PO Box 2345 Beijing 100023, China
Fax: +86-10-85381893
Email: wcjd@wjgnet.com www.wjgnet.com

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抑制NO诱生对BCG免疫性肝损伤中CYP1A2表达的影响

薛永志, 章国良, 步秀云, 王 昕, 李 丹

薛永志, 章国良, 步秀云, 王昕, 李丹, 北京大学医学部基础医学院药理学系 北京市 100083
薛永志, 男, 1968-09-20生, 内蒙古赤峰市人, 汉族, 2003年北京大学医学部硕士, 副教授, 主要从事肝脏药理学研究.
国家自然科学基金, No. 30171097, No. 30371665和北京大学985项目-人类疾病相关基因研究中心资助课题基金, No. A2000-1
项目负责人: 章国良, 100083, 北京市海淀区学院路38号, 北京大学医学部基础医学院药理学系, 药物代谢与遗传药理学研究室. zhanggl168@mail.bjmu.edu.cn
电话: 010-82802725 传真: 010-82802725
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Effect of inducible nitric oxide synthesis inhibitor on CYP1A2 protein expression in BCG-immune liver damage in mice

Yong-Zhi Xue, Guo-Liang Zhang, Xiu-Yun Bu, Xin Wang, Dan Li

Yong-Zhi Xue, Guo-Liang Zhang, Xiu-Yun Bu, Xin Wang, Dan Li, Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing 100083, China
Supported by the National Natural Science Foundation of China, No. 30171097, No. 30371665; Peking University Center For Human Disease Genomics Research Foundation, No. 2000-A-1
Correspondence to: Guo-Liang Zhang, Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing 100083, China. zhanggl168@mail.bjmu.edu.cn
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Abstract

AIM: To study the effect of nitric oxide production on CYP1A2 protein expression in immune liver damage induced by *Mycobacterium* Calmette-Guerin (BCG) in mice.

METHODS: Immune liver damage was induced by intravenous injection of BCG (125 mg/kg) for 2 weeks *in vivo*. The hepatic tissues injury was estimated by histopathological H-E staining. The protein expression of CYP2E1 and iNOS in hepatic tissues was determined by the method of immunohistochemistry. The correlation between iNOS inducing and liver injury degree was observered by the method of demi-quantification image analysis.

RESULTS: Two weeks after of BCG injection, granuloma was easily observed, and over-expression of iNOS protein was detected in the granulomas. The decrease of CYP1A2 protein expression was observed in mice hepatic tissues. Aminoguanidine, a selective iNOS inhibitor, significantly inhibited iNOS protein expression, and reversed down-regulation of CYP1A2 protein induced by BCG-immune liver damage in mice.

CONCLUSION: Under the BCG-stimulated condition, nitric oxide production participates in the down-regulation of CYP1A2 protein expression induced by immune hepatic injury in mice.

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摘要

目的: 研究卡介苗(BCG)所致小鼠免疫性肝损伤中, 一氧化氮(NO)诱生对肝脏细胞色素P450药物代谢酶系CYP1A2亚型表达的影响.

方法: 采用尾静脉注射BCG诱发小鼠产生免疫性肝损伤, HE染色法观察肝脏病理组织学变化, 采用免疫组化法测定肝组织诱导型一氧化氮合酶(iNOS)及其CYP1A2的蛋白表达, 采用图像梯度灰度扫描法对肝脏病理损伤与iNOS形成进行半定量相关性分析.

结果: 尾静脉注射BCG14 d后, 可致小鼠肝脏形成大量肉芽肿, iNOS蛋白呈团块状棕色强阳性表达, 表达部位与肉芽肿部位相一致, CYP1A2蛋白表达减少; 应用选择性iNOS抑制剂氨基胍抑制NO合成, 可逆转BCG所致CYP1A2蛋白表达的下调.

结论: BCG免疫刺激条件下, iNOS诱生参与了CYP450药物代谢酶系1A2亚型表达下调机制.

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0 引言

CYP1A2是肝脏细胞色素P450(cytochrome P450, CYP450)药物代谢酶系超家族中重要的参与I相氧化反应的酶亚型之一, 在肝脏CYP450酶总含量中所占比例仅次于CYP3A和CYP2C亚家族而居于第3位[1-2]. CYP1A2可参与多种外源性药物及内源性类固醇激素的代谢, 也参与多种前毒物或前致癌物质如芳香胺类、黄曲霉素及杂环胺类的代谢活化过程[3-5]. 以往对CYP1A2代谢功能的研究多着重于该酶在化学毒物代谢中的作用及机制, 而对CYP1A2在免疫性肝损伤过程中确切的作用

及调节机制知之甚少. 近年来的研究表明[6-8]，肝实质细胞可经免疫刺激产生诱导型一氧化氮合酶(iNOS)，所生成大量的一氧化氮(NO)是重要的非特异性免疫效应分子，但NO对肝脏药物代谢酶系中CYP1A2的影响及作用环节尚未明确. 本研究以BALB/C小鼠为实验对象，观察免疫刺激条件下肝脏病理损伤与iNOS诱生相关性、iNOS对在肝脏表达的亚型CYP1A2表达的影响，旨在探讨免疫性肝损伤对CYP450药物代谢酶系及其在肝脏表达的亚型CYP1A2可能的作用及调节机制.

1 材料和方法

1.1 材料 BALB/C近交系♂小鼠，体质量18-22 g，购自北京大学医学部实验动物中心. 卡介苗冻干粉(BCG)，购自中国药物生物制品鉴定所. 兔抗人NOS2 (M-19)IgG抗体购自北京中山生物技术有限公司. Sheep Anti-Rat CYP1A2抗体，购自美国Research Diagnostics公司. Powervision二抗，美国Santa Cruz产品，购自北京中山生物技术有限公司. 氨基胍(aminoguanidine，AG)，L-精氨酸甲酯(L-NAME)，及细菌脂多糖(LPS)均为美国Sigma公司产品. 其他试剂：联二亚硫酸钠，苯巴比妥钠，三羟甲基氨基甲烷，蔗糖，KCl，$MgCl_2$，考马斯亮兰，磷酸，均为国产，化学纯.

1.2 方法 取BALB/C ♂小鼠42只，随机分为7组，每组6只. 即空白对照组、AG对照组、BCG免疫性肝损伤模型组、BCG+LPS组、BCG+AG各剂量组(AG为25、50、100 mg/kg). 将BCG刺激各组小鼠经尾静脉1次注射给予BCG (125 mg/kg，2 wk)，制备免疫性肝损伤动物模型. BCG刺激1 wk后，给予BCG + AG各剂量组隔日1次(共3次)经ip上述剂量的AG. 于BCG刺激2 wk后(第13晚)，给予BCG+LPS组经尾静脉1次性注射LPS (125 μg/kg)刺激12 h. AG对照组于实验第1 wk后隔日1次ip给予空白鼠AG(50 mg/kg)，共给予3次. 在BCG刺激2 wk后(第14晨)，各组动物取肝脏经40 g/L多聚甲醛固定24 h，石蜡包埋. 每只肝脏在同一部位取材后，连续制备下列肝组织切片(5 μm)，即空白对照、HE染色、免疫组化空白对照(无iNOS或CYP1A2一抗)、iNOS或CYP1A2免疫组化染色. 在光镜下观察肝脏病理组织学或免疫组织化学变化.

1.2.1 肝组织iNOS及CYP1A2免疫组化染色[9-11] 石蜡包埋切片常规系列乙醇脱蜡至水. 30 mL/L过氧化氢封闭，微波加热法修复抗原，50-100 mL/L山羊血清封闭后，分别滴加CYP 1A2一抗(4 ℃过夜)、生物素标记链酶卵白素二抗，PBS冲洗5 min，用DAB(染iNOS)及AEC(染CYP1A2)显色，苏木精复染核后透明封片. 光镜下观察免疫组化染色结果.

1.2.2 半定量图像灰度梯度扫描肝病理学及免疫组化结果 采用Olympus光学显微镜及LEICA-Q/550IW图像分析系统(德国LEICA公司)，在光镜下每张切片随机选取3个视野，对各组HE染色肝病理组织片及免疫组化片进行图像灰度梯度扫描，以单位面积内淋巴细胞浸润所致深蓝色肉芽肿团块、及iNOS阳性棕色染色团块的密度与面积的乘积作为观察指标，进行半定量相关性分析.

统计学处理 实验结果以平均值±标准差(mean±SD)表示，显著性分析采用ANOVA，Duncan's test，SPSS11.0进行方差分析处理，组间比较采用双侧t检验.

2 结果

2.1 BCG免疫性肝损伤与小鼠肝组织中iNOS表达的相关性 肝脏病理组织学结果显示，BCG免疫刺激组小鼠肝组织中有大量淋巴细胞浸润所致蓝色肉芽肿团块形成(图1)；在BCG刺激的基础上给予LPS刺激后，肉芽肿形成面积更为增大. 将图像进行灰度梯度扫描所得肉芽肿阳性染色密度与阳性染色面积的乘积(S × OD)作为观察指标，结果显示BCG组与空白对照组、BCG+LPS组与BCG组相比均有显著性差异(表1，$P<0.05$). 免疫组化结果显示，在BCG刺激组的肉芽肿形成部位，可见大量iNOS强阳性棕色染色团块(图2)；在BCG刺激的基础上给予LPS刺激后，iNOS表达亦进一步增多. 图像灰度梯度半定量扫描结果显示，BCG组与空白对照组、BCG+LPS组与BCG组各组之间iNOS阳性染色均有显著性差异(表1，$P<0.05$).

表1 氨基胍对BCG免疫损伤性小鼠肝组织肉芽肿形成及iNOS表达灰度扫描面积的影响. (mean±SD, n =6)

Group	S×OD(μm²/ view)	
	Granuloma formation	iNOS expression
Control	0 ± 0	0 ± 0
BCG (125 mg/kg)	184 523 ± 24 634[c]	43 554 ± 5 248[c]
BCG+LPS (125 μg/kg)	437 163 ± 52 654[a,c]	88 501 ± 21 311[a,c]
BCG+AG (25 mg/kg)	218 294 ± 22 847[c]	39 038 ± 6 375[c]
BCG+AG (50 mg/kg)	169 588 ± 19 380[c]	30 158 ± 6 007[c]
BCG+AG (100 mg/kg)	125 548 ± 67 99[a,c]	24 226 ± 4 466[a,c]
AG (50 mg/kg)	0 ± 0[a]	0 ± 0[a]

[a]$P<0.05$ *vs* BCG组；[c]$P<0.05$ *vs* Control组.

2.2 BCG免疫性肝损伤对小鼠肝脏CYP1A2表达的影响 CYP1A2表达免疫组织化学结果显示，CYP1A2在空白对照组小鼠肝实质细胞浆中呈少量阳性固有表达，BCG免疫性刺激可导致小鼠肝实质细胞中CYP1A2表达明显减少(图3)，BCG+LPS刺激则使CYP1A2蛋白表达进一步减少.

2.3 AG对BCG免疫性肝伤条件下CYP1A2表达的影响 与空白对照组相比，单独给予选择性iNOS抑制剂AG未见肝实质细胞内CYP1A2红色阳性表达染色强度有明显改变；在BCG免疫性刺激条件下，给予25-100 mg/kg剂量的AG可使被BCG抑制的肝实质细胞内CYP1A2阳

性表达染色增强(图 4).

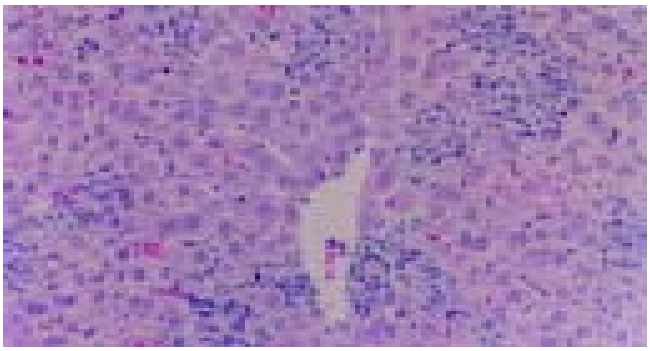

图1 BCG(125 mg/kg, iv)免疫性肝损伤组大鼠肝组织大量肉芽肿形成(×200).

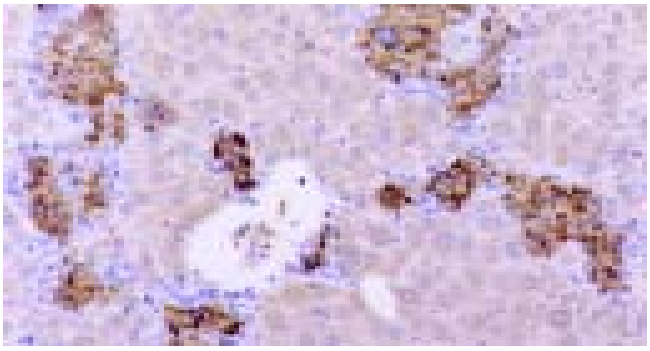

图2 BCG(125 mg/kg)免疫性肝损伤组 iNOS 高表达(×200).

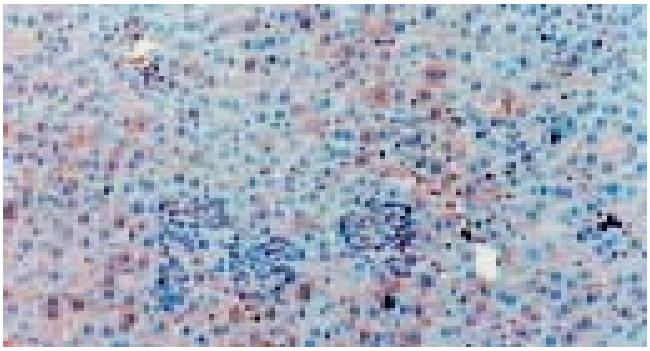

图3 BCG(125 mg/kg)免疫性肝损伤组 CYP1A2 低表达(×200).

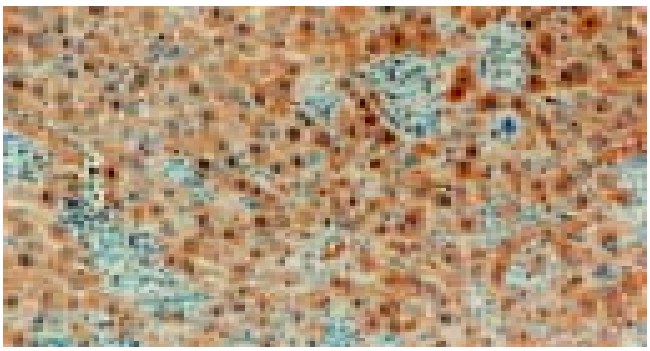

图4 BCG(125 mg/kg)+ 氨基胍(100mg/kg)组 CYP1A2 高表达(×200).

3 讨论

在我国人群中发生的肝炎多为病毒、原虫等微生物感染所致免疫性肝损伤[12], 但以往对肝损伤条件下药物代谢动力学调控机制的研究多以化学性肝损伤模型为基础[13-14]. 根据文献[15-16]报道以及本室以往的研究, 经尾静脉注射BCG或BCG+LPS可诱发啮齿类动物如小鼠或大鼠的免疫性肝损伤, 此过程由大量炎性细胞因子诱生所介导, 可较方便地作为肝脏免疫损伤条件下药代动力学机制研究的评价手段. 本研究结果进一步表明, 在 BCG 免疫损伤性刺激所致肝脏炎症条件下, 肝组织间炎性细胞浸润形成的大量肉芽肿, 与iNOS表达增加呈明显正相关. 在BCG刺激的基础上加用LPS可见肝损伤进一步加重, iNOS 表达亦随之增加, 提示 NO 作为肝脏中重要的非特异性免疫效应分子参与了细胞因子所致肝脏免疫损伤性反应机制. 在BCG损伤性刺激条件下, 肝脏匀浆或肝微粒体样本中的 CYP450 全酶含量较之对照组均显著降低[17]. 我们通过观察BCG免疫性肝损伤对 CYP1A2 酶蛋白表达的影响, 进一步明确了BCG免疫性刺激可对主要在肝脏固有表达的CYP1A2亚型产生抑制作用. 本研究免疫组化结果还显示, CYP1A2 酶蛋白在空白对照组小鼠肝实质细胞中可有一定量的固有表达, BCG 免疫性刺激可导致小鼠肝实质细胞中 CYP1A2 表达减少, BCG+LPS 刺激则使 CYP1A2 蛋白表达进一步减少. 选择性iNOS抑制剂氨基胍[17-18]在25-100 mg/kg 剂量范围内, 可使被 BCG 抑制的肝实质细胞内 CYP1A2 表达染色增强, 提示 BCG 或 BCG+LPS 对CYP1A2酶蛋白表达的抑制作用至少部分是通过NO诱生机制实现的.

本结果提示, 多种临床常用的 NO 供体类药物(如硝酸甘油等)均有可能对 CYP450 酶系的代谢能力产生重要影响[19-22], 亦为肝脏感染及炎症条件下采用 NOS 抑制剂对CYP450代谢能力进行选择性调控提供了新思路. 已知CYP1A2代谢能力与肝脏炎症及癌发生过程密切相关, 故 CYP1A2 与 NO 相互作用和调节机制近年来引起了人们的关注. 由于CYP450酶系与NOS本身均为含血红素蛋白, 二者都以亚铁血色素为辅基[23-28]. 有学者观察到在免疫损伤性刺激条件下, 肝脏iNOS和亚铁血红素合成酶均过表达, NO 作用于氧合血红蛋白, 引起血管收缩, 导致肝功能紊乱; 在选择性抑制iNOS的基础上, 合用亚铁血红素合成酶抑制剂可抑制上述改变, 推测其机制可能是NO作用于CYP450血色素的铁-硫反应中心, 从而下调CYP450mRNA 表达所致[29-30]. 故今后尚需进一步观察 NO 对 CYP1A2 在酶代谢活性或 mRNA 表达水平的影响, 以阐明 NO 下调 CYP450 酶系及亚型的分子机制和调控环节.

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