

News Release

Title

Hydrogen sulfide attenuates lymphedema via the induction of lymphangiogenesis through a PI3K/Akt-dependent mechanism

Key Points

- Secondary lymphedema which causes a significant decline in quality of life, is expected to increase with advances in cancer treatment. However, it is difficult to provide fundamental treatment for it, and is currently treated mainly by physical symptomatic therapy.
- In this study, we found that administration of DATS, which is contained in garlic odor components and generates hydrogen sulfide in the body, increased the concentration of hydrogen sulfide in the edematous tissue, and promoted lymphangiogenesis to reduce lymphedema.
- Hydrogen sulfide is expected to be a novel molecular target in therapeutic lymphangiogenesis in the future and become a fundamental treatment for patients with secondary lymphedema.

Summary

A research group led by Junya Suzuki, graduate student, Yuuki Shimizu, assistant professor, and Toyoaki Murohara, professor of department of cardiology at Nagoya University Graduate School of Medicine, has investigated the possibility that hydrogen sulfide could promote lymphangiogenesis and provide a novel treatment for lymphedema.

Secondary lymphedema is a condition in which the lymphatic system malfunctions due to lymph node dissection or radiation therapy during cancer treatment, causing swelling of the extremities. Lymphedema causes not only visual problems but also functional impairment of the extremities, repeated pain, and inflammation, resulting in a significant decline in quality of life (QOL). In addition, as the prognosis of cancer patients improves with advances in cancer treatment, the number of patients with lymphedema is expected to increase in the future. However, current treatment for lymphedema is mainly symptomatic physical therapy such as compression with elastic stockings, exercise, and skin care, and it is difficult to provide a fundamental treatment.

Therefore, this study focused on therapeutic lymphangiogenesis with hydrogen sulfide. Hydrogen sulfide is a gas with a rotten egg smell, known to exist in the body in trace amounts and is widely involved in antioxidant effects, immunomodulation, metabolism, angiogenesis, and vasodilation as a gas transmitter molecule. The group discovered that administration of H₂S donor: DATS, a substance that is abundant in the garlic smell and generates hydrogen sulfide when taken into the body, promotes lymphangiogenesis in lymphedema tissue and

reduces edema. This finding suggests that hydrogen sulfide may be a useful treatment for refractory secondary lymphedema in the future. The results of this study were published in the American scientific journal *JAMA*. (electronic edition in 2022).

Research Background

Secondary lymphedema is a condition in which the lymphatic system malfunctions due to lymph node dissection or radiation therapy during cancer treatment, causing swelling of the extremities. It is not only a cosmetic problem, but also causes significant deterioration in quality of life due to dysfunction of the extremities, skin ulcers, recurrent pain, and infection. The number of patients with lymphedema is expected to increase in the future because advances in cancer treatment would improve the prognosis. However, lymphedema is difficult to treat fundamentally and is mainly treated symptomatically with physical therapy such as elastic stockings, exercise, and skin care, and new treatment methods are expected to be developed.

Hydrogen sulfide is a gas molecule that exists in the body in very small amounts similar as nitric oxide and carbon monoxide, and known to act as a gas transmitter in the body. Hydrogen sulfide is produced by enzymes (e.g., CSE) in the body and plays an important role in maintaining homeostasis, including antioxidant effects, immune and metabolic regulation, angiogenesis, and vasodilation. It is also known that DATS, a substance abundant in garlic odorants, generates hydrogen sulfide *in vivo*. This research group has previously reported that administration of DATS in ischemic tissues promotes angiogenesis and improves lower limb ischemia. In this study, we sought to elucidate the mechanism by which hydrogen sulfide modulates lymphangiogenesis, and to evaluate and verify whether DATS administration could be a novel treatment for secondary lymphedema.

Research Results

Secondary lymphedema model was created by cauterizing and dissecting the lymphatic network in the superficial layer of subcutaneous tissue in the tail of mice. First, lymphedema was induced in mice genetically engineered to lack CSE, an enzyme that generates hydrogen sulfide *in vivo* [CSE knockout (KO) mice] and in normal wild-type mice. The hydrogen sulfide concentration in the lymphedema tissue was decreased in the CSE-KO mice. Next, after 4 weeks of lymphedema induction, the CSE-KO mice had poor lymphatic vessel development and the degree of lymphedema was exacerbated.

On the other hand, when DATS was administered intraperitoneally into the murine lymphedema model, hydrogen sulfide levels in the blood and lymphedema tissue were higher than in mice that were treated with the vehicle. Furthermore, after 4 weeks of lymphedema induction, the degree of lymphedema was found to be reduced in the DATS-treated group. Next, immunofluorescence staining of the lymphedema tissue under a microscope showed that more neointimal lymphatic endothelial cells were observed in the DATS-treated mice, indicating that lymphangiogenesis was promoted. This suggests that administration of DATS induces the

production of hydrogen sulfide in vivo, which stimulates lymphangiogenesis, improves lymphatic dysfunction, and reduces lymphedema.

In order to elucidate the detailed therapeutic mechanism, experiments were conducted at the cellular level using cultured human lymphatic endothelial cells. The results showed that the treatment of DATS to lymphatic endothelial cells produced hydrogen sulfide, which promoted more active proliferation of lymphatic endothelial cells as well as their migration and tube formation abilities. We also demonstrated that hydrogen sulfide acts directly on lymphatic endothelial cells during the process, partly through PI3K/Akt signaling.

This study indicates that in vivo generation of hydrogen sulfide promotes lymphangiogenesis and reduces secondary lymphedema. This finding suggests that a therapeutic approach targeting hydrogen sulfide could be a potential novel treatment for secondary lymphedema.

Research Summary and Future Perspective

Present study demonstrated that targeting hydrogen sulfide in mice promotes lymphangiogenesis and improves secondary lymphedema. Based on these results, further translational research will be conducted to verify the safety and efficacy of this treatment in larger animals as a preclinical study, and eventually establish it as a treatment for actual patients.

Publication

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