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论著

硝基油酸对肾急性缺血再灌注模型小鼠肾的保护作用及其机制

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摘要 目的 探讨硝基油酸(OA-NO_2)对肾急性缺血再灌注(I/R)模型小鼠肾的保护作用。方法 C57小鼠分为假手术、I/R模型对照、I/R+OA-NO_2 500 μg·kg^{-1}和I/R+油酸(OA)500 μg·kg^{-1}组。I/R小鼠使用乙醚麻醉后,开腹采用夹闭双侧肾动脉30 min,去除血管夹,再灌注24 h制备肾I/R模型。I/R+OA-NO_2和I/R+OA组在去除血管夹后分别ip给予OA-NO_2和OA 500 μg·kg^{-1},每6 h注射1次。假手术和I/R模型组ip给予乙醇0.8 ml·kg^{-1}。24 h后处死小鼠,取血和肾组织,用全自动生化检测仪检测小鼠血浆尿素氮(BUN)和肌酐(Cr)水平,HE染色检测肾组织病理改变,ELISA检测血浆肿瘤坏死因子α(TNF-α)浓度,实时PCR检测肾组织细胞黏附分子1(ICAM-1)、白细胞介素1β(IL-1β)、烟酰胺腺嘌呤二核苷酸磷酸氧化酶胞浆亚基(p^{47})和烟酰胺腺嘌呤二核苷酸磷酸氧化酶催化亚基(gp^{91})基因表达,Western 蛋白质印迹法检测肾组织TNF-α和IL-1β蛋白表达,ELISA检测肾组织丙二醛(MDA)含量。结果 I/R模型组小鼠血浆BUN和Cr水平较假手术组明显增高(P<0.01);OA-NO_2处理后,与I/R模型组比较,血浆BUN水平降低36%(P<0.01),Cr水平降低44%(P<0.01);I/R+OA组BUN和Cr水平无明显变化。I/R模型组小鼠肾组织出现肾小管上皮细胞坏死、细胞结构消失、肾小管管腔扩张和肾小管管腔管型堵塞等改变,血浆TNF-α浓度、肾组织ICAM-1, IL-1β, p^{47}和gp^{91} mRNA表达、TNF-α和IL-1β蛋白表达及MDA含量均较假手术组明显升高(P<0.01);应用OA-NO_2处理后,与I/R模型组比较,肾组织病理改变减轻,肾小管管腔扩张明显改善,未发现明显的肾小管管腔管型堵塞;血浆TNF-α浓度由I/R模型组的(590±73)ng·L^{-1}降低至(259±71)ng·L^{-1}(P<0.01),肾组织ICAM-1, IL-1β, p^{47}和gp^{91}基因表达以及TNF-α和IL-1β蛋白表达降低(P<0.01),MDA含量由I/R模型组的(3.6±0.7)mol·g^{-1}组织降低至(1.8±0.4)mol·g^{-1}组织(P<0.01);应用OA处理后,上述指标与I/R模型组比较均无明显差异。结论 OA-NO_2对肾I/R导致的急性肾损伤具有明显的治疗作用,其作用机制可能与抗炎通路有关。

关键词 硝基油酸 再灌注损伤 肾损伤

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Protection of nitro-oleic acid against acute kidney injury of mice induced by renal ischemia/reperfusion injury and its mechanism

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Abstract

OBJECTIVE To evaluate the potential therapeutic effect of nitro-oleic acid (OA-NO_2) on acute kidney injury. **METHODS** C57 mice were subjected to bilateral renal ischemia for 30 min, followed by 24 h of reperfusion. After ischemia, the mice were ip given OA-NO_2 500 μg·kg^{-1} or oleic acid (OA) 500 μg·kg^{-1} every 6 h during the 24-h recovery period, while those of ischemia/reperfusion(I/R) model group were ip given 0.8 ml·kg^{-1} ethanol every 6 h. The blood and kidney tissue of mice were collected after 24 h reperfusion. Plasma urea nitrogen (BUN) and creatinine(Cr) were tested by the automatic biochemical detector. Histopathological changes of the kidney were detected by HE staining. The plasma concentration of tumor necrosis factor-α(TNF-α) and renal tissue malondialdehyde (MDA) content was detected by ELISA, and the mRNA expression of renal tissue intercellular adhesion molecule-1 (ICAM-1), interleukin-1β(IL-1β), nicotinamide adenine dinucleotide phosphate oxidase cytoplasm subunit (p^{47}) and nicotinamide adenine dinucleotide phosphate oxidase catalytic subunit (gp^{91}) was examined by real-time PCR. The protein expression of renal tissue TNF-α and IL-1β was determined using Western blotting. **RESULTS** Compared with sham group, the plasma BUN and Cr level was elevated in the mice of I/R model group (P< 0.01), but decreased after adminstration by OA-NO_2 by 36% and 44%, respectively, compared with I/R model group (P<0.01). Morphology changes of the kidney in I/R model group, including renal tubular epithelial cell necrosis, cell structure collapse, tubular expansion and tube cast jam, were observed. The concentration of TNF-α and MDA, the mRNA expression of ICAM-1, IL-1β, p^{47} and gp^{91} and the protein expression of TNF-α and IL-1β were significantly increased in I/R model group compared with sham group (P<0.01). After treatment

with OA-NO_2, the pathological changes in renal tissue were attenuated, while renal tubular lumen expansion was decreased compared with the I/R model group, but without obvious renal tubular cast jam. The plasma TNF-α concentration decreased from (590±73)ng • L^{-1} in I/R model group to (259±71)ng • L^{-1} in I/R+OA-NO_2 group (P<0.01). The renal tissue MDA content was decreased from (3.6±0.7)mol • g^{-1} tissue in I/R model group to (1.8±0.4)mol • g^{-1} tissue in I/R+OA-NO_2 group (P<0.01), while the level of BUN, Cr and MDA, the expression of ICAM-1, IL-1β, p^{47}, gp^{91}, TNF-α and IL-1β and histological damage were not significantly different between I/R model and I/R+OA groups. **CONCLUSION** OA-NO_2 attenuates kidney I/R injury likely by inhibiting the inflammatory response.

Key words nitro-oleic acid reperfusion injury kidney injury

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