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·基础研究·

二十二碳六烯酸对氧糖剥夺环境下大鼠脑星形胶质细胞凋亡及血管生成因子分泌的影响*

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摘要 目的:评价二十二碳六烯酸(Docosahexaenoic Acid,DHA)预处理对氧糖剥夺环境下(Oxygen and glucose deprivation,OGD)大鼠脑星形胶质细胞凋亡及血管生成因子分泌的影响。**方法:**大鼠脑星形胶质细胞传代培养,第3~4代用于实验。采用随机数字法将培养的细胞分为6组：正常对照组、OGD组、OGD+10 μMDHA组、OGD+40 μMDHA组、OGD+10 μMDHA+GW9662组、OGD+40 μMDHA+GW9662组。在所有缺氧模型组（除正常对照组外）先用无糖、无血清的DMEM液置换原培液；其次在OGD+10 μMDHA、OGD+40 μMDHA、OGD+10 μMDHA+GW9662、OGD+40 μMDHA+GW9662组加入相应浓度的DHA,同时在OGD+10 μMDHA+GW9662组和OGD+40μMDHA+GW9662组加入5 μM GW9662（过氧化物酶体增殖物激活受体PPARγ的抑制剂)。预处理完成后,正常对照组和其余各组分别在5% CO_2:95%空气和94%N_2:5% CO_2:1%O_2条件下培养24 h。采用流式细胞技术检测细胞凋亡率，酶联免疫吸附剂测定（Enzyme linked immunosorbent assay,ELISA）检测培养上清液中的促血管生成素1（Angiopoietin-1,Ang1)、促血管生成素2(Angiopoietin2,Ang2)、血管内皮生长因子(vascular endothelial growth factor,VEGF)的分泌量,Western blotting法检测Bax、Bcl-2、caspase-3的表达。**结果:**与正常对照组比较,其余组细胞凋亡率、Bax、Caspase-3表达水平明显增加($P<0.01$),而Bcl-2、Bcl-2/Bax表达明显降低($P<0.01$)。与OGD组比较,OGD+10 μMDHA组和OGD+40 μMDHA组细胞凋亡率、Bax、Caspase-3表达水平明显降低($P<0.01$),而Bcl-2、Bcl-2/Bax表达明显增加($P<0.01$);Ang1分泌量明显增加($P<0.01$),而Ang2和VEGF分泌量明显降低($P<0.01$);上述各指标的差异在OGD+40 μMDHA组里更加显著($P<0.01$)。与OGD组比较,OGD+10 μMDHA+GW9662和OGD+40 μMDHA+GW9662组各个观察指标均无明显差异($P>0.05$)。相关性统计分析结果显示细胞凋亡率与Ang1水平呈显著负相关($P<0.01$),与Ang2和VEGF的水平呈显著正相关($P<0.01$)。**结论:**二十二碳六烯酸(DHA)预处理能够减少大鼠脑星形胶质细胞在氧糖剥夺(OGD)环境下的凋亡,其机制与增加Ang1分泌,减少Ang2和VEGF分泌,进而调控Ang/Tie2信号通路相关。

关键词:二十二碳六烯酸;氧糖剥夺;血管生成因子;星形胶质细胞

中图分类号:R-33;Q593.2;R741.02 **文献标识码:**A **文章编号:**1673-6273(2019)21-4001-05

Effects of Docosahexaenoic Acid on the Apoptosis and Secretion of Angiogenic Factors in the Rat Brain Astrocytes under Oxygen-glucose Deprivation Environment*

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ABSTRACT Objective: To evaluate the effects of docosahexaenoic acid(DHA) on the apoptosis and secretion of angiogenic factors in the rat brain astrocytes under oxygen-glucose deprivation (OGD) environment. **Methods:** Rat astrocytes were cultured in vitro and randomly divided into six groups using a random number table: normal control group, OGD group, OGD+10 μM DHA group, OGD+40 μM DHA group, OGD+10 μM DHA+GW9662, OGD+40 μ MDHA+GW9662. The concentration of DHA was 10 μM or 40 μM in the corresponding group according to the design of experiment. In addition, 5 μM GW9662 was added in OGD+10 μM DHA+GW9662 group and OGD+40 μMDHA+GW9662 group. Except of the normal control group that cultured under the condition of 5% CO_2:95% air. The other groups were cultured under the condition of 94%N_2:5%CO_2:1%O_2 for 24 h. The cell apoptosis rate was detected with flow cytome-

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try technology. The content of Ang1, Ang2, and VEGF in the culture medium were detected by ELISA.The expression of Bax, Bcl-2, Caspase-3 were detected by Western Blot. **Results:** Compared with the normal control group, the cell apoptosis rate, expression of Bax, Caspase-3 in the OGD group were significantly increased (P<0.01), and the expression of Bcl-2, Bcl-2/Bax were decreased(P<0.01). Compared with OGD group, the cell apoptosis rate, expression of Bax, and Caspase-3 and the secretion of Ang2 and VEGF were all significantly decreased in the OGD+10 μMDHA group and OGD+40 μMDHA group (P<0.01). and the expression of Bcl-2, Bcl-2/Bax and Ang1 secretion were increased (P<0.01). The difference of the above respective indexes in OGD+40 μMDHA group were more significant than that in the OGD+10 μMDHA group (P<0.01). Compared with OGD group, all measurements showed no significant statistical difference in OGD+10 μM DHA+GW9662 group and OGD+40 μMDHA+GW9662 group(P＞0.05). The cell apoptosis rate has positive correlation with the level of Ang2 and VEGF(P<0.01), but negative correlation with the level of Ang1(P<0.01). **Conclusion:** Pretreatment with DHA could reduce the apoptosis of rat brain astrocytes under OGD environment, it may be related to regulate the Ang/Tie2 signal pathway by increasing the secretion of Ang1, and reducing the secretion of Ang2 and VEGF.

Key words: DHA; Oxygen-glucose deprivation; Angiogenesis; Astrocytes

Chinese Library Classification(CLC): R-33; Q593.2; R741.02 Document code: A

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前言

研究表明在缺血性脑损伤早期，血管生成素 1(Angipoietin1，Ang1)水平降低而血管生成素 2(Angipoietin2，Ang2)的水平明显增加[1-4]。Ang1/Ang2 之间平衡失调导致对血管生成素受体 Tie2 激活能力下降[5-7]。Tie2 激活作用的降低影响了其下游 PI3/Akt 等与细胞生存紧密相关的信号通路的功能[8]。因此，在脑损伤早期，增加 Ang1 或降低 Ang2 的表达，调控 Ang/Tie2 信号通路已成为早期脑保护非常有前景的方法[9,10]。

过氧化物酶体增殖剂激活受体(peroxisome proliferators-activated receptors，PPARs)是核受体超家族的成员，其三个亚型 α、β/δ、γ 均参与脑损伤的病理生理机制，其中 PPAR-γ 与脑损伤具有最密切的关系，PPAR-γ 激动剂能减轻神经炎和帕金森病相关的病理变化[11,12]。二十二碳六烯酸(DHA)是 PPARs 的激动剂之一。DHA 能否能通过调控 Ang1/Tie2 信号通路减轻缺血缺氧性脑损伤目前尚未见研究报道。因此，本研究主要探讨了 DHA 对脑星形胶质细胞在氧糖剥夺环境下凋亡和血管生成因子生成的影响，以期明确 DHA 对缺血缺氧性脑损伤的保护作用。

1 材料和方法

1.1 主要试剂和仪器

Model-550 酶标仪、TCSSP5 激光共聚焦显微镜 (Leica，德国)、Nu47-E 可调氧浓度孵箱 (Nuaire, 美国)。特异性 Ang1、Ang2、VEGF ELISA 检测试剂盒 (伊莱瑞特生物科技有限公司)；鼠抗羊 bax、bcl-2 一抗(Cell signaling)；兔抗羊 Capase-3 一抗(Santa Cruz 公司)。

1.2 脑星形胶质细胞的分离与培养

脑星形胶质细胞取自 2-3 周、体重 40-60gSD 大鼠，雌雄均可(华中科技大学实验动物中心)，按照文献中描述的方法进行分离、培养和纯化。Ⅷ因子相关抗原抗体作免疫细胞化学鉴定，取第 3 代 95%以上为脑星形胶质细胞的细胞用于实验。

1.3 脑星形胶质细胞氧糖剥夺模型(OGD)的建立

原代培养的细胞在 5%CO_2 条件下培养 24 h 后进行细胞同步化，然后用无糖、无血清的 DMEM 液置换原培液后置于 94%N_2:5%CO_2:1%O_2 低氧条件下培养 24 h[13]。

1.4 实验分组及处理

采用随机数字法将脑星形胶质细胞样本随机分为 6 组：正常对照组 (C 组)、OGD 组、OGD+10 μMDHA 组、OGD+40 μMDHA 组、OGD+10 μMDHA+ GW9662 组、OGD+40 μMDHA+GW9662 组。取第 3 代细胞，消化制成单细胞悬液，调整细胞密度为 1× 10^5/mL，接种于 6 孔培养板中，每组设 3 个复孔。OGD+10μ MDHA、OGD+40 μM DHA、OGD+10 μM DHA+GW9662、OGD+40 μM DHA+GW9662 组加入相应浓度的 DHA，同时在 OGD+10 μM DHA+GW9662 组和 OGD+40 μM DHA+GW9662 组还加入 5 μM GW9662，以上各组在 5%CO_2 和 95%空气条件预处理 1 h 后，正常对照组在 5% CO_2 和 95%空气条件下继续培养，其余各组均置于 94%N_2:5%CO_2:1%O_2 低氧条件下培养 24 h。

1.5 细胞凋亡检测

收集不含 EDTA 的 0.25%胰酶消化后的细胞，1500 rpm 离心 5 min 后去上清，PBS 重悬及润洗 2 次后按照 AnnexinV&PI 细胞凋亡检测试剂盒操作说明进行细胞凋亡检测：各样品分别加入 500 μL Binding Buffer，5 μL AnnexinV，5 μL PI，混匀，室温避光反应 5~15 min 后流式细胞仪上机检测，每个实验重复 3 次统计数据并进行分析。

1.6 ELISA 检测 Ang1、Ang2、VEGF 的含量

培养 24h 后收集各组培养上清液，-20℃保存备 ELISA 用，用 ELISA 试剂盒检测上清液中的 Ang1、Ang2、VEGF，按照说明书进行操作。

1.7 Western blotting 法检测 Bax、Bcl-2、caspase-3 的表达

适量 RIPA 裂解液裂解细胞后测蛋白浓度，取 50 μg 总蛋白上样 8%的 PAGE 胶电泳，PVDF 膜用甲醇浸泡数秒后和滤纸一同浸泡于电转缓冲液中湿转 90 min，5% 脱脂牛奶室温封闭 1 h，一抗(1：1000)4 度摇床孵育过夜后 TBST 洗膜 5-6 次，相应荧光二抗室温避光孵育 1h，TBST 洗膜 3 次后奥德赛扫膜，根据目的条带及内参条带的灰度值得出目的蛋白的相对表达量。

1.8 统计学处理

采用 GraphPad Prism 5.0 软件进行数据分析，正态分布的

计量资料以均数± 标准差($\bar{x}$± s)表示，多组间比较采用单因素方差分析，两组间比较采用 SNK-q 检验，以 P<0.05 为差异有统计学意义。

2 结果

2.1 DHA 预处理对 OGD 大鼠脑星形胶质细胞凋亡的影响

与正常对照组比较，氧糖剥夺使细胞凋亡明显增加(P<0.01)；与 OGD 组比较，OGD+10 μMDHA 和 OGD+40 μMDHA 组细胞凋亡明显降低(P<0.01)，而 OGD+10 μMDHA+GW9662 和 OGD+40 μMDHA+GW9662 组细胞凋亡无明显差异(P>0.05)；与 OGD+10 μMDHA 组比较，OGD+40 μDHA 组细胞凋亡有明显降低(P<0.01)，见图 1。

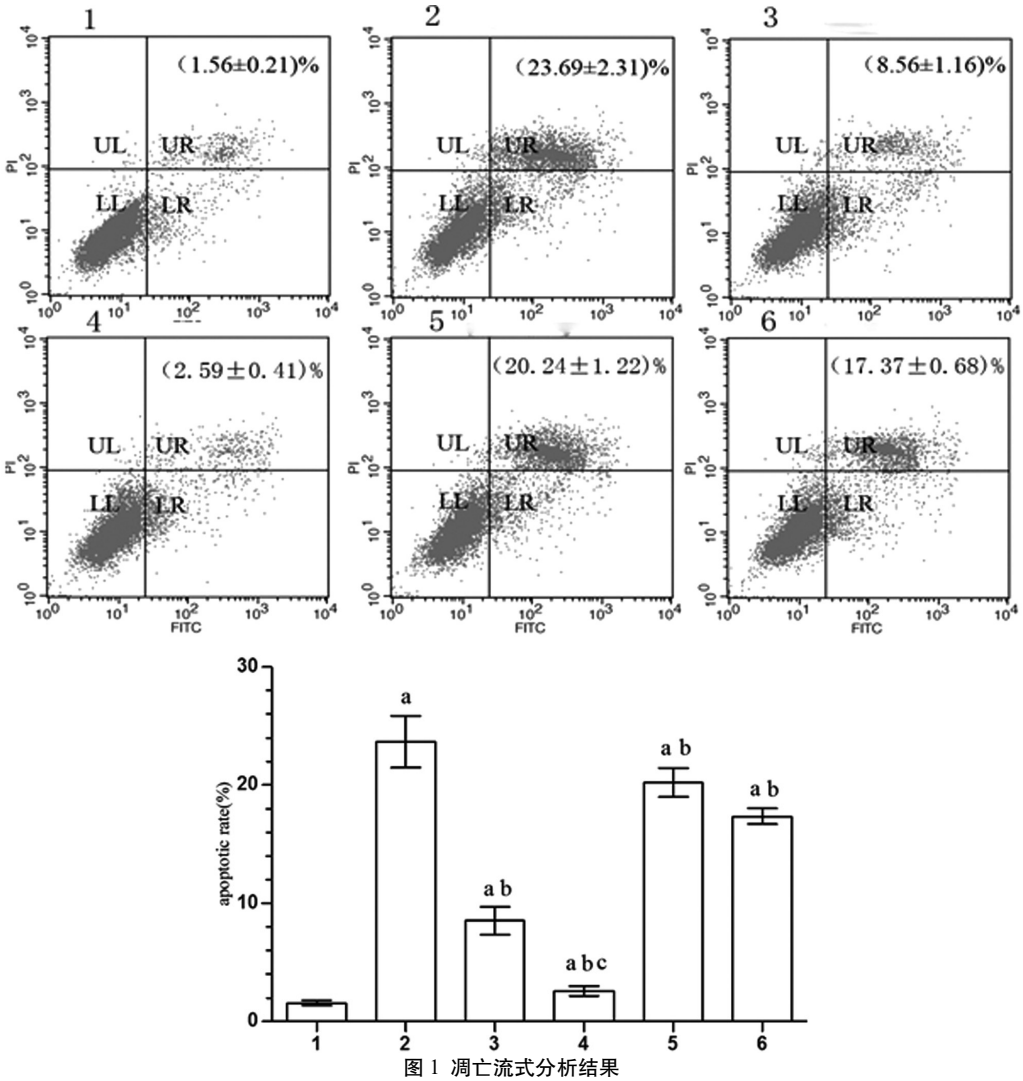

图 1 凋亡流式分析结果

Fig.1 Apoptotic flow analysis results

1 C 组;2 OGD 组;3 OGD+10 μMDHA;4 OGD+ 40 μMDHA;
5 OGD+10 μMDHA+GW9662; 6 OGD+40 μMDHA+GW9662;

Note: Compared with group C, [a]P<0.01; Compared with group OGD, [b]P<0.01; Compared with group OGD+10 μMDHA, [c]P<0.01.

2.2 DHA 预处理对 OGD 大鼠脑星形胶质细胞 Bax、Bcl-2、caspase-3 蛋白表达的影响

Western blotting 结果(图 2)显示：与正常对照组相比，氧糖剥夺可以使大鼠脑星形胶质细胞 Bax、Caspase-3 表达明显增加(P<0.01)，而 Bcl-2、Bcl-2/Bax 的表达则明显降低(P<0.01)；与 OGD 组比较，OGD+10 μMDHA 和 OGD+40 μMDHA 组 Bax、Caspase-3 表达明显降低 (P<0.01)，Bcl-2、Bcl-2/Bax 表达明显增加 (P<0.01)，而 OGD+10 μMDHA+GW9662 和 OGD+40 μMDHA+GW9662 组上述检测指标无明显差异 (P>0.05)；与 OGD+10 μMDHA 比较，OGD+40 μMDHA 组 Bax、Caspase-3 表达明显降低(P<0.01)，cl-2、Bcl-2/Bax 表达明显增加(P<0.01)，见表 1。

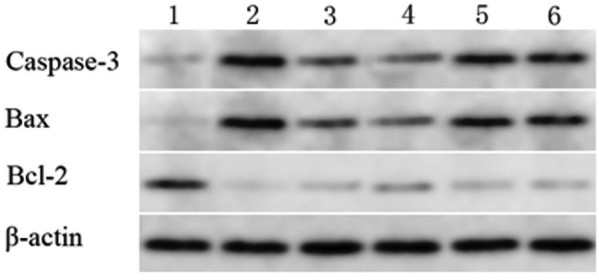

图 2 Western blotting 免疫印迹发光图

Fig.2 Result of Western blotting

Note: 1 C; 2 OGD; 3 OGD+10 μMDHA; 4 OGD+40 μMDHA;
5 OGD+10 μMDHA+GW9662 ;6 OGD+40 μMDHA+ GW9662;

表 1 Western blotting 检测 Caspase-3、Bax、Bcl-2、Bcl-2/Bax 的表达(n=3, $\bar{x}\pm s$)

Table 1 Expression of Caspase-3, Bax, Bcl-2 and Bcl-2/Bax detected by Western blotting

Groups	Caspase-3	Bax	Bcl-2	Bax/Bcl-2(%)
C	0.17± 0.01	0.15± 0.01	0.32± 0.01	209.13± 7.07
OGD	0.78± 0.05[a]	0.65± 0.03[a]	0.09± 0.01[a]	15.82± 1.66[a]
OGD+10 μMDHA	0.62± 0.04[ab]	0.52± 0.03[ab]	0.18± 0.01[ab]	31.03± 1.59[ab]
OGD+40 μMDHA	0.54± 0.02[abc]	0.42± 0.03[abc]	0.25± 0.02[abc]	59.83± 2.32[abc]
OGD+DHA+GW9662	0.72± 0.03[a]	0.68± 0.02[a]	0.11± 0.01[a]	16.12± 1.53[a]
OGD+DHA+GW9662	0.75± 0.02[a]	0.63± 0.02[a]	0.12± 0.01[a]	17.23± 1.57[a]

Note: Compared with group C, [a]$P<0.01$; Compared with group OGD, [b]$P<0.01$; Compared with group OGD+10 μMDHA, [c]$P<0.01$.

2.3 DHA 预处理对 OGD 大鼠脑星形胶质细胞 Ang1 分泌水平的影响

与正常对照组比较,Ang1 在 OGD+40 μMDHA 组的分泌量明显增加($P<0.01$),而在 OGD+10 μMDHA 组则无差异($P>0.05$),OGD 组、OGD+10 μMDHA+GW9662 组、OGD+40 μMDHA+GW9662 组分泌量均降低 ($P<0.01$);与 OGD 组比较,OGD+10 μMDHA 和 OGD+40 μMDHA 组 Ang1 分泌量均增加($P<0.01$),OGD+10 μMDHA+GW9662 组和 OGD+40 μMDHA+GW9662 组 Ang1 分泌量无差异 ($P>0.05$);与 OGD+10 μMDHA 组比较,OGD+40 μMDHA 组 Ang1 分泌量明显增加($P<0.01$),见表 2。

2.4 DHA 预处理对 OGD 大鼠脑星形胶质细胞 Ang2 分泌水平的影响

与正常对照组比较, OGD 组、OGD+10 μMDHA 组、OGD+10 μMDHA+GW9662、OGD+40 μMDHA+GW9662 组 Ang2 分泌量均明显增加($P<0.01$),但 OGD+40 μMDHA 组无差异 ($P>0.05$);相比较于 OGD 组,Ang2 分泌量在 OGD+10 μMDHA 组和 OGD+40 μMDHA 组所有时间点的均明显降低($P<0.01$),OGD+10 μMDHA+GW9662 和 OGD+40 μMDHA+GW9662 组 Ang2 分泌量无差异 ($P>0.05$);与 OGD+10 μMDHA 组比较, OGD+40 μMDHA 组 Ang2 分泌量明显降低 ($P<0.01$),见表 2。

2.5 DHA 预处理对 OGD 大鼠脑星形胶质细胞 VEGF 分泌水平的影响

与正常对照组比较,OGD 组、OGD+10 μMDHA 组、OGD+40 μMDHA 组 、OGD+10μMDHA+GW9662、OGD+40 μMDHA+GW9662 组 VEGF 分泌量均明显增加 ($P<0.01$);与 OGD 组比较,OGD+10 μMDHA 组和 OGD+40 μMDHA 组 VEGF 分泌量均明显降低 ($P<0.01$),OGD+10 μMDHA+GW9662 和 OGD+40 μMDHA+GW9662 组 VEGF 分泌量无明显差异 ($P>0.05$)。与 OGD+10 μMDHA 组比较,OGD+40 μMDHA 组 VEGF 分泌量均明显降低($P<0.01$),见表 2。

表 2 ELISA 检测 Ang1、Ang2、VEGF 的分泌水平(n=3, $\bar{x}\pm s$,pg/mL)

Table 2 Secretion of Ang1, Ang2 and VEGF detected by ELISA(n=3, $\bar{x}\pm s$, pg/mL)

Groups	Ang1	Ang2	VEGF
C	1279.33± 16.27	69.97± 7.85	146.75± 7.38
OGD	1073.67± 24.55[a]	252.51± 23.19[a]	881.87± 55.33[a]
OGD+10μMDHA	1363.33± 28.94[ab]	116.78± 13.68[ab]	760.85± 22.34[ab]
OGD+40μMDHA	1758.01± 33.18[abc]	56.53± 8.65[abc]	628.29± 21.23[abc]
OGD+DHA+GW9662	1109.12± 89.85[a]	242.59± 25.15[a]	860.46± 34.31[a]
OGD+DHA+GW9662	1123.21± 42.14[a]	235.75± 22.69[a]	850.54± 40.21[a]

Note: Compared with group C, [a]$P<0.01$; Compared with grou OGD, [b]$P<0.01$; Compared with group OGD+10 μMDHA, [c]$P<0.01$.

2.6 OGD 大鼠脑星形胶质细胞凋亡与血管生成因子水平的相关性分析

脑星形胶质细胞凋亡率与 Ang1 分泌水平呈负相关,相关系数为 -0.78($P<0.01$);与 Ang2、VEGF 分泌水平呈显著正相关,相关系数分别为 0.86 和 0.73($P<0.01$)。

3 讨论

氧糖剥夺 - 复氧复糖损伤模型可模拟在体脑缺血再灌注损伤的病理生理过程[14,15],在本实验中,我们利用大鼠脑星形胶质细胞的原代培养,在氧糖剥夺环境下进行培养 24h 造成模拟体内缺血缺氧环境模型, 以研究揭示 DHA 对星形胶质细胞在氧糖剥夺状态下细胞凋亡的影响及血管生成因子分泌的变化。

DHA 是神经元膜不可或缺的组成部分, 体内和体外实验均显示其对神经系统有多方面的保护作用[16-19],有益于婴幼儿的视力、学习和行动能力[20],延缓老年人的中枢神经系统退变和老化[21,22]。本实验结果显示氧糖剥夺环境下,脑星形胶质细胞凋亡明显增加,给予 DHA 预处理后能明显减轻细胞的凋亡,并且呈一定的剂量依赖性,40 μM DHA 组的抗凋亡效果比 40 μM DHA 组的效果更显著,这与 Juhyun Song[23]、Pan 等[24]报道的 DHA 对离体神经细胞的保护作用相一致。

Ang-1 可促进血管成熟，稳定血管内皮细胞间缝隙连接，减轻缺血所致的血脑屏障损 伤 ,Ang2 是 Ang-1 的拮抗剂,有介导血管舒张、重构基质薄板、血管内皮细胞增殖迁徙等作用。通过上调 Ang-1 或下调 Ang-2 的表达,使两者比例升高有利于维持血脑屏障的完整性，减轻脑损伤 2,Ang/Tie2 信号通路能够诱导细胞促生存 / 凋亡作用[25-27]。Ang1 的结合,使 Tie2 发生自身磷酸化,激活下游的磷脂酶肌醇 3 激酶(PI3K)和 Akt。后者的激活进一步激活下游的多个信号通路，发挥促生存作用,包括哺乳动物雷帕霉素靶蛋白、抑制 caspase3、7、9，增加 BCl-2 表达和抗凋亡作用[28]。在本研究中可以看到氧糖剥夺条件下脑星形胶质细胞 Ang2 和 VEGF 分泌增加，而 Ang1 分泌水平下降,DHA 预处理后能够明显降低 Ang2 和 VEGF 的分泌,而增加脑星形胶质细胞 Ang1 的分泌,尤其是后者的分泌水平明显升高,甚至高于正常氧含量状态下的基础分泌值。同时脑星形胶质细胞的凋亡率与 Ang1 分泌水平呈负相关，而与 Ang2、VEGF 分泌水平呈显著正相关,这与 Chen X[29]等在脑微血管内皮细胞及本课题组在人脑血管周细胞[30]方面的研究结论相一致。加入 PPARs 抑制剂 GW9662 后,DHA 对脑星形胶质细胞的抗凋亡作用和对 Ang1、Ang2、VEGF 分泌的影响均被拮抗。我们推测 DHA 对缺氧状态下脑星形胶质细胞的保护作用是在与 PPARs 结合后,促进 Ang1 分泌,降低 Ang2、VEGF 分泌,恢复 Ang1/Ang2 之间平衡,进而调控 Ang/Tie2 信号通路有关。但 DHA 是通过何种途径影响到 ANG 和 VEGF 的分泌尚有待进一步研究明确。

综上所述,二十二碳六烯酸(DHA)预处理能够减少大鼠脑星形胶质细胞在氧糖剥夺 (OGD) 环境下的凋亡，其机制与 DHA 通过增加 Ang1，降低 Ang2 和 VEGF 分泌，调控 Ang/Tie2 信号通路相关。

参 考 文 献(References)

[1] Nag S, Papneja T, Venugopalan R, et al. Increased angiopoietin 2 expression is associated with endothelial apoptosis and blood-brain barrier breakdown [J]. Laboratory investigation; a journal of technical methods and pathology, 2005, 85(10): 1189-1198

[2] Ohishi M, K. Yamamoto, H. Rakugi, et al. Angiotensin (1-7) and other angiotensin peptides[J]. Curr Pharm Des, 2013, 19(17): 3060-3064

[3] 陈小波,余艳丽,方海滨,等. 二十二碳六烯酸对大鼠氧糖剥夺脑微血管内皮细胞 Ang-2 和 VEGF 表达的影响 [J]. 中华麻醉学杂志, 2016, 36(9): 1142-1145

[4] Alfieri A, Ong AC, Kammerer RA, et al. Angiopoietin-1 regulates microvascular reactivity and protects the microcirculation during acute endothelial dysfunction: Role of eNOS and VE-cadherin [J]. Pharmacol Res, 2014, 80: 43-51

[5] Abbott NJ, Dolman DE, Drndarski S, et al. An improved in vitro blood-brain barrier model: rat brain endothelial cells co-cultured with astrocytes[J]. Methods in molecular biology, 2012, 814: 415-430

[6] Gurnik S, Devraj K, Macas J, et al. Angiopoietin-2-induced blood-brain barrier compromise and increased stroke size are rescued by VE-PTP-dependent restoration of Tie2 signaling [J]. Acta Neuropathol, 2016, 131(5): 753-73

[7] Chittiboina P1, Ganta V, Monceaux CP, et al. Angiopoietins as promising biomarkers and potential therapeutic targets in brain injury [J]. Pathophysiology, 2013, 20(1): 15-21

[8] Zhang, W, Wang Y, Bi G. Limb remote ischemic postconditioning-induced elevation of fibulin-5 confers neuroprotection to rats with cerebral ischemia/reperfusion injury: activation of the AKT pathway[J]. Clin Exp Pharmacol Physiol, 2017, 44(6): 656-663

[9] van der Heijden M, van Nieuw Amerongen GP, Chedamni S, et al. The angiopoietin-Tie2 system as a therapeutic target in sepsis and acute lung injury [J]. Expert opinion on therapeutic targets, 2009, 13(1): 39-53

[10] Matijs van Meurs, Philipp Kümpers, Jack JM Ligtenberg. Bench-to-bedside review: Angiopoietin signalling in critical illness - a future target?[J]. Crit Care, 2009, 13(2): 207

[11] Benedusi V, Martorana F, Brambilla L, et al. The peroxisome proliferator-activated receptor gamma (PPARgamma) controls natural protective mechanisms against lipid peroxidation in amyotrophic lateral sclerosis [J]. The Journal of biological chemistry, 2012, 287(43): 35899-35911

[12] L Hunter Randy, Bing Guoying. Agonism of Peroxisome Proliferator Receptor-Gamma may have Therapeutic Potential for Neuroinflammation and Parkinson's Disease [J]. Current neuropharmacology, 2007, 5(1): 35-46

[13] Chin-Yi Cheng, Yu-Chen Lee. Anti-Inflammatory Effects of Traditional Chinese Medicines against Ischemic Injury in In Vivo Models of Cerebral Ischemia [J]. Evid Based Complement Alternat Med, 2016, 2016: 5739434

[14] Li C, Wang X, Cheng F, et al. Geniposide protects against hypoxia/reperfusion-induced blood-brain barrier impairment by increasing tight junction protein expression and decreasing inflammation, oxidative stress, and apoptosis in an in vitro system [J]. Eur J Pharmacol, 2019, 854: 224-231

[15] Kokubu Y, Yamaguchi T, Kawabata K. In vitro model of cerebral ischemia by using brain microvascular endothelial cells derived from human induced pluripotent stem cells[J]. Biochem Biophys Res Commun, 2017, 486(2): 577-583

[16] Pu H, Jiang X, Hu X, et al. Delayed Docosahexaenoic Acid Treatment Combined with Dietary Supplementation of Omega-3 Fatty Acids Promotes Long-Term Neurovascular Restoration After Ischemic Stroke[J]. Transl Stroke Res, 2016, 7(6): 521-534

[17] Lim SN, Huang W, Hall JC, et al. Improved outcome after spinal cord compression injury in mice treated with docosahexaenoic acid[J]. Experimental neurology, 2013, 239: 13-27

[18] Quartu M, Serra MP, Boi M, et al. Effect of acute administration of Pistacia lentiscus L. essential oil on rat cerebral cortex following transient bilateral common carotid artery occlusion[J]. Lipids Health Dis, 2012, 11: 8

[19] LinY, M XU, J WAN, et al. Docosahexaenoic acid attenuates hyperglycemia-enhanced hemorrhagic transformation after transient focal cerebral ischemia in rats[J]. Neuroscience, 2015, 301: 471-479

[20] Connye N, Kuratko, Erin Cernkovich Barrett, et al. The Relationship of Docosahexaenoic Acid (DHA) with Learning and Behavior in Healthy Children: A Review[J]. Nutrients, 2013, 5(7): 2777-2810

[21] Irene Paterniti, Daniela Impellizzeri, Rosanna Di Paola, et al. Docosahexaenoic acid attenuates the early inflammatory response following spinal cord injury in mice: in-vivo and in-vitro studies [J]. J Neuroinflammation, 2014, 11: 6

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[18] 蔡建华,邓虎平,申传安,等.瘢痕切除结合负压整复烧伤患儿增生性瘢痕的效果[J].中华烧伤杂志, 2017, 33(7): 410-414

[19] Yang JH, Yoon JY, Moon J, et al. Expression of inflammatory and fibrogenetic markers in acne hypertrophic scar formation: focusing on role of TGF-β and IGF-1R [J]. Arch Dermatol Res, 2018, 310(8): 665-673

[20] Chen Q, Zhao T, Xie X, et al. MicroRNA-663 regulates the proliferation of fibroblasts in hypertrophic scars via transforming growth factor-β1[J]. Exp Ther Med, 2018, 16(2): 1311-1317

[21] Zhang YK, Yang H, Zhang JY, et al. Comparison of intramuscular compound betamethasone and oral diclofenac sodium in the treatment of acute attacks of gout[J]. Int J Clin Pract, 2014, 68(5): 633-638

[22] Rodriguez JS, Zürcher NR, Keenan KE, et al. Prenatal betamethasone exposure has sex specific effects in reversal learning and attention in juvenile baboons[J]. Am J Obstet Gynecol, 2011, 204(6): 545.e1-10

[23] 焦健,范玉.δ-氨基酮戊酸光动力疗法对瘢痕疙瘩成纤维细胞增殖及胶原分泌的影响[J].中华整形外科杂志, 2015, 31(1): 49-53

[24] 姚建,苏开新,王海华,等.光动力疗法联合复方倍他米松局部封闭治疗增生性瘢痕疗效观察[J].中国美容医学, 2018, 27(2): 81-83

[25] Collier NJ, Allan D, Diaz Pesantes F, et al. Systemic photodynamic therapy in folliculitis decalvans [J]. Clin Exp Dermatol, 2018, 43(1): 46-49

[26] Anand S, Rollakanti KR, Brankov N, et al. Fluorouracil Enhances Photodynamic Therapy of Squamous Cell Carcinoma via a p53-Independent Mechanism that Increases Protoporphyrin IX levels and Tumor Cell Death[J]. Mol Cancer Ther, 2017, 16(6): 1092-1101

[27] Tong M, Wang J, Jiang N, et al. Correlation between p-STAT3 overexpression and prognosis in lung cancer: A systematic review and meta-analysis[J]. PLoS One, 2017, 12(8): e0182282

[28] Tiunova AA, Bezriadnov DV, Komissarova NV, et al. Recovery of Impaired Memory: Expression of c-Fos and Egr-1 Transcription Factors during Restoration of Damaged Engram in the Chick Brain [J]. Biochemistry (Mosc), 2018, 83(9): 1117-1123

[29] Jablonski SA, Robinson-Drummer PA, Schreiber WB, et al. Impairment of the context preexposure facilitation effect in juvenile rats by neonatal alcohol exposure is associated with decreased Egr-1 mRNA expression in the prefrontal cortex [J]. Behav Neurosci, 2018, 132(6): 497-511

[30] Kim YB. The effects of platelet-rich plasma on hypertrophic scars fibroblasts[J]. Int Wound J, 2018, 15(4): 547-554

（上接第 4005 页）

[22] Simon C, Dyall. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA[J]. Front Aging Neurosci, 2015, 7: 52

[23] Juhyun Song, Joohyun Park, Jae Hwan Kim, et al. Dehydroascorbic Acid Attenuates Ischemic Brain Edema and Neurotoxicity in Cerebral Ischemia: An in vivo Study[J]. Exp Neurobiol, 2015, 24(1): 41-54

[24] Pan HC, Kao TK, Ou YC, et al.Protective effect of docosahexaenoic acid against brain injury in ischemic rats[J]. The Journal of nutritional biochemistry, 2009, 20(9): 715-725

[25] Huang J, Bae JO, Tsai JP, et al. Angiopoietin-1/Tie-2 activation contributes to vascular survival and tumor growth during VEGFblockade. Int J Oncol, 2009, 34: 79-87

[26] Hai Tao Yuan, Eliyahu V, Khankin S, et al. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium [J]. Mol Cell Biol, 2009, 29(8): 2011-2022

[27] Chan W, Ismail H, Mayaki D, et al. Fibulin-5 Regulates Angiopoietin-1/Tie-2 Receptor Signaling in Endothelial Cells [J]. PLoS One, 2016, 11: e0156994

[28] Harfouche R, Gratton JP, Yancopoulos GD, et al. Hussain SN: Angiopoietin-1 activates both anti- and proapoptotic mitogen-activated protein kinases[J]. FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 2003, 17(11): 1523-1525

[29] Chen X, Wang Q, Zhan LY, et al. Effects and mechanisms of docosahexaenoic acid on the generation of angiopoietin-2 by rat brain microvascular endothelial cells under an oxygen- and glucose-deprivation environment[J]. SpringerPlus, 2016, 5(1): 1518

[30] 余艳丽,夏中元,方海滨,等.二十二碳六烯酸调节人脑血管周细胞氧糖剥夺/复氧复糖时血管生成素表达的机制：与 SSeCKS 的关系[J].中华麻醉学杂志, 2017, 37(2): 188-191