Inhibition of Tumor Necrosis Factor alpha (TNFα) Production by Macrophages in Response to Lipopolysaccharide Following Treatment with Zinc or Copper-Doped Bioactive Glass

Lisa M. Flick, Elizabeth A. Varmette, Matthew M. Hall.
Alfred University.

Introduction: Bioactive glasses are silica-based materials which are capable of osseointegration. In their active form, these materials have been shown to stimulate osteoblast activity in vitro. Previous research has investigated the potential uses for sol-gel derived bioactive glass compositions in bone regeneration, although little is known about the nature of the immune response to bioactive glass (Rectenwald JE. Shock. 2002:17:135-138.). Other studies have indicated that sol-gel bioactive glasses have antimicrobial properties. One in vivo study demonstrated a decrease in gum inflammation associated with the use of zinc-doped bioactive glass (Eberhard J. Biomaterials. 2005:26:1545-1541). This study examines the effect of zinc or copper-doped bioactive glass on two markers of the inflammatory response, tumor necrosis factor alpha (TNFα) and nitric oxide (NO), produced from lipopolysaccharide (LPS) stimulated macrophages.

Methods: Bioactive glass compositions were prepared using an acid-catalyzed sol-gel method as previously described (Oki A. J Biomed Mater Res, Part A. 2004:69A:216-221). Spherical particles 10-20 mm in diameter were produced by consolidating the gelled material at 625°C for 24 hours then vibratory milling for 5 minutes. RAW 264.7 murine macrophages were maintained using standard culture conditions. Multi-well culture plates were treated at various times with 1 μg/mL lipopolysaccharide. Cell culture media was collected and analyzed for TNFα and nitric oxide. A standard sandwich ELISA technique was used to measure TNFα concentrations using antibody pairs from Pierce and recombinant protein standard from R&D Systems. Nitric oxide concentrations were estimated based on the nitrite concentration determined by Griess assay.

Results and Discussion: The therapeutic benefit of metal-doped bioactive glass was investigated by exposing the RAW 264.7 macrophages to LPS followed by bioactive glass treatment at various intervals. Treatment with the 5mol% glasses showed no anti-inflammatory effect over time as seen in the steady levels of TNFα and NO. Although the total concentration of TNFα remained nearly unchanged regardless of the time period between LPS addition and bioactive glass treatment, macrophages treated with either 5 or 10 mol% copper or zinc glasses showed consistently lower levels of TNF-α expression at each timepoint. To determine if the bioactive glass could prevent initiation of the inflammatory cascade, cells were pre-treated with the glasses followed by LPS stimulation. All samples exhibited an increase in TNFα production over time with the exception of the macrophages pre-treated with the 10mol% Zn doped bioglass which completely inhibited TNFα production. This indicates that the Zn-doped bioactive glass has a protective effect. No significant changes were observed in the nitric oxide levels following bioglass pre-treatment and LPS stimulation. In an effort to determine if the inhibition of TNFα production was mediated by phagocytosis of the bioglasses or stimulation of the macrophages by the dissolution products of the glass, additional macrophage samples were stimulated with LPS and then treated with conditioned media obtained from soaking the bioactive glass compositions in cell culture media for 24 hours. Dissolution studies of the glasses showed particularly high release of copper compared to zinc although both ion concentrations peaked at 16 hours. All the compositions showed evidence of precipitation once a critical ion concentration was reached. Cells treated with the dissolution products of copper-doped bioglasses exhibited lower levels of TNFα and NO compared to the Zn-doped, and undoped bioglasses.

Conclusions: The decreases in TNFα production observed following LPS stimulation and bioactive glass treatment indicate that the Zn or Cu ions released from the bioactive glass do have an anti-inflammatory effect on RAW 264.7 cells. This is most likely due to the ability of Zn ions to activate metallothioneins and the transcription factor A20 which regulates NfκB, another transcription factor involved in regulating inflammation and apoptosis. Cu-doped bioactive glass also showed an improvement in the inflammatory biomarker expression, which may be a result of increased metallothionein activity. Further research is necessary to examine the activation state of the NFκB, metallothionein, and A20 in RAW 264.7 cells treated with these bioactive glasses. Alternatively, the effect of the copper-doped bioactive glass may in fact be due to Cu-induced cytotoxicity as a result of increased reactive oxygen species production. Nitric oxide is an example of a reactive oxygen molecule however levels of NO did not seem to correlate well (proportionately or inversely) with Cu-doped bioactive glass treatment. Additional experiments demonstrating the effect of the copper-doped bioactive glass on superoxide anion concentrations and superoxide dismutase enzyme activity are needed to elucidate this further.