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Hot topics in reactive oxygen therapy: antimicrobial and immunological mechanisms, safety and clinical applications

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Highlights

- Reactive oxygen species (ROS) delivered by engineered honey or gel.
- Novel antimicrobial with activity against all bacteria as well as fungal and viral activity.
- Topical treatment with antibiofilm activity.
- Huge therapeutic implications not just for wound healing but possibly mucosal infection in respiratory and urinary tract.
- Topical and local application, but could be applied to internal mucosal structures.

ABSTRACT

Reactive oxygen species (ROS), when combined with various delivery mechanisms, has the potential to become a powerful novel therapeutic agent against difficult-to-treat infections, especially those involving biofilm. It is important in the context of the global antibiotic resistance crisis. ROS is rapidly active in vitro against all Gram-positive and Gram-negative bacteria tested. ROS also has antifungal and antiviral properties. ROS prevents the formation of biofilms caused by a range of bacterial species in wounds and respiratory epithelium. ROS has been successfully used in infection prevention, eradication of multiresistant organisms, prevention of surgical site infection, and intravascular line care. This antimicrobial mechanism has great potential for the control of bioburden and biofilm at many sites, thus providing an alternative to systemic antibiotics on epithelial/mucosal surfaces, for wound and

cavity infection, chronic respiratory infections and possibly recurrent urinary infections as well as local delivery to deeper structures and prosthetic devices. Its simplicity and stability lend itself to use in developing economies as well.

Keywords:

Reactive oxygen species, Novel antimicrobial

1. Introduction

The solutions to the global antimicrobial resistance crisis require a reduction in the volume of antimicrobial use to reduce selection pressure, improved infection prevention to reduce transmission, and new antimicrobial agents [1–4]. The first entirely novel antimicrobial agents to reach early clinical use employ reactive oxygen species (ROS) as their mechanism of action. Surgihoney (SHRO), an engineered honey, is the first such product for topical use delivering sustained release of ROS as an entirely novel solution to controlling and eradicating bacteria [5]. Other ROS antimicrobial agents and delivery systems employing this mechanism are under development and will be available for clinical use in due course. ROS is rapidly active in vitro against all Gram-positive and Gram-negative bacteria tested [6]. ROS also has antifungal and antiviral properties. ROS prevents the formation of biofilms caused by a range of bacterial species in wounds [7] and in respiratory epithelium, and this will be discussed in greater detail in a subsequent Hot topics review.

ROS has been successfully used in infection prevention, eradication of multiresistant organisms [8], prevention of surgical site infection [9] and intravascular line care [10]. This antimicrobial mechanism has great potential for the control of bioburden and biofilm at many sites, thus providing an alternative to systemic antibiotics on

epithelial/mucosal surfaces, for wound and cavity infection, chronic respiratory infections and possibly urinary infections.

ROS is a novel solution to controlling bacterial growth (preventing and treating) at many clinical sites and to treating localised infection. In addition, the one delivery system, Surgihoney (SHRO), currently licensed as a medical device also delivers healing properties (moist barrier, local nutrition, slough control, and possibly angio- and neurogenerative properties) to wounds based on the additional properties of honey [11,12]. It has great potential to reduce inappropriate antibiotic use, to support antimicrobial stewardship and to reduce antimicrobial resistance. It is simple to administer and can be applied to any healthcare system anywhere in the world.

2. Reactive oxygen species: antimicrobial mechanism and role in healing

Oxygen (O_2) is the essential substrate required for high mitochondrial-driven adenosine triphosphate (ATP) yields and, in the context of wound healing, it supplies the increased amount of energy required for tissue renewal [13]. ROS also act as secondary messenger-signalling molecules. The term 'ROS' applies to molecules containing O_2 but that have been reduced with added electrons to become a highly reactive radical format. Examples of ROS include superoxide anion ($\cdot O^{-2}$), peroxide ($\cdot O_2^{-2}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and hydroxyl (OH^-) ions. All have different actions and kinetics in cellular metabolism.

ROS is directly antimicrobial. H_2O_2 appears to elicit its antimicrobial action by reaction with thiol groups in enzymes and proteins, DNA and bacterial cell membranes. It possesses concentration-dependent activity and toxicity. In vitro studies have demonstrated high activity of SHRO against all Gram-positive and Gram-negative organisms tested, including those with multiple antibiotic resistance mechanisms, using techniques that included minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination and time–kill curves [6].

SHRO is a modified honey that has been engineered to provide a constant level of H_2O_2 when applied to a wound [14]. The main mechanism appears to be constant production of H_2O_2, whose instability releases ROS [5]. The availability of ROS from SHRO can be enhanced by the level of the engineering process. In two enhanced SHRO prototypes, the ROS activities at 12 h were 7 and 10 times, respectively, the value for SHRO alone. There is a striking linear relationship between antimicrobial activity and the maximum output of ROS H_2O_2 from the three honey prototypes [5].

Any new health technology innovation, such as SHRO, is required to satisfy satisfactory pharmacodynamics with appropriate dose–response characteristics. It needs to demonstrate satisfactory pharmacokinetics, which, as a topical agent, SHRO achieves. In clinical studies, SHRO has demonstrated satisfactory safety and tolerance as well as clinical efficacy and cost effectiveness in practice [6,8–10].

Infections are a major problem in wound healing, initiated by microbial colonisation resulting in microbial overgrowth and biofilm formation. Antimicrobial-resistant

organisms may be selected by injudicious use of broad-spectrum antimicrobials that are either ineffectual or toxic [14]. Antimicrobial stewardship is therefore important in wound care, and ROS therapy can contribute to this [15].

Following a wound injury, a number of homeostatic processes are triggered [13]. These include: haemostasis, where early ROS-mediated vasoconstriction occurs following platelet exposure to extracellular materials and collagen plus platelet and thrombin activation to induce thrombus formation; lymphocyte recruitment, where there is rapid migration of neutrophils from local blood vessels towards the injury site via ROS signalling; pathogen defence, where there is killing of bacteria and fungi by ROS-driven phagocytosis and bacteriostatic H_2O_2 by platelets and neutrophils; lymphocyte recruitment, where monocytes migrate to the wound site via ROS signalling; and tissue repair, with ROS-mediated cell division and migration of keratinocytes, endothelial angiogenesis and fibroblast and collagen formation.

Thus, ROS are pivotal in the normal wound healing response. They act as secondary messengers to many immunocytes and non-lymphoid cells in regulation of angiogenesis and perfusion into the wound area. ROS act in early host defence against infection through phagocytes and ROS burst. These roles could be exploited in clinical practice in therapeutic strategies to treat wounds, particularly when there is stalled healing, e.g. in chronic leg ulcers, pressure injury and infected/dehisced surgical wounds and burns. Emerging concepts associated with ROS modulation have the potential for clinical practice.

New agents such as SHRO offer a radical solution for preventing and managing infection in wounds. ROS offers a very useful clinical activity against antimicrobial-resistant organisms and reduces the dependency on systemic antimicrobials.

3. Safety of SHRO and cytotoxicity to host cells

There are no published animal studies of SHRO toxicity or cytotoxicity. High levels of ROS have been documented to cause cellular damage to host cells, specifically through oxidative stress. Oxidative stress can result both in direct and indirect ROS-mediated damage of nucleic acids, proteins and lipids, which can lead to disease states including inflammation, cancer, neurodegeneration and aging [16,17]. Although it is also possible that debridement of damaged and infected cells can aid in the wound healing process. Low levels of ROS produced by host cells are also increasingly recognised to play a critical role in regulating microbial colonisation, host immune responses and cellular function [18].

A study reported at a national symposium on ROS investigated the cytotoxicity of SHRO [19]. Three SHRO prototypes (S1, S2 and S3), which display increasing production of H_2O_2, were used to evaluate their dose–response in host cells compared with a non-engineered Acacia honey and a no-treatment control. Concentrations used included 100, 40, 10 and 1 g/L at a range of time points spanning 24 h to observe any changes in the percentage of live and dead cells from each group.

Three separate immortalised cell lines, namely HMC-1 (mast cells), ccl-30/RPMI2650 (nasal epithelial cells) and U937 (monocytes), were used to carry out

a live/dead cell assay. These were chosen because of their importance in the protection of the host from pathogens such as bacteria. Mast cells are sentinel cells located just underneath the epithelium in tissues with close contact to the external environment, such as the skin, airways and intestines; because of this they are ideally located to contribute to early recognition of bacteria. Following activation due to various challenges, mast cells can undergo degranulation and release a variety of soluble factors that act to modulate the immune response. However, they have also been found to harbour viable intracellular bacteria, specifically *Staphylococcus aureus*, which may act to promote infection by enabling the bacteria to evade the host's immune system. Equally important, epithelial cells play a role as a barrier to the external environment, which often serves as the first line of defence against evading pathogens. Nasal epithelial cells have also been found to harbour *S. aureus.* Monocytes are fundamental to the host immune response, as once they migrate from the bloodstream into various tissues they undergo differentiation into tissue-resident macrophages and dendritic cells. These cells have important roles in phagocytosis of pathogens, antigen presentation and cytokine production, which modulate important downstream immune pathways.

Study findings showed low cell death in all treatment groups when exposed to SHRO for <3 h, although some increase in cell death was always present when cells were treated for a longer time period. However, the lower concentrations of S2 and S3 showed lower cell death than the equivalent concentration in the less active S1, while maintaining a higher antibacterial activity as they have been engineered to produce a greater amount of H_2O_2. In addition, when using the higher concentration of SHRO (100 g/L), a shrinking of the cells occurred, which was attributed the

osmolality of the honey as it was also observed with the Acacia honey with no significant difference between both types of honey, but when 10 g/L of S1, S2 and S3 were used the cells did not shrink.

It is important to remember that a certain level of cell death can be beneficial in wound healing in order to remove cells with damaged DNA and cells such as mast cells and nasal epithelial cells that can harbour intracellular stores of pathogens and so help them to evade the immune system [17,18]. The data produced here are important as they form a guideline for dosage use in the future clinical trials as well as the usage of analogues in future therapeutic applications.

In clinical studies, ROS has been well tolerated and safe. SHRO has been used extensively as topical treatment of wounds [5,6,8–10,20]. In these studies and in routine use, comprising a total of ca. 1000 patients, there have been no reports of any serious adverse effects. Patients treated with SHRO included those with multiple co-morbidities, the elderly and diabetics. There has been no associated disturbance of glucose metabolism with topical SHRO use. There were a small number of reports of localised itching or stinging.

4. Reactive oxygen species clinical potential in developed health economies

There are many clinical conditions where bacterial overgrowth and biofilm production produce low-grade, chronic inflammation and persistent intractable or recurrent symptoms [21–23]. Multiple courses of prolonged antibiotics are often employed in

these situations to little clinical effect and usually result in progressive colonisation, infection and transmission of resistant bacteria. Examples of these conditions are: in soft tissues, they include chronic ulcers (varicose, ischaemic, diabetic, pressure sores, open cavities and burns); in the respiratory tract, chronic sinusitis, chronic 'wet' ear, chronic bronchitis, bronchiectasis, cystic fibrosis and empyema; in the urinary tract, recurrent persistent cystitis; in the peritoneum, peritoneal soiling after surgery and chronic pancreatitis; and in orthopaedics, complex prosthetic joint reconstruction. In some or all of these, ROS therapy may have a role in preference to conventional antibiotics and sparing the use of systemic antibiotics.

SHRO has been evaluated in chronic wounds in an open-label multicentre study and has shown, through its ROS activity, to reduce bacterial bioburden and biofilm and to support healing [20]. SHRO has been shown to eradicate multiresistant organisms from superficial sites and intravascular catheters [8,10] and to prevent surgical site infection [9]. This last study was a temporal study comparing surgical site infection rates in Caesarean section wounds before and after an intervention with a single application of SHRO at wound closure. A striking 60% reduction in wound infection was recorded and, whilst this study has significant limitations, it nevertheless paves the way for future randomised controlled trials of ROS in surgical prophylaxis. ROS might work with or in some cases replace surgical antibiotic prophylaxis.

To date, SHRO has been used internally in limited clinical cases for inhalation in nebulised form to reduce respiratory tract bacterial colonisation [19] and on complex prosthetic joint reconstruction [24]. It has demonstrated no safety concerns and in the limited cases suppression of infection in prosthetic joints has been demonstrated

for up to 12 months post-surgery. These preliminary data are important as they pave the way for further trials on the control of infection of internal structures and devices, providing ROS can be delivered to these sites by some practical means, be it by direct surgical intervention, injection, instillation or via a catheter. ROS instilled into the bladder may be useful in chronic recurrent multidrug-resistant (MDR) cystitis where biofilm may contribute to pathogenesis. However, there are no clinical data for this indication yet.

ROS use in the respiratory epithelium by nebulised delivery has numerous potential clinical uses to reduce bioburden and biofilm in chronic respiratory conditions, cystic fibrosis, bronchiectasis and ventilator-associated pneumonia. There are anecdotal cases where nebulised ROS has been demonstrated to reduce bacterial load in the respiratory tract, but full trials have yet to be carried out. ROS has not yet been used clinically in other upper respiratory tract chronic inflammatory processes, but ROS use in in vitro models demonstrates efficacy in antimicrobial and biofilm control, supporting clinical use in chronic sinusitis and chronic ear infection [19]. The in vitro efficacy of SHRO on nasal methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) isolates in planktonic and biofilm form has been demonstrated. The data are currently being prepared for publication [19]. The aim is to extend these findings into the clinical setting by developing SHRO as a novel adjunctive antibiofilm therapy in *S. aureus*-related chronic rhinosinusitis, as well as potential use as part of a novel MRSA decontamination regimen.

Table 1 summarises the existing and potential clinical applications for ROS technology.

5. Reactive oxygen species therapy in developing health economies

Wound management in the developing world has many challenges. Living conditions are often poor and overcrowded and the environment is hot and dusty or in the rainy season wet and muddy. Keeping skin clean and free from dirt and pathogens is difficult. It can be achieved by washing with soap and water, but in many parts of the world accessing water is difficult and soap may be too expensive. Approximately 2.5 billion people globally do not have access to an improved water supply, which is one that comes from a stand pipe or protected well or spring or rain water [25]. This water often has to be carried some distance from its source to their homes. It is heavy work as a 25 L container of water weights 25 kg and it is often undertaken by women and children. Owing to lack of easy access to water, priority is given to using it for drinking, cooking and giving it to animals rather than washing the skin. But even if water from an improved water source is used to clean skin it may not necessarily be free of pathogens and of drinkable quality [26], so even minor wounds often become infected.

There is also the difficulty of low literacy and low health literacy levels. These, together with the distances required to access healthcare facilities, sometimes result in local healers being accessed to give advice on wound care. This, at times, results in harmful treatments being used with subsequent deterioration in the condition of the wound and of the patient.

Payment is sometimes required for health care so often the very poor will delay seeking treatment until their wound is grossly infected and very painful. Tetanus is not uncommon in children in some areas. Malnutrition and anaemia often due to malaria are other factors that delay or prevent wound healing, together with undiagnosed and untreated co-morbidities such as diabetes.

The wound cleansers used in health facilities usually consist of lotions such as Savlon™ in various dilutions or diluted bleach (NaOCl), neither of which is now used in the UK, or sterile saline that is expensive. In the developed world, drinkable quality water is generally used for cleaning wounds [27].

Wound dressings are mainly made of gauze cut from rolls and then made into swabs by nurses. They are then autoclaved in drums, but once the drums are opened the swabs inside do not remain sterile for very long. The swabs may also loosen cotton fibres, which may provide a focal point for infection in the wounds. For exuding wounds, the gauze is covered with cotton wool padding. Autoclaved Vaseline™ gauze is placed on burns. Gauze bandages or tape is used to secure dressings. Both gauze and Vaseline™ gauze tend to stick to the wound, causing pain and trauma on removal and also the removal of any new tissue. Pain is an issue, especially for those with burns where painkillers may not be given due to cost.

A trial of SHRO was undertaken in two hospitals in Africa (video). One was a rural hospital in Uganda and the other a town hospital in Ethiopia. The hospital staff were given supplies of the product, taught the benefits of honey in wound management, the quantities required to apply to different sized wounds and the frequency of

dressing changes. This was followed by practical demonstrations and written instructions. A tablet computer was given to each hospital to anonymously record details of the patients and photographs of their wounds over a period of time on pre-written forms. Approximately 20 patients in each hospital were treated with SHRO. The wounds included extensive burns, ulcers including diabetic ulcers, wounds caused by trauma and post-operative wounds. All of the wounds improved significantly in terms of slough, exudate and healing with less wound trauma. Measurement of bioburden by culture was not possible due to lack of facilities. Some wounds required no oral antibiotics, thus reducing antibiotic use and making an important cost-saving. The dressings were easy to remove, resulting in less pain. Only one patient who had surgical amputation of her arm following a road traffic accident complained of stinging on application. There were no other adverse effects.

There is a huge need in developing countries for wound products that are sterile, easy to apply, and that do not cause trauma to the wound, pain on removal or leave fibres in the wound that may be a source of infection or inflammation. Dressings also need to be antimicrobial and antifungal in order to treat infected wounds and potentially reduce the need for antibiotics and antifungals. They need to be easy to store with a long shelf-life and not be affected by high ambient temperatures. Finally, they need to be low cost. SHRO fits these criteria. The potent antimicrobial activity of SHRO [5,6,10], negligible toxicity and its efficacy in reducing wound pain and exudate [20] and disrupting biofilms that delay healing [7,20] gives SHRO considerable potential for improving wound care in the developing world.

6. Conclusions

SHRO and ROS in other delivery formats is the only entirely novel antimicrobial strategy to reach clinical use in several decades. This review has demonstrated the mechanism, efficacy, safety and wide range of existing and potential clinical applications for ROS technology. The implications for global health are immense, particularly as the therapy is simple to produce, safe to use, possibly cheap, easy to transport and store, and simple to administer to treat a very common clinical problem, namely colonised and infected soft tissue defects from all causes. ROS therapy may reduce the requirement for systemic antibiotics.

The use of ROS as a chronic wound dressing has demonstrated wound healing and a reduction in bioburden and wound biofilm [5–7,20]. Chronic wounds are a huge global health burden and a reason for much inappropriate antibiotic use. ROS could provide a solution to this problem. ROS used prophylactically has shown a reduction in surgical site infections [9]. Further randomised clinical trials are required but ROS clearly has the potential to be used for all surgery to reduce infection and spare antibiotic use. Limited use in Caesarean wounds and complex orthopaedic joint surgery shows clinical efficacy with no disadvantages.

ROS has been successful in clearing MDR organisms, including MRSA and carbapenemase-producing *Escherichia coli*, from wounds and vascular line sites [8,10], and work currently underway shows antiviral and antifungal properties [19]. Nebulised ROS has been evaluated in limited subjects to assess reductions in bioburden in chronically colonised respiratory tracts. A subsequent review on the activity of ROS on bacterial biofilm may have great implications for the treatment of a

variety of persistent respiratory conditions. These are just the sort of conditions where conventional antibiotics are overused with limited clinical benefit and where ROS could play an important role in control of bioburden and biofilm. ROS technology could help patients with chronic colonisation and infection of the bladder with MDR bacteria. ROS used locally on internal structures from deep cavities, pleural lining, peritoneum, prosthetic devices and shunts could help control infection that has hitherto not been possible. The simplicity and stability of the treatment delivery systems may lend themselves well to use in developing health economies.

ROS therapy is a unique and novel technology with wide clinical applications that can provide a solution to help resolve the global crisis of infections caused by MDR microbes.

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Table 1

Reactive oxygen species (ROS) technology: clinical uses and therapeutic potential

Clinical applications of ROS	Therapeutic benefits	Evidence
Wounds, skin and soft tissue	Reduction in bacterial load and biofilm. Healing promotion	Large observational study [20] In vitro studies [5–7,11,13]
Surgical prophylaxis	Reduction in rates of surgical site infection	Temporal observation study [9] RCTs required
Infection prevention	Eradication of multiresistant and pathogenic organisms	Observational reports describing effective eradication and control [8,10]
Antimicrobial stewardship	Great potential for antibiotic-sparing around the world, particularly early use in soft tissue lesions. May have potential in respiratory and urinary mucosa to prevent colonisation with MDR bacteria and requirement for last-resort antibiotics	Large observational study [20] Further studies required
Prosthetic joint infection	Use as topical suppression therapy on joint	Small series of case reports demonstrate efficacy and safety. Further studies required [24]
Infected surgical cavities	Potential use in infected cavities (peritoneum, thorax, deep wounds, abscesses)	No studies as yet

Upper respiratory tract	Reduction in bacterial load and biofilm. Healing promotion in sinusitis	In vitro and clinical studies in progress [19]
Chronic lower respiratory tract conditions	Potential to reduce bacterial load and biofilm and to prevent exacerbations in chronic obstructive airway disease, bronchiectasis, cystic fibrosis and ventilator-associated infection	Limited in vitro data and anecdotal clinical cases [19] Further studies required
Recurrent urinary tract infection	Potential for ROS use via urinary/nephrostomy catheters to reduce bacterial load and biofilm and to eradicate MDR organisms	No studies as yet. In vitro efficacy of ROS against MDR pathogens [6]

RCT, randomised controlled trial; MDR, multidrug-resistant.