低氧/厌氧产品应用案例——糖氧剥夺（OGD）模型

文章题目： Activation of Wnt3α/β-catenin signal pathway attenuates apoptosis of the cerebral microvascular endothelial cells induced by oxygen-glucose deprivation 激活 WNT3α/β-catenin 信号通路可减轻缺氧缺糖诱导的脑微血管内皮细胞凋亡。

文章出处： 西安交通大学神经生物学研究所，Biochem. Biophys. Res. Commun. 2017 08 19;490(2) DOI：10.1016/j.bbrc.2017.03.130
工作站使用情况：bugbox

摘要： Brain microvascular endothelial cells (BMECs) play vital roles in cerebral ischemia, during which many signal pathways mediate BMECs apoptosis. In this study, we explored the potential role of Wnt3α/β-catenin signal in BMECs apoptosis induced by ischemia. Here, we found that oxygen-glucose deprivation (OGD) could induce apoptosis of BMECs with Wnt3a mRNA expression decrease. Meanwhile, activation Wnt3a/β-catenin signal with exogenous Wnt3α protein (100 ng/ml) or Lithium Chloride (LiCl, 4 mM) decreased significantly apoptosis of BMECs induced by OGD with increasing expression of Bcl-2 in the whole cell and β-catenin in the nucleus. But, inhibition Wnt3a/β-catenin signal with DKK1 (100 ng/ml) or 2.4-diamino quinazoline (DQ, 0.2 μM) increased apoptosis of BMECs with decreasing expression of Bcl-2. These results suggest that activation Wnt3α/β-catenin signal attenuate apoptosis of BMECs induced by ischemia.

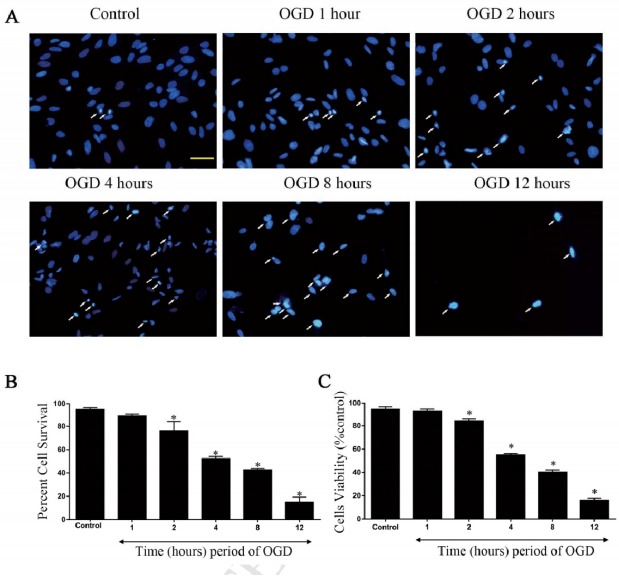

Fig.1. Time-dependent effect of oxygen and glucose deprivation(OGD)on rat brain microvascular endothelial cells (BMECs) apoptosis and viability.
(A) Representative fluorescence photomicrographs of rat BMECs with nuclear fragmentation

stained with Hoechst 33258 exposed to OGD for 0hour (control group), 1hour, 2hours, 4hours, 8hours, and 12hours (arrow). (B) Quantification of BMECs percent survival data using A showed that cell survival decreased by OGD in the time dependence. (C) MTT assay also showed that cell viability decreased by OGD in the time dependence. Results represent mean and SEM from the three different independent experiments (*p < 0.05 vs. Control, Control group: normal culture, Scale bar represents 25μm).

伴随着 OGD 时间的增加，BMECs 的凋亡增加，存活率降低。结果表明糖氧剥夺(OGD)可诱导 BMECs 凋亡。

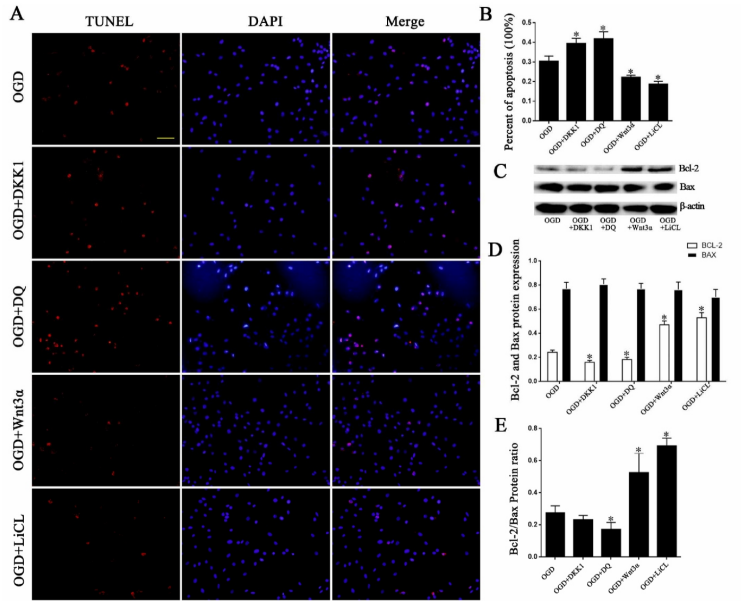

Fig.2. Effect of Wnt/β-catenin signal pathway agonists or antagonists on BMECs apoptosis induced by ODG 4hours. A. Representative fluorescence photomicrographs for TUNEL-positive cells (red, TUNEL-positive cells and blue, DAPI; scale bar, 40μm). B. Apoptotic cells were quantified and expressed as the percentage of TUNEL-positive cells to DAPI-positive cells. The quantification illustrated that percentage of TUNEL-positive cells induced by ODG 4hours increased in the treatment with antagonists of DKK1 (100ng/ml) or DQ (0.20μM), but decreased in the treatment with agonists of Wnt3α (100ng/ml) or LiCl (4mM). C.D.E. Relative protein levels of Bcl2 and ratio of Bcl2/Bax determining by western blot decreased in treatment with the antagonists of DKK1 (100ng/ml) or DQ (0.20μM), increased in the treatment with agonists of Wnt3α (100ng/ml) or LiCl (40mM). Results represent mean and SEM from the three different independent experiments (*p < 0.05 vs. OGD, OGD group: OGD culture for 4hours).

外源性 Wnt3α蛋白(100 ng/ml)或氯化锂(LiCl,4mM)能够激活 Wnt3α/β-catenin 信号，明显减少 OGD 诱导的 BMECs 凋亡，增加 Bcl-2 的表达。但 DKK1(100 ng/ml)或 DQ(0.2 ng/ml)抑制 Wnt3a/β-catenin 信号，并增加 BMECs 凋亡，降低 Bcl-2 的表达。表明激活 Wnt3α/β-catenin 信号可减轻缺血诱导的 BMECs 凋亡。

脑微血管内皮细胞(BMECs)在脑缺血中起重要作用，在此过程中有多条信号通路介导 BMECs 凋亡。我们研究了激活或抑制 Wnt /β-catenin 信号对体外缺氧和缺糖诱导的 BMEC 细胞凋亡的影响，这可能为脑缺血性损伤提供了一种潜在的治疗策略。

文章题目：Brusatol Protects HepG2 Cells against Oxygen-Glucose Deprivation-Induced Injury via Inhibit – ing Mitochondrial Reactive Oxygen Species- Induced Oxidative Stress 布鲁塞尔醇通过抑制线粒体活性氧诱导的氧化应激保护 HepG2 细胞免受缺氧缺糖诱导的损伤

文章出处： 苏州大学附属第一医院普外科，Pharmacology 2019 Dec 11; DOI：10.1159/000504482

BACKGROUND:It has been reported that brusatol (BRU) reduces cellular reactive oxygen species (ROS) level under hypoxia; here the protective effect of BRU against oxygen-glucose deprivation/reoxygenation (OGD-R)-induced injury in HepG2 cells and against anoxia/reoxygenation (A/R)-induced injury in rat liver mitochondria was investigated.

MATERIALS AND METHODS:OGD-R-induced HepG2 cell viability loss was detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide and trypan blue staining. Mitochondrial ROS level in HepG2 cells was measured by MitoSOX staining. The cellular malondialdehyde and adenosine triphosphate level was measured by commercial kits. The mitochondrial membrane potential in HepG2 cells was measured by JC-1 staining. The protein level was detected by Western blotting. Rat liver mitochondria were separated by differential centrifugation. A/R-induced injury in isolated rat liver mitochondria was established by using a Clark oxygen electrode. The ROS generation in isolated mitochondria was evaluated using Amplex red/horseradish peroxidase.

RESULTS:BRU reduced mitochondrial ROS level and alleviated oxidative injury in HepG2 cells, thereby significantly inhibited OGD-R-induced cell death. During OGD-R, BRU improved mitochondrial function and inhibited the release of cytochrome c. Furthermore, BRU showed a clear protective effect against A/R-induced injury in isolated rat liver mitochondria. When isolated rat liver mitochondria were pretreated with BRU, A/R-induced ROS generation was significantly decreased, and mitochondrial respiratory dysfunction was ameliorated.

CONCLUSIONS:BRU pretreatment attenuated OGD-R-induced injury in HepG2 cells and A/R-induced injury in isolated rat liver mitochondria by inhibiting mitochondrial ROS-induced oxidative stress.

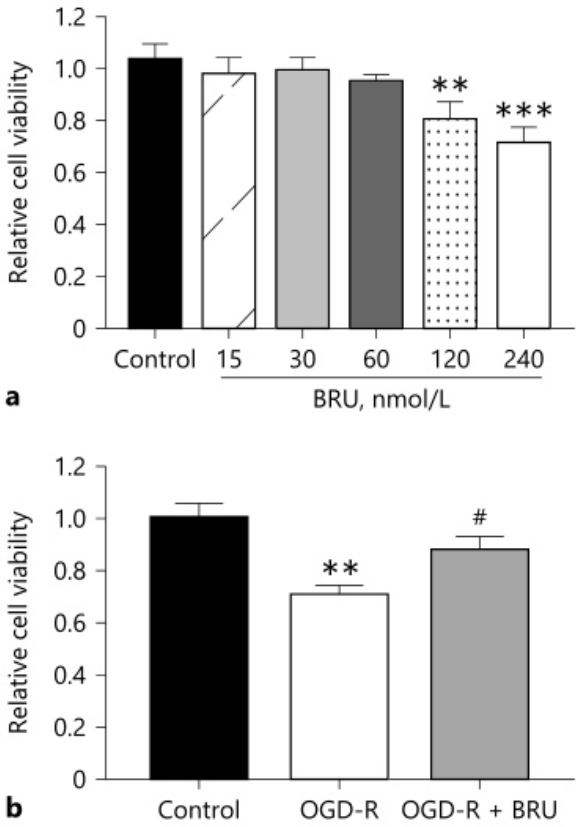

Fig. 1. BRU protected HepG2 cells against OGD-R-induced injury.
a HepG2 cells were treated with various concentrations of BRU for 24 h, and cell viability was detected by MTT assay. Data are expressed as mean ± SD (n = 6). **p < 0.01, ***p < 0.001 versus control. b HepG2 cells were exposed to 60 nmol/L BRU and then were treated with OGD-R, and the viability of HepG2 cells was detected by MTT assay. Data are expressed as mean ± SD (n = 6). **p < 0.01 versus control, # p < 0.05 versus OGD-R-treated group.BRU, brusatol; OGD-R, oxygen-glucose deprivation /re – oxygenation

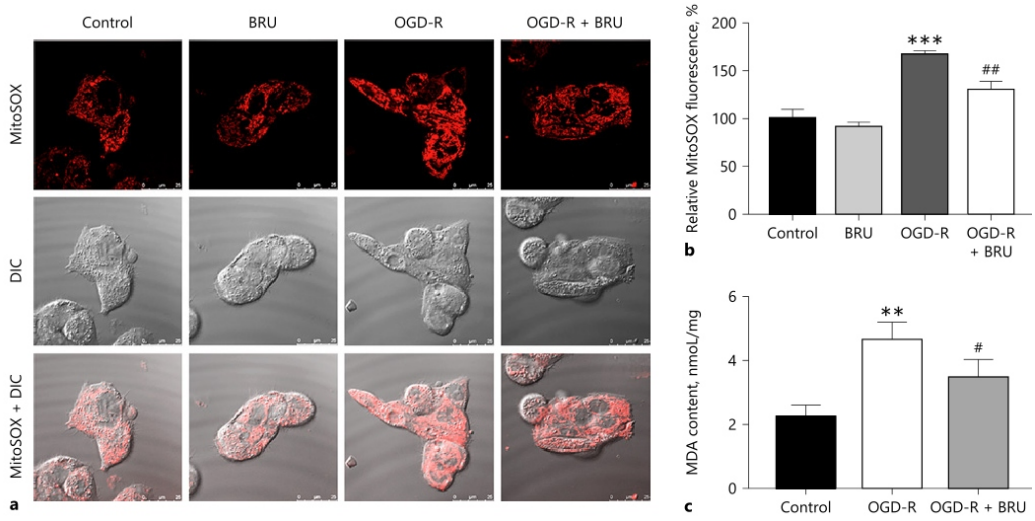

Fig.2. BRU decreased mitochondrial ROS level induced by OGD-R and alleviated cellular oxidative stress injury in HepG2 cells.
a HepG2 cells were exposed to 60 nmol/L BRU and then were treated with OGD-R and mitochondrial ROS level was analyzed by MitoSOX staining using a confocal microscopy. Cells were also observed by DIC microscopy. b Quantitative analysis of mitochondrial ROS level

described in (a). Data are expressed as means ± SD (n = 3). *** p < 0.001 versus control, ## p < 0.05 versus OGD-R-treated group. c HepG2 cells were exposed to 60 nmol/L BRU and then were treated with OGD-R, and the cellular content of MDA was detected. Data are expressed as means ± SD (n = 6). *** p < 0.001 versus control, # p < 0.05 versus OGD-R-treated group. DIC, differential interference contrast; BRU, brusatol; OGD-R, oxygen-glucose deprivation/re oxygenation; MDA, malondialdehyde

在 OGD-R 处理组中发现 MitoSOX 荧光显着增加，表明线粒体 ROS 水平升高。当用 BRU 预处理 HepG2 细胞时，OGD-R 诱导的线粒体 ROS 水平显着降低。OGD-R 处理组的 MDA 含量显着增加。相反，BRU 预处理减弱了 OGD-R 诱导的细胞 MDA 含量的增加，表明 BRU 降低了 OGD-R 诱导的氧化损伤。

大量的体外和体内缺血/再灌注（I / R）模型显示，I / R 诱导的组织损伤主要是由线粒体中活性氧（ROS）的产生引起的。因此，在再灌注早期抑制线粒体 ROS 的产生可能有利于减轻 I / R 诱导的损伤。所以本实验研究了 BRU 对缺氧/复氧(OGD-R)诱导的 HepG2 细胞损伤和大鼠肝线粒体缺氧/复氧(A/R)损伤的保护作用。实验结果表明：BRU 保护 HepG2 细胞免受 OGD-R 诱导的损伤，BRU 降低了 OGD-R 诱导的线粒体 ROS 水平并减轻了 HepG2 细胞的细胞氧化损伤。因此，我们认为适当剂量的 BRU 在预防 I / R 引起的损伤中可能具有充当线粒体保护剂的潜力。

Ruskinn 工作站——OGD 文献列表（部分）

1 Ghrelin Inhibits Apoptosis in Hypothalamic NeuronalCells during Oxygen-Glucose Deprivation
文章出处：Endocrinology 2007 Jan;148(1) DOI：10.1210/en.2006-0991

2 Induction of ER stress in response to oxygen-glucosedeprivation of cortical cultures involves the activation of the PERK and IRE-1 pathways and of caspase-12
文章出处：Cell Death Dis 2011 Apr 28;2 DOI：10.1038/cddis.2011.31；工作站使用：Invivo$_2$ 400

3 Involvement of SSAO/VAP-1 in Oxygen-Glucose Deprivation-Mediated Damage Using the Endothelial hSSAO/VAP-1-Expressing Cells as an Experimental Model of Cerebral Ischemia
文章出处：erebrovasc. Dis. 2014;37(3) DOI：10.1159/000357660；工作站使用：Invivo$_2$

4 Protective effect of TSG against oxygen-glucose deprivation in cardiomyoblast cell line H9c2: involvement of Bcl-2 family, Caspase 3/9, and Akt signaling pathway
文章出处：Int J Clin Exp Pathol ；工作站使用：Invivo$_2$ 500

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