# EphA2-ephrinA1 system in acute mesenteric inflammation due to ischemia/reperfusion in mice

V. Vivo, S. Bertoni, L. Flammini, A. Grandi, M. Tognolini, V. Ballabeni, E. Barocelli

Dept. of Pharmacy, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma. www.unipr.it

**Background and Aim**

Erythropoietin-producing hepatocellular (Eph) tyrosine kinases, activated upon binding to their membrane-associated ephrin ligands, are crucially involved in embryogenesis but also in pathogenesis in adults. Emerging evidence suggests that the Eph/ephrin receptor-ligand system has multiple roles in cell adhesion-based processes during inflammation and immunity, although with apparently contradictory outcomes depending on cell type and context (1,2). While EphA2 receptor activation seems to contribute to the inflammatory responses by promoting vascular permeability, as observed in different models of lung injury (3), ephrinA1-Fc administration has shown beneficial effects, such as neutrophil density reduction, in a model of myocardial infarction in mice (4).

The aim of this study was therefore to investigate the involvement of the Eph/ephrin system in the inflammatory responses caused by 45 min of mesenteric ischemia, induced by occlusion of the superior mesenteric artery, followed by 5h reperfusion (I/R) in mice.

**Materials and Methods**

In female Swiss mice, the effects produced by intravenous administration of the recombinant proteins ephrinA1-Fc (50, 100 microg×$Kg^{-1}$), EphA2-Fc (180 microg×$kg^{-1}$), Fc (50 microg×$kg^{-1}$), monomeric EphA2 (60, 120, 240 microg×$kg^{-1}$), or vehicle (saline 10 ml×$kg^{-1}$) on I/R-induced inflammatory responses were investigated. Sham operated animals receiving only vehicle served as controls. All experiments were performed according to Guiding Principles in the Care and Use of Animals (DLGS 26/2014).

**Results**

I/R mice displayed an increased gut (50.1±5.2 *vs* 1.2±0.7 U MPO/g dry tissue, $P<0.01$) and lung (294.1±25.1 *vs* 144.6±45.1 U MPO/g dry tissue, $P<0.05$) myeloperoxidase activity, index of neutrophil infiltration, intestinal malondialdehyde levels (617.4±91.9 *vs* 203.4±62.6 nmol MDA/g dry tissue, $P<0.05$), index of reactive oxygen species formation, and plasma extravasation (4.5±0.1 vs 3.4±0.3 wet to dry weight ratio, $P<0.001$) compared to controls.

Monomeric EphA2 (240 microg/kg), interfering with Eph-ephrin signalling, was able to significantly reduce intestinal edema formation (3.3±0.2 vs 4.5±0.1, $P<0.01$). EphrinA1-Fc (100 microg/kg) clearly decreased gut leukocyte recruitment (24.9±2.9 vs 50.1±5.1 U MPO/g dry tissue, $P<0.05$) resulting from SMA occlusion, while neither equimolar EphA2-Fc nor Fc alone were effective in modifying I/R-induced inflammatory responses.

**Conclusions**

These results suggest that modulation of Eph-ephrin signalling may be advantageous in controlling local inflammatory responses, such as leukocytes infiltration and plasma extravasation, induced by mesenteric I/R.

**References**

1. Coulthard MG et al., Am J Pathol 2012; 181(5):1493-503.
2. Funk SD et al., Pharmacol Res 2013; 67(1):42-52.
3. Carpenter TC et al., Am J Respir Cell Mol Biol 2012; 46(1):40-7.
4. Dries JL et al., J Physiol 2011; 589:1725-40.