Poster # 0630

In Vitro Impact of Dual Zinc plus Arginine (DZA) on Bacterial Endotoxin (LPS)-Induced Inflammation

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Objective

Assess the role of zinc oxide, zinc citrate, arginine, and their combination (Dual Zinc with Arginine, DZA) in inhibiting TLR4-bound bacterial endotoxin-induced inflammation.

Conclusions

Through multiple human cell and tissue experiments, we have shown that the majority of the reduction in inflammation is contributed by the zinc compounds and the effect is retained in the presence of arginine. This study suggests that formulations containing zinc oxide, zinc citrate, and arginine display strong anti-inflammatory efficacy in the presence of pathogenic bacterial endotoxins.

Results

Figure 1. TNFα output is reduced in HEK-hTLR4 cell supernatants with zinc solutions and standard P. gingivalis LPS (0.1µg/mL). Pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα), was significantly reduced in the presence of zinc citrate, dual zinc, and DZA at 300ppm and 600ppm total zinc. Dual zinc and DZA contain a ratio of 85mM zinc oxide to 15mM zinc citrate.

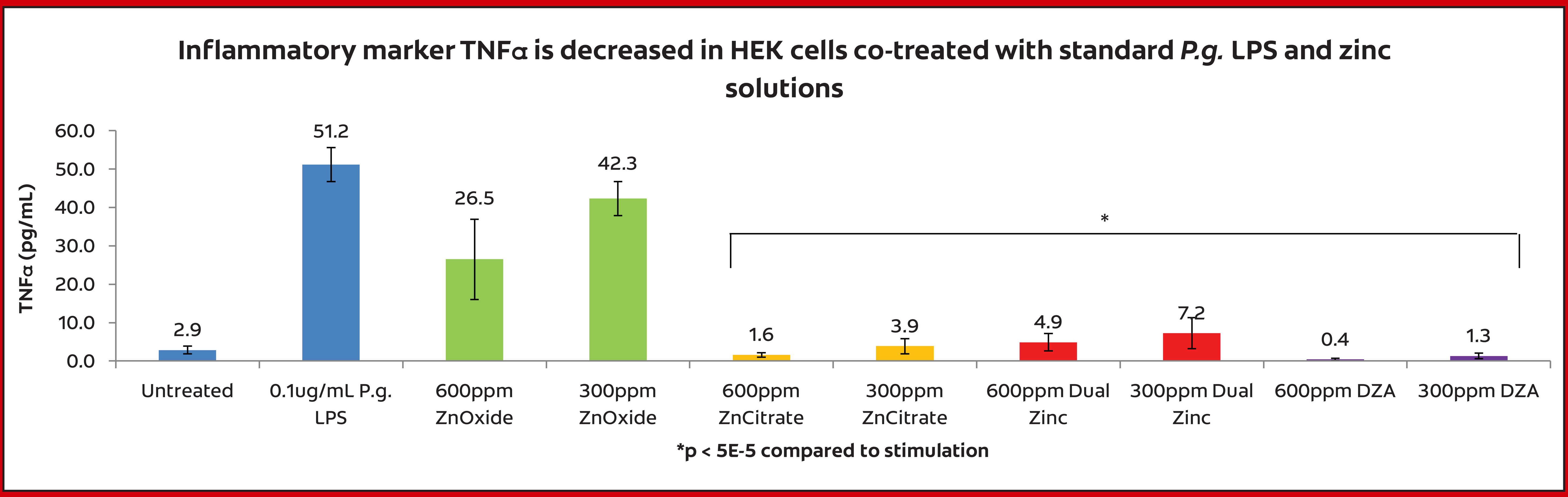


Figure 2. IL-8 expression is reduced in HEK-hTLR4 cells co-incubated with ultrapure *P. gingivalis* LPS (1µg/mL) and zinc and arginine combinations. Pro-inflammatory cytokine, interleukin 8 (IL-8), was significantly reduced in the presence of various dilutions of use-level zinc citrate, dual zinc, and DZA, but not arginine alone. See Table S1 for detailed description of samples.

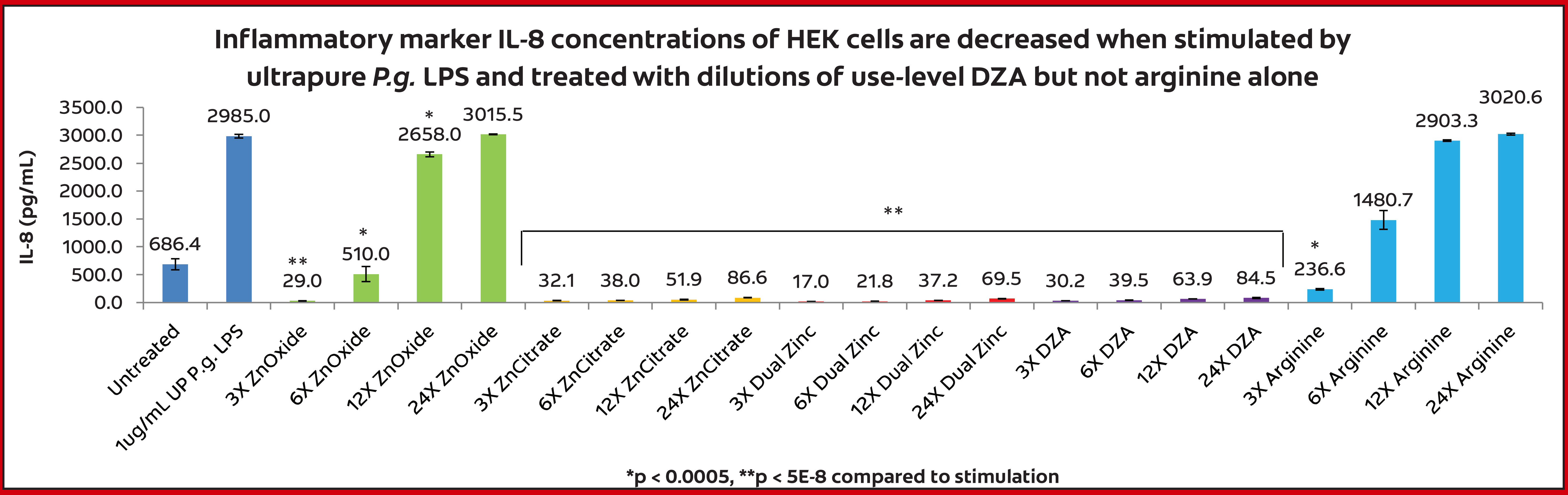
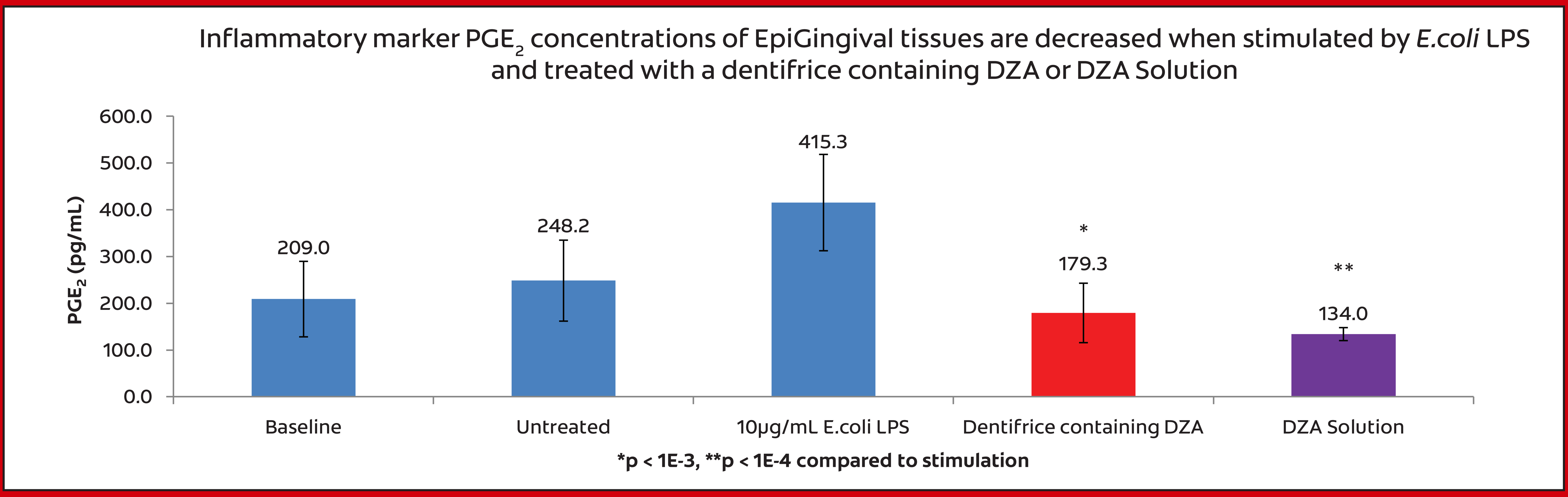


Figure 3. Concentrations of PGE_2 in medium from human gingival 3D MatTek tissue co-treated with a dentifrice containing DZA or DZA solution and *E. coli* LPS (10µg/mL). A dentifrice containing DZA and DZA solution both demonstrated a significant reduction of PGE_2 in 3D human gingival tissues.



Methods and Materials

- **Cell Studies:**
  - HEK-hTLR4 cells were grown in 96-well plates until confluent at 37 °C with 5% CO_2 in HEK-blue selection media
  - Cells were co-incubated overnight with varying concentrations of two different zinc actives as well as arginine and stimulated with either 0.1µg/mL standard *P .gingivalis* LPS or 1µg/mL ultrapure *P. gingivalis* LPS (standard LPS targets both TLR2 and TLR4 receptors, ultrapure LPS specifically targets the TLR4 receptor) (n=4)
- **Tissue Study:**
  - Human Gingival tissues were co-treated with 10µg/mL *E. coli* LPS, DZA solution, and a dentifrice containing DZA (n=3)
- **Analysis:**
  - Cell and tissue supernatant was collected for human inflammatory cytokine panels using Multiplex and ELISA analyses
  - All data is normalized to post-treatment cell or tissue viability and statistical significance was calculated using a one-way t-test

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