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Targeting the delivery of systemically administered haematopoietic stem/progenitor cells to the inflamed colon using hydrogen peroxide and platelet microparticle pre-treatment strategies

Adrian Yemm [a], David Adams [a,b], Neena Kalia [a,*]

[a] Centre for Cardiovascular Sciences, Institute of Biomedical Research, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[b] School of Immunity and Infection, Institute of Biomedical Research, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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ABSTRACT

Haematopoietic stem and progenitor cell (HSC) therapy may be promising for the treatment of inflammatory bowel disorders (IBDs). However, clinical success remains poor, partly explained by limited HSC recruitment following systemic delivery. The mechanisms governing HSC adhesion within inflamed colon, and whether this event can be enhanced, are not known. An immortalised HSC-like line (HPC7) was pre-treated with hydrogen peroxide (H_2O_2), activated platelet releasate enriched supernatant (PES) or platelet microparticles (PMPs). Subsequent adhesion was monitored using adhesion assays or in vivo ischaemia–reperfusion (IR) and colitis injured mouse colon intravitally. Integrin clustering was determined confocally and cell morphology using scanning electron microscopy. Both injuries resulted in increased HPC7 adhesion within colonic mucosal microcirculation. H_2O_2 and PES significantly enhanced adhesion in vitro and in the colitis, but not IR injured, colon. PMPs had no effect on adhesion. PES and PMPs induced clustering of integrins on the HPC7 surface, but did not alter their expression. Adhesion to the colon is modulated by injury but only in colitis injury can this recruitment be enhanced. The enhanced adhesion induced by PES is likely through integrin distribution changes on the HPC7 surface. Improving local HSC presence in injured colon may result in better therapeutic efficacy for treatment of IBD.

1. Introduction

Inflammatory and ischaemic bowel injuries such as Crohn's and colitis are difficult to treat, with current treatments rapidly losing effectiveness. Hence there is a pressing need for new, effective options to be identified. Evidence suggests that haematopoietic stem cell (HSC) therapy may be an effective treatment option for inflammatory gastrointestinal disorders (Singh et al., 2010; Lanzoni et al., 2008; Garcia-Bosch et al., 2010). HSCs confer benefit in experimental models of colitis and in Crohn's disease patients. This was originally thought to be due to direct repair of tissue by HSCs transdifferentiating into non-haematologic cells of the gut, but is now considered to be primarily due to combatting the excessive immune responses triggered by these inflammatory injuries through paracrine immunomodulatory mechanisms (Oyama et al., 2005; Wei et al., 2009). Administration of adult $CD34^+$ stem cells (SCs) improves clinical scores and significantly reduces mortality in dextran sodium sulphate (DSS)-induced murine colitis (Khalil et al., 2007). Furthermore, coeliac patients have increased circulating $CD34^+$ cells when compared with healthy controls (Mastrandrea et al., 2008). There have also been several promising trials utilising HSCs as a therapy for Crohn's disease, with initial reports suggesting some improvement although recurrence rates remained high (Burt et al., 2010; Hawkey 2012).

Despite ongoing trials, it is clear that clinical efficacy of cellular therapies is either minor or transitory. Poor success has been partially explained by a limited number of systemically transplanted SCs interacting with local microvessels (Karp and Teo, 2009; Kavanagh and Kalia, 2011). Considering the extension of the inflamed intestine, systemic infusion of cellular therapy offers a less invasive mode of delivery than multiple localised injections directly into the bowel and thus requires less HSCs (Karp and Teo, 2009). Furthermore, this preferred route of SC administration most closely mimics how endogenous bone marrow-derived HSCs may participate physiologically in tissue regeneration. Capture of exogenously administered circulating HSCs by injured tissue microvasculature is therefore a prerequisite event for successful therapy, regardless of the subsequent mechanisms underlying HSC-mediated repair. However, systemic injection is associated with low levels of SC retention in injured tissues. Indeed,

* Corresponding author.
E-mail address: n.kalia@bham.ac.uk (N. Kalia).

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within infarcted heart, endothelial progenitor cell retention is <5%, with most cells not demonstrating sustained engraftment (Aicher et al., 2003). Furthermore, difficulties remain in obtaining sufficiently large quantities of high purity HSCs using existing culture conditions. Therefore, identification of methodologies that improve or enhance SC recruitment to injured tissue microcirculation is a current high priority for cellular therapies (Karp and Teo, 2009; Kavanagh and Kalia, 2011). Although attempts have been made to enhance stem/progenitor cell recruitment using genetically modified cells or by increasing the local concentration of potent SC chemoattractants within injured tissue, non-invasive techniques, not requiring genetic manipulation, is more appealing.

It is well established that a host of factors within the injured microenvironment are capable of activating circulating leukocytes and platelets and thus permitting their endothelial adhesion during inflammatory processes (Mori et al., 2005a; Vowinkel et al., 2007; Sprague and Khalil, 2009; Santen et al., 2010). A potent leukocyte pro-adhesive modulator found within many inflammatory environments, particularly the ischaemic and colitis gut, is the free radical hydrogen peroxide (H_2O_2), generated locally by endothelium and infiltrating neutrophils (Fraticelli et al., 1996; Damiani et al., 2007). Similar factors most likely activate trafficking SCs as they circulate through damaged tissue. We hypothesised that pre-treatment of HSCs with such factors would increase the likelihood of their adhesion. Indeed, we have previously demonstrated that H_2O_2 pre-treatment enhances adhesion of an immortalised HSC-like line (HPC7) within the ischaemia-reperfusion (IR) injured small intestine (SI) in vivo (Kavanagh et al., 2013a). This increase was mediated by clustering α4 and β2 integrins on the HPC7 surface, increasing their affinity for endothelial counterligands such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and inducing HPC7 pro-migratory filopodia formation. Whether H_2O_2 pre-treated HSCs can adhere within the injured colon more efficiently than naïve HSCs is not known. An alternative strategy could involve HSC pre-treatment with the use of biological factors such as platelet derived microparticles (PMPs). On activation, platelets release these small (<1 μm) membranous vesicles that express many of the surface receptors found on platelets (Burnouf et al., 2014). PMPs are the biggest source of microparticles within humans and are known to be increased in various pathologies, including Crohn's disease (Chamouard et al., 2005). Initially, it was thought that PMPs were cellular debris without any distinct physiological or pathological function. However, it is increasingly evident that PMPs have many roles, including an ability to increase neutrophil adhesion by transfer of adhesion molecules to their surface (Jy et al., 1995). The use of PMPs as a pre-treatment option for HSCs is attractive as they have not only been shown to enhance HSC recruitment to bone marrow (Janowska-Wieczorek et al., 2001), but also increase cellular repair efficiency independently of affects on adhesion (Burnouf et al., 2014; Mause et al., 2010).

Determining whether systemic administration of transplanted HSCs can efficiently deliver cells to intestinal tissues, and whether this phenomenon can be enhanced, has received little attention, with no studies conducted in the injured colon in vivo. Therefore in this study, we used intravital microscopy, a methodology with single-cell sensitivity, to firstly detail the homing kinetics of an immortalised HSC-like line (HPC7) to the murine colon following two distinctly different injuries, namely an acute IR injury and a more chronic colitis injury. We further elucidated the molecular adhesive mechanisms governing HSC homing to injured colonic microvessels. Having already demonstrated an ability for H_2O_2 to improve HPC7 retention within IR injured SI, we tested this pre-treatment strategy within the two colon injury models. The efficacy of this chemical strategy was also compared with HPC7s pre-coated with PMPs. This is the first study to have tested HSC homing to two models of colonic injury and also utilised two distinctly different pre-treatment strategies in an attempt to enhance their local presence.

2. Materials and methods

2.1. Animals

Male C57Bl/6 mice (Harlan, UK) were used for procedures in accordance with the Animals (Scientific Procedures) Act of 1986 (Project Licence: 40/3336, 40/3226 or 30/2721). Anaesthetized animals (ketamine/xylazine administered intraperitoneally) underwent carotid artery cannulation to facilitate infusion of fluorescently labelled HPC7s and maintenance anaesthesia. The colon was exteriorised by gently moving the caecum out of the abdominal cavity and held in place with atraumatic hooks. A longitudinal cautery incision, made in the ascending colon along the anti-mesenteric border, was held open using atraumatic hooks. The exposed mucosa was cleaned gently with saline and a coverslip placed on it to improve image clarity when imaging microscopically. The mucosa was visualised using an upright fluorescent intravital microscope (×10 objective; BX61WI, Olympus). Images were captured and analysed using Slidebook V.5 (Intelligent Imaging Innovations, USA). Colonic blood flow was quantitated using the same preparation and monitored using a Moor FPLI laser speckle imager (Moor Instruments, UK).

2.2. Colon injury models and intravital microscopy

Intestinal IR injury was induced by occlusion of the superior mesenteric artery (SMA) with a non-traumatic clamp for 45 min and subsequent removal to initiate reperfusion. Control animals underwent sham surgery in which the SMA was isolated but not clamped. A single bolus dose of 2×10^6 CFSE labelled HPC7s was injected via the carotid artery cannula at 1 h reperfusion. Colitis injury was induced by allowing ad libitum access to 3% dextran sodium sulphate (DSS, MW36-50 kDa; MP Biomedicals, UK) in drinking water for 5 days. All colitis mice met a threshold injury score to ensure injury status as previously described (Cooper et al., 1993). For tracking HPC7s in the colitis colon, fluorescently labelled cells were infused via the carotid artery once the surgical preparation for intravital imaging was completed and the colon had been allowed to stabilise for 30 min. In both IR and colitis mice, a single pre-selected field of view was imaged intravitally every 5 min for 1 h. Adherent HPC7s were defined as those that remained stationary for >30 s. Free flowing HPC7s were defined as those that passed through the field of view without slowing or becoming stationary. At no point were HPC7s seen to be rolling in the colonic microvasculature. For all experiments, the colon was excised at the end of intravital imaging and 5 additional fields of view were monitored ex vivo. This was to ensure the adhesive events in the pre-selected area for IR and colitis mice were representative of events taking place in the whole colon. The lungs were also analysed ex vivo to quantitate HPC7 presence in at least 5 random fields of view.

2.3. Platelet microparticle generation

Platelets were isolated from the descending aorta of donor C57BL/6 mice following CO_2 narcosis, into 100 μl acid citrate dextrose (120 mM sodium citrate, 110 mM glucose, 80 mM citric acid). Blood was diluted in Tyrode's-HEPES buffer (134 mM NaCl, 0.34 mM Na_2HPO_4, 2.9 mM KCl, 12 mM $NaHCO_3$, 20 mM HEPES, 5 mM glucose, 1 mM $MgCl_2$, pH 7.3) and centrifuged (200 ×g, 5 min) to obtain a platelet rich plasma (PRP). The PRP was spiked with 10 μg/ml of prostacyclin and centrifuged at 1000 ×g for 6 min, the platelet pellet washed and counted and finally resuspended in Stem Pro SFM 34 (Invitrogen, UK) at 3×10^7/ml. Platelets were subsequently activated with 2 U/ml of thrombin for 30 min and then centrifuged at 1000 ×g for 6 min. The pellet was discarded and the supernatant kept and classed as a platelet microparticle enriched supernatant (PES). This contained not only the platelet microparticles but also the soluble factors released by platelets following their activation. To generate purified platelet microparticles (PMP), the