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Novel Transdermal Curcumin Therapeutic Preserves Endothelial Barrier Function in a Rat Model of Sepsis.

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INTRODUCTION

Sepsis

Systemic inflammatory event triggered by pathogen or damage-associated activation of the toll-like receptor (TLR) system.

Mechanisms of TLR Activation

- Classic Sepsis: Infection via lipopolysaccharide **(LPS)** and lipoteichoic acid (Gram positive).
- Sterile Sepsis: Poly-trauma and ischemia from DNA, histones and other components of necrotic cells

Stages and Symptoms

- Mild Sepsis: Temperature, tachycardia, hyperventilation
  - **Endothelial Dysfunction** – blood vessel leakiness
- Severe Sepsis: Hypotension, organ hypoperfusion, altered mental state
- Septic Shock: Hypotension, hypoperfusion, elevated lactate. 50% mortality.

Curcumin

Primary curcuminoid found in turmeric root. Long implicated as a potent anti-inflammatory agent.

Anti-inflammatory Mechanisms

- Downregulation of TLR-4
- Blockade of NF-κB
- Increases endothelial nitric oxide production to improve vascular function
- May alter epigenetic modifier (DNMT-1, SIRT1, etc) expression

Therapeutic Challenges

- Poor solubility in water
- Difficult to reach bioactive potency

VASCEPTOR™

Transdermal platform that facilitates systemic delivery of curcuminoids

- Applied topically as a cream
- High concentration of curcuminoids
- Rapid delivery and prolonged anti-hypertensive action

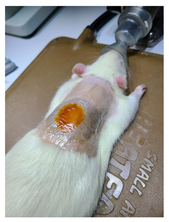
METHODS & MATERIALS

Endotoxemic Modelling of Sepsis

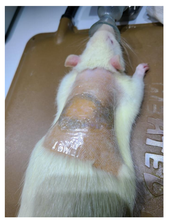
- Daily (every 24h) IP injections of LPS (Sigma Aldrich, St. Louis, MO; 10 mg/kg) for 72h

Treatment

- Anesthetized with Isoflurane
- LPS administered
- Back shaved and cleaned
- 0.1 mL of topical agent applied
- Tegaderm affixed over agent
- Treatments concurrent with LPS (Once every 24h for 72h)



CUR



VC

Groups

- LPS+VC (Vehicle Control; LPS+0.1 mL topical agent without curcumin; N = 7)
- LPS+CUR (VASCEPTOR; LPS+0.1 mL topical agent with curcumin; N = 7)
- No LPS (Healthy Control; no LPS, no topical agent; N = 8)

Extravasation Protocol

- At 72h: animals anesthetized and surgically cannulated for cardiovascular monitoring, blood sampling, and fluorescence microscopy of the exteriorized spinotrapezius muscle.
- 67 kDa TRITC bovine serum albumin admin administered after baseline parameters
- Fluorescence was measured in the interstitial fluid of the spinotrapezius for 30 minutes
- Phenylephrine was administered at the end of the study to measure arteriolar responsiveness

FLOURESCENT 67 KDA ALBUMIN EXTRAVASATION

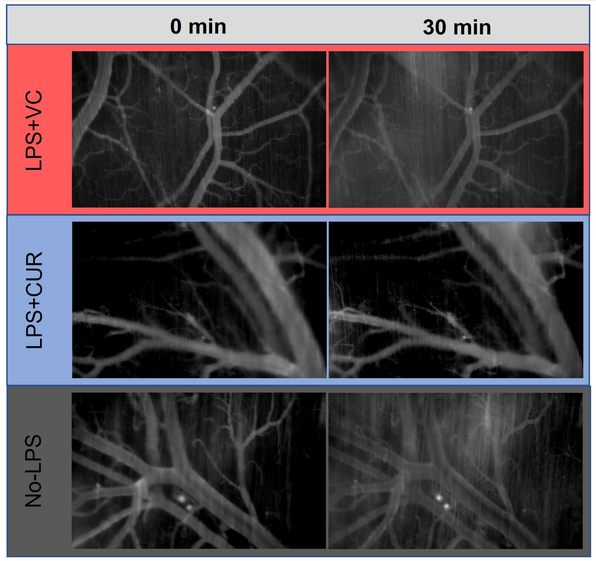


Figure 1: Accumulation of Fluorescent Albumin in the Spinotrapezius

Images (5X magnification) were captured by a monochromatic digital camera using identical flash intensity and exposure times for each series. Contrast and brightness were adjusted for presentation using the Zen II software and standardized for all images.

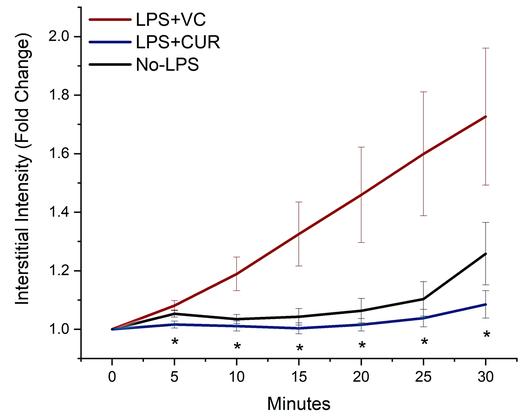


Figure 2: Albumin Extravasation

TRITC bovine serum albumin was injected IV and monitored with fluorescence microscopy of the spinotrapezius microvasculature. Accumulation in the interstitial fluid was measured as intensity, normalized to BL, and expressed as fold change. Exposure times varied slightly between experiments (1500-2000 ms) to account for differing tissue thicknesses but were not changed within each time course. Data are mean ± SEM.

***** p < 0.05 vs LPS+VC

VASCULAR RESPONSE TO PE

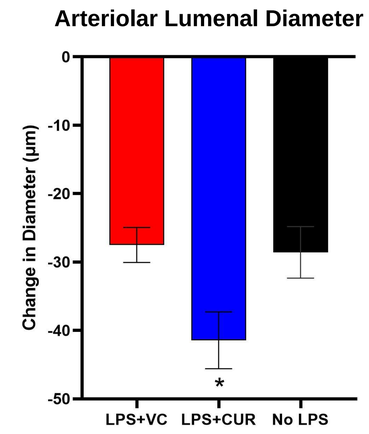


Figure 3: Arteriolar Response to Phenylephrine

Pressers are used to relieve hypotension in sepsis through peripheral vasoconstriction. Since they inevitably lead to distal ischemia, co-treatments that can limit their use are desirable. After 72 h of endotoxemia and the extravasation test, a site containing second order arterioles 100-30 µm was selected and imaged. 0.1 M PE was infused, and maximal vessel contraction measured. Data are mean ± SEM.

* p < 0.05 vs No LPS and LPS+VC

CARDIOVASCULAR

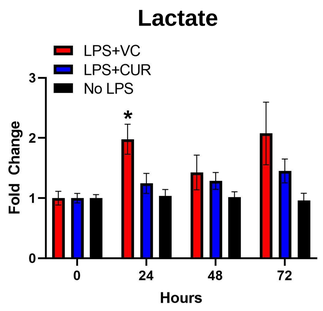
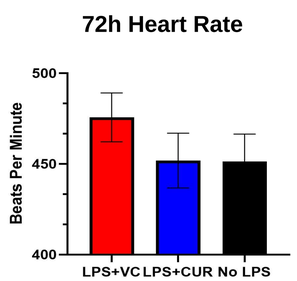
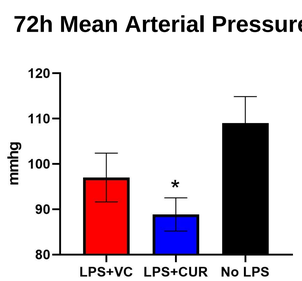


Figure 4: Physiological profile of LPS regimen.

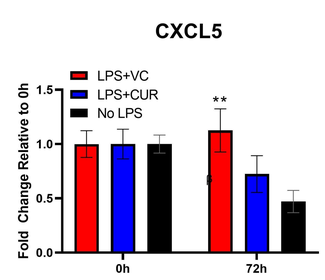
Lactate, an indicator of hypoperfusion, was elevated for Vehicle after 24 h of treatment. LPS+CUR animals trended lower than LPS+VC but higher than No LPS. Heart Rate and Mean Arterial Pressure were assessed at 72h before extravasation experiments. Heart Rate trended higher for Vehicle, consistent with tachycardia expected in sepsis. Mean Arterial Pressure was low for both LPS-treated groups, but lowest for LPS+CUR. Data are mean ± SEM.





***** p < 0.05 vs LPS+VC

INFLAMMATORY MARKERS



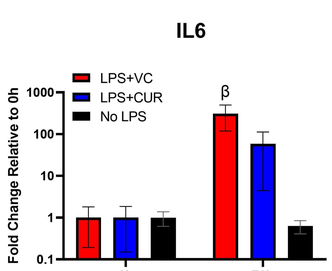


Figure 5: Protein expression of cytokine IL-6 and neutrophil chemokine CXCL5 (LIX)

Plasma samples were quantified with ELISA. Values for both CXCL5 and IL6 proteins (pg/ml) were normalized to baseline (before initial LPS treatment) at 0 h for a comparison of relative change within groups. For CXCL5, differences between LPS+VC and LPS+CUR were trending at 72 h (p = 0.14), but not resolved at N values of 7. The Y-axis for IL6 was set to Log10 and a significant rise was seen for LPS+VC compared to 0h. For LPS+VC vs. LPS+CUR, p=0.25. Data are mean ± SEM.

β p < 0.05 versus 0 h | ** p < 0.01 versus No LPS

CONCLUSIONS

Curcumin:

1. Lowered cardiovascular burden of endotoxemia
2. Prevented LPS-induced extravasation
3. Reduced inflammatory markers
4. Increased vascular responsiveness to PE

Hypotension was noted for LPS+CUR, but may be a contaminant effect of enhanced NO production rather than an indicator of illness

Results suggest efficacy of VASCEPTOR in mitigating dysregulated inflammatory disorders and enhancing effectiveness of pressor treatments.

INSTITUTIONS

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