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

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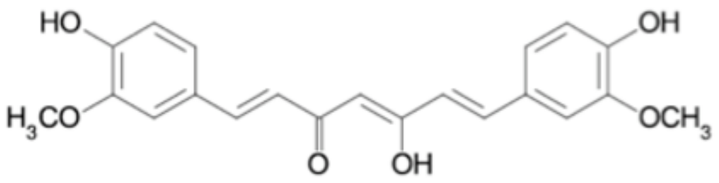
Abstract

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-1 β , IL-6, IL-8, and TNF- α .

Curcumin



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

Suppresses 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™ 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™ on stimulated cellular surrogates for clinical cytokine storm.

Study Design

Stimulation of cytokine production/release from lymphocytes and macrophages by lipopoly-saccharide(LPS) and a complex glycolipid consisting of glucosamine, 3-OH fatty acids, and 3-deoxy-D-*manno*-octulonsonic acid (Kdo_2-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.

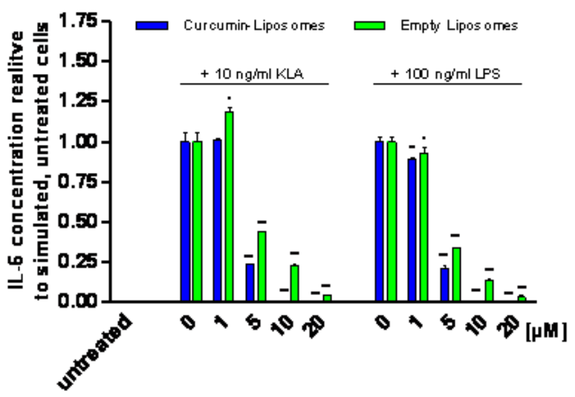
Male SD rats (n=8) received empty liposomes or LipocurcTM intravenously (i.v) 5 min prior i.p injection of LPS at 125 μg/kg.

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-1 β and IL-6 were quantified 6 h post LPS by ELISA.

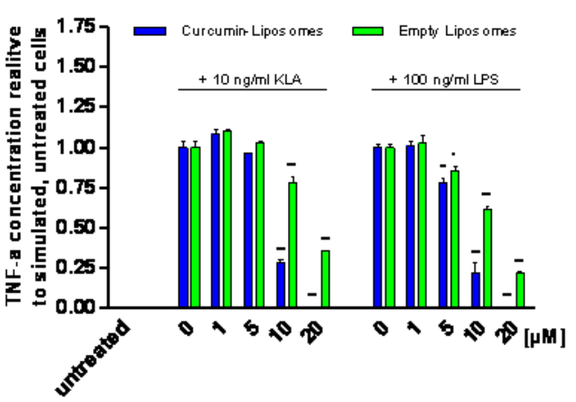
Results

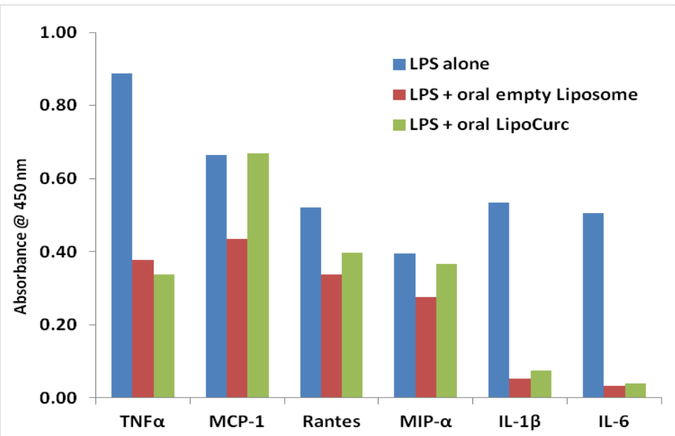
In Kdo2-lipid A or LPS-stimulated macrophages, LipocurcTM (5μM) suppressed IL-6 production/release at ~75% (A). Empty liposomes blocked IL-6 to a similar extent (A). TNF- α production was diminished by LipocurcTM at 10 μM (B). Empty liposomes showed similar effects at 20μM (B). LipocurcTM up to 5μM did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

A

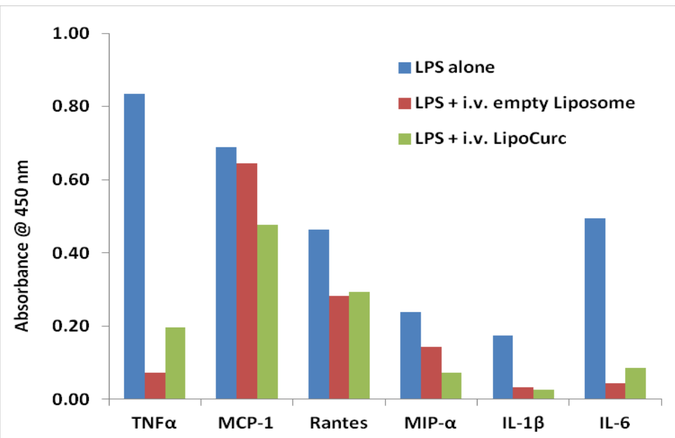


B





Lipocurc™ administered orally:
- blocked TNF- α production by 62%
- blocked IL1 β production by 86%
- blocked IL6 production by 92%



Lipocurc™ administered intravenously:
- blocked TNF- α production by 77%
- blocked IL1 β production by 85%
- blocked IL6 production by 83%

Conclusions

Therapeutic levels of intravenous liposomal curcumin (Lipocurc™) 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

1. Anti-inflammatory and apoptotic effects of the polyphenol curcumin on human fibroblast-like synoviocytes. *Int Immunopharmacol.* 2013, 15:400-405. Kloesch_B, Becker T, Dietersdorfer E, Kiener H, Steiner G.

2. Curcumin Suppression of Cytokine Release and Cytokine Storm. A potential therapy for patients with Ebola and other Severe Viral infections. *In Vivo* 2015: 29: 1-4. Sordillo P, Helson L.