Complementing our immune system: the antimicrobial mode of action of exogenous nitric oxide and its potential in wound care

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Introduction

Nitric oxide (NO) is an innate molecule of the human immune response to invading pathogens. NO is produced in the body by nitrogen synthases (NOS) from L-arginine through a series of oxidation reactions.[1] Whilst NO has potent antimicrobial activity against bacteria, its effects on mammalian cells are lessened due to diffusion down the concentration gradient out of cells and into red blood cells. Here it is broken down into nitrate via oxyhemoglobin faster than it can react with intracellular components.[2] NO could be employed in hard-to-heal wounds that are infected or at risk of infection due to the lack of bacterial resistance mechanisms to NO and tolerance by human cells. We conducted a narrative review of the evidence underlying the mechanisms of action of NO as an antimicrobial agent in wound dressings for the treatment of hard-to-heal wounds, such as diabetic foot ulcers (DFUs).

Aim: To evaluate the evidence for nitric oxide (NO) as an antimicrobial agent for the treatment of hard-to-heal wounds.

Interactions at the cell wall

Thiol and tyrosine group disruption (Figure 1A and 1B)

- Reactive nitrogen species (RNS), such as dinitrogen trioxide (N_2O_3) and peroxynitrite ($OONO^-$), are formed from a reaction between NO (●–●) and superoxide (O_2^-) (●–●$^-$).[2,3]
- N_2O_3 has a strong affinity for thiol groups in proteins embedded within the lipid bilayer,[3] adding nitrous groups, resulting in misshapen proteins, which eventually destroys their function.[4]
- $OONO^-$ will also react with tyrosine groups on internal cell wall-bound proteins. The end-product, 3-nitrotyrosine, is an established biomarker of cellular nitro-oxidative stress.[3,4]

→ Loss of function will result in bacterial death.

Diffusion of NO through the cell wall (Figure 1C)

- NO gas freely diffuses through bacterial cell walls[5] due to its small diameter, low polarity, and lipophilic property.[6] The rate of diffusion is affected by whether the bacteria has a thick peptidoglycan layer on the outer cell wall (Gram positive), a thicker layer slows the rate of diffusion.[7]

→ NO gas can effectively penetrate bacterial cells

Eventual breakdown of the cell wall (Figure 1D)

- The tightly spaced lipid bilayer that makes up the cell wall acts as a barrier to large and charged molecules, preventing them from entering the bacteria. As NO disrupts both internal and external bound proteins, the cell wall begins to break down.

→ The disruption of the cell wall increases its permeability to antibiotics and other large antimicrobial molecules.[8]

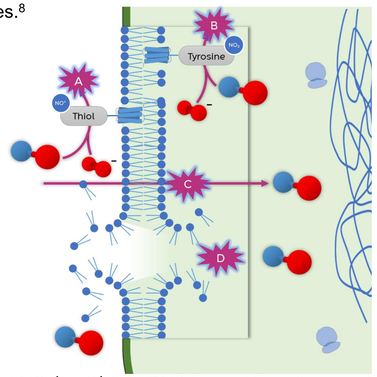

Figure 1. Interactions of NO (●–●) and RNS formed from the reaction of NO with O_2^- (●–●$^-$) at the cell wall. **(A)** Thiol group disruption. **(B)** Tyrosine group disruption. **(C)** Diffusion of NO through the cell wall. **(D)** Eventual breakdown of the cell wall.

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Disruption of DNA processes

Production of peroxynitrite ($OONO^-$) (Figure 2A)

- As NO moves into the bacterial cell, it will form other RNS such as $OONO^-$. RNS react with DNA, causing oxidative damage and loss of amine groups.
- $OONO^-$ has been shown to cause strand breaks more efficiently than NO alone, as it reacts directly with the sugar backbone of the DNA breaking it down.[9]

→ Breakdown of the backbone will eventually lead to DNA cleavage and an instability of the genome[3,4] **(Figure 2C)**

Inhibition of ribonucleotide reductase (Figure 2B)

- Ribonucleotide reductase (RNR) is an enzyme for the conversion of ribonucleotides (NTP; the building blocks of RNA), into deoxyribonucleotides (dNTP; the building blocks of DNA). dNTP in bacterial cells is essential for DNA repair and replication and overall stability of the genome.[10]
- NO has strong reactivity towards iron containing amino groups in the RNR enzyme, which results in its inhibition [10].

→ Without the ability to repair and create new DNA strands, bacteria will become unstable, unable to reproduce, and ultimately die.[10,11]

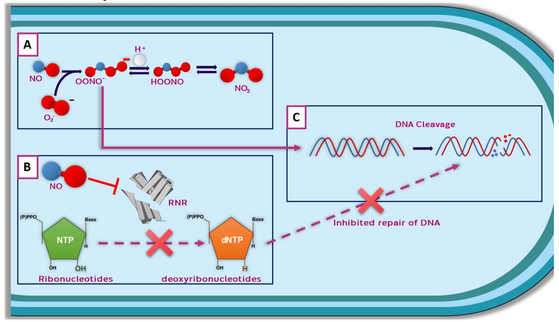

Figure 2. Disruption of DNA processes. **(A)** The production of the RNS $OONO^-$ from NO and O_2^-. **(B)** Inhibition of RNR. **(C)** Cleavage of DNA by $OONO^-$ and inhibition of repair.

Breakdown of iron-sulphur clusters

- Iron-sulphur (Fe-S)-containing proteins are one of the primary targets of NO. Fe-S clusters are essential enzyme co-factors required for fundamental biological processes within bacteria such as oxidative phosphorylation and ribosome assembly.[12] Bacteria also use Fe-S clusters for electron transport, which is susceptible to NO attack.[13]
- NO is attracted to sulphur and reacts with it, breaking the molecule into two smaller iron complexes called dinitrosyl iron complexes (DNICs), which are biomarkers for NO toxicity [12] **(Figure 3).**
- DNICs then donate nitrogen and react with oxygen to form additional RNS species.[14] DNICs negatively affect bacteria cells through the release of RNS.[15]
- Fe released from Fe-S clusters binds to bacterial DNA which is then oxidized by peroxide leading to DNA cleavage.[13]

→ Destruction of key Fe-S cluster-containing proteins disrupts pathways needed for the survival and proliferation of bacteria.

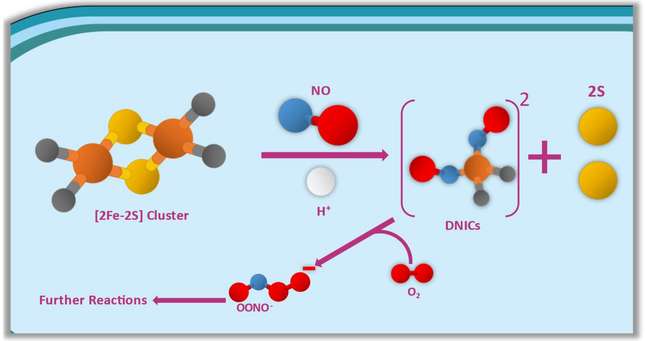

Figure 3. The breakdown of iron-sulphur (2Fe-2S) clusters by NO into dinitrosyl iron complexes (DNICs) and further reduction into $OONO^-$.

Discussion

The diverse mechanisms of action of NO and other RNS against bacteria means there is less opportunity for bacterial evasion of their action, decreasing risk of resistance or reduced susceptibility. The exploitation of NO generation from exogenous sources represents a promising strategy as an antimicrobial agent, which has the potential to be used in wound dressings, for the treatment of hard-to-heal wounds such as DFUs.

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