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ScienceDirect
European Journal of Cell Biology 87 (2008) 81–90
European Journal of Cell Biology
www.elsevier.de/ejcb

Characterisation of the interaction between circulating and in vitro cultivated endothelial progenitor cells and the endothelial barrier

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Received 12 April 2007; received in revised form 8 August 2007; accepted 31 August 2007

Abstract

In vitro cultured endothelial progenitor cells (cEPC) are used for intracoronary cell therapy in cardiac regeneration. The aim of this study was to investigate whether cEPC and circulating mononuclear cells (MNC), which include a small number of in vivo circulating EPC, are able to transmigrate through the endothelial barrier into the cardiac tissue. MNC and EPC were isolated from the peripheral blood from healthy male volunteers ($n = 13$, 25 ± 6 years) and stained with a fluorescent marker. The cells were perfused in vitro through organs with endothelial layers of different phenotypes (rat aorta, human umbilical vein, isolated mouse heart). The endothelium and the basal lamina were then stained by immunofluorescence and the cryo-sections analysed using a confocal laser scanning microscope. After perfusion through the rat aorta, an adhesion/integration of MNC was observed at the endothelial layer and the basal lamina beneath endothelial cells. However, no migration of MNC over the endothelial barrier was found. This remained true even when the cell numbers were increased (from 0.5 to 10 million cells/h), when the time of perfusion was prolonged (1.5–4 h) and when the aorta was cultivated for 24 h. In the Langendorff-perfused mouse heart with intact endothelium, no migration of MNC (1×10^7) or cEPC (1×10^6) was observed after 0.5 and 2 h. In conclusion, MNC and cEPC do not possess any capacity to transmigrate the endothelial barrier. In the context of stem cell therapy, these cells may therefore serve as endothelial regenerators but not as cardiomyocyte substitutes.

Keywords: Cell therapy; Endothelial barrier; Transmigration

Introduction

The importance of stem and progenitor cells for the regeneration and adaptation of definite tissues, for example skeletal muscle and cell lines such as haematopoietic cells, has been known for a long time. Over the past few years, several studies have shown that stem cells and progenitor cells play an important role in cardiac (Oh et al., 2003) and vascular regeneration (Dimmeler and Zeiher, 2004). The importance of the role of endothelial progenitor cells (EPC) in this context has become widely recognised.

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doi:10.1016/j.ejcb.2007.08.002

EPC are bone marrow-derived cells that circulate in the peripheral blood. A small number of them are found among the mononuclear cells (MNC). Their mobilisation from the bone marrow to the peripheral circulation, as well as their proliferation, occurs in response to stimuli such as growth factors and cytokines. In this context, vascular endothelial growth factor (VEGF) and stromal-derived factor 1 (SDF-1), both of which have been shown to be released by ischemic tissue, seem to play an important role in the mobilisation of EPC (Asahara et al., 1999; Askari et al., 2003; Ceradini et al., 2004).

EPC are able to proliferate and differentiate into mature endothelial cells when grown under appropriate conditions in vitro. Asahara et al. (1997) showed that CD34-positive cells from human peripheral blood grew into cells with endothelial characteristics after being plated on a fibronectin-coated surface. Tso and et al. (2006) have shown that EPC adhere to endothelium in response to an inflammation in the thoracic aorta of mice and differentiate into mature endothelial cells.

Endothelial health reflects a balance between endothelial injury and repair. The endothelium is at least partly regenerated by circulating adult endothelial cells. However, this pool is very limited, proliferates infrequently (Schwartz et al., 1976) and is not sufficiently active to be able to satisfy this regeneration process. It is well known that the amount of circulating EPC in the blood is decreased in patients with cardiac risk factors (Hill et al., 2003; Vasa et al., 2001) and is increased in patients with acute myocardial infarction and active ischemic heart disease (Massa et al., 2005; Shintani et al., 2001). Thus, it seems promising that EPC could be used to develop therapies for the treatment of cardiovascular diseases (e.g. after atherosclerotic or cardiac events).

There is also evidence that EPC trans-differentiate into cells of the myocardial lineage (Murasawa et al., 2005) and may thereby contribute to myocardial repair. In this context, it may be assumed that EPC not only adhere to, but also transmigrate through the endothelial barrier. The aim of this study was to investigate the interaction between EPC and the endothelial barrier. For this purpose, co-culture and perfusion experiments were performed with MNC, cultivated EPC (cEPC) and endothelial cells of rat aorta, murine heart and umbilical vein.

Materials and methods

Isolation of mononuclear cells

MNC were isolated from the peripheral blood of thirteen healthy, male human, non-smoking volunteers (25 ± 6 years old). After Ficoll-density gradient centrifugation, these cells were washed twice with Dulbeccós phosphate-buffered saline (DPBS; 9.5 mM phosphate without calcium and magnesium, Invitrogen, Paisley, UK) and prepared for further analysis.

Culturing of EPC

Immediately after isolation of MNC, 1×10^7 cells were plated onto culture dishes coated with fibronectin and maintained in endothelial basal medium MV2 (PromoCell, Heidelberg, Germany) supplemented with 5% foetal calf serum (FCS; Invitrogen), epidermal growth factor, hydrocortisone, VEGF, basic fibroblast growth factor (hbFGF), IGF-1, ascorbic acid, gentamycin, and amphotericin B. After 3 days in culture (95% humidity, 5% CO_2), non-adherent cells were removed by washing with DPBS and the remaining adherent cells (i.e. cEPC) were incubated in fresh medium for 24 h before perfusion through isolated organs (Urbich et al., 2005). For staining, cells were removed gently from the dishes with 3 ml accutase® (PAA, Pasching) and washed with DPBS.

PKH cell staining

Vital cell staining of MNC and cEPC was carried out using a MINI 67 staining kit with PKH-67 (Sigma-Aldrich, Steinheim, Germany), a green fluorescent cell tracking dye. Its long alkyl-chains rapidly partition into cell membranes and provide strong anchorage in the lipid bi-layer of living cells. The cells (MNC or cEPC) were resuspended in diluent C and PKH-67 diluted in diluent C was added. Staining was performed at 25 °C for 5 min in the dark and then stopped by adding FCS for 1 min. The cells were washed with MV2 medium and counted in a Neubauer chamber.

'Static' stem cell aorta model for co-culture

Male Wistar rats (8–12 weeks old) were sacrificed by cervical dislocation. The aorta was removed immediately after death, cut into rings, opened and attached to 12-mm glass cover slips with the endothelium facing the top using a two-component fibrin sealant (Tissucol Kit, Baxter, Vienna, Austria). The aortas were transferred in well plates and incubated with 6×10^4 PKH67 vital stained MNC for 105 min in endothelial basal medium MV2. These co-culture experiments between MNC and endothelial cells of rat aorta were performed three times.

MNC perfusion through rat aorta

The aorta was removed from rats as described above and cannulated for perfusion. The stained MNC were