involved in the pathogenesis of vascular calcification. VPO1 utilizes chloride through HO2 to produce HOCl, which possesses a powerful oxidizing capacity and will further accelerate oxidative stress. The aims of this study were to explore whether VPO1 plays a role in osteogenic differentiation of VSMCs induced by oxidized low-density lipoprotein (ox-LDL) and the underlying mechanism.

**METHODS**

VSMCs were cultured in medium supplemented with ox-LDL for 10 days. The role of VPO1 was assessed by knockdown with small interfering RNA (siRNA). Osteogenic transdifferentiation was assessed by gene expression, matrix mineralization and alkaline phosphatase activity.

**RESULTS**

We found that ox-LDL accelerated mineralization, increased alkaline phosphatase (ALP) activity, promoted a phenotypic switch of VSMC from contractile to osteogenic phenotype up-regulated the expression of VPO1 in VSMCs in a concentration- and time-dependent manner, increased the generation of HOCl and the phosphorylation of AKT, while knockdown of VPO1 in VSMCs potently suppressed ox-LDL induced osteogenic differentiation of VSMCs, HOCl production and AKT phosphorylation. Furthermore, HOCl treatment facilitated phosphorylation of AKT and runt-related transcription factor 2 (Runx2) expression, which is a key osteogenic transcription factor. Preincubation with the LY294002 (a specific inhibitor of PI3K) significantly inhibited ox-LDL and HOCl-induced up-regulation expression of Runx2.

**CONCLUSIONS**

VPO1 promotes vascular calcification by accelerating ox-LDL-induced osteogenic transition of VSMCs through VPO1/HOCl/P3K/AKT/Runx2 signaling pathway, these results firstly suggest that VPO1 may act as a novel endogenous regulator of vascular calcification.

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**GW26-e2333**

**Study About the Mechanism of Intimal Calcification in Diabetic Atherosclerosis of ApoE-/- Mice**

Xiaoyu Yang,1 Zhongqun Wang2

1Department of Pathology, Xinning Medical College, Xinning, Henan;
2Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang

**OBJECTIVES**

To investigate the role of endoplasmic reticulum stress (ERS)-mediated apoptosis in atherosclerotic calcification of apoE−/− mice.

**METHODS**

Male apoE−/− mice were first rendered diabetic by the administration of 5 daily intraperitoneal injections of streptozocin (STZ, 40 mg/kg), and then given a semi-synthetic high-fat diet (HFD) plus daily injections of CML (10 mg/kg/day). The mice were euthanized and analyzed at 0 month (group 0M, n=10), 2 months (group 2M, n=10), 4 months (group 4M, n=10), and 4 months (group 4M, n=10) after the triple administrations of STZ-CML-HFD.

**RESULTS**

Morbid analysis showed that early atherosclerotic plaques appeared 2 months after the triple administrations of STZ-CML-HFD, and that typically advanced plaques with extensive calcified lesions, abundant cholesterol crystals, and proliferative collagen were formed 4 months after the triple administrations of STZ-CML-HFD. The intrinsic phenotype of aortic smooth muscle cells was gradually lost and osteoblast-like phenotypes (BMP-2, cbfβ) were increased. Furthermore, CML deposition signals and the expression of receptor for advanced glycation end-products (RAGE) in the aortic wall were mainly restricted in the atherosclerotic plaques. Western blot assay showed the expression of CML, RAGE and CD36 in aortic wall of diabetic apoE−/− mice was significantly up-regulated, but the expression of ABCA1 firstly displayed a compensated increase and then reduced near the baseline. Experiment of TUNEL staining and cleaved caspase-3 immunohistochemical staining found intra-plaque apoptotic rate rised with the progression of diabetic atherosclerosis. Immunohistochemical location analysis showed GFP, a molecular chaperone of endoplasmic reticulum stress and CHOP were mainly restricted in the lipid poor of atherosclerosis. Furthermore, compared with CHOP, the distribution signal of GRP78 in group 4M appeared more basal in lipid pool. With the extension of diabetic course in apoE−/− mice, related indexes of endoplasmic reticulum stress (ERP) increased and the aortic wall were mainly restricted in the atherosclerotic plaques. Western blot assay showed the expression of CML, RAGE and CD36 in aortic wall of diabetic apoE−/− mice was significantly up-regulated, but the expression of ABCA1 firstly displayed a compensated increase and then reduced near the baseline. Experiment of TUNEL staining and cleaved caspase-3 immunohistochemical staining found intra-plaque apoptotic rate rised with the progression of diabetic atherosclerosis. Immunohistochemical location analysis showed GFP, a molecular chaperone of endoplasmic reticulum stress and CHOP were mainly restricted in the lipid poor of atherosclerosis. Furthermore, compared with CHOP, the distribution signal of GRP78 in group 4M appeared more basal in lipid pool. With the extension of diabetic course in apoE−/− mice, related indexes of endoplasmic reticulum stress (ERP) increased and the aortic wall were mainly restricted in the atherosclerotic plaques.

**CONCLUSIONS**

Early- and apoE−/− mice which were first rendered diabetic by the administration of 5 daily intraperitoneal injections of streptozocin (STZ, 40 mg/kg), and then given a semi-synthetic high-fat diet (HFD) plus daily injections of CML (10 mg/kg/day). The mice were euthanized and analyzed at 0 month (group 0M, n=10), 2 months (group 2M, n=10), 4 months (group 4M, n=10), and 4 months (group 4M, n=10) after the triple administrations of STZ-CML-HFD.

**GW26-e2359**

**Study About the Mechanism of Intimal Calcification in Diabetic Atherosclerosis of ApoE-/- Mice**

Xiaoyu Yang,1 Zhongqun Wang2

1Department of Pathology, Xinning Medical College, Xinning, Henan;
2Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang

**OBJECTIVES**

To investigate the role of endoplasmic reticulum stress (ERS)-mediated apoptosis in atherosclerotic calcification of apoE−/− mice.

**METHODS**

Male apoE−/− mice were first rendered diabetic by the administration of 5 daily intraperitoneal injections of streptozocin (STZ, 40 mg/kg), and then given a semi-synthetic high-fat diet (HFD) plus daily injections of CML (10 mg/kg/day). The mice were euthanized and analyzed at 0 month (group 0M, n=10), 2 months (group 2M, n=10), 4 months (group 4M, n=10), and 4 months (group 4M, n=10) after the triple administrations of STZ-CML-HFD.

**RESULTS**

Morbid analysis showed that early atherosclerotic plaques appeared 2 months after the triple administrations of STZ-CML-HFD, and that typically advanced plaques with extensive calcified lesions, abundant cholesterol crystals, and proliferative collagen were formed 4 months after the triple administrations of STZ-CML-HFD. The intrinsic phenotype of aortic smooth muscle cells was gradually lost and osteoblast-like phenotypes (BMP-2, cbfβ) were increased. Furthermore, CML deposition signals and the expression of receptor for advanced glycation end-products (RAGE) in the aortic wall were mainly restricted in the atherosclerotic plaques. Western blot assay showed the expression of CML, RAGE and CD36 in aortic wall of diabetic apoE−/− mice was significantly up-regulated, but the expression of ABCA1 firstly displayed a compensated increase and then reduced near the baseline. Experiment of TUNEL staining and cleaved caspase-3 immunohistochemical staining found intra-plaque apoptotic rate rised with the progression of diabetic atherosclerosis. Immunohistochemical location analysis showed GFP, a molecular chaperone of endoplasmic reticulum stress and CHOP were mainly restricted in the lipid poor of atherosclerosis. Furthermore, compared with CHOP, the distribution signal of GRP78 in group 4M appeared more basal in lipid pool. With the extension of diabetic course in apoE−/− mice, related indexes of endoplasmic reticulum stress (ERP) increased and the aortic wall were mainly restricted in the atherosclerotic plaques. Western blot assay showed the expression of CML, RAGE and CD36 in aortic wall of diabetic apoE−/− mice was significantly up-regulated, but the expression of ABCA1 firstly displayed a compensated increase and then reduced near the baseline. Experiment of TUNEL staining and cleaved caspase-3 immunohistochemical staining found intra-plaque apoptotic rate rised with the progression of diabetic atherosclerosis. Immunohistochemical location analysis showed GFP, a molecular chaperone of endoplasmic reticulum stress and CHOP were mainly restricted in the lipid poor of atherosclerosis. Furthermore, compared with CHOP, the distribution signal of GRP78 in group 4M appeared more basal in lipid pool. With the extension of diabetic course in apoE−/− mice, related indexes of endoplasmic reticulum stress (ERP) increased and the aortic wall were mainly restricted in the atherosclerotic plaques.