Globotriaosylceramide Induces Endothelial Dysfunction in Fabry Disease

Kimio Satoh

The endothelium critically regulates the contractile status of the vascular smooth muscle cells. Dysfunction of endothelial cells (ECs) induces the increased expression of adhesion molecules for inflammatory cells. Inflammatory cell migration and vascular inflammation generate an oxidizing environment. The accumulating inflammatory cells produce abundant reactive oxygen species (ROS) and secrete inflammatory cytokines/chemokines and growth factors that contribute to EC dysfunction and vascular smooth muscle cell proliferation. Therefore, oxidants were once principally considered agents of vascular injury and disease, and numerous studies have corroborated this role for ROS. However, this has become an outdated theory considering recent evidence suggesting that hydrogen peroxide (H$_2$O$_2$) also serves as an important signaling molecule in the vascular system when found at low concentrations. At low concentrations, H$_2$O$_2$ can act as a secondary messenger, transducing the oxidative signal into a biological response through post-translational protein modification. These structural changes ultimately lead to altered cellular function.

See accompanying article on page 81

Oxygen derivatives, including superoxide (O$_2^-$), H$_2$O$_2$, and hydroxyl radical (OH$^-$), are called ROS. Strictly controlled ROS formation mediates the physiological functions of the vasculature. Therefore, ROS contribute to vascular protection as well as vascular diseases. Vascular ECs themselves produce small amounts of O$_2^-$ and H$_2$O$_2$, which play a crucial role in EC protection under physiological conditions. EC-dependent relaxation is mediated primarily by prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factors (EDHFs). We and others have demonstrated that H$_2$O$_2$ is an EDHF that contributes as a signaling molecule in the vasculature and protects EC function. However, the mechanism of EDHF is complex and varies according to blood vessel type, one of which is also through the opening of calcium (Ca$^{2+}$)-activated K$^+$-channels ($\text{K}_\text{Ca}$) $\text{K}_\text{Ca}3.1$. The initial endothelial hyperpolarization results from the opening of $\text{K}_\text{Ca}$ including $\text{K}_\text{Ca}3.1$ and $\text{K}_\text{Ca}2.3$, both of which are activated by Ca$^{2+}$ and are voltage independent.

Fabry disease is a lysosomal storage disease caused by $\alpha$-galactosidase deficiency that leads to the accumulation of glycosphingolipids such as globotriaosylceramide (Gb3). The accumulation of Gb3 is often observed in the ECs of patients with Fabry disease, which results in the clinical manifestation of poor organ perfusion. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Choi et al. examined the detailed mechanism of Gb3-induced $\text{K}_\text{Ca}3.1$ degradation, which is implicated in the endothelial dysfunction observed in Fabry disease. In this study, the authors demonstrated that the glycosphingolipid Gb3 modulates $\text{K}_\text{Ca}3.1$ expression in ECs via clathrin-dependent and early endosome antigen 1–enriched endosome-mediated lysosomal degradation (Figure). Specifically, inhibition of Gb3-induced $\text{K}_\text{Ca}3.1$ degradation through clathrin knockdown suggested that the $\text{K}_\text{Ca}3.1$ protein is internalized by Gb3 via a clathrin-dependent process (Figure). Furthermore, Gb3-induced $\text{K}_\text{Ca}3.1$ degradation was prevented by lysosome blockers, suggesting that Gb3 induces the lysosomal degradation of $\text{K}_\text{Ca}3.1$ (Figure).

To prove the hypothesis that accumulated Gb3 induces endothelial $\text{K}_\text{Ca}3.1$ degradation in $\alpha$-galactosidase A (Gla) knockout mice, the authors measured age-dependent changes in early endosome antigen 1, lysosomal-associated membrane protein 2, and $\text{K}_\text{Ca}3.1$ expression in ECs from Gla-knockout mice and compared them with those induced by exogenously added Gb3. Importantly, the authors found similar changes in the expression of $\text{K}_\text{Ca}3.1$, early endosome antigen 1, Rab5, and lysosomal-associated membrane protein 2 consistently in both aged Gla-knockout and Gb3-treated wild-type ECs. Finally, the authors concluded that accumulated Gb3 induces endothelial $\text{K}_\text{Ca}3.1$ degradation in Gla-knockout mice and subjects with Fabry disease. This finding is particularly important in the clinical setting.

Several studies have reported that enzymatic replacement therapy is of limited impact in the prevention of cardiovascular complications in Fabry disease. Abnormalities in arterial blood flow were reported in patients with Fabry disease. Here, the authors identified clathrin and Rab5 as a critical component in $\text{K}_\text{Ca}3.1$ degradation. Based on the present study, the abnormalities in Fabry disease can be explained through Gb3-induced impairment of endothelium-dependent vasodilation. Endothelial dysfunction leads to decreased endothelium-dependent vasodilation involving endothelial $\text{K}_\text{Ca}3.1$ dysfunction. $\text{K}_\text{Ca}3.1$ activation increases the endothelial Ca$^{2+}$-driving force and induces endothelium-dependent hyperpolarization of vascular smooth muscle cell. An increase in the Ca$^{2+}$-driving force increases Ca$^{2+}$ influx and thereby NO synthesis by activating Ca$^{2+}$-dependent endothelial NO synthase. Furthermore, the authors demonstrated that $\text{K}_\text{Ca}3.1$ recovery
by Rab5C knockdown leads to the recovery of Gb3-induced endothelial dysfunction. Thus, the authors demonstrated that Gb3-induced KCa3.1 degradation played a crucial role in endothelial dysfunction in a mouse model of Fabry disease and in Fabry disease in humans. Because of these findings, the authors proposed a novel mechanism underlying Gb3-mediated EC dysfunction, which is associated with the suppression of EDHF.

**Clinical Perspectives**

Physiological ROS levels are important in the regulation of EC function. The involvement of EC dysfunction in all stages of vascular diseases is generally accepted. Importantly, Choi et al. demonstrated that Gb3 significantly attenuates the activities of endothelial KCa3.1. However, several issues remain regarding the interpretation of this study. First, in the present study, among major glycosphingolipids, only Gb3 was confirmed to attenuate KCa3.1 activity in EC. Second, although the authors derived results by using primary cultured mouse aortic ECs and human umbilical vein ECs, the endothelial response varies according to blood vessel type. Therefore, the EC responses in smaller arteries are of interest because the contribution of EDHF is prominent, especially in microvessels rather than in large vessels. Third, the authors did not measure the effects of Gb3 on vascular smooth muscle cell. Further studies aimed at elucidating the mechanism of Gb3-mediated vascular dysfunction will provide an important clue to the treatment of Fabry disease.

**Sources of Funding**

This work was supported in part by the grants-in-aid for Scientific Research (21790698, 23659408, and 24390193), all of which are from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan, and the grants-in-aid for Scientific Research from the Ministry of Health, Labour, and Welfare, Tokyo, Japan (10102895).

**Disclosures**

None.

**References**


**Key Words:** Editorials ■ endothelium-dependent hyperpolarization factor ■ Fabry disease ■ hydrogen peroxide ■ oxidative stress ■ vascular diseases
Globotriaosylceramide Induces Endothelial Dysfunction in Fabry Disease
Kimio Satoh

Arterioscler Thromb Vasc Biol. 2014;34:2-4
doi: 10.1161/ATVBAHA.113.302744
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/34/1/2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/