Episode 72 – Metronidazole Enhancement of Oral Innate Immunity

November 11, 2022

Introduction

- Periodontitis affects the supporting structures of teeth due to interactions between subgingival biofilm and host immune response.
- Periodontal therapy in severe periodontitis often uses antimicrobial agents with some potential to improve host defense responses. [1]

Metronidazole is a nitroimidazole antibiotic used against:

- Anaerobic bacteria (e.g., *Porphyromonas gingivalis*, *Clostridium difficile*).
- Some microaerophilic bacteria[1] (e.g., *Helicobacter pylori*, *Gardnerella vaginalis*).
- Certain protozoan parasites (e.g., *Trichomonas vaginalis*). [1] [2]

Polymorphonuclear neutrophils[2] (PMNs), also called granular leukocytes or granulocytes, are a type of leukocyte that contain granules with enzymes that are released (i.e., degranulation) during infections, allergic reactions, and asthma. [3]

PMNs use various mechanisms to eliminate target pathogens, such as:

- Engulfing microbes by phagocytosis.
- Killing via reactive oxygen species (ROS).[3]
- Secreting the toxic contents from their granules, such as proteins (e.g., lactoferrin, cathepsins) and enzymes (e.g., myeloperoxidase [MPO], elastase).
- Releasing neutrophil extracellular traps (NETs).[4] [1] [4] [5] [6] [7]

P. gingivalis, a gram-negative anaerobe, is a keystone pathogen in the initiation, development, and persistence of periodontitis. [1]

P. gingivalis has developed several mechanisms allowing them to avoid and modulate the PMN response of the host, such as:

- Impaired recruitment and chemotaxis. A delay of neutrophil recruitment may allow time for initial colonization and formation of bacterial communities that can

[1] Microaerophilic bacteria grow in environments containing low oxygen concentrations and are unable to grow at normal atmospheric oxygen tensions. [12]
[2] Refer to Episodes 44 & 45 for more information on PMNs and immune response.
[3] ROS is a generic term that defines a wide variety of oxidant molecules, with very different properties and biological functions, ranging from signalling to causing cell damage to killing bacteria.
[4] NETs are a meshwork of extracellular fibres formed by the release of granule proteins and chromatin fibres. NETs immobilize and kill invading bacteria to help prevent them from spreading.

subsequently subvert neutrophils, leading to defective killing capacity and dysregulated inflammatory responses.

- Resistance to granule-derived antimicrobial agents and oxidative burst. The capacity of *P. gingivalis* to inactivate granular enzymes and antimicrobial peptides and thus evade neutrophil-mediated killing may be an important means for its persistence in periodontal pockets. To kill pathogenic bacteria, neutrophils phagocytose the bacteria. Phagocytosis is followed by increased neutrophil oxidative metabolism and release superoxide as part of the oxidative burst process. Superoxide is converted to hydrogen peroxide, which is a main component of bactericidal activity of neutrophils.
- Inhibition of phagocytic killing through various mechanisms, including the ability of *P. gingivalis* to inactivate elastase, bacterial-permeability increasing factor, and defensins (type of antimicrobial peptide).
- Delayed PMN NETosis.[5]
- Delayed PMN apoptosis[6]. Neutrophil apoptosis, followed by subsequent phagocytosis of dead neutrophils, is important to resolve inflammation. Thus, a delay in apoptosis caused by periodontal bacteria may prolong inflammatory responses and increase tissue damage. [1] [8]

In addition, *P. gingivalis*:

- Can persist in periodontal pockets despite pro-inflammatory recruitment of PMNs by subverting the host's immune response, resulting in a chronic dysbiotic state.
- Supports periodontal biofilm growth by collaborating with other periodontal pathogens.
- Presence is linked to unresolved periodontal lesions and progressive bone loss, even after periodontal treatment.
- Persistence with other keystone periodontal pathogens (e.g., *T. forsythia*, *Prevotella* spp.) does not respond favourably to mechanical debridement, the gold standard in periodontal disease treatment. [1] [9] [10] [11]

Metronidazole enhances killing of *Porphyromonas gingivalis* by human PMNs

Research objectives

To investigate the *in vitro* effect of metronidazole at concentrations observed clinically in periodontal pockets on PMN activation and PMN mediated killing of *P. gingivalis*.

Materials and methods

Flow cytometry-based assays were used to measure the impact of metronidazole on PMN degranulation, NET formation, and MPO release and phagocytosis in response to *P. gingivalis*. Functional assays for PMN mediated killing of *P. gingivalis* and ROS production in PMN were also completed.

Results

Findings demonstrated PMNs pretreated with metronidazole displayed enhanced killing of *P. gingivalis* compared to untreated PMNs. At concentrations achieved physiologically in periodontal pockets, metronidazole induced PMN surface expression

[5] NETosis is a type of cell death dependent on formation of neutrophil extracellular traps (NET). [13]
[6] Apoptosis is the process of programmed cell death which eliminates unwanted cells.

of two activation markers. Metronidazole did not alter *P. gingivalis*-induced NETosis, but suppressed *P. gingivalis*-induced ROS production and phagocytosis. [1]

Take home messages

- Metronidazole showed a positive interaction with PMNs potentiating their ability to kill *P. gingivalis* and may therefore contribute to its beneficial effects in treatment of periodontitis initiated by *P. gingivalis* infections, including cases refractory to conventional periodontal treatment. [1]
- Metronidazole is not the first line of treatment against periodontal disease. Phase one therapy (e.g., scaling and root planing) should be initiated first.
- A team approach is important for periodontitis unresponsive to therapy, including consultation with a periodontist to determine if antibiotics are necessary.

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Additional Resources

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