New insight in sepsis capillary leak syndrome: alpha 1 AMPK, from the comprehension of key molecular mechanisms to the exploration of a new therapeutic approach

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Introduction

Sepsis is a major health concern worldwide, and is defined as a syndrome of dysregulated host response to infection causing life-threatening organ dysfunction.

Despite significant advances in the understanding of the disease, the therapeutic management of septic patients primarily relies on supportive care and mortality rates remain unacceptably high, around 40% (1). Sepsis capillary leak syndrome (SCLS), mainly caused by vascular hyperpermeability, is a critical process in sepsis pathophysiology and has been demonstrated to be an independent prognostic factor of survival (2). Moreover, growing evidence supports that maintenance of vascular barrier integrity improves sepsis outcome (3). However, no therapeutic proposal that targets SCLS has so far reached the clinical trial stage. SCLS is caused by vascular barrier disruption. Under healthy conditions, endothelial cells are sealed to one another by inter-endothelial junctions (IEJs) that effectively control the passage of molecules in a size-selective manner. Vascular endothelial cadherin (VE-Cad), the major component of adherens junctions (AJs), is a protein essentially involved in this regulation (4). Its stability depends on the actin cytoskeleton, whose polymerization is notably regulated by the phosphorylation of heat-shock protein of 27 kDa (HSP27), downstream of the p38 MAP kinase (p38MAPK) (5). Upon sepsis, stress mediators trigger signalling cascades that induce actin cytoskeleton contraction, AJs disruption, and loss of endothelial barrier function (6). This event is characterized by the formation of intercellular gaps, leading to plasma leaking through the endothelium and resulting in widespread oedema, finally compromising microcirculation (7).

The catalytic subunit of AMP-activated protein kinase (AMPK) is primarily expressed under its α1-isoform within the microvascular endothelium; there, it acts as a major regulator of the actin cytoskeleton and IEJs (8).

Canagliflozin, an inhibitor of sodium-glucose co-transporter 2 (SGLT2i), is currently prescribed as oral glucose-lowering agent to patients with diabetes. Independently of modulating glucose transport, clinically relevant canagliflozin concentrations also activate AMPK in different cell types, including human endothelial cells (9).

Interestingly, in addition to increasing renal glucose excretion, strong evidence supports that canagliflozin exerts significant cardiovascular protective effects, whose exact mechanisms are still poorly understood (10). On account of its effect on AMPK activity, we hypothesized that canagliflozin may constitute a new therapeutic option to target SCLS. In this thesis, we aimed (a) to characterize the role of endothelial α1AMPK in endothelial barrier function during sepsis, (b) to identify the molecular mechanisms involved in this regulation (c) to demonstrate the potential (AMPK dependent) protective effect of canagliflozin against SCLS.

Methods

α1AMPK expression and/or activity was modulated in human dermal microvascular endothelial cells (HMECs) using either α1AMPK-targeting small interfering RNA or the direct pharmacological AMPK activator 991, prior to lipopolysaccharide (LPS) treatment. Western blotting was used to analyse the expression and/or phosphorylation of proteins that compose cellular junctions (zonula occludens-1 (ZO-1), vascular endothelial cadherin (VE-Cad), connexin 43 (Cx43)), or that regulate actin cytoskeleton (p38 MAPK; heat shock protein 27 (HSP27)). Functional endothelial permeability was assessed by *in vitro* Transwell assays, and quantification of cellular junctions in the plasma membrane was assessed by immunofluorescence. Actin cytoskeleton remodelling was evaluated through actin fluorescent staining.

A mouse model of specific and conditional endothelial α1AMPK deletion was generated (e-AMPK WT/KO). Canagliflozin was administered by oral gavage, and endotoxemia was induced by intraperitoneal injections of sublethal doses of LPS. Capillary leak was monitored with Evans Blue Dye (EBD) and plasmatic albumin levels.

Results

First, we have demonstrated the pivotal role of α1AMPK in the regulation of endothelial barrier function. *In vitro*, we described that α1AMPK invalidation is associated with increased endothelial permeability, while AMPK activation by 991 leads to endothelial barrier reinforcement against LPS injury. In vivo, EBD detection on myocardial sections showed that specific endothelial α1AMPK deletion is associated with increased vascular leakage in response to endotoxemia, while its pharmacological activation protects against this mechanism in e-AMPK WT, but not KO animals.

Second, we investigated the underlying molecular mechanisms of this protective effect, and demonstrated that α1AMPK deficiency is associated

with reduced expression of CX43, ZO-1, and VE-Cad. The drastic loss of CX43 is likely responsible for the subsequent decreased expression and localization of ZO-1 and VE-Cad in the plasma membrane of endothelial cells. Moreover, α1AMPK activation by 991 protects against LPS-induced endothelial barrier disruption by reinforcing cortical actin cytoskeleton. This is due to a mechanism that involves the phosphorylation of p38 MAPK and HSP27, which is nonetheless independent of the small GTPase Rac1.

Third, we described protectives effects of canagliflozin on endothelial barrier function submitted to sepsis conditions. *In vitro*, we reproduced the protective effects previously described with the pharmacological activator 991. We described that their abrogation appears inconstant in AMPK depleted cells, indicating that AMPK-independent mechanisms seem involved. In vivo, canagliflozin administration drastically reduced LPS-induced myocardial oedema and maintained albumin plasma levels. Endotoxemia-induced myocardial oedema and hypoalbuminemia persisted despite canagliflozin treatment in e-AMPK KO animals, demonstrating that canagliflozin protection involves endothelial α1AMPK. We confirmed that this protection involves both activation of p38MAPK/HSP27 pathway and preservation of VE-Cad integrity.

Finally, we validated these results in human endothelial cells submitted to human plasma collected from volunteers (HV) or septic shock (SS) patients. Immunostainings show that both HV and SS plasma affect VE-Cad architecture, with SS plasma inducing higher VE-Cad disruption. Of major interest, canagliflozin importantly preserved VE-Cad integrity while slightly enhancing its membrane expression in HMECs exposed to both HV and SS plasma.

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