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论著

大蒜素对慢性铁中毒大鼠氧化应激与肝细胞自噬的抑制作用

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摘要 目的 探讨大蒜素(allicin)对慢性铁中毒大鼠氧化应激与肝细胞自噬的抑制作用及可能机制。方法 36只SD大鼠按体质量随机分为6组, 即正常对照(饲喂基础饲料)、慢性铁中毒模型(饲喂高铁饲料)、正常+大蒜素40 mg·kg^{-1}、慢性铁中毒+大蒜素30 ,40和60 mg·kg^{-1}组, 每组6只。持续饲喂6周后, 取血及肝、结肠和肾组织用试剂盒测定铁含量、总铁结合力、丙二醛(MDA)含量及总超氧化物歧化酶(T-SOD)活性。透射电镜和免疫组织化学方法观察肝细胞形态结构、自噬以及胱天蛋白酶3凋亡蛋白、Ki-67细胞增殖抗原和LC3-B微管相关蛋白表达的变化。结果 与正常对照组比较, 模型组血清铁含量增加(P<0.01), 总铁结合力下降(P<0.01), 肝和结肠组织铁含量增加(P<0.05)。与模型组比较, 饲喂不同剂量大蒜素大鼠血清铁含量显著下降(P<0.05), 总铁结合力显著增加(P<0.05), 结肠组织铁含量显著下降(P<0.05); 饲喂大蒜素40和60 mg·kg^{-1}肝组织铁含量显著下降(P<0.05)。与正常对照组比较, 模型组肝和结肠组织MDA含量显著增加(P<0.05), 肝组织T-SOD活性增加(P<0.01), 结肠组织T-SOD活性下降(P<0.01)。与模型组比较, 饲喂大蒜素组肝组织MDA含量显著下降(P<0.05), 结肠和肾组织T-SOD活性显著增加(P<0.05)。与正常对照组比较, 模型组肝实质细胞和肝非实质细胞Ki-67增殖抗原表达显著增加(P<0.05), 胱天蛋白酶3凋亡蛋白表达无明显变化(P>0.05)。观察肝细胞超微结构, 模型组大鼠肝细胞有大量铁蛋白颗粒累积, 线粒体和内质网膜膨胀, 胞质中形成自噬前体。模型组大鼠肝细胞LC3-B微管相关蛋白有较强表达, 正常对照和正常+大蒜素组肝细胞未发现LC3-B免疫阳性染色。结论 大蒜素能有效清除体内自由基, 增强机体抗氧化能力, 降低肝中铁诱导的氧化压力、线粒体膜的改变和细胞自噬, 对慢性铁中毒肝细胞具有一定的保护作用。

关键词 大蒜素 铁中毒 氧化应激 自噬

分类号 R151.1, R285.5

Protective effect of allicin against oxidative stress and hepatocyte autophagy in iron-overloaded rats

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Abstract

OBJECTIVE To determine the inhibitory effect of allicin on oxidative stress, and hepatocyte autophagy and defence mechanisms. METHODS Thirty-six SD rats were randomly divided into normal (basal diet, Fe 50 mg·kg^{-1}), iron model (high iron diet, Fe 1000 mg·kg^{-1}), normal+allicin 40 mg·kg^{-1} (basal diet, ig given allicin), iron model+allicin 30, 40 and 60 mg·kg^{-1} (high iron diet, ig given allicin) groups. These rats were fed for 6 weeks. Serum and tissue (the liver, colon and kidneys) iron concentrations, total iron-binding capacity (TIBC), the level of malondialdehyde (MDA) and total superoxide dismutase (T-SOD) were quantified by reagent kit method. At the same time, the morphological changes of the liver, hepatocyte autophagy and caspase 3, Ki-67 and LC3-B protein expression were evaluated through ultrastructural observation and immunohistochemistry. RESULTS Compared with normal group, the serum iron concentration, TIBC and the liver and colon iron concentrations increased significantly in the iron model group (P<0.05, P<0.01). Compared with the iron model group, serum and colon iron concentrations decreased (P<0.05) and TIBC increased (P<0.05) in iron model + allicin groups; liver iron concentrations decreased significantly (P<0.05) in iron

model+allicin 40 and 60 mg • kg^{-1} groups. Compared with normal group, the level of MDA in liver and colon and T-SOD activities in the liver increased ($P<0.05$, $P<0.01$) while T-SOD activities in the colon decreased significantly ($P<0.01$). Compared with iron model group, the level of MDA in the liver decreased significantly ($P<0.05$) while T-SOD activities in the colon and kidneys increased significantly ($P<0.05$). Compared with normal group, cell proliferation increased significantly in hepatocytes and in non-parenchymal cells induced by iron supplementation in the iron model group ($P<0.05$), but caspase 3 immunostaining had no significant difference between these groups. In addition, iron was accumulated within the hepatic lysosomes where it triggered autophagy as evidenced by the formation of autophagic vesicles in the iron model group. It also induced morphologic alterations of the mitochondrial and endocytoplasmic reticulum membranes. In addition, LC3-B was strongly expressed in the iron model