Low-dose ionizing radiation induces therapeutic neovascularization in a pre-clinical model of hindlimb ischemia

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Authors
Augusto Ministro, Paula de Oliveira, Raquel J. Nunes, André dos Santos Rocha, Adriana Correia, Tânia Carvalho, José Rino, Pedro Faísca, Jorg D. Becker, João Goyri-O'Neill, Filomena Pina, Esmeralda Poli, Bruno Silva-Santos, Fausto Pinto, Marc Mareel, Karine Serre, Susana Constantino Rosa Santos

Abstract

Aims

We have previously shown that low-dose ionizing radiation (LDIR) induces angiogenesis but there is no evidence that it induces neovascularization in the setting of peripheral arterial disease. Here, we investigated the use of LDIR as an innovative and non-invasive strategy to stimulate therapeutic neovascularization using a model of experimentally induced hindlimb ischemia (HLI).

Methods and results

After surgical induction of unilateral HLI, both hindlimbs of female C57BL/6 mice were sham-irradiated or irradiated with four daily fractions of 0.3 Gy, in consecutive days and allowed to recover. We demonstrate that LDIR, significantly improved blood perfusion in the murine ischemic limb by stimulating neovascularization, as assessed by laser Doppler flow, capillary density, and collateral vessel formation. LDIR significantly increased the circulating levels of VEGF, PlGF, and G-CSF, as well as the number of circulating endothelial progenitor cells (EPCs) mediating their incorporation to ischemic muscles. These effects were dependent upon LDIR exposition on the ischemic niche (thigh and shank regions). In irradiated ischemic muscles, these effects were independent of the recruitment of monocytes and macrophages. Importantly, LDIR induced a durable and simultaneous up-regulation of a repertoire of pro-angiogenic factors and their receptors in endothelial cells (ECs), as evident in ECs isolated from the irradiated gastrocnemius muscles by laser capture microdissection. This specific mechanism was mediated via vascular endothelial growth factor (VEGF) receptor signaling, since VEGF receptor inhibition abrogated the LDIR-mediated gene up-regulation and impeded the increase in capillary density. Finally, the vasculature in an irradiated non-ischemic bed was not affected and after 52 week of LDIR exposure no differences in the incidence of morbidity and mortality were seen.

Conclusions

These findings disclose an innovative, non-invasive strategy to induce therapeutic neovascularization in a mouse model of HLI, emerging as a novel approach in the treatment of critical limb ischemia patients.

Keywords:

Neovascularization, Critical limb ischemia, Ionizing radiation, Endothelial progenitor cells, Hindlimb ischemia