

BRYOSTATIN-1 LIMITS NEUTROPHIL TRANSENDOTHELIAL MIGRATION FOLLOWING ISCHEMIA-REPERFUSION INJURY: IMPACT FOR THERAPY

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Introduction and Background: Ischemia-reperfusion injury (IRI) is an inherent component of solid organ transplantation and axiomatically linked to graft damage. In the kidney, vascular endothelial cells (EC) are highly vulnerable to IRI. These cells are the first site of graft injury, while neutrophils are the first line of host defense after reperfusion. The degree of renal EC damage predicts the severity of neutrophil transendothelial migration (TEM), with neutrophils in turn orchestrating the influx of subsequent leukocytes waves into the graft. Therefore, EC integrity and neutrophil TEM represent promising targets to attenuate IRI. One drug known to stabilize EC integrity and to limit neutrophil TEM is Bryostatin-1, an activator of the EC second messenger protein kinase C delta. Therefore, we examined the role of Bryostatin-1 on neutrophil TEM in an in vitro IRI model.

Methods: We used an in vitro IRI model with human umbilical vein ECs (HUVECs) and human neutrophils (approved by the ethic committee (STUDY00000261) to study the role of Bryostatin-1 in IRI-induced neutrophil TEM. HUVECs were exposed to either normoxic (21% O₂) or hypoxic (1.5% O₂) conditions for 20 hours (h) with and without Bryostatin-1 (1-100 nM) followed by 2 h exposure to Calcein-AM dye labeled neutrophils. TEM to saline or the chemoattractant leukotriene B₄ (LTB₄) was determined by measuring fluorescence intensity and myeloperoxidase (MPO) production.

Results and Conclusions: Bryostatin-1 dose-dependently inhibited human neutrophil TEM under normoxic and hypoxic conditions. Bryostatin-1 (100 nM) blocked 75% (P < 0.05) of TEM toward LTB₄ in normoxic conditions; this was intensified when HUVECs were placed in hypoxic conditions (83%, P < 0.001). These data were further supported by a mirrored effect when MPO production (a marker of neutrophil activation) was measured. In summary, these promising in vitro results demonstrate that our model recapitulates IRI-induced EC damage, and most importantly that Bryostatin-1 alters neutrophil TEM in an in vitro IRI model.