Biobran (MGN-3) Enhances Host-Pathogen Interaction in an In Vitro Hyperglycaemic Model of an Infected Diabetic Foot Ulcer via the CD14/TLR-4 Pathway.

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Background

Low grade prolonged inflammation is a key feature of chronic hyperglycaemia (CH) and a pathophysiological characteristic attribute of chronic wounds. CH affects multiple inflammatory pathways leading to dysregulated signalling. Pathogen recognition receptors (PRRs) are a vital component of the innate immune system that recognise Pathogen associated molecular patterns (PAMPs) and Danger associated molecular pathogens (DAMPS), thus enabling an immune response to be elicited. Toll-like receptors (TLRs), particularly TLR-4, is upregulated in patients with an infected diabetic foot ulcer (DFU). Lipopolysaccharide (LPS), a primary ligand for CD14 and its co-receptor TLR-4, has structural similarity to Biobran (MGN-3) in terms of molecular weight and chemical structure, suggesting TLR-4 and/or CD14 may be key regulators of innate immune responses mediated by MGN-3. Thus, this study investigated whether the TLR-4 pathway mediates the effects of MGN-3 on phagocyte function 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 U937-derived 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 endotoxin (LPS: 5ug/ml) were used as negative and positive controls respectively, and bacterial clearance was compared against untreated M1 macrophages (untreated control). The TLR-4 pathway was interrogated using

TLR-4, myeloid differentiation primary response protein (MYD88) and Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) inhibitors. CD14 was analysed using both flow cytometry to measure membrane-associated CD14 (mCD14) levels and enzyme-linked immunosorbent assay (ELISA) to measure soluble CD14 (sCD14) levels. Host-pathogen interactions were visualised by a combination of scanning electron and confocal microscopy.

Results

Increasing levels of hyperglycaemia significantly ($P<0.05$) impaired M1-mediated phagocytosis in a dose-dependent manner. MGN-3 and LPS supplementation reversed the detrimental effect of hyperglycaemia by significantly ($P<0.01$) increasing M1-mediated phagocytosis of both MRSA and PA01 in a dose dependent manner compared to untreated controls. Inhibition of TLR-4, MYD88 or TRIF significantly ($P<0.05$) blocked the beneficial effects of MGN-3 on the clearance of both MRSA and PAO1, regardless of the glycaemic state. Flow cytometry showed neither LPS nor MGN-3 affected mCD14 levels under euglycemic conditions. However, hyperglycaemia resulted in a significant increase in mCD14 when M1 macrophages were stimulated with LPS, which was confirmed by immunofluorescence staining. Concomitant treatment of M1 macrophages with MGN-3 and LPS significantly ($P<0.01$) reversed the effects of hyperglycaemia on LPS-mediated stimulation of mCD14 expression. A significant ($P<0.01$) increase in sCD14 levels was detected from LPS-activated M1 macrophages at all glucose concentrations. In contrast, MGN-3 treatment (significantly ($P<0.05$) decreased M1 macrophage-derived sCD14 levels at lower glucose concentrations (11 and 15mM), with sCD14 levels only increasing compared to the untreated control at higher glucose concentrations (20 and 30mM).

Conclusion

MGN-3 significantly reversed the detrimental impact of hyperglycaemia on M1-mediated phagocytosis of both MRSA and PA01. Inhibition of TLR-4, MYD88 or TRIF blocked the stimulation of phagocytosis observed with MGN-3, confirming the TLR-4 pathway is (at least in part) fundamental for the beneficial activity of MGN-3 on bacterial clearance. MGN-3 was able to reverse the LPS-induced increase in mCD14 expression under hyperglycaemic

conditions. Unlike LPS which increased sCD14 at all glucose concentrations, MGN-3 treatment reduced sCD14 levels at lower glucose (11 and 15mM) concentrations. Collectively, these findings suggest TLR-4 and CD14 may be key regulators of innate immune responses mediated by MGN-3, warranting further investigation of MGN-3 as a potential therapeutic strategy for the treatment of patients with an infected DFU.