

过氧化氢酶对SW480细胞线粒体和凋亡的影响

越来越多的研究发现, 炎症过程中可伴随大量氧活性物质(reactive oxygen species, ROS)的产生, 其中过氧化氢(H_2O_2)裂解后产生的氢氧根离子(OH^-)对生物膜可产生过氧化损伤作用。因此消除 H_2O_2 就可以阻断 OH^- 的产生, 从而有效防止 OH^- 对线粒体的损伤。近年来ROS在凋亡中的作用日益受到重视, 很多证据[1][2]提示在促凋亡信号的作用下, ROS的升高可能作为第二信使上调促凋亡蛋白表达, 促进线粒体通透性转变孔的开放, 激活天门冬氨酸特异的半胱氨酸蛋白酶(caspase), 参与 Ca^{2+} 途径等诱导细胞凋亡。许多环境因素都可引起氧化应激介导的凋亡, 而这一诱导作用又可以被多种抗氧化剂(内源性的或外源性的)所阻止。正常情况下, 核转录因子(nuclear factor, NF) κ B存在于细胞浆内, 与抑制蛋白I κ B结合而无活性。在一定刺激作用下, NF κ B释放进入细胞核激活靶基因。众多研究发现NF κ B与肿瘤细胞抵抗凋亡有关。因此我们设想通过应用过氧化氢酶作为抗氧化剂来保护肠上皮细胞内的线粒体, 观察其对线粒体形态和细胞凋亡的影响及细胞内NF κ B表达的变化。

1 材料与方法

1.1 细胞

SW480 细胞株购自中国典型培养物保藏中心。

1.2 试剂

牛肝过氧化氢酶(catalase, CAT, Sigma公司), NF κ B多克隆抗体, 免疫组化试剂盒(北京中山公司)。

1.3 细胞培养及分组

SW480细胞在37 °C, 5% CO_2 饱和湿度的 CO_2 培养箱中培养, 培养液为含10% 热灭活小牛血清、青霉素100 U/ml和链霉素100 μ g/ml的RPMI1640培养基。待细胞铺满瓶底, 生长至对数生长期时, 随机分为正常对照组、脂多糖组(liposaccharide, LPS组)、过氧化氢酶预孵组(CAT预孵组)、过氧化氢酶治疗组(CAT治疗组)。正常对照组不加任何处理因素, 其它3组均加入LPS, 浓度为100 ng/ml。CAT预孵组在加入LPS前30 min加入浓度为200 U/ml的牛肝CAT, CAT治疗组则在加入LPS 同时加入浓度为200 U/ml的牛肝CAT, 孵育30 min后3组同时终止。

1.4 透射电镜观察

将上述处理后的SW480细胞进行胰酶消化、离心, 冷PBS液洗2次, 加入新鲜戊二醛固定1周, 做透射电镜。

1.5 免疫组化分析

采用SABC法, 细胞培养片经多聚甲醛固定, 加入抗NF κ B多抗, 湿盒内4 °C过夜, 依次与生物素标记的羊抗鼠IgG和SABC反应, 最后用DAB显色。

2 结果

2.1 电镜结果

经过CAT预孵处理的细胞在电镜下观察可见线粒体较正常对照组无明显变化, 亦未见凋亡发生, LPS组的线粒体明显肿胀, 线粒体嵴不清楚, 可以见到典型的凋亡小体, 而CAT治疗组的线粒体也有轻微肿胀, 并且可以见到凋亡早期现象, 表现为细胞变圆, 核染色质密度增高, 胞浆浓缩(图1)。

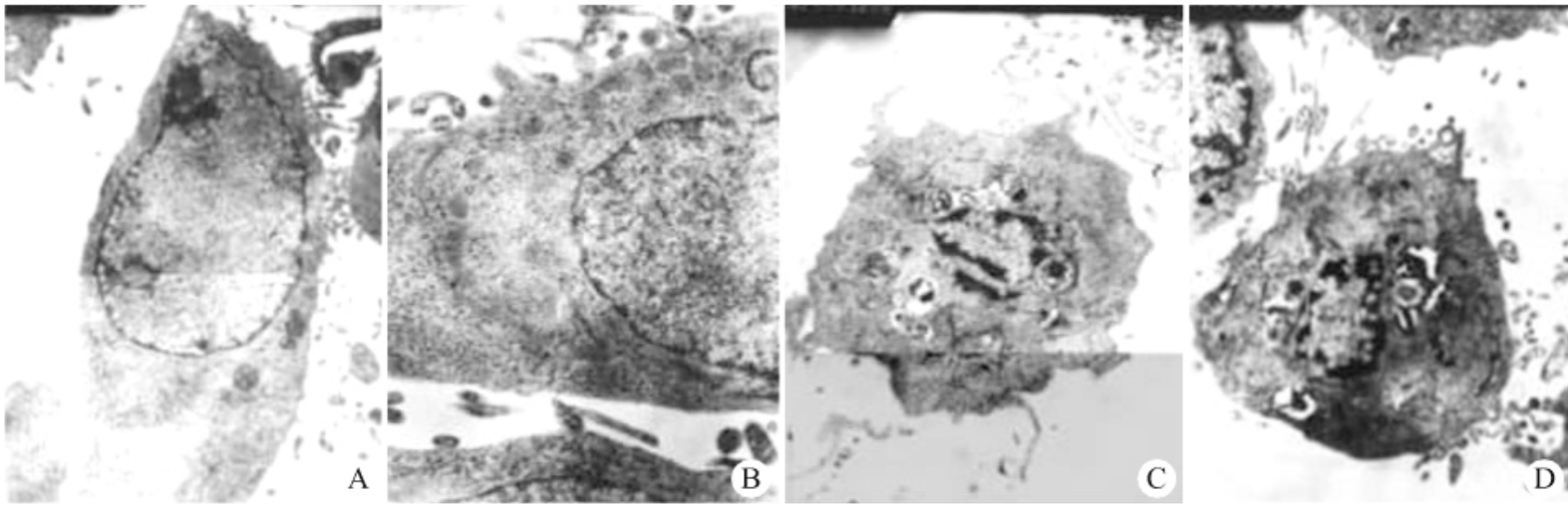


图1 透射电镜结果

Fig.1 Transmission electron microscopic observation of the morphology of SW480 cells with LPS and/or catalase treatment

A: Normal cells and mitochondria, without apoptosis (Original magnification: $\times 6\ 000$); B: Catalase-pretreated cell with normal mitochondrial and without apoptosis (Original magnification: $\times 1\ 200$); C: LPS-stimulated cells with obvious mitochondrial swelling and cell apoptosis; D: Catalase-treated cells with LPS stimulation with slight mitochondrial swelling and early-stage cell apoptosis; LPS: Liposaccharide

2.2 免疫组织化学结果

正常对照组细胞免疫组化染色主要表现为胞质染色阳性，胞核染色弱阳性；在LPS组可以看到大部分细胞内NF- κ B被激活，主要表现为胞核染色强阳性，胞质染色弱阳性，CAT预孵组也有表达，但较LPS组表达明显减少($P < 0.01$)，CAT治疗组的表达与LPS组比较未见明显差别(图2, 3)。

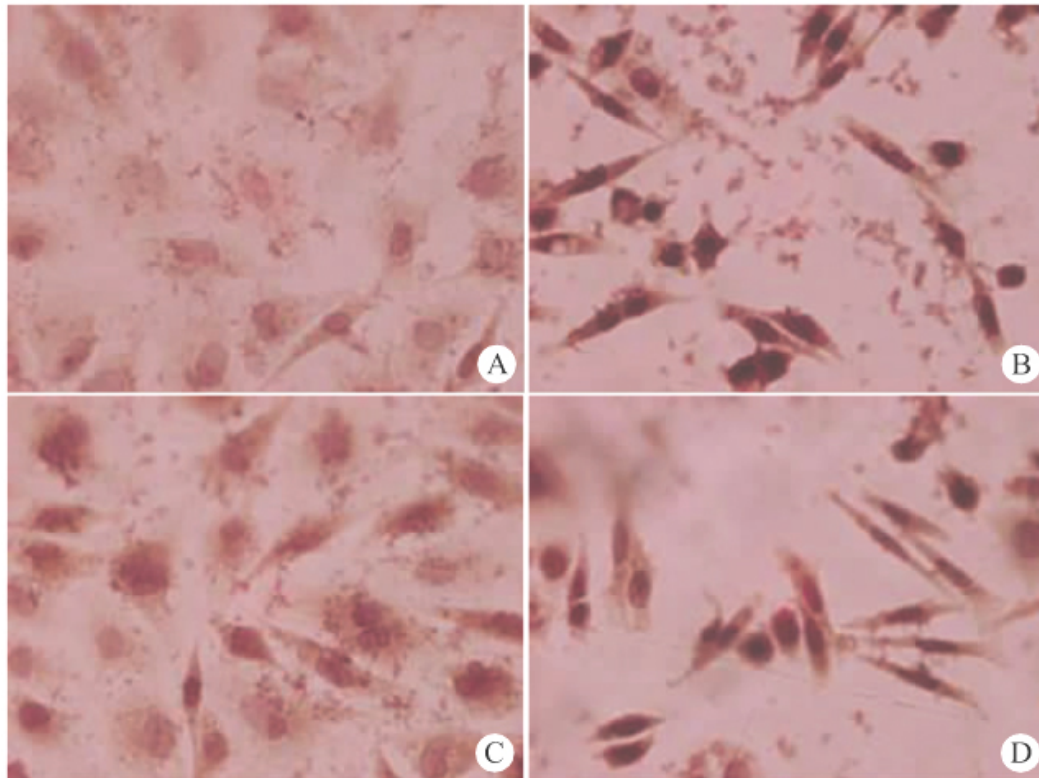


图2 免疫组化结果

Fig.2 Results of immunohistochemical staining of the SW480 cells treated with LPS and/or catalase;

A: Control group (Original magnification: $\times 400$); B: LPS group (Original magnification: $\times 400$); C: Catalase-pretreated group (Original magnification: $\times 400$); D: Catalase therapy group (Original magnification: $\times 400$)

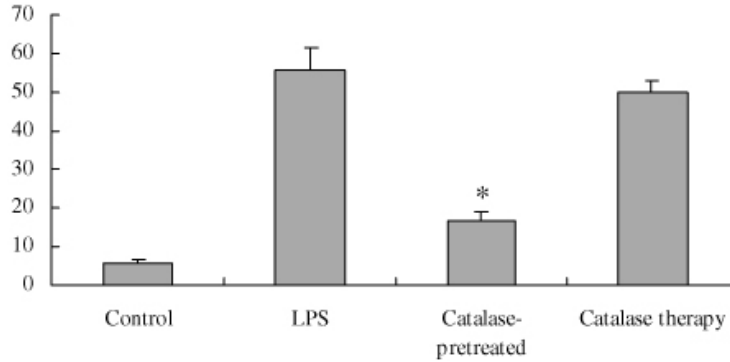


图3 NF-κB 阳性细胞数

Fig.3 Positivity rate of NF-κB in SW480 cells after LPS and/or catalase treatment

3 讨论

许多研究表明, 肠道炎症与ROS的产生密切相关。ROS可损伤蛋白质、脂质、核酸等。许多病理生理现象: 衰老、肿瘤、炎症、脑缺血等均与氧自由基有密切关系。线粒体既是能量代谢的主要场所, 又是调节细胞内钙稳态的重要细胞器之一, 同时也会产生自由基[3]。线粒体作为细胞内主要的产能场所, 对缺血缺氧极为敏感。在缺血缺氧时, Na^+ 大量进入使正常情况下的钠钙交换出现翻转, 导致细胞内 Ca^{2+} 超载进一步加重, 从而激活一系列的蛋白水解酶, 引起迟发性细胞死亡。ROS攻击线粒体膜脂、蛋白和DNA, 而且 Ca^{2+} 超载引起线粒体功能异常。SOD和GSH-Px是细胞内的两种主要抗氧化酶, 可增加自由基的清除, 它们是体内自由基平衡的酶性调节剂, 可调动或激活机体中的内源性抗氧化系统, 有利于预防或减轻自由基引发的细胞和组织损伤 [4]。

肠黏膜是人体氧化保护机制较完善的器官之一。LPS是革兰氏阴性菌细胞壁的主要成分之一, 由特异性多糖、核心多糖和脂类A组成。LPS可增加机体NO、TNF- α 等细胞因子的产生, 继而通过细胞因子介导组织损伤, 引起严重的炎症反应, 导致肠粘膜屏障功能不全[5]。国外学者[6]已证实, 在沙门氏菌感染、慢性腹泻和溃疡性结肠炎动物模型中, 肠黏膜的抗氧化系统受损, 表现为上述几种主要抗氧化酶的含量降低, 因而提出了氧自由基清除剂以辅助治疗上述疾病的设想[6][7][8][9][10]。

我们研究发现, 经过CAT预孵的肠上皮细胞内的线粒体在细胞受到刺激的过程中损伤明显减轻, 凋亡也不明显, 说明CAT对线粒体有保护作用, 但这种保护作用在CAT的治疗过程中却不明显, 其原因还有待进一步探讨。

NF-κB在炎症刺激时明显激活, 进入细胞核内与靶序列结合调控下游基因的转录, 其中包括许多炎症细胞因子, 如TNF- α , IL-1 β , IL-8等。有研究证实, 在转染入人CAT基因的内皮细胞内, 高表达的CAT可以减少ROS的产生, 这一保护作用是通过下调JNK和上调ERK1/2磷酸化和AP-1实现的[11]。通过我们的实验可以发现, 应用CAT预孵的细胞, 其核内NF-κB表达明显减少, 说明NF-κB的激活也减少, 由此可以假设CAT是通过抑制NF-κB的激活来减轻LPS引起的应激反应的, 而这一作用是通过清除ROS这一途径来实现的。

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