BENEFICIAL EFFECTS OF PHARMACOLOGICAL MODULATION OF EPHA-EPHRINA SYSTEM ON MESENTERIC I/R-INDUCED INFLAMMATION IN MICE

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BACKGROUND

Mesenteric ischemia/reperfusion (I/R), a life-threatening condition caused by the depletion of oxygen in the intestinal tract followed by recovery of blood supply, triggers a severe local and systemic inflammation that is hardly controlled by pharmacological interventions (1).

Since the discovery that pro-inflammatory cytokines stimulate ephrinA1 and EphA2 genes expression, an increasing number of studies has investigated the role played by Eph/ephrin system in the modulation of inflammatory and immune responses (2), suggesting that it may critically affect vascular permeability and leukocytes trafficking (3). However, up to now the involvement of Eph-ephrin system in mesenteric I/R injury has been scarcely explored.

AIM

To investigate the effects produced by exogenous activation (ephrin-A1-Fc) or blockade (EphA2) of EphA-ephrinA signalling on the local and systemic inflammatory responses induced in our model of mesenteric I/R injury in mice (4).

METHODS

Female Swiss mice, 6-8 weeks old, were randomly assigned to the following groups:

-S: sham operated mice + vehicle (NaCl 0,9% 10ml/kg)

-I/R: I/R + vehicle

-ephrinA1: I/R + chimeric protein ephrin-A1-Fc 200 µg/kg

-EphA2: I/R + equimolar monomeric EphA2 240 µg/kg

All the treatments were applied intravenously 5 minutes prior to 45 min occlusion of the superior mesenteric artery (SMA) followed by 5h reperfusion (I/R). Gut and lung myeloperoxidase (MPO) activity, index of neutrophils recruitment, intestinal oedema, index of fluid extravasation, and lung TNF-α and IL-1β levels were assayed and gut and lung histo-pathological evaluation was performed. Finally, lung EphA2 and ephrinA1 expression was determined in vehicle- and ephrin-A1-Fc-treated I/R and sham-operated mice. All experiments were performed in accordance with the Guiding Principles in the Care and Use of Animals (DL26/2014).

RESULTS

I/R mice showed remarkable intestinal oedema (P<0.05), consistent neutrophils infiltration both in the gut (P<0.01) and in the lungs (P<0.05), increased pulmonary levels of TNF and IL-1 (P<0.01) and severe intestinal and pulmonary histological injury with respect to S mice; both ephrin-A1 and, especially, EphA2 protein expression was increased by SMA occlusion. Blockade of EphA-ephrinA signalling by EphA2 significantly reduced intestinal oedema (P<0.05 vs I/R) and IL-1 levels (P<0.05 vs I/R), mitigating also TNF increase and histological injury in the lungs, especially limiting congestion of the septae. Activation of EphA forward signalling by ephrin-A1-Fc significantly prevented pulmonary leukocyte recruitment (P<0.05 vs I/R), attenuated the increase in pro-inflammatory cytokines levels and markedly protected both intestinal villi architecture and pulmonary tissue integrity. Interestingly, ephrin-A1-Fc treatment strongly down-regulated I/R-induced EphA2 over-expression at the pulmonary level.

CONCLUSIONS

Our findings showed that interference with ephrin-A1-EphA2 interactions by monomeric EphA2 may be useful in limiting vascular alterations and exogenous activation of EphA2 receptors by ephrin-A1-Fc could promote epithelial barrier integrity, efficiently dampening intestinal and pulmonary I/R-related injuries. A fine pharmacological manipulation of this signaling pathway may therefore represent a potential novel strategy in controlling mesenteric I/R-induced dysfunctions.

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