EXPERIMENTAL COLITIS PROTECTION BY A SMALL MOLECULE ANTAGONIZING EPH/EPHRIN SYSTEM

Carmine Giorgio[1], Simone Palese[1], Massimiliano Tognolini[1], Riccardo Castelli[1], Lisa Flammini[1], Miriam Corrado[1], Elisabetta Barocelli[1], Simona Bertoni[1]

[1]Dipartimento di Scienze degli Alimenti e del Farmaco, Università degli studi di Parma, Parma - Italy

Background and aim: Eph receptors, the largest family of tyrosine kinase receptors in mammals, comprise A and B classes according to sequence homology and preferences for cell-bound ephrin ligands. Eph/ephrins system was primarily related to carcinogenesis and developmental biology; yet, its involvement in the regulation of inflammatory and immune responses is gradually emerging. Interestingly, ephrinB2mRNA was found to be up-regulated in the mucosal lesions of Crohn's disease patients; moreover, we have recently demonstrated the beneficial action of monomeric protein EphB4, interfering with EphB/ephrinB signalling pathways, in a murine model of TNBS-induced colitis. Starting from these encouraging results, the aim of the present work was to verify the protective effects of UniPR1331, a newly synthesised small molecule Eph/ephrin antagonist, in TNBS-induced colitis and to investigate its mechanism of action on explanted murine splenic lymphocytes.

Methods: Colitis was induced in female C57BL/6mice by enema administration of 5mg/mouse 2,4,6-TriNitroBenzene Sulfonic acid (TNBS) in 50% ethanol. Normal mice (N) received only saline solution. UniPR1331(10, 25mg/kg b.i.d.) and sulfasalazine (50 mg/kg), used as positive control, were orally administered daily starting from TNBS enema till suppression, 3days later; control mice (C) received only saline solution. Disease Activity Index (DAI), colonic macroscopic score (MS), colon length and thickness and colon and lung myeloperoxidase (MPO) activity were determined. Splenic and mesenteric lymph nodes (MLN) $CD4^+$ and $CD8^+$ T lymphocytes were counted by flow cytometry. Explanted murine splenic mononuclear cells were stimulated by ionomycin and phorbol 12-myristate 13-acetate (P+I) in the absence or presence of UniPR1331(10-30mM) and TNFa levels were determined by means of ELISA kits. All experiments were performed according to the guidelines for the Care and Use of Animals (DL26/2014).

Results: UniPR1331reduced remarkably and dose-dependently DAI and MS, colon shortening and thickening, colon and lung MPO ($P<0.05$vs. C), and acted even more effectively than sulfasalazine, which was unable to revert TNBS-induced colon shortening and lung neutrophil recruitment. Moreover, at the highest tested dose, UniPR1331attenuated the hapten-induced reduction of splenic $CD4^+$ and $CD8^+$ T cells count and at 30mM significantly lowered the levels of TNFa produced by stimulated splenic mononuclear cells ($P<0.05$vs. P+I).

Discussion and conclusions: Our findings represent a further proof-of-concept that blockade of Eph/ephrin signalling pathways is a promising pharmacological strategy in the management of IBD and highlight UniPR1331as a novel, favourable drug candidate, seemingly working through modulation of lymphocytes responses.