Hypoxic Preconditioning
– A Cutting “Aged” Remedy for Impaired Angiogenesis –
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Since the discovery of endothelial progenitor cells (EPCs) approximately 15 years ago, many scientists and clinicians have attempted to establish a successful procedure for cell-based therapy using EPCs in the field of cardiovascular medicine. Indeed, the use of EPCs for “therapeutic angiogenesis” was found to be effective for some patient populations, such as those with Buerger’s disease. However, for patients with comorbid diseases (ie, diabetes mellitus or end-stage renal disease (ESRD)), the efficacy of therapeutic angiogenesis is lower. The underlying molecular mechanisms of this inadequacy, as well as any interventional remedies, have yet to be clarified.

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Aging is one of the crucial factors that retards angiogenesis. In this issue of the Journal, Kubo et al have demonstrated that hypoxic preconditioning reverses age-induced angiogenesis dysfunction in bone marrow-derived monocellular cells (BMCs), both in vitro and in vivo. They used a mouse model with the aim of clarifying the characteristics of aged BMCs by comparing them to BMCs obtained from younger counterparts. In the aging group, the number of CD34+ or c-kit-positive BMCs was markedly reduced and the aged BMCs exhibited functional impairments (ie, adhesion, survival and angiogenic potency with concomitant higher p53 expression and lower telomerase activity). Most patients with critical limb ischemia or inoperable ischemic heart disease who have undergone cell-based therapy are of an advanced age (eg, the average age of the enrolled patients in the TACT trial was 66±12 years). Accordingly, the preclinical data demonstrated by Kubo et al prompt us to take a new look at hypoxic preconditioning as a conventional and feasible remedy for refractory cases of cell-based therapy.

To date, there have been several milestone studies regarding the use of hypoxic preconditioning for peripheral blood mononuclear cells (PB-MNCs) and/or EPC activation. The first report, presented by Akita et al, demonstrated primary evidence supporting the interventional utility of hypoxic preconditioning in PB-MNCs/EPC activation. Based on the notion that local ischemia (ie, hypoxia) facilitates the differentiation and function of EPCs, Akita et al demonstrated that the vascular endothelial growth factor (VEGF) and kinase-insert-domain receptor/VEGFR-2 (KDR) axis played an essential role in hypoxia-mediated augmentation of angiogenesis via enhanced VEGF production in PB-MNCs, which accelerated both the differentiation of the PB-MNCs into EPCs in an autocrine manner and the angiogenic properties of the EPCs. The transcription factor, hypoxia-inducible factor-1α (HIF-1α), contains the VEGF gene promoter (ie, the hypoxia-responsive element), and thereby enhances the transcription of VEGF under hypoxic conditions. In addition, it is worth noting that the local overexpression of HIF-1α by viral vector enhanced angiogenesis in the absence of ischemia, and that in addition to VEGF production, activation of HIF-1α upregulated various proangiogenic chemokines, including basic fibroblast growth factor, angioptietin-1, and SDF-1α. Overall, HIF-1α is essential for hypoxia-mediated proangiogenic signaling.

HIF-1α is also important for the regulation of p53 expression levels. P53 has been demonstrated not only to function as a tumor suppressor but also to modulate angiogenesis, apoptosis, and senescence. Interestingly, the synthesis of HIF-1α is regulated via O2-independent mechanisms, whereas its degradation is regulated primarily via O2-dependent mechanisms. Severe hypoxia induces an abnormal expression of p53, which binds to HIF-1α and promotes its degradation, thereby inhibiting its transactivation properties. Kubo et al focused on the role of p53 in terms of cell senescence and found that p53 was upregulated in aged EPCs and showed a concomitant decline in telomerase activity.

A simple question regarding the impact of hypoxic preconditioning is its effect(s) on the “stemness (ie, stem cell potential)” of BMCs. As Kubo et al demonstrate in their study, hypoxia preconditioning increased the number of VE-cadherin-positive BMCs, suggesting that the stemness of BMCs may be modulated by hypoxic preconditioning, and therefore an increased number of further differentiated BMCs was observed. However, a previous report using cardiac stem cells demonstrated that low O2 culture conditions reduced the level of intracellular reactive oxygen species, cell senescence, and promoted higher resistance to oxidative stress compared with cells grown in normal O2 conditions, although the expression of stem cell antigens and adhesion molecules was comparable between the groups. Taken together, these data suggest that cardiac stem cells and BMCs/EPCs respond differently to hypoxia modulation for cell differentiation, including stemness. Further evaluations, including the effect of p53 and telomerase on the BMCs/EPCs stemness/differentiation under hypoxia, are still underway.

It is worth mentioning that physiological hypoxia, induced...
by exercise training, promotes EPC activation and mobilization. Cheng et al clearly demonstrated that exercise induced by swimming augmented neovascularization in response to hypoxia via a phosphatidylinositol-3-kinase-dependent mechanism that was mediated by the HIF-1α/VEGF/MMP-2 pathway in advanced age. Exercise is distinct from any of the disease conditions, so its regulatory mechanism is presumably independent of the p53-mediated pathway, suggesting the primary role of tissue hypoxia-mediated HIF-1α upregulation in the reactive angiogenesis induced by both physiological and pathological stimuli (Figure B). The data demonstrated by Kubo et al, in addition to the results by Cheng et al, may be applied to an extended interpretation that can be used to describe the mechanisms underlying exercise therapy for peripheral artery disease (PAD).

In conclusion, the study by Kubo et al provides a novel and feasible concept that suggests that hypoxic preconditioning is a safe, conventional, and versatile procedure that could serve as a “cutting-edge” breakthrough for the refractory cases seen in current cell-based therapy caused by aging and presumably by comorbidity.

References