus removal. The choice of primary and secondary dressing was at the discretion of the clinician, as were dressing changes (on average, these took place every 2 days).

If a wound was infected, the peri-wound skin was protected, where applicable, with a zinc cream.

Fig 2. Wound healing per wound type, comparing results at day 0 and day 42



LU=leg ulcer; DFU=diabetic foot ulcer; PU=pressure ulcer; VLU=venous leg ulcer

Treatment was according to wound phase. For instance, highly exuding wounds received an absorbent dressing; deep wounds were treated with a wound filler (such as an alginate) and covered with a secondary dressing (such as a foam).

Follow up study

On completion of the study, the wound healing clinics kept AM as part of their treatment protocol. A further study was conducted between June 2009 and October 2010 at the same four wound healing clinics, using the same methodology and the same wound types as the present cohort study. Data were selected from 1158 patients, treated with AM, with various wound types, to assess the effectiveness of AM treatment and confirm its safety.

Results

All patients that were included (n=73) completed the study, and no adverse events were recorded. The mean age of participants was 68.8 years (SD±7.97) (range: 9–95 years). 35 were female. The duration of wounds before the start of this treatment ranged from 0.5 months to 221 months. Prior to entry into the study, the cleansing regimen for wounds was just PHMB. The same moist wound healing dressings were used. Table 1 shows patient characteristics, concomitant diseases, wound types and wound locations.

In 90% of cases, standard AM rinsing solution was used. The median treatment period was 46.04 days (range: 3–197 days). At 42 days, 33% (n=24) of included wounds had healed, 58% (n=42) had improved (with at least a 20% reduction in wound area), 3% (n=2) remained stagnant and 7% (n=5) had deteriorated. All of the wounds that remained stagnant or deteriorated had issues with microcirculation, or an arterial component. Results are given at 42 days as this is the time by which wounds of the included categories might be expected to improve or heal.[2,5,9] There were no differences in healing rates between treatment centres. For details of wound healing results by wound type, see Fig 2.

At the beginning of the study (day 0) peri-wound skin inflammation was present in all of the included wounds, with a mean score of 3.6 (SD±3.12) on the four-point scale. By the end of the study (day 42), this had resolved in 60% (n=44), with a mean score of 1 (SD±1.02); was minimal in 33% (n=23), with a mean score of 1.7 (SD±1.14); and was moderate in 7% (n=6), with a mean score of 2.8 (SD±2.62).

Clinicians and patients both noted a reduction of offensive odour within 10 minutes of applying AM. On day 0, very offensive odour was present in 36% (n=26) with a mean score of 4.6 (SD±4.32) on the five-point scale used. By the second dressing change, the odour score had reduced to a mean of 2.1 (SD±2.02) in 18 of these patients (25% of the total) and in the remaining 8 patients (11%) odour scores were moderate, with a mean of 3.2 (SD±3.18). At the end of the study, offensive odour had been resolved in all cases, with a mean score of 1.2 (SD±0.96). No significant differences were found between the scores given by clinicians and patients.

Results concerning the impact of wound odour on quality of life were positive, with patients reporting an improvement in their everyday quality of life as a result of odour reduction. At day 0, the mean score was 4.2 (±3.8), whereas by day 42 this had reduced to 1.8 (±1.6).

On day 0, 42% of patients (n=31) had wounds with symptoms and signs of critical colonisation and/or infection. Infection was confirmed in 12 cases (16% of the total) by wound swab. One patient received systemic antibiotics. Local wound treatment with AM was sufficient for the other patients.

Clinicians reported that handling AM during dressing changes was easy. The majority (84.2%) scored its clinical efficacy as 'very good' and stated that they would recommend AM for wound cleansing.

AM was successfully applied in combination with various dressings, including absorbent pads, alginates, Hydrofiber, hydrocolloid, collagen, foam, films and superabsorbent dressings.

Follow up study results

The follow up study found similar cleansing efficacy. Of the 33% (n=386) of patients that had wounds with symptoms and signs of critical colonisation and/or infection at day 0, 28% (n=108) had resolved within 14 days of AM treatment, which is in line with the results of the cohort study. Eradication of infection was confirmed by comparing wound cul-

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tures taken on days 0 and 14, together with the clinical picture of symptoms and signs. No adverse events were reported when performing wound cleansing and/or wound disinfection with AM, which suggests that it is safe to use in various wound types.

Discussion

The results of our study indicate that AM reduces signs of infection and peri-wound skin inflammation and that it supports wound cleansing.[9] Using the wet-to-dry phase with AM and absorbent dressings, there was a shift from chronic inflammation to proliferation, which shows that stagnating wounds moved on to granulation and epithelialisation. This suggests a reduction of proinflammatory cells, such as MMPs.[15]

The wounds that remained stagnant and/or deteriorated all had reduced microcirculation or an arterial component, which might explain their lack of response to treatment.[15,16] AM's wound cleansing and disinfection efficacy was nonetheless demonstrated for various wound types.

It is thought that a major factor enhancing inflammation in stagnating wounds is an imbalance of oxidants and antioxidants.[15,16,19] The stagnating wound microenvironment induces oxidative stress.[15,16] Following wounding, leukocytes, such as neutrophils, release various ROS into the wound environment, such as superoxide anions, hydroxyl radicals, singlet oxygen and hydrogen peroxide.[15,16] Endothelial cells and fibroblasts — in particular senescent fibroblasts, which are prominent in stagnating wounds — are also a potential source of ROS.[19] The redox activity of AM's singlet oxygen may help to restore the balance of oxidants and antioxidants.[10,11,15]

The effect of this product on wound odour reduction and its compatibility with dressings such as alginates, Hydrofiber and foams, warrants further investigation of singlet oxygen in the treatment of chronic wounds. Offensive odour can be a big problem in oncology wounds[20,21] and current strategies to reduce it involve the use of topical antimicrobials (such as metronidazole, cadexomer iodine and polihexanide) together with reducing the amount of dead tissue.[22–24] These approaches are not always effective and can cause complications such as bleeding.[25,26] Wound odour may be attributed to the size or irregular shape of the wound, the liquefaction of dead tissue or the management of exudate.[26] The current management options are not sufficient, as they do not stay activated long enough, are too toxic to be used on large surfaces, or they do not penetrate far enough, to anaerobic bacteria located beneath the surface.[26] Here, the application of AM has shown its potential. Especially as it may be combined with various dressings and it is appropriate for use on fragile tissues.

Limitations

As with many new commercially available cleansing products, direct comparative data on the use of AM is not yet available. Because there is no comparison or control group, cause and effect relationships cannot be inferred from the present study. However, before the study treatment was initiated, all of the included patients had previously been treated, unsuccessfully, with other therapeutic modalities, which may be considered as a historical control.

Conclusion

AM demonstrated effective wound cleansing, removing debris and slough from the wound bed. The product was easy to apply and can be safely used in both hospital and community settings. AM showed good tolerability and high levels of user satisfaction and patient comfort. ■

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