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Title: Effect of exendin-4 on human umbilical vein endothelial cells with endoplasmic reticulum stress

作者: 胡小春; 刘浩宇; 覃数; 张冬颖; 刘剑
重庆医科大学附属第一医院心血管内科

Author(s): Hu Xiaochun; Liu Haoyu; Qin Shu; Zhang Dongying; Liu Jian
Department of Cardiology, First Affiliated Hospital, Chongqing Medical University, Chongqing, 400016, China

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摘要: 目的 研究exendin-4（Ex-4）对人脐静脉内皮细胞（human umbilical vein endothelial cells，HUVECs）内质网应激相关凋亡的拮抗作用。 方法 RPMI1640培养基培养HUVECs，待细胞长到培养瓶底部80%左右后，分4组干预细胞，每组8瓶，共32瓶。实验分组：①正常对照组（*n*=8）：不做任何处理；②Ex-4组（*n*=8）：（Ex-4，8 μg/mL，45 min）；③EPBS组（*n*=8）：（EPBS，100 μL/mL，30 min）；④双药组（*n*=8）：Ex-4预处理（8 μg/mL，45 min），后加EPBS（100 μL/mL，30 min）。免疫荧光法检测确认HUVECs上存在胰高血糖素样肽-1受体（glucagon-like peptide-1 receptor，GLP-1R）；内源性过氧化氢酶抑制物（endogenous peroxidase blocking solution，EPBS）诱导HUVECs的凋亡，流式细胞仪检测细胞凋亡率变化；加入Ex-4预处理后，观察EPBS诱导HUVECs凋亡率的变化。Western blot检测细胞Calnexin、（C/EBP homologous protein，

CHOP）和Bax表达情况，探讨内质网应激相关凋亡的机制。　结果　① HUVECs上有GLP-1受体表达；②与正常对照组相比，EPBS组HUVECs的凋亡率显著增加[（52.27±3.66）%*vs*（7.25±0.62）%，*P*<0.01）]；与EPBS组比较，Ex-4的预处理可明显降低EPBS诱导的HUVECs凋亡[（37.77±2.86）% *vs*（52.27±3.66）%，*P*<0.01）]。与正常对照组相比，EPBS能增加Calnexin [（1.76±0.16）*vs*（1.00±0.05），*P*<0.01]、CHOP [（4.58±0.32）*vs*（1.00±0.12），*P*<0.01]和Bax [（2.25±0.10）*vs*（1.00±0.06），*P*<0.01]的表达；与EPBS组比较，Ex-4的预处理可显著降低Calnexin [（0.42±0.05）*vs*（1.76±0.16），*P*<0.01]、CHOP [（0.64±0.10）*vs*（1.65±0.12），*P*<0.01]和Bax[（1.48±0.08）*vs*（2.25±0.10），*P*<0.01）]的表达。　结论　Ex-4对EPBS诱导的HUVECs凋亡有明显抑制作用，其抑制作用可能与EX-4减少内质网应激相关蛋白Calnexin、CHOP和Bax相关。

Abstract: Objective To study the antagonistic effect of exendin-4 (Ex-4) on human umbilical vein endothelial cells (HUVECs) with endoplasmic reticulum (ER) stress-induced cell apoptosis. Methods HUVECs were cultured with RPMI 1640 medium. When the HUVECs grew to around 80% at the bottom of culture flasks, the cells were divided into 4 groups (*n*=8) including: a control group (without any treatment), an Ex-4 group (Ex-4, 8 μg/ml for 45 min), an endogenous peroxidase blocking solution (EPBS) group (EPBS, 100 μL/mL for 30 min), and an Ex-4+EPBS group [Ex-4 pretreatment (8 μg/mL for 45 min) and EPBS treatment (100 μL/mL for 30 min)]. The glucagon-like peptide-1 receptor (GLP-1R) was verified by immunofluorescence method. The cell apoptosis was detected by flow cytometry. The expression of calnexin, C/EBP homologous protein (CHOP) and Bax were detected by Western blotting. Results GLP-1R existed in HUVECs. EPBS could significantly increase the apoptosis of HUVECs compared with the control group [(52.27±3.66)% *vs* (7.25±0.62)%, *P*<0.01)]. The rate of cell apoptosis in the Ex-4+EPBS group was significantly lower than that in the EPBS group [(37.77±2.86)% *vs* (52.27±3.66)%, *P*<0.01)]. Compared with the control group, EPBS group could increase the expression of calnexin [(1.76±0.16) *vs* (1.00±0.05), *P*<0.01], CHOP [(4.58±0.32)*vs*. (1.00±0.12), *P*<0.01] and Bax [(2.25±0.10) *vs* (1.00±0.06), *P*<0.01]. Compared with the EPBS group, Ex-4 group could significantly reduce the expression of calnexin [(0.42±0.05) *vs* (1.76±0.16), *P*<0.01], CHOP [(0.64±0.10)*vs* (1.65±0.12), *P*<0.01] and Bax [(1.48±0.08) *vs* (2.25±0.10), *P*<0.01)]. Conclusion Ex-4 can inhibit EPBS-induced apoptosis in HUVECs through down-regulating the expression of ER stress-related proteins such as calnexin, CHOP and Bax.

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