中国药理学与毒理学杂志 2013 27 (5): 836-842 ISSN: 1000-3002 CN: 11-1115/R

论著

一氧化氮在双氯芬酸钠致大鼠急性肝损伤中的作用

赵莉萍[1,2]，梅俏[2]，胡咏梅[2]，许建明[2]，姚强[2]

1. 安徽淮北职业技术学院医学系，安徽 淮北 235000；
2. 安徽医科大学第一附属医院消化内科，安徽省消化病重点实验室，安徽 合肥 230022

收稿日期 2012-12-28 修回日期 2013-8-31 网络版发布日期 2013-10-18 接受日期

摘要 目的 探讨一氧化氮(NO)在双氯芬酸钠(DCF)诱导大鼠急性肝损伤中的作用。方法 大鼠随机分为正常对照、DCF模型、DCF+N-硝基-*L*-精氨酸甲酯(*L*-NAME)2，10和50 mg·kg^{-1}组。DCF模型组大鼠一次性ip给予DCF100 mg·kg^{-1}，DCF+*L*-NAME组大鼠在给予DCF前10 min一次性ip给予*L*-NAME。给药24 h后处死大鼠，制备血清，用全自动生化分析仪检测谷丙转氨酶(GPT)、谷草转氨酶(GOT)和总胆红素(TBIL)水平，化学法检测NO水平。同时制备肝组织匀浆，用化学法检测NO水平，黄嘌呤氧化酶法测定超氧化物歧化酶(SOD)活性，硫代巴比妥酸法测定丙二醛(MDA)含量，四甲基联苯胺法测定髓过氧化物酶(MPO)活性，Eutler改良法检测还原型谷胱甘肽(GSH)含量，酶促法检测GSH过氧化物酶(GSH-Px)活性。HE染色观察肝组织病理变化。免疫组化法检测肝组织诱导型一氧化氮合酶(iNOS)蛋白表达。提取肝细胞线粒体，硫酸甲酯吩嗪反应法检测琥珀酸脱氢酶(SDH)活性，用紫外可见分光光度计检测ATP酶活性，自体荧光光度计检测还原型烟酰胺腺嘌呤二核苷酸(NADH)水平，罗丹明123法检测膜电位，线粒体肿胀度采用在520 nm处吸光度降低值表示。结果 与正常对照组比较，DCF模型组血清GPT，GOT和TBIL水平明显升高(P<0.05)，血清和肝匀浆NO水平升高(P<0.01)。肝组织iNOS蛋白表达增强(P<0.01)。组织病理观察发现，肝细胞肿胀坏死，炎症细胞浸润。肝匀浆内MDA(P<0.01)和MPO(P<0.05)含量增高，GSH含量(P<0.01)，GSH-Px(P<0.05)和SOD(P<0.01)活性降低。肝细胞线粒体NADH水平、SDH及ATP酶活性明显降低(P<0.01)，同时线粒体膜电位异常，肿胀的敏感性下降。与模型组比较，*L*-NAME 2，10和50 mg·kg^{-1}组血清GPT，GOT和TBIL水平降低，血清和肝匀浆NO含量减低(P<0.05)。肝组织iNOS 表达明显减弱(P<0.05)。组织病理学检测发现，肝细胞肿胀坏死及炎症细胞浸润程度减轻。肝匀浆中MDA和MPO含量降低(P<0.05)，GSH含量及GSH-Px和SOD活性显著升高(P<0.05)，线粒体NADH水平、SDH和ATP酶活性升高(P<0.05)，并且线粒体膜电位恢复，线粒体肿胀的敏感性显著升高。结论 NO对DCF致急性肝损伤起促进作用，其机制可能与线粒体功能障碍有关。

关键词 一氧化氮 双氯芬酸钠 肝损伤 一氧化氮合酶 线粒体

分类号 R99 R971.1

Role of nitric oxide in rats with acute liver injury induced by diclofenac

ZHAO Li-ping[1,2], MEI Qiao[2], HU Yong-mei[2], XU Jian-ming[2], YAO Qiang[2]

1. Medical Department, Huaibei Vocational and Technical College, Huaibei 235000, China;
2. Department of Gastroenterology, the Key Laboratory of Digestive Disease of Anhui Province, the First Affiliated Hospital, Anhui Medical University, Hefei 230022, China

Abstract

OBJECTIVE To investigate the mechanism of nitric oxide (NO) on diclofenac(DCF)-induced liver injury in rats. **METHODS** Rats in DCF model group were once ip injected DCF 100 mg·kg^{-1}, while those in DCF+N^{G}-nitro-*L*-arginine methyl ester(*L*-NAME) 2,10 and 50 mg·kg^{-1} groups were once ip injected *L*-NAME 10 min prior to DCF injection. Twenty-four hours after injection, serum glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT) and total bilirubin (TBIL) levels were analyzed by the automatic biochemistry analyzer, and content of NO in serum was determined by a chemical method. The liver tissue homogenates were prepared, their content of NO was determined by a chemical method, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was determined by a xanthinoxidase method and enzyme method, and the content of malondialdehyde (MDA), glutathione (GSH) and myeloperoxidase (MPO) was detected respectively by thiobarbituric acid, Eutler and tetramethyl benzidine methods. Pathological changes were observed after HE staining. Immunohistochemical SP method was used to investigate the expression of inducible nitric oxide synthase (iNOS). Isolated hepatocyte mitochondria were prepared, the activity of succinodehydrogenase(SDH) and denosine triphosphatase (ATPase) was detected respectively by the methylsulfate method and spectrophotometric analysis, the level of dinucleotide-reduced adenosine triphosphatase (NADH) was monitored by measuring their auto fluorescence, and the mitochondrial membrane potential (MMP) and mitochondrial swelling were also measured. **RESULTS** Compared with normal group, the level of GPT, GOT and TBIL in serum increased in DCF model group(P<0.05). The total production of NO in serum and tissue homogenates also increased (P<0.01). The expression of iNOS was significantly negatively regulated(P<0.01). DCF resulted in liver hepatocyte necrosis,swelling and leukocyte infiltration, and decrease in SOD(P<0.01), GSH(P<0.01) and GSH-PX(P<0.05), while the content of MDA(P<0.01) and MPO increased(P<0.05). The content of NADH and activity of SDH and ATPase were all

decreased(P<0.01). MMP and the sensitivity of mitochondrial swelling were reduced. After *L*-NAME pretreatment, the level of GPT, GOT and TBIL was reduced(P<0.05). The content of NO in serum and liver tissue decreased. The expression of iNOS significantly decreased(P<0.05). Centrilobular hepatic necrosis with leukocyte infiltration was also reduced. The content of MDA and MPO decreased evidently(P<0.05), whereas the activity of SOD(P<0.05), GSH(P<0.05) and GSH-Px(P<0.05) increased in DCF+*L*-NAME groups. Furthermore, the level of mitochondrial NADH increased, and the activity of SDH and ATPase was promoted(P<0.05). MMP was recovered, and the sensitivity of mitochondria swelling was increased. **CONCLUSION** NO contributes to the DCF-induced liver injury via mitochondrial dysfunction.

Key words nitric oxide diclofenac liver injury nitric oxide synthase mitochondria

DOI: 10.3867/j.issn.1000-3002.2013.05.012

通讯作者 梅 俏, E-mail: meiqiaomq@yahoo.com.cn meiqiaomq@yahoo.com.cn