Liposomal curcumin (Lipocurc<sup>TM</sup>) and in vitro/in vivo surrogates for cytokine storm associated with uncontrolled EBOLA infection.

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Abstract

Massive over-production and persistent elevation of inflammatory cytokines over time by the body's immune system can trigger a dangerous syndrome known as a cytokine storm. Frequently occurs in advanced or terminal stages of Ebola infection. Dysregulation of normal immune response characterized by high levels of circulating cytokines can induce potentially fatal pathologic changes in cells, tissues, and organs leading to multiple organ failure. Uncontrolled Ebola virus (EBOV) infection of peripheral blood mononuclear cells (PBMCs) results in induction of excessive IL-6 and TNF-α production designated as cytokine storm. Important pro-inflammatory cytokines: IL-1β, IL-6, IL-8, and TNF-α.

**Curcumin**

![Curcumin](image)

Suppresses release of IL-1β, IL-8, TNF-α, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 α (MIP-1α) from monocytes and macrophages.

**Supresses release of IL-6, IL-8, TNF-α, MCP-1 from monocytes in high-glucose environment.**

**Curcumin Suppresses Release of Other Key Cytokines:** IL-2, IL-12, Interferon γ, GRO α (CXCL1), GRO β (CXCL2), IP-10 (CXCL10), SDF-1 (CXCL12), IL-5, IL-11, and IL-17.

**Curcumin Anti-Viral Activity:** HIV-1, HIV-2, HSV, HPV, HTLV-1, HBV, HCV, Japanese encephalitis virus and H1N1 in culture, Hepatitis B in culture.

**Liposomes**

The liposome in Lipocurc<sup>TM</sup> is composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phosphoric-1-glycerol sodium salt.

**Study Objectives**

To demonstrate the effect of Lipocurc<sup>TM</sup> on stimulated cellular surrogates for clinical cytokine storm.

**Study Design**

Stimulation of cytokine production/release from lymphocytes and macrophages by lipopolysaccharide (LPS) and a complex glycolipid consisting of glucosamine, 3-OH fatty acids, and 3-deoxy-D-manno-octulosonic acid (Kdo2-lipid A): the principle and essential component of the outer leaflet of the outer cell wall of Gram-negative bacteria.

**Materials & Methods**

**In vitro study**

IL-6 and TNF-α production in mouse macrophages: RAW264 cells were pre-incubated for 24h with empty liposomes or LipocurcTM (1-10µM) before being stimulated for 24h with Kdo2-lipid A (10ng/ml) or LPS (100ng/ml). IL-6 and TNF-α release was quantified by ELISA. Cell viability and cell proliferation were analyzed by XTT-assay.

**In vivo study**

Male Sprague-Dawley (SD) rats (n=8) received empty liposomes or LipocurcTM by gavage 1h prior intraperitoneal (i.p) injection of LPS at 125 µg/kg.

Male SD rats (n=8) received empty liposomes or LipocurcTM intravenously (i.v) 5 min prior i.p injection of LPS at 125 µg/kg.

Blood samples were taken 2 and 6 h following LPS injection. TNF-α was quantified 2 h post LPS by ELISA. MCP-1, Rantes, MIP-α, IL-1β and IL-6 were quantified 6 h post LPS by ELISA.

**Results**

In Kdo2-lipid A or LPS-stimulated macrophages, LipocurcTM (5µM) suppressed IL-6 production/release at ~75% (A). Empty liposomes blocked IL-6 to a similar extent (A). TNF-α production was diminished by LipocurcTM at 10 µM (B). Empty liposomes showed similar effects at 20µM (B). LipocurcTM up to 5µM did not affect cell growth. Cell viability significantly decreased at 10µM whereas empty liposomes did not negatively influence cell viability (data not shown).

**Lipocurc<sup>TM</sup> administered orally:**
- blocked TNF-α production by 62%
- blocked IL1β production by 86%
- blocked IL6 production by 92%

**Lipocurc<sup>TM</sup> administered intravenously:**
- blocked TNF-α production by 77%
- blocked IL1β production by 85%
- blocked IL6 production by 83%

**Conclusions**

Therapeutic levels of intravenous liposomal curcumin (Lipocurc<sup>TM</sup>) may prevent mortality in patients with Ebola exhibiting signs and symptoms of cytokine storm, and prevent uveitis in patients successfully rehabilitating from the disease.

**References**
