Does Helium Act on Vascular Endothelial Function in Humans?

To the Editor:

Smit *et al.*[1] have demonstrated intriguing results that preconditioning with helium can preserve acetylcholine-induced vasodilator responses in the human forearm exposed to ischemia–reperfusion. This study seems to have many questions regarding the mechanisms of helium's action, whereas we would congratulate their impressive results. First, Smit *et al.* used a nonselective nitric oxide synthase inhibitor, NG-monomethyl-L-arginine, to document that the acetylcholine-induced enhancement of forearm blood flow is due to the activation of endothelial nitric oxide synthase.[1–3] However, they failed to show that the inhibition by NG-monomethyl-L-arginine in the control condition, as well as that with the effect of helium-preconditioning.[1] Therefore, it is quite difficult to conclude that nitric oxide, derived from endothelial enzyme, is a source of acetylcholine-induced vasodilator effects in their study. Second, it is unclear that the dosage of NG-monomethyl-L-arginine, used by Smit *et al.*, is comparable with those are sufficiently effective to inhibit acetylcholine-induced vasodilation in humans (32–64 μmol/min or 5 mg/min).[1–3] Third, Smit *et al.* did not evaluate roles of oxygen-derived free radicals including superoxide, which should modify vasodilator function in several pathological conditions including ischemia–reperfusion.[4–6] Indeed, it is possible to obtain endothelial cells from patients using J-wires to determine the degree of oxidative stress.[4] Fourth, it is possible that deflation of a blood pressure cuff placed on the arm to a pressure of 200 mmHg for 20 min induces flow-mediated dilation, which is mediated by endothelium-derived nitric oxide.[7] This possibility has to be ruled out because this dilation may play a role in the protective effects of helium on forearm blood flow after ischemia–reperfusion.[7] Therefore, further studies are needed to clarify the mechanistic insight into helium's protective effects on the vascular function exposed toward ischemia–reperfusion.

Hiroyuki Kinoshita, M.D., Ph.D.,* Noboru Hatakeyama, M.D., Ph.D., Yoshihiro Fujiwara, M.D., Ph.D. *Aichi Medical University School of Medicine, Aichi, Japan. hkinoshi@aichi-med-u.ac.jp

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In Reply:

We thank Kinoshita *et al.* for their valuable comments on our article regarding helium preconditioning in human endothelium.[1] Kinoshita *et al.* state that there still remain questions regarding the underlying mechanism of the observed endothelial protection after helium-induced late and early preconditioning. In our experiments,[1] we have shown significant endothelial dysfunction after 20 min of forearm ischemia induced by inflating a blood pressure cuff. This endothelial dysfunction was prevented by helium early and late preconditioning. The experiments using the nonselective nitric oxide synthase inhibitor, NG-monomethyl-L-arginine, during helium preconditioning were performed to investigate whether endothelial nitric oxide synthase is crucially involved in the mechanism of the observed helium preconditioning. Because co-infusion of NG-monomethyl-L-arginine did not block the protective effect of helium, we concluded that endothelial nitric oxide synthase is not a central mediator of helium-induced preconditioning in this model. In contrast to the reference[2] cited by Kinoshita *et al.*, we did not use NG-monomethyl-L-arginine as vasoconstrictive agent during acetylcholine infusion. Therefore, it is correct that we cannot conclude that nitric oxide derived from endothelium is the source of the acetylcholine-induced vasodilatory effect. The dosage of NG-monomethyl-L-arginine, we used equates to that, maximally reduces basal forearm blood flow in healthy volunteers without a possible systemic effect.[3] Unfortunately, we were unable to directly obtain endothelial cells from our volunteers to measure oxidative stress in those cells. The last comment raised by the authors pertained to reactive

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