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Review

Reactive oxygen species: a novel antimicrobial

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ABSTRACT

The main solution to the global antibiotic resistance crisis is to reduce the volume of antibiotic use in medicine, agriculture and the environment. However, there is also a pressing need for novel antimicrobials. Despite much rhetoric, there are few entirely novel agents in development. One such therapy to reach clinical use is an agent using Reactive Oxygen Species (ROS), oxygen radicals, as an antimicrobial mechanism. ROS can be delivered to the site of infection in various formats. ROS are highly antimicrobial against Gram-positive and Gram-negative bacteria, viruses and fungi. They also prevent and break down biofilm. These functions make ROS potentially highly suitable for chronic inflammatory conditions, where antibiotics are frequently overused and relatively ineffective, including: chronic wounds, ulcers and burns; chronic rhinosinusitis, chronic bronchitis, bronchiectasis, cystic fibrosis and ventilated airways; recurrent cystitis; and prosthetic device infection. ROS could have an important role in infection prevention and antimicrobial stewardship. Much clinical investigation remains to be delivered on ROS therapy, but in vitro work on infection models and early clinical evaluations are extremely promising.

1. A solution to a global crisis

Although resistance to antimicrobials (antibacterials, antifungals and antivirals) is now a major global concern [1], practical solutions effective across the world have been in short supply. Antimicrobial resistance (AMR) has considerable clinical and financial consequences and the financial burden of AMR has been estimated to be huge [2]. Governments are developing AMR strategies that include programmes of antimicrobial stewardship, and encourage calls for the urgent development of novel antibiotics [3]. The solutions to the global antimicrobial resistance crisis require a reduction in the demand for, and volume of, antimicrobials to reduce selection pressure, improved infection prevention to reduce transmission, and new antimicrobial agents. Several new agents based on existing classes of antibiotics are being developed, but there has been little progress on entirely novel agents. One entirely novel antimicrobial agent to reach early clinical use is an agent employing reactive oxygen species (ROS) as its mechanism of action (Table 1).

Bacterial and fungal biofilms are a significant problem in many clinical settings because of their increased tolerance towards conventionally-prescribed antimicrobials [4]. Antibiotic use in such conditions (chronic wounds, burns, chronic respiratory conditions and cystic fibrosis, recurrent cystitis) leads to intense selective pressure, which often results in further antibacterial resistance. Therefore, there is a pressing need for the development of alternative therapeutic strategies that can improve antimicrobial efficacy towards biofilms, thereby limiting antibiotic use and reducing the development of further resistance [5]. Therapies involving ROS as a mechanism of action are already available in clinical use for wounds and are being developed for clinical use in other settings [6]. Encouraging research into, and the development of, the clinical therapeutic applications of ROS should be a major focus of the campaign against global antibiotic resistance.

2. What is ROS?

The term 'ROS' applies to molecules that contain O_2, but which 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 ·OH, and hydroxyl OH– ions. All have different actions and kinetics in cellular metabolism [6].

ROS are directly antimicrobial. H_2O_2 appears to elicit its antimicrobial action by a reaction with thiol groups in enzymes and proteins, DNA and bacterial cell membranes [6]. Although H_2O_2 can be used as a cleansing, antiseptic agent, the duration of its activity is too short to be of use as a therapeutic agent. However, ROS gels have been manufactured to release ROS slowly over a prolonged period of time, allowing sustained continuous release of ROS to a

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Table 1
Summary of a clinical register of use of Surgihoney Reactive Oxygen (SHRO) on compassionate grounds in various complex infections.

Clinical site	Dosing regimen	Clinical details	Outcome	Adverse reports
Respiratory tract	Daily nebulized SHRO in respiratory nebulizer	Bronchiectasis. Several patients One patient—recurrent exacerbations with secondary infection with *Mycobacterium avium*	Reduction in bacterial load and temporary eradication of *M. avium* (one patient)	None
Scalp	Daily topical application for 6 weeks	Fungal kerion. *T. tonsurans*. Patient intolerant of oral antifungals	Complete resolution	None
Intraperitoneal	50–100 g daily via abdominal drain	Severe four-quadrant peritonitis following intra-abdominal infection and corrective surgery. Patients also on systemic antibiotics. Microbiology often polymicrobial with MDR strains and *Candida* spp.	Variable, but general control of peritonitis	None related to SHRO use
Abdominal wall deep soft tissue	SHRO into open cavity with each dressing	Used both prophylactically and therapeutically in around 20 patients.	Prevention of infection and effective therapy in infected cavities	None
Prosthetic joints	Single dose around prosthetic joint at surgery. Numerous patients	Mixed microbiology including *Staph ludenensis* PJI. Given in conjunction with systemic antibiotics	Good adjunct to existing management	None
Prepatellar bursitis	Single application at debridement	On immunosuppression for psoriasis. *M. malmoense* isolated from prepatellar pus. Put on clarithromycin, rifampicin and ethambutol + SHRO topically	Complete healing and no further isolation of *M. malmoense*	None
Bladder	Twice-weekly instillation via suprapubic catheter	Several patients with long-term urethral or suprapubic catheters.	Reduction in urosepsis	None
External auditory canal	Daily with wick or cotton wool	Pseudomonas otitis externa	Resolved	None
Oral infections	Daily oral application of 10 g SHRO	Recurrent apthous ulcer, gingivitis, geographic tongue. No microbiology	Reported reduction in duration of symptoms	None
Helicobacter gastritis	Once daily 10 g SHRO for 10 days	Confirmed H*elicobacter pylori* gastritis, MDR strain and no response to antibiotic eradication regimens	Continuation of symptoms. Therapeutic failure	None

target site. In such a format, and there is the potential for many delivery formats, ROS can be employed as a therapeutic antimicrobial agent [7].

ROS have potent antimicrobial activity against bacteria, fungi and viruses. ROS are rapidly active in vitro against all Gram-positive and Gram-negative bacteria tested, including multidrug-resistant (MDR) strains, which are causing significant infection control and therapeutic concern [8]. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) to ROS are consistent amongst isolates of the same bacterial species, whether the isolates are MDR or highly sensitive. MICs and MBCs to ROS are well below concentrations that can be achieved with topical delivery. Cidal activity is very swift, with 3-fold log reduction in colony forming units in 30 minutes of exposure, and complete eradication in 2 hours when the lowest potency of ROS gel was used against *Staphylococcus aureus* [8].

The first ROS therapeutic agent was in the form of a pharmaceutical honey wound gel, Surgihoney Reactive Oxygen (SHRO). SHRO is a modified honey that has been engineered to provide a constant level of ROS over a prolonged period of time when applied to a wound [9]. The availability of ROS from SHRO, or indeed an alternative synthetic delivery system, can be enhanced and is scalable. Other ROS antibiotic agents and delivery systems, such as gels, sprays, nebulizers and infusions, that employ this mechanism are being developed and may be particularly useful for delivery of ROS to other clinical sites.

3. Antibiofilm activity

In addition, ROS agents are effective at preventing the formation of biofilm and disrupting established biofilm. SHRO and ROS prototypes of increased antimicrobial activity were compared with pharmaceutical grade honeys (Activon manuka honey and Medihoney manuka honey) and five antimicrobial dressings (AMDs) in their ability to prevent biofilm formation in vitro by 16 bacterial isolates [10]. In serial dilution, SHRO and ROS prototypes were most effective in disrupting established biofilm. In addition, SHRO had superior antibacterial potency to three commercially-available antimicrobial dressings (AMDs). Similar results have been found in respiratory epithelium models [5,7].

4. Biofilms in clinical practice

Antibiotics are most effective in acute infections. Acute infections are caused by planktonic bacteria invading blood or tissues, which react with an innate inflammatory response characterised by polymorphonucleocytes. Antibiotics are usually effective in resolving such acute infections quickly and efficiently. In contrast, biofilm infections do not respond well to antibiotics, although antibiotics in high dose and for prolonged periods are often used in an attempt to treat these conditions [4]. This therapeutic approach is not very successful, and patients with biofilm infections tend to become progressively colonised with increasingly resistant bacteria [7].

Biofilm infections include dental plaque and periodontal disease, chronic wounds and ulcers, burns, diabetic wounds, chronic rhinosinusitis and otitis, chronic bronchitis, cystic fibrosis, bronchiectasis, chronic osteomyelitis, chronic recurrent cystitis, bacterial vaginosis, healthcare catheter- and shunt-related infections and prosthetic device infections [7].

RO technologies may be the ideal agents to treat biofilm infections. ROS can be delivered topically to the site of biofilms via delivery mechanisms, such as SHRO or RO gels for wounds, ears, operative sites, catheters and shunts, and to many prosthetic devices. ROS agents can be added to douches and wash outs for rhinosinusitis or infiltrated in liquid form to catheters, shunts and bladders [5,7]. It may be possible to develop ROS particles for inhalation to coat the respiratory tract in patients with chronic respiratory conditions, or in ventilated patients. Slow, continuous ROS production through such delivery mechanisms can control the bacterial load and break down the biofilm, probably reducing the need for systemic antibiotics and reducing the selection pressure, which so often results in such patients acquiring MDR bacteria. These are entirely novel and exciting therapeutic approaches that are worthy of much further investigation.