Although recent procedural advances in revascularization such as percutaneous coronary intervention and coronary artery bypass grafting improve quality of life and prognosis of the patients with ischemic diseases, these is still a subset of patients who are refractory to these conventional therapies and have poor prognosis. Therapeutic angiogenesis might offer a novel approach to these patients. Therapeutic angiogenesis involves an intervention to induce the formation of new blood vessels to restore the arterial blood and oxygen supply to ischemic tissues. Here, the term “angiogenesis” represents the process of new blood vessel formation in general (although the same term is also used to describe a more specific biological process in which the new capillaries sprout from preexisting vessels).

Evolving knowledge of mechanisms of new blood vessel formation has raised the expectations for therapeutic angiogenesis as a treatment option. Recent studies have identified many angiogenic growth factors, vascular transcription factors, and the cells involved in neovascularization. Current potential strategies for therapeutic angiogenesis include delivering an angiogenic factor as a protein or a gene, and supplying cells which themselves are vascular progenitors or are releasing angiogenic factors. These strategies have worked in animal studies and in initial small scale open-labeled clinical trials. However, in larger, double-blinded controlled trials, therapeutic angiogenesis approaches have failed to show clinical benefits. Why? Perhaps there are subtle differences in angiogenesis between animals and humans, or the ischemic pathophysiology of animal models and human diseases are dissimilar. Another possibility is technical difficulties in translating the biology into the practice.

In this issue of Circulation Research, Leon-Poi and colleagues report that targeted delivery of vascular endothelial growth factor (VEGF) gene using ultrasound-mediated destruction of cationic lipid microbubbles restores microvascular blood flow in a rat model of chronic hindlimb ischemia. Ultrasound-targeted microbubble destruction uses ultrasound contrast agents, mostly perfluorocarbon bubbles stabilized with albumin or a lipid shell. When insonified at high acoustic power, these agents oscillate, resulting in microbubble disintegration. This microbubble destruction has been shown to induce biophysical effects in the vicinity of contrast agents, and the therapeutic use of this phenomenon has been proposed for delivery of genes or drugs, and direct mechanical effects. One of the advantages of this method is that these bubbles cross the pulmonary circulation, so that the agents can be administered intravenously, and reach any part of the body with arterial blood supply. And, at the desired sites, the agents can be activated or delivered by ultrasound. Leon-Poi et al coated the microbubbles with VEGF-expressing plasmid, intravenously administrated these bubbles, and then transferred the gene at the site of ischemia with ultrasound in hindlimb ischemia model. This model of ischemia is chronic, because the gene was transferred 14 days after common iliac artery ligation.

Leon-Poi et al observed a significant increase in tissue perfusion mainly through the process of arteriogenesis, rather than angiogenesis in a narrow sense. Arteriogenesis refers to the maturation or de novo synthesis of collateral vessels. The effect of VEGF gene transfer with this method on arteriogenesis rather than angiogenesis is intriguing, as remodeling or development of collaterals could be much more effective than an increase in capillary bed to restore the blood flow in the setting of flow-limiting proximal conduit artery lesions. It will be an important issue to determine whether the preferential effect on arteriogenesis is associated with the method of gene delivery, ultrasonic destruction of microbubble destruction (Figure).

Although the gene delivery is an important application of ultrasound-mediated microbubble destruction, use of this method is not limited to gene therapy. Acoustic cavitation leads to microbubble oscillation and collapse. Electron microscopic analysis showed transient pore formation on cell membrane immediately after microbubble destruction, called sonoporation. These mechanical effects facilitate entry of gene into the cells. At the same time, these mechanical forces affect nearby cells and tissues in vicinity of microbubble destruction. For example, microbubble destruction can facilitate thrombolysis in combination with thrombolytic agents such as urokinase and tissue plasminogen activator. Furthermore, ultrasound-mediated microbubble destruction itself can be angiogenic, as ultrasound-mediated microbubble destruction has been shown to be able to induce capillary rupture and increase the density of arterioles in ischemic muscle with local recruitment of VEGF producing inflammatory cells. Although capillary rupture with higher energies of ultrasound is just a step from adverse tissue damage, even with ultrasound without capillary rupture, ultrasound-mediated microbubble destruction has direct effects on vasculature and surrounding tissues. These effects can be
used for potential therapies, especially when combined with other modalities. One such example is microbubble destruction in combination with cell therapy. We have shown that targeted delivery of bone marrow-derived mononuclear cells by ultrasound-mediated microbubble destruction significantly enhances angiogenic response both in an ischemic hindlimb model and in a δ-sarcoglycan deficient cardiomyopathy model. In the ischemic hindlimb model, ultrasound-mediated microbubble destruction induces platelet activation on the surface of endothelium, and subsequent induction of adhesion molecules on endothelium, which in turn stimulates recruitment of angiogenic mononuclear cells and enhances new vessel formation. Ultrasound-mediated targeted cardiac delivery of marrow-derived mononuclear cells also efficiently enhances regional blood flow in myopathic hamsters, leading to improvement of cardiac function.

Recent proof-of-principal studies show that ultrasound-mediated microbubble destruction has great potential to target various substrates including genes, proteins, drugs and cells to the desired sites. However, before this strategy is adopted in the clinic, many issues have to be resolved. Which patients are ideal subjects? What tissues are ideal target locations? What proteins and cells are ideal substrates to be delivered by microbubbles? Furthermore, technical issues of ultrasound-mediated microbubble destruction such as microbubble composition and ultrasound application must be refined. More collaboration between clinicians, biologist, chemists and engineers is needed to bring this exciting technique into the clinic.

Sources of Funding
This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Grants-in-Aid from the Ministry of Health, Labor, and Welfare of Japan.

Disclosures
None.

References

Key Words: therapeutic angiogenesis ■ gene therapy ■ ultrasound ■ microbubbles