

JOINT EVENT

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Agaricus brasiliensis sulfated polysaccharide prevents DENV-2 NS1 induced endothelial barrier dysfunction *in vitro*

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Introduction: Vascular leakage is an adverse outcome in response to dengue virus (DENV) infection, resulting in depleted intravascular volume and hypotension, which may evolve into hypovolemic shock. Dengue non-structural protein 1 (DENV NS1) has been shown to contribute to pathogenesis by directly triggering endothelial glycocalyx layer (EGL) degeneration, in a cytokine independent pathway, as well as by inducing the release of vasoactive cytokines from PBMCs, both leading to plasma leakage. *Agaricus brasiliensis* is a basidiomycete fungus native to Brazil widely consumed and studied due its therapeutic properties, most of them related to its polysaccharidic content. A (1→6)-(1→3)-beta-D-glucan isolated from *A. brasiliensis* fruiting bodies (FR) was chemically modified to produce its corresponding sulfated derivative (FR-S). Since there is no specific treatment for DENV and the current available Sanofi dengue vaccine does not confer full protection to the disease, reversing or preventing EGL degeneration has therapeutic potential on severe dengue.

Methodology & Theoretical Orientation: FR-S was tested against DENV NS-1 induced endothelial barrier disruption by measuring trans-endothelial electrical resistance (TEER). The effect of FR-S on NS-1 binding to endothelial cells was evaluated by immunohistochemistry.

Findings: Figure 1 show that FR-S completely inhibited TEER reduction induced by DENV-2 NS1 treatment on HPMECs at the two higher concentrations tested (0.25 and 0.12 µg/mL). At the two lower tested concentrations (0.06 and 0.03 µg/mL), no protection against NS1-induced TEER reduction was observed. Results in figure 2 show that the mechanism by which FR-S prevents endothelial barrier dysfunction includes inhibition of NS1 binding to HPMECs at a concentration dependent manner.

Conclusion & Significance: The findings indicate that FR-S inhibits NS1 binding to endothelial cells and prevents NS1 induced endothelial dysfunction. FR-S may have an anti-vascular leak effect since NS1 is in part responsible for the plasma leakage occurring in patients with severe dengue.

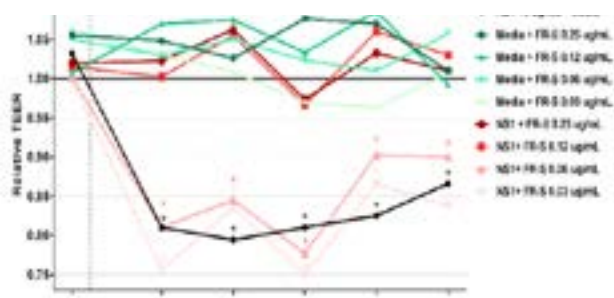


Figure 1: Effect of FR-S on endothelial dysfunction induced by DENV-2 NS1. Human pulmonary microvascular endothelial cells (HPMECs) monolayers in contact or not with 10 µg/mL of DENV-2 NS1 were treated with FR-S at four different concentrations. Trans-Endothelial Electrical Resistance (TEER) was measured at sequential 2-hour time points following the treatments. The groups were compared through two-way ANOVA + Dunnett's test. *Groups NS1+media, NS1+FR-S 0.06 µg/mL, NS1+FR-S 0.03 µg/mL displayed statistically significant lower TEER values when compared to the other groups ($p < 0.05$) with no differences among them.

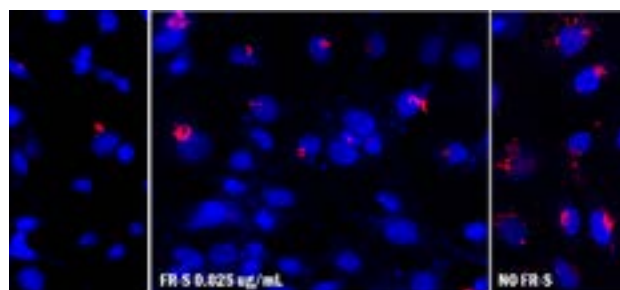


Figure 2: Effect of FR-S on DENV-2 NS1 binding to HPMECs. Human pulmonary microvascular endothelial cells (HPMECs) monolayers grown in gelatin coated coverslips were treated or not with two different concentrations of FR-S (0.25 and 0.025 µg/mL) for 1 h. Then 10 µg/mL of DENV-2 NS1 histidine tag was added to pre-chilled cells. After 1 h of contact with NS1, monolayers were fixed and stained with Hoechst (blue) and anti-His Tag Alexa 547 (red). Fluorescence intensity, measured by ImageJ software, indicate that FR-S 0.25 and 0.025 µg/mL treatments respectively reduced 93.05 % and 81.97 % of NS1 binding to HPMECs when compared to untreated controls (No FR-S).

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Recent Publications

1. Martina B E E, Koraka P and Osterhaus A D M E (2009) Dengue virus pathogenesis: An integrated view. *Clinical Microbiology Reviews* 22(4):564-81.
2. Avirutnan P, Punyadee N, Noisakran S, Komoltri C, Thiemmecca S, Auethavornanan K, et al. (2006) Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. *The Journal of infectious diseases* 193(8):1078-88.
3. Cardozo F T G S, Camelini C M, Cordeiro M N S, Mascarello A, Malagoli B G, Larsen I et al. (2013) Characterization and cytotoxic activity of sulfated derivatives of polysaccharides from *Agaricus brasiliensis*. *International Journal of Biological Macromolecules* 57:265-72.

Biography

Francielle Tramontini Gomes de Sousa has her expertise on dengue pathogenesis and evaluation of antiviral activity and mechanism of action of natural and semi-synthetic compounds. She has developed an *in vitro* co-culture model of endothelial cells and monocytes to screen compounds with therapeutic potential for management of endothelial extravasation in severe dengue. In a previous work, she had shown that serum from acute severe dengue patients differentially affects endothelial cells barrier function *in vitro* and found some correlations between immunomediators and vascular leakage. Her current interests include studying the pathogenic mechanism of dengue vascular leak as well as searching for therapeutic candidates against dengue virus by using *in vitro* (endothelial cells) and *in vivo* (murine) models.

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