Angiotsensin II Deteriorates Endothelial Progenitor Cells
Good Intentions With Bad Consequences
Anton J.M. Roks

See related article, pp 394–403

Approximately 15 years ago it was discovered that the bone marrow and blood harbor stem cells with angiogenic potential.1 Because some of these stem cells express endothelial markers, it is believed that they are endothelial progenitor cells (EPCs) that will contribute to the maintenance of the circulatory system and develop into adult endothelial cells. Many studies followed to unravel the physiological role and the therapeutic application of EPCs. All of these research endeavors did not yet lead to a consensus regarding the identification of true EPCs, and EPC quantification remains subjective.2 To complicate matters even more, it has now been proposed that nonendothelial CD31+ bone marrow cells participate in neovascularization.3 It is, therefore, not only complicated for the investigator to compose a satisfactory study design but also for the reader to evaluate the results. Preferably, such studies investigate circulating, as well as cultured EPCs, and provide proof of their involvement in (patho)physiology through functional studies. Identification of circulating EPCs should be identified by combining stem cell markers with endothelial cell markers, whereas EPCs obtained from cultured blood, bone marrow, or spleen-derived mononuclear cells (MNCs) should be identified on the basis of endothelial markers and variables of angiogenic function.2

It is accordingly these favorable requirements that, in this issue of Hypertension, Endtmann et al4 show that treatment of adherent, cultured human blood MNCs with angiotensin (Ang) II decreases early outgrowth EPC numbers, colony formation, and migration via Ang II type 1 receptor (AT1R) stimulation and that a 12-day infusion of Ang II reduces the numbers of circulating EPCs in wild-type mice. Novel evidence for the involvement of AT1R signaling in the regulation of vascular repair function of progenitor cells was obtained by studying the effect of wild-type versus AT1R−/− spleen-derived MNCs on neointima formation and re-endothelialization after carotid artery injury in mice. Through an elegant series of experiments, Endtmann et al4 show that AT1R signaling inhibits the endothelial repair function of MNCs. In addition, bone marrow replacement studies in apolipoprotein E−/− mice suggest that bone marrow AT1R+ cells play a role in atherogenesis. The in vivo studies do not illustrate whether the AT1R-mediated effects are conferred by EPCs, by other MNCs, or bone marrow cell types. However, because the angiogenic potential of progenitor cells does not entirely depend on true EPCs,3 this question seems more academic than of clinical interest. The in vitro results (see Figure 1 of the article by Endtmann et al4) show that non-EPC MNC types are also affected and emphasize the importance of studying the immunomodulatory role of Ang II in vascular disease, a field of research that is gaining attention. In all likelihood, an interplay between EPCs and inflammatory cells exists that is of utmost importance for the vasoprotective effects of pharmacological renin-angiotensin system modulation.

Importantly, Endtmann et al4 show that in vitro Ang II affects EPC numbers through apoptosis, a process mediated by the apoptosis signal-regulating kinase 1–c-Jun N-terminal kinase/p38 mitogen-activated protein kinase–Bax/Bcl2 signaling axis and subsequent caspase 3 activation, and not because of an effect on proliferation. This is a novel finding that complements the observation that Ang II can also decrease EPCs through induction of senescence (Figure).2,5,6 Both processes involve production of reactive oxygen species (ROS) and require several days (≥5 days) of exposure to Ang II. This is in sharp contrast with the antiapoptotic, proangiogenic effect of Ang II on EPCs that was observed in other studies.2,7,8 These stimulatory effects of Ang II takes place on a shorter term (≤5 days). Therefore, the paradoxical findings seem to be based on a temporal difference in exposure to Ang II. Although Ang II–AT1R signaling leads to an immediate angiogenic response, and is meant to operate with good intentions, the chronic activation of this pathway has bad consequences.

The putative differential effect on the basis of exposure time can be explained from the distinct manner through which signaling pathways are activated by Ang II in EPCs (Figure of this commentary). The more acute stimulatory effects in cultured EPCs depend on AT1R-mediated facilitation of vascular endothelial growth factor–mediated NO release, involving an increased vascular endothelial growth factor receptor and endothelial NO synthase expression, as well as antiapoptotic phosphatidylinositol 3/Akt signaling.2,7 The chronic, deleterious effects on cultured EPCs depend on ROS release, and accumulation of damage to macromolecules, in particular to DNA, might be a necessary, time-consuming interlude between ROS release and induction of
apoptosis or senescence. In addition, Endtmann et al observed that ROS production in EPCs is increasing during the first 48 hours of Ang II treatment. Perhaps the induction of deleterious Ang II effects in EPCs requires a high degree of ROS production, for example, to circumvent the protective effect of antioxidant pathways. As a consequence, harmful amounts of ROS are only produced after 2 days, and the damage threshold leading to apoptosis or senescence is reached only after prolonged Ang II stimulation. Although this is still a singular observation, it is interesting to investigate what the reason is for the increase in ROS production after 48 hours, because this might represent a therapeutic target. In this respect, the reciprocal regulation of NAPDH oxidase and of the opposing antioxidant pathways would be of interest.

Apart from the above issues, the present study raises several other questions. First, there is the possibility that in vivo effects of Ang II infusion are dependent on blood pressure. Because Ang II affects EPC both in vivo and in vitro, the effect of the peptide does not seem to depend strictly on blood pressure. Unfortunately, there are no studies that use nonpressure doses of Ang II to confirm this presumption. At least the in vivo increase of EPC levels by hydralazine treatment does associate with its antihypertensive properties, indicating that blood pressure, per se, might not affect EPCs. Second, the present study does not provide evidence for a role of EPC apoptosis and migratory capacity in the deleterious effects of Ang II in vivo on progenitor cell repair function. This would have required immediate quantification of apoptosis in freshly obtained progenitor cells and acute testing of their migratory capacity. Also, it would have been interesting to know whether the decreased colony forming units and migratory capacity of EPCs (Figure 4 in the article) are a direct result of the apoptotic effect by counting the numbers of apoptotic nonadherent MNCs during reseeding for the respective assays. This seems most relevant for CFU assays, for which MNCs are reseeded after 48 hours and less so for migration (reseeding at 96 hours).

In conclusion, Endtmann et al here show that ROS-induced apoptosis of EPCs or functionally related progenitor cells is a mechanism that possibly contributes to the deleterious effect of Ang II on vascular health. Exact identification of the progenitor cell types that undergo apoptosis in vivo will be necessary for establishment and further understanding of this process. The interplay between these progenitor cell types and inflammatory cells that are affected by Ang II can be an important new topic of research. Finally, as noted by Endtmann et al, the identified apoptosis signaling pathway provides a foundation for the development of novel pharmacological interventions.
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None.

References
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