Nanocurcumin attenuates inflammation by decreasing Toll-like receptor 2 and 4 expression and activity and promoting an anti-inflammatory macrophage phenotype

Xinpu Chen¹, Venugopal Chenna², Anirban Maitra², Sridevi Devaraj¹
¹ Baylor College of Medicine, Houston, TX, ² UT MD Anderson, Houston, TX

Abstract
Curcumin, the yellow polyphenol extracted from turmeric (Curcuma longa), has potent anti-inflammatory properties. However, due to poor aqueous solubility and thus limited systemic bioavailability, it has not been used clinically. Nanoparticle-based drug delivery approaches have the potential for rendering hydrophobic agents like curcumin dispersible in aqueous media. We have synthesized a polymeric nanoparticle encapsulated curcumin - nanocurcumin (NC) and have shown that it is readily dispersed in aqueous media and demonstrates comparable in vitro therapeutic efficacy to free curcumin. Obesity and diabetes are pro-inflammatory states characterized by increased monocyte Toll like receptors (TLR) 2 and 4 and a propensity to a M1 pro-inflammatory phenotype. In this study, we aimed to examine the effects of NC on monocyte TLR2 and 4 and M1 and M2 phenotype.

Methods
• Monocyte THP-1 cells were maintained in RPMI-160 media. THP-1 cells were pretreated with 5μM nanocurcumin or void vehicle. 2 hours later, 1 ng/ml lipopolysaccharide (LPS) was added to the growth media. Cells were harvested after 16-18 hours of LPS treatment.
• NE-PER Nuclear and Cytoplasmic Extraction Reagents was used for the separation and preparation of cytoplasmic and nuclear extracts from the harvested THP-1 cells for the western blot analysis of MyD88 or pp65.
• The harvested cells were also incubated with fluorescently labeled anti-TLR2, TLR4, or isotype-matched IgG. Followed by flow cytometry
• Monocyte subtypes, i.e. M1 and M2 phenotypes were also characterized by flow cytometry and populations were gated by positivity for IL-1, IL-12/23, CCR2 for M1 and CD206, IL-10, for M2 phenotype.
• Supernatant from the cells treated with LPS and vehicle control or Nanocurcumin respectively was also saved at ~80°C and cytokines were measured using a Multiplex assay (Millipore) according to the manufacturer’s instruction.
• Statistical Analyses was performed using Minitab 16 statistical software.

Results
Effect of Nanocurcumin on Monocyte TLR2 and TLR4 Surface Expression by Flow Cytometry

Effect of Nanocurcumin on Inflammatory Cytokines

Conclusion
Incubation of monocytes with NC (5μM) compared to vehicle control resulted in 50% inhibition of TLR2 and 60% inhibition of TLR4 expression, a 35% reduction in downstream signaling, ie. MyD88 expression and NFKb activity. Furthermore, incubation with NC resulted in conversion of monocytes to a M2 macrophage phenotype.

Background
• Curcumin, the yellow polyphenol extracted from turmeric (Curcuma longa), has potent anti-inflammatory properties. However, due to poor aqueous solubility and thus limited systemic bioavailability, it has not been used clinically.
• Nanoparticle-based drug delivery approaches have the potential for rendering hydrophobic agents like curcumin dispersible in aqueous media.
• Obesity and diabetes are pro-inflammatory states characterized by increased monocyte Toll like receptors (TLR) 2 and 4 and a propensity to a M1 pro-inflammatory phenotype. There are no studies on the effect of NC on monocyte TLR2 and 4 expression and activity and on macrophage phenotype.

Aims
• Examine the effects of nanocurcumin on monocyte TLR2 and TLR4 expression and monocyte subtype.
• Examine the effects of nanocurcumin on the expression of pro-inflammatory cytokines.

*pp<0.001 compared to Control
#p<0.05 compared to LPS