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Original article

Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system

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Abstract

Enalapril, an angiotensin-converting enzyme (ACE) inhibitor, reduces cardiovascular events in patients with acute myocardial infarction. However, whether the beneficial effect of enalapril is mediated in part through endothelial progenitor cells (EPCs) has yet to be elucidated. This study investigated the role of the CD26/dipeptidylpeptidase IV (DPP IV) system in enalapril-modulated EPC mobilization. C57 BL/6 mice were divided into control and enalapril-treated groups. Peripheral EPCs were enumerated before and after ischemic stress. CD26/DPP IV activity and stroma-derived factor-1α (SDF-1α) levels were measured in the blood and the bone marrow. In response to ischemic stress, the enalapril group displayed a significant increase in circulating EPCs (with a 3.6-fold increase of sca-1^+KDR$^+$ cells and a 2.2-fold increase of c-kit$^+$CD31$^+$ cells versus controls at 12 h). Enalapril also caused a sixfold increase in the contribution of bone marrow-derived EPCs to the ischemia-induced neovascularization. In the bone marrow, enalapril did not alter CD26$^+$ cell numbers; however, it did amplify DPP IV activity. In the blood, through the anti-inflammatory effect, enalapril significantly decreased CD26$^+$ cell numbers, leading to a decrease in total DPP IV activity. These phenomena were associated with a lower SDF-1α concentration in the bone marrow but higher in the blood in the enalapril group, compared to the controls. All these findings were not demonstrated without ischemic stress. The effect of enalapril on EPC mobilization could be substantially blocked by Diprotin-A, a DDP IV antagonist. This study demonstrates that one of the pleiotropic effects of enalapril on the cardiovascular system involves the modulation of circulating EPC numbers via the CD26/DPP IV system, which may serve as a potential target for mobilizing EPCs for therapeutic purposes.

Keywords: Angiotensin-converting enzyme; CD26/dipeptidylpeptidase IV; Endothelial progenitor cell; Ischemia; Enalapril

1. Introduction

Endothelial progenitor cells (EPCs) have recently been identified from adult species and shown to possess therapeutic potential in a variety of diseases caused by atherosclerosis [1,2]. EPCs are mobilized from the bone marrow into the circulation, home to the site of vessel injury in response to physiological and pathological stimuli, and then differentiate into endothelial lineage cells, thus contributing to postnatal neovascularization. To date, EPCs have been applied in tissue engineering for improving the biocompatibility of vascular grafts [3,4,], have been used to promote neovascularization of the adult brain following stroke [5] and of ischemic limbs [6], and have been demonstrated to preserve left ventricular function following myocardial ischemic injury [7]. Although the mobilization of EPCs for regenerative medicine has attracted keen interest, the controlling mechanisms of EPC mobilization remain to be elucidated.

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CD26 (dipeptidylpeptidase IV [DPP IV]) is a membrane-bound extracellular peptidase with the ability to cleave chemokines containing the essential N-terminal X-Pro or X-Ala motif [8], such as stroma-derived factor-1α (SDF-1α)/CXCL12, which serves as a chemoattractant for human $CD34^+$ cells and stem/progenitor cell populations. Recent studies have demonstrated that endogenous CD26/DPP IV expression on donor cells regulates homing and engraftment of transplanted stem/progenitor cells [9]. The inhibition or deletion of CD26/DPP IV activity in bone marrow greatly increases the efficiency of stem/progenitor cell engraftment back to the bone marrow. These observations suggest that CD26/DPP IV may serve as a novel target for modifying stem/progenitor cell mobilization. Furthermore, DPP IV and ACE belong to the dipeptidyl peptidase family and exert their proteolytic activities in a balanced fashion, regulating the metabolism and function of peptide hormones. Accordingly, DPP IV can be assumed to complement the function of ACE in regulating the activation of various chemokines [10]. This study, using a hindlimb ischemic stress model, demonstrated that enalapril, an ACE inhibitor, could regulate the activity of CD26/DPP IV, and thus modulate the functioning of CXCL12/SDF-1α and influence the level of EPC mobilization.

2. Methods

2.1. Animal studies

Male C57BL/6 wild-type mice (Jackson Laboratory, Bar Harbor, Me) or bone marrow-reconstituted FVB mice ($BMT^{Gfp\rightarrow Wild}$ mice) underwent severe hindlimb ischemia surgery under general anesthesia. Animals were treated subcutaneously with different combinations of medications, including enalapril (an ACE inhibitor, 1 mg/kg/day), Diprotin-A (a DPP IV antagonist, 5 μmol twice a day), Losartan (an angiotensin receptor type 1 antagonist, 10 mg/kg/day), and PD123319 (an angiotensin receptor type 2 antagonist, 3 mg/kg/day). Control animals were injected with normal saline. Treatments started 4 days before surgery and maintained until sacrifice. The study protocol was reviewed and approved by the Committee on Ethics on Animal Experiments, Chang Gung Memorial Hospital Faculty of Medicine, and the experiments were conducted according to the Guidelines of the American Physiological Society.

2.2. Hindlimb ischemia model

Mice underwent surgery to induce severe unilateral hindlimb ischemia. The animals were anesthetized using isoflurane inhalation. Under sufficient anesthesia, the left external iliac artery and vein, the deep femoral and circumflex arteries and veins, and the entire left superficial femoral artery and vein (from just below the deep femoral arteries to the popliteal artery and vein) were ligated, cut, and excised. To estimate limb perfusion 2 weeks after ischemia induction, ischemic (right)/normal (left) limb blood flow ratio was measured with a laser Doppler imager (MoorLDI-Mark 2, Moor Instruments). Following 2 laser Doppler image recordings, average perfusion for the ischemic and nonischemic limbs was calculated on the basis of colored histogram pixels.

2.3. Bone marrow transplantation model

Recipient FVB mice at 8 weeks of age were lethally irradiated with a total dose of 900 rad, 9.0 Gy. eGFP transgenic mice (FVB background) that ubiquitously expressed enhanced GFP were used as the donors (Level Biotechnology Inc., Taipei, Taiwan) [11]. After irradiation, the recipient mice received unfractionated bone marrow cells (5×10^6) from eGFP mice by tail vein injection. At 8 weeks after injection, surgery of hindlimb ischemia was performed. Repopulation by eGFP-positive bone marrow cells was measured by flow cytometry to be 95%.

2.4. Assessment of white blood cells in peripheral blood

At different time points (baseline, 6 h, 12 h, 24 h, 48 h, 7 and 14 days), blood samples were obtained by cardiac puncture. The total number of white blood cells (WBCs) was assessed with a Neubauer hematocytometer (Fisher Scientific). Moreover, differential leukocyte counts (lymphocytes, monocytes and polymorphonuclear leukocytes) were obtained by examining blood smears, stained with Wright-Giemsa, from each mouse (200 cells counted/smear).

2.5. Measurement of cytokines

Whole blood, with EDTA as an anticoagulant, was centrifuged to isolate plasma and stored at −80 °C for future analysis. Bone marrow from bilateral femurs of each mouse was flushed out using 1 ml IMDM. Following centrifugation, the supernatant was stored for cytokine analysis. Plasma and bone marrow SDF-1α concentrations were measured with a mouse SDF-1α ELISA kit (R&D Systems). The plasma concentrations of VEGF (vascular endothelial growth factor), SCF (stem cell factor), and G-CSF (granulocyte-colony stimulating factor) (R and D Systems) were assessed at different time points following surgery.

2.6. Human mononuclear cell isolation and culture

Mononuclear cells were isolated from the blood of healthy volunteers by density gradient centrifugation using Ficoll separating solution (Becton Dickinson). After resuspension in EGM-2 medium (containing vascular endothelial growth factor [VEGF] 10 ng/ml), 1×10^6 mononuclear cells/cm^2 were plated on each chamber of fibronectin-coated, 2-chamber, cell culture slides. Following culturing for 0, 7, 14, and 21 days, cells were collected for quantification of angiotensin II receptor type 1 (AT1R) and AT2R mRNA expression. Additionally, to estimate the effect of AT II on EPC differentiation, cells were grouped as follows ($n = 4–5$ for each group): (1) control group, (2) AT II treated groups (from 5 pM to 1 μM), (3) AT II with Losartan (10 μM), (4) AT II with PD123319 (10 μM), and (5) AT II with Losartan and PD123319. Medications were administered on day 4 and cells were analyzed using with flow cytometry on day 7.