News Release

Title

Endothelial cellular senescence is inhibited by liver X receptor activation with an additional mechanism for its atheroprotection in diabetes

Key Points

- Our studies demonstrate that the regulation of endothelial cellular senescence emerges as a new additional mechanism underlying the anti-atherogenic properties of liver X receptor (LXR) agents. Furthermore, our data suggest that combination therapy with LXR agents and metformin may provide a clinically-useful therapeutic strategy to alleviate a LXR activation-mediated adverse effect on liver triglyceride metabolism.
- The beneficial effects of LXR activation appear to be reduced reactive oxygen species levels and increased endothelial nitric oxide synthase activity, both of which will lead to increased nitric oxide actions.
- Among the elderly population, atherosclerosis is a growing problem, leading to an increased risk of mortality by cardiovascular events. Adoption of combination therapy with LXR agents and widely-prescribed metformin may be of clinical significance.

Summary

Senescence of vascular endothelial cells leads to endothelial dysfunction and contributes to the progression of atherosclerosis. Liver X receptors (LXRs) are nuclear receptors whose activation protects against atherosclerosis by transcriptional regulation of genes important in promoting cholesterol efflux and inhibiting inflammation. Here we found that LXR activation with specific ligands reduced the increase in senescence-associated-β-galactosidase (SA-β-gal) activity, a senescence marker, and reversed the decrease in telomerase activity, a replicative senescence marker, in human endothelial cells under high glucose. This effect of LXR activation was associated with reduced reactive oxygen species and increased endothelial nitric oxide synthase activity. A series of experiments using small interfering RNAs indicated that LXRβ mediates the prevention of endothelial cellular senescence, and that sterol regulatory element binding protein-1 (SREBP-1), which was up-regulated as a direct LXRβ target gene, may act as a brake of endothelial cellular senescence. Although oral administration of the LXR ligand led to severe fatty liver in diabetic rats, concomitant therapy with metformin avoided the development of hepatic steatosis. However, the preventive effect of the LXR ligand on SA-β-gal-stained cells in diabetic aortic endothelium was preserved even if metformin was co-administered. Taken together, our studies demonstrate that a new additional mechanism, such as the regulation of endothelial cellular senescence, is related to the anti-atherogenic properties of LXRs, and concomitant treatment with metformin may provide a clinically-useful therapeutic strategy to alleviate a LXR activation-mediated adverse effects on liver triglyceride metabolism.

Research Background

Nuclear receptors are ligand-activated transcription factors that play an important role in the regulation of cellular metabolic function such as lipid and glucose metabolism. Dysregulation of these processes causes development of metabolic diseases such as hyperlipidemia, diabetes, and cardiovascular disease. In humans, 48 different types of nuclear receptors have been identified. These include: the receptor for a metabolite of vitamin A, retinoic acid, retinoic acid receptor (RAR); the vitamin D receptor (VDR); the fatty acid receptor, peroxisome proliferator-activated receptor γ (PPARγ); the oxysterol receptor, liver X receptor (LXR); and their obligate heterodimeric partner, the retinoid X receptor (RXR). LXRs act as potent transcriptional switches for the coordinated regulation of genes involved in the control of hepatic lipid and cholesterol metabolism, and have a crucial role in reverse cholesterol transport, thereby stimulating of cholesterol efflux from the peripheral tissue to the liver. However, most LXR agonists, while lowering cholesterol, have the concomitant induction of lipogenic genes that leads to hypertriglyceridemia and liver steatosis, which have limited further clinical development.

Endothelial cellular senescence is an important contributor to the pathogenesis of age-associated vascular disorders. The senescent phenotype of endothelial cells can be transformed from anti-atherosclerotic to pro-atherosclerotic. We have also shown that senescent endothelial cells are observed in human atherosclerotic lesions but not in non-atherosclerotic lesions. Furthermore, endothelial cellular senescence is considered as an important cause of diabetes-associated vascular aging. Hyperglycemia is well established to accelerate senescence in endothelial cells. Accordingly, prevention of high glucose-associated endothelial cellular senescence may be a new potential target to arrest the development of atherosclerosis in diabetes.

Very little is known about the precise role of nuclear receptors in the regulation of endothelial cellular senescence.

Research Results

Specific ligands for eight representative nuclear receptors were given to human umbilical venous endothelial cells (HUVECs) cultured for 72 h under a high glucose condition (22 mM), and their effects on high glucose-induced endothelial cellular senescence were examined using SA-β-gal as a quantitative indicator of senescence. The VDR agonist calcitriol (vitamin D3), the famesoid X receptor (FXR) agonist GW4064, the RAR agonist all-trans-retinoic acid (ATRA), the PPARα agonist Wy-14643, the PPARδ agonist GW501516, the PPARγ agonist rosiglitazone, and the RXR agonist bexarotene did not prevent the increase in SA-β-gal activity that was induced by high glucose conditions. In contrast, the LXR-activating ligands T0901317 and GW3965 significantly reduced SA-β-gal activity under high glucose. Telomerase activity was significantly decreased in a high glucose environment, and T0901317 significantly prevented the decrease in telomere length, suggesting that LXR activation can suppress a process termed replicative senescence, a limitation in the number of times that somatic cells can divide.

In HUVECs, we observed that T0901317 and GW3965 strikingly up-regulated the ABCA1 gene not only under normal but also high glucose conditions. On Western blots, expression of the ABCA1 protein was evidently increased by T0901317 and GW3965 in HUVECs under normal glucose conditions.

Increased reactive oxygen species (ROS) and decreased endothelial nitric oxide synthase (eNOS) under high glucose play a critical role in endothelial cellular senescence. The LXR agonist T0901317 prevented the increase in ROS-induced intracellular fluorescence under high glucose conditions, which was completely blocked by the LXA antagonist 5CPPSS-50. T0901317 also showed increases in eNOS expression and phosphorylation, and caveolin-1 expression, which were damped under high glucose. LXRs consist of LXRα (NR1H3) and LXRβ (NR1H2). LXRβ is ubiquitously expressed, whereas LXRα expression is highest in the liver, kidney, intestine, and adrenal ligand. LXRβ is functionally important in vascular endothelial cells and mediates the prevention of endothelial cellular senescence. T0901317 significantly reduced SA-β-gal activity under high glucose regardless of whether ABCA1 or ABCG1 siRNAs were applied. Transfection of SREBP-1 siRNAs greatly augmented high glucose-stimulated SA-β-gal activity, suggesting that SREBP-1 may act as a brake on endothelial cellular senescence.
Oral administration of T0901317 (20 mg/kg) for 7 days did lead to severe fatty liver in streptozotocin (STZ)-induced diabetic rats, which STZ was injected 7 days before oral administration. However, when metformin (50 mg/kg), widely given to patients with type 2 diabetes, was concomitantly used, hepatic steatosis did not apparently occur upon treatment with T0901317. Plasma glucose levels are 428.3±21.9, 403.0±43.9, 391.2±39.0, and 352.2±39.9mg/dl in STZ, STZ+metformin, STZ+ T0901317 and STZ+metformin+T0901317 rats (no significant differences). In diabetic rats, significant SA-β-gal-stained cells in the aortic endothelium were detected. Diabetic rats treated with T0901317 exhibited a significantly decreased ratio of SA-β-gal-stained cells regardless of whether metformin was coadministered.

We also examined the combined effect of T0901317 and metformin using Zucker diabetic fatty rats (ZDFs), an insulin-resistant type 2 diabetes model. Oil red O staining showed lipid accumulation in the aortic section from ZDFs fed a high-fat diet. In addition, consumption of a high-fat diet resulted in increased vascular cell adhesion molecule-1 (VCAM-1) expression in the aortas of ZDFs. Treatment with T0901317 effectively reduced lipid deposition and VCAM-1 expression in the aortas of ZDFs on a high-fat diet, regardless of co-administration of metformin.

Research Summary and Future Perspective

Activation of LXRs can prevent endothelial cellular senescence accelerated by hyperglycemia. This beneficial effect on endothelial cellular senescence would be a new additional mechanism by which LXRs inhibit the development of atherosclerosis. We believe that LXRs could be a

good target for the development of therapy to limit atherosclerosis in diabetes. Metformin can contribute to a reduction in LXR-mediated hepatic steatosis. Therefore, combination treatment with metformin may hold the promise for the key to the clinical use of LXR-activating ligands.

Toshio Hayashi, Hitoshi Kotani, Tomoe Yamaguchi, Kumiko Taguchi, Mayu Iida, Koichiro Ina, Morihiko Maeda, Masafumi Kuzuya, Yuichi Hattori and Louis J. Ignarro. Endothelial cellular senescence is inhibited by liver X receptor activation with an additional mechanism for its atheroprotection in diabetes. *Proceedings of the National Academy of Sciences of the United States of America 2014(To be published on Jan. 7,2014)*

Japanese ver.

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