Determining the Cellular and Molecular Mechanisms by which Biobran (MGN-3) Mediates Innate Immunity in a Hyperglycaemic Model of a Diabetic Foot Ulcer Infection.

Sana Shah, Mohamed El Mohtadi and Jason Ashworth

Centre for Bioscience, Department of Life Sciences, Manchester Metropolitan University, Manchester, United Kingdom.

Sana Shah (sana.shah@stu.mmu.ac.uk)

Jason Ashworth (J.Ashworth@mmu.ac.uk)

Background

Diabetic foot ulcer (DFU) infections frequently become infected by polymicrobial communities, leading to significant morbidity and mortality. Antibiotics are the first line of defence against DFU infections, but over-usage has led to widespread antibiotic resistance. To reduce the reliance on antibiotics, novel therapies are desired that promote infection clearance by stimulating innate host immune responses, thereby either replacing or working alongside antibiotic intervention. This study investigated the use of Biobran (MGN-3) to mediate innate host clearance of typical wound pathogens in a diabetic (hyperglycaemic) model of an infected DFU.

Methods

Host-pathogen interaction assays (n=12) were used to assess the effect of Biobran (MGN-3: 0.5, 1.0, 2.0 mg/ml) on M1 macrophage-mediated phagocytosis of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA01) under increasing glycaemic (glucose: 11, 15, 20 and 30mM) culture conditions. Treatment of M1 macrophages with rice starch (RS: 2.0 mg/ml) or bacterial lipopolysaccharide (LPS: 5μg/ml) were used as negative and positive controls respectively, and bacterial clearance was compared against untreated M1 macrophages (untreated control). Furthermore, gentamycin assays were conducted to elucidate whether M1 cells were successfully killing or sequestering bacteria. Host pathogen interactions were visualised by a combination of scanning electron and confocal microscopy. Inflammatory cytokine, nitric oxide and reactive oxygen species (ROS)

production were determined by enzyme-linked immunosorbent assay (ELISA), the Griess assay and a membrane permeable fluorescent immunoassay respectively.

Results

Increasing levels of hyperglycaemia significantly ($p<0.05$) impaired M1-mediated phagocytosis. MGN-3 and LPS supplementation reversed the detrimental effect of glucose by significantly increasing ($p<0.05$) M1-mediated phagocytosis of both MRSA and PA01 in a dose dependent manner compared to untreated and RS-treated controls. MGN-3 and LPS supplementation significantly ($p<0.05$) induced tumour necrosis factor–alpha (TNFα) and Interferon beta (IFN-β) secretion by M1 macrophage and reversed the glucose-induced reduction in nitric oxide production. Elevated levels of glucose lead to pronounced ROS production by M1 macrophages, which was significantly ($p<0.05$) alleviated by MGN-3 in a dose-dependent manner.

Conclusion

MGN-3 significantly reversed the detrimental impact of hyperglycaemia on M1-mediated effector functions including phagocytosis, cytokine secretion and NO/ROS production. These findings highlight the beneficial dose-dependent effects of MGN-3 on promoting bacterial clearance under hyperglycaemic conditions, warranting further investigations to evaluate the use of MGN-3 in local wound dressings as a potential cost-effective therapeutic strategy to resolve clinical DFU infections.