Myeloperoxidase leads the way toward safe and efficient antiseptics

- There is a vital need for antiseptics that work well in physiological conditions. All currently known antiseptics, however, are inhibited by blood.
- Robert C Allen shows how myeloperoxidase (MPO), a key enzyme in the human immune system, kills pathogens in the body through in situ generation of highly reactive singlet oxygen species.
- Based on these findings, he has developed a myeloperoxidase-based antiseptic which is effective in blood and systemically safe.
- This new microbicidal agent also exhibits remarkably selective anticancer properties.
- Together with Jackson T Stephens, the challenge now is to overcome obstacles restricting the widespread use of MPO antiseptics in therapy.

The immune system is vital for survival and health, protecting the body from pathogens and preventing serious infections. Neutrophils, a type of white blood cell, are essential contributors in this defence. They act as the first line of defence by quickly responding to infections and inflammation, engulfing and destroying pathogens through phagocytosis and combustive phagolysosomal oxygenations.

The importance of oxygen

Robert C Allen has devoted over 50 years of research to understanding how neutrophils act in the body. Through a series of elegant experimental studies and quantum mechanical considerations, he has identified the key player in the extraordinarily complex and efficient physiological processes carried out by the neutrophils. He has shown that, in the presence of pathogens, neutrophils generate a highly oxidative environment that destroys bacteria and other microorganisms. This is achieved through the conversion of normally inert triplet oxygen molecules ($^3O_2$) from the atmosphere into their highly reactive singlet form ($^1O_2$*), which rapidly attacks and decomposes the pathogens. $^1O_2$* is a short-lived (metastable electronically excited) species and its lifetime is in the order of microseconds. $^1O_2$* has powerful antiseptic activity targeted to the proximity of its nascence avoiding collateral damage to healthy body cells.

Chemiluminescence

According to Allen, the processes through which neutrophils kill bacteria are analogous to combustion, and they are, therefore, highly exergonic. As $^1O_2$* reacts with a pathogen's organic matter, it generates carbonyl products in electronically excited states, which decay to their ground state emitting visible light. Allen has shown that these oxygenation activities can be measured accurately and with high sensitivity using chemiluminigenic probes, small organic molecules that react with the $^1O_2$* generated by neutrophils resulting in enhanced light emission. This has led him to create an exquisitely sensitive method to monitor in real time the functionality of the immune system during an infection.

A vital enzyme

Within the body, cells involved in specific functions, like immune response and secretion, are equipped with granules, which are small membrane-bound compartments containing enzymes, proteins, or other molecules. In neutrophils, the so-called azurophilic (or primary) granules contain enzymes that are involved in the initial response to an infection. Myeloperoxidase, or MPO, is one of the most important of such enzymes for the immune system's ability to destroy pathogens. Allen has been studying the physiological role of MPO since 1971. Using chemiluminescence and metabolic studies he has been able to study the complex and finely regulated mechanism of NADPH oxidase driven MPO action in microbicidal activity.

> **MPO-based E-101 is the first wound and systemically safe antiseptic, with a safety profile comparable to saline, that is effective in physiological conditions.**

Combustive elimination of pathogens

When neutrophils reach an infected area of the body, they engulf external pathogens in membrane-bound vesicles, known as phagosomes. The neutrophil granules, which in addition to MPO also contain lysosomal enzymes that promote the degradation of large macromolecules, fuse with the phagosomes, producing a phagolysosomal vacuole. It is inside these vacuoles that the microbicidal action of MPO occurs. MPO catalyses the conversion of hydrogen peroxide (H_2O_2) into hypochlorous acid (HOCl), which reacts with another H_2O_2 generating $^1O_2$*. This aggressive chemical species promptly reacts with the pathogen's molecular structure, triggering its oxidation and eventual destruction. 'Phagocytosis is linked to the activation of NADPH oxidase resulting in respiratory

Myeloperoxidase-based antiseptic is an effective and selective microbicidal agent with potential to mitigate the challenges associated with antibiotic-resistant bacteria such as *S. aureus*.

burst metabolism and reduction of oxygen to radical products such as HO_2 and O_2', explains Allen, 'disproportionation of these doublet multiplicity intermediates yields $^1O_2$* and the hydrogen peroxide (H_2O_2) that drives MPO oxidation of chloride (Cl^-) to hypochlorite (OCl^-). Reaction of OCl^- with an additional H_2O_2 yields the $^1O_2$* that is directed to the combustive elimination of the infectious microbes.'

Selective bacterial killing and endotoxin inhibition of MPO

Neutrophils circulate in the blood for less than one day. After leaving the blood stream, they migrate to body cavities, such as the mouth, the gastrointestinal tract, and the vaginal vault, transporting MPO with them. In these environments, MPO can act as a powerful microbicidal agent, killing potentially harmful bacteria. However, MPO is also highly selective: it does not bind to and thus does not damage the lactic acid bacteria that constitute the natural flora. Allen and Stephens have shown that the targeted microbicidal action of MPO is related to its ability to bind selectively to very specific classes of microorganism. When the concentration of MPO is limiting only microbes or cells that binds MPO are susceptible to combustive oxidation driven by $^1O_2$*, whereas cells that do not bind MPO experience no or minimal damage.

MPO binds to all gram-negative, endotoxin-positive microbes tested. Allen and collaborators have reported that MPO, and to a lesser degree eosinophil peroxidase (EPO), inhibit lipopolysaccharide and lipid A measured by the Limulus assay. Such inhibition does not require haloperoxidase action. Likewise, endotoxin lethal dose 90 (LD90) studies in mice demonstrate that MPO and EPO increase survival.

E-101: A powerful and selective antiseptic

MPO is a very stable enzyme, which can be coated onto surfaces or maintained in solution for extended periods of time. Allen discovered that combining MPO with a suitable source of H_2O_2 provides a powerful antimicrobial, which is as efficient and selective as the MPO naturally occurring in the human body. The E-101 solution developed at Exoxemis, Inc makes use of glucose oxidase, an enzyme that catalyses the oxidation of glucose, to produce H_2O_2, which, in the presence of MPO and Cl^-, produces $^1O_2$*. E-101, trade named Zempia®, can be prepared in the absence of O_2, for instance under a controlled nitrogen atmosphere, and can be activated simply by exposing it to atmospheric oxygen, which initiates the generation of $^1O_2$*.

> **MPO's focused cytotoxicity against cancer cells arises from its preferential binding to negatively charged cancer cell membranes.**

E-101 is the first antiseptic of its class that retains its activity in blood and can be used to clean and disinfect wounds with minimal systemic toxicity and no adverse effects on the human body.

Antitumoural activity

MPO has been found to act not only as a powerful antiseptic, but also as an anticancer agent. In collaboration with Mayo Clinic, C-202, a solution with the same pharmaceutical ingredients of E-101, showed focused cytotoxicity against bladder cancer cells, with no damage for healthy cells. The results demonstrate selective toxicity of MPO for cancer cells that is related to their anomalous physiology, ie, Warberg effect, which causes their membrane to acquire an anionic (negative) charge, unlike healthy cells, which are charge neutral. Since MPO is a positively charged macromolecule, it binds preferentially to cancer cells and, in the presence of H_2O_2, generates $^1O_2$*, which oxidises and destroys cell membrane components essential for their survival. In a letter of recommendation to the Food and Drug Administration, C-202 has been put forward as a potential treatment for non-muscle invasive bladder cancer patients. Not only does C-202 have potential to improve treatment outcomes, it could also mitigate the supply/demand issue facing current FDA-recommended therapy (BCG).

Personal response

What are the principles of action of MPO in vivo and as a therapeutic antiseptic?

The microbicidal and antineoplastic actions of MPO are the consequence of H_2O_2 and Cl^- dependent haloperoxidase generation of $^1O_2$*. $^1O_2$* is a metastable electronically excited singlet multiplicity state of oxygen that has potent electrophilic reactivity like chlorine gas, but its lifetime restricts reactivity to within a radius of about 0.3 micron from its point of nascence. Thus, combustive oxygenating activity is short-lived and focused with minimal bystander damage.

You have strong data demonstrating the efficacy and safety of MPO, formulated as an antiseptic agent in E-101 (Zempia®). What is the next step in moving MPO forward as a therapeutic for routine use in human patients?

Overcoming the regulatory gaps which abound in approved antiseptics. E-101 is effective against all organisms tested, including antibiotic resistant microbes and does not select for resistance. It is highly effective in blood and in animal models. E-101 shows no systemic statistical safety differences compared to saline when applied in open, human, surgical wounds and does not hinder those wounds' healing compared to saline. No other antiseptic can make these claims.

Can you describe your experiences in getting E-101 to the point where it can be used by patients?

The journey began in 1987 with the purification of sufficient quantities of MPO for in-depth study. A significant breakthrough was identifying the selective surface binding mechanisms of MPO, allowing the enzyme to specifically target and kill pathogens in the presence of blood while sparing normal flora and human tissue. Subsequent work involved resource-intensive formulation development and scaling up production.

Steve, you've described your and Bob's journey with myeloperoxidase as a 'Pareto superior move'. Can you explain what you mean by this?

The development process of MPO epitomises a Pareto superior move, which in economic terms, is an act of wealth creation that benefits at least one individual without making another worse off. Rigorous development, including human clinical trials of E-101 (NCT01297959), demonstrated pathogen binding and killing with excellent safety data. Additionally, MPO/C-202 shows selective and efficient killing of bladder cancer cells with minimal cytotoxicity against non-cancer urothelial cells. These innovations promise to enhance public health without compromising safety, aligning with the principles of a Pareto superior move.

In stark contrast, the FDA's general regulatory approach implicitly relies on Pareto-optimal decision-making. A state is Pareto-optimal when no further incremental improvements can be made without making someone else worse off. For antiseptics, there is no baseline for FDA's Pareto-optimal incremental improvement in their approval process. Even with a baseline as with bladder cancer, the FDA favours approved antineoplastic agents on the market with a less effective mode of action and known side effects.

MPO represents a significant advancement due to its combined benefits: focused antiseptic action, systemic and wound safety, targeted endotoxin inactivation. In general, cationic MPO shows targeted antineoplastic (anti-cancer) action based on the increased anionic (negative) surface charge of cancer cells (Warberg effect).

What are the remaining obstacles and challenges that restrict the widespread use of MPO antiseptic or antineoplastic formulations in therapy?

The primary challenges are bureaucratic. The FDA has imposed standards on E-101 that other antiseptics have never met, creating an uneven playing field and prohibiting its market introduction in the US. Existing antiseptics were grandfathered by the FDA in 1976 without the rigorous safety and efficacy studies required for MPO/E-101. Additionally, the investigation of MPO/C-202 cancer therapy is restricted by the FDA to human trials in BCG-unresponsive patients only complicating the situation further. BCG, an FDA-approved cancer therapy, is in limited supply and is expected to remain so at least through 2025. Given these challenges, regulatory authorities in other countries are being asked to review the data and consider the benefits of MPO. Efforts will continue down the available FDA avenues for E-101 and C-202.

Details

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Bio

Professor Robert C Allen's published research started in 1972, with the report that neutrophils engaged in microbe killing emit light in the visible range of the spectrum. In a seminal paper in 1986, he demonstrated that neutrophil oxidase and haloperoxidase-dependent oxygenation activities could be differentially quantified in real time and with high sensitivity using chemiluminigenic probes (luminol and lucigenin). In recent decades, Allen has applied luminescence measurement techniques and discriminant statistical analysis to evaluate host systemic inflammation and diagnosis of infectious states. His work with Exoxemis, Inc began in 1987 and focused on improving blood neutrophil luminescence analysis, and on haloperoxidase microbe killing. Breakthroughs in MPO and EPO isolation and purifications allowed experimentation that demonstrated the selective binding of haloperoxidases to microbes and cancer cells. The microbicidal agent of peroxide-driven haloperoxidase action, ie, metastable electronically excited singlet molecular oxygen ($^1O_2$*), has a microsecond lifetime that restricts combustive oxygenation action to the proximity of its point of nascence. Thus, damage is focused to the site of haloperoxidase binding with minimal bystander damage. Consequently, such haloperoxidase action satisfies Fleming's position that an ideal antiseptic agent must selectively kill infecting microbes with minimal host cellular damage.

Jackson T 'Steve' Stephens, Jr, Founder and CEO of Exoxemis, Inc.

Competing interest statement

Jackson T Stephens, Jr serves as President and CEO of Exoxemis, Inc.

Further reading

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