

Dietary Apigenin in the Prevention of Endothelial Cell Dysfunction

Ned J. Lamb, PhD and Florence Gizard, PhD

This Commentary relates to the article by Kazuo Yamagata et al on pages 558–565.

The polyphenol apigenin (4', 5, 7-trihydroxyflavone, Figure 1) is a major dietary flavonoid found in many fruits (eg, oranges), vegetables (eg, celery, rutabagas, and onions), and herbs (eg, parsley, wheat sprouts, and chamomile tea). It has been identified as an active ingredient in traditional herbal medicines and wine.¹ Although numerous studies converge to highlight the anticarcinogenic properties of apigenin, its potential use against other chronic disorders such as cardiovascular diseases has recently received increased attention.

In mice or rats, apigenin was reported to prevent hyperlipidemia, weight gain, and atherosclerosis^{2,3} and favor postischemic functional recovery.⁴ Different types of vascular cells (monocytes/macrophages, vascular smooth muscle cells, and endothelial cells) were involved in the regulation of cholesterol homeostasis and inflammation by apigenin.^{2,3}

Yamagata et al and others have detailed the benefits of apigenin in endothelial dysfunction and diabetes-related vascular pathophysiology.^{2,5} In these studies, endothelial dysfunction was elicited by either palmitic acid, high glucose and/or tumour necrosis factor alpha, or reoxygenation after hypoxia. Several apigenin-elicited protective responses against endothelial dysfunction were identified: These involve I κ B kinase/NF κ B,⁴ estrogen receptor,⁶ lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1),⁵ apelin (an endogenous ligand for the G-protein-coupled APJ receptor) and fatty acid transport protein1,⁵ PKC β II, reactive oxygen species, and nitric oxide.^{1,5,7}

In the current issue (vol 00, pp 00), Yamagata et al further identify new inflammation and metabolic syndrome pathways regulated by apigenin in endothelial cells.

As previously, Yamagata et al have analyzed the effects of apigenin in a model endothelial cell line established from a human metastatic hemangiosarcoma (ISO-HAS). Although the transformed nature of these cells may present differences to wild-type endothelial cells, these tumor cells retain several authentic endothelial-cell properties including gene expression of characteristic markers such as von Willebrand factor, CD31, vascular endothelial growth factor (VEGF) and its 2 receptors VEGF receptor-1 (VEGFR-1) and VEGFR-2 (also known as kinase insert domain receptor, KDR). Breaking new ground, cells were treated with trimethylamine N-oxide (TMAO), a metabolite derived from the gut microbiota inducing vascular inflammation. The authors found that in this context, apigenin decreases the gene and protein expression of the scavenging receptor LOX-1, the adhesion molecule ICAM-1 and the inflammasome factor NLRP3 [nucleotide-binding oligomerization domain-like receptor (NLR) pyrin-containing receptor 3, also named cryopyrin]. Corroborating previous studies,¹ the uptake of acetylated LDL by endothelial cells and adhesion of the human monocytic cell line U937 to these cells also decreased. The potential contributions of NLRP3 in the production of inflammasome-related cytokines (eg, IL-1 β and IL-18), adhesion molecules, and/or scavenger receptors still remain to be investigated.

Received for publication July 5, 2019; accepted July 16, 2019.

From the Mammalian Cell Biology group, Institute of Human Genetics UMR 9002, CNRS-University of Montpellier, Montpellier cedex 05, France.

The authors report no conflicts of interest.

Comment on "Dietary apigenin reduces induction of LOX-1 and NLRP3 expression, leukocyte adhesion, and acetylated low-density lipoprotein uptake in human endothelial cells exposed to trimethylamine-N-oxide" *J Cardiovasc Pharmacol*.

Reprints: Florence Gizard, PhD, Mammalian Cell Biology group, Department of Genetics and Development, Institute of Human Genetics UMR 9002, 141 Rue de la Cardonille, 34090 Montpellier, France (e-mail: FGizard@yahoo.com).

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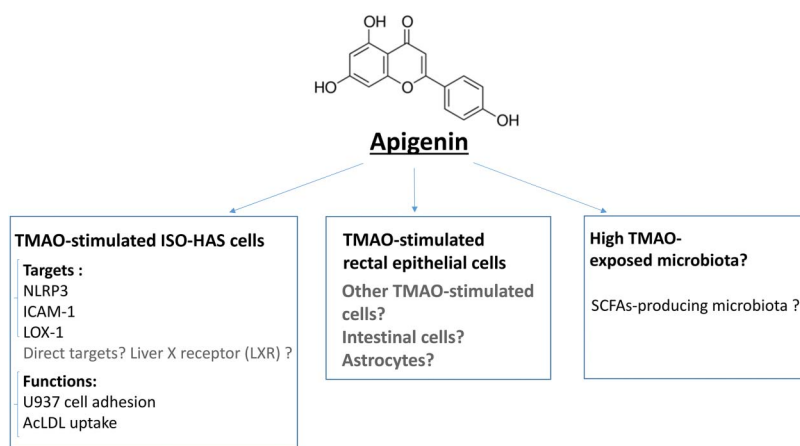


FIGURE 1. Interactions between apigenin- and TMAO-stimulated pathways: results from this study and further questions. SCFAs, short chain fatty acids.

In addition to genes encoding LOX-1, ICAM-1 and NLRP3, the authors show that other genes are inversely regulated by TMAO and apigenin. These encode the adhesion and chemotactic VCAM-1 and MCP-1, the cytokine and/or scavenger receptors SCARF-1 and CXCL16, and the inflammasome regulators PYCARD and TXNIP. Whether these regulations translate to protein levels remains to be studied. In line with this, further studies are warranted to assess the potential contributions of LOX-1 and other scavenger receptors in apigenin-dependent regulation of acetylated LDL uptake. Finally, the occurrence of these regulatory pathways in primary endothelial cells may also be ascertained.

There is no doubt that the use of TMAO in this study will open the route to more discoveries in the cardiovascular field and beyond. TMAO is a molecule generated from dietary phosphatidylcholine (found in meat, eggs, fish, and crustaceans) and L-carnitine (found in red meat) through gut microbial metabolism: Choline from phosphatidylcholine and L-carnitine are metabolized by trimethylamine (TMA) lyase into TMA, which is further oxidized in the liver by flavin-containing monooxygenases (FMO) into circulating TMAO. Plasma TMAO levels have been associated with an increased risk of atherosclerosis and major adverse cardiovascular events.⁸ Concurring with this, this study identifies numerous TMAO-target genes that are involved in atherosclerotic processes.

Considering the current knowledge of structure–activity relationships of flavonoids,⁵ the present report also prompts further studies aiming to identify the molecule(s) directly interacting with apigenin in TMAO-treated endothelial cells (Figure 1). They may represent (a) novel therapeutic target(s) against endothelial dysfunction and TMAO-dependent atherosclerosis.

After the previous report of Fischer et al showing a cooperative interaction between apigenin and TMAO pathways in the correction of Cl-transport defect in a mouse model of cystic fibrosis,⁹ the present data also encourage studies evaluating the interactions between both pathways at other sites (Figure 1).

Importantly, apigenin might also influence atherosclerotic development at the microbiota level. It inhibits the growth of several laboratory strains of model bacterial species and also affects the relative abundance of bacteria in a fecal

inoculum simulating a human colon microbial community.¹⁰ This latest effect was associated with an increased production of short-chain fatty acids, mainly acetate, propionate, and butyrate. These short-chain fatty acids have been shown to display beneficial effects on inflammatory and metabolic disorders, notably atherosclerosis.⁸ Interestingly, in ApoE-deficient mice fed a high-fat/cholesterol diet, an increase in the microbial production of acetate after supplementation with plant sterol ester was accompanied by a decrease in the production of TMA (precursor of TMAO), serum total cholesterol, and atherosclerosis.

Apigenin efficiency may depend on its *in vivo* metabolic transformation,¹¹ and its absorption and metabolism in humans is currently being investigated. Clinical trials also evaluate its potential health benefits when administered alone or in association against insomnia, cancer, and allergic rhinoconjunctivitis in pediatrics (ClinicalTrials.gov).

By highlighting molecular pathways targeted by apigenin, the present report further emphasizes the need to consider new formulation(s) containing apigenin or a derivative that may prevent atherosclerosis and associated disorders.⁸ Given the variability of the average daily intake of major flavones, including apigenin, throughout the world,¹⁰ it also encourages epidemiologic studies and public action to favor adequate consumption.

ACKNOWLEDGMENTS

The authors express their deep gratitude to Dr Jonathan Clifton (Université Polytechnique Hauts-de-France) and Dr Anne Fernandez (Mammalian Cell Biology group, Institut de Génétique Humaine UMR 9002, Montpellier) for their critical proofreading of the article.

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