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

World Chin J Digestol 2003 August;11(8):1182-1184
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R




# PD98059对乙醛刺激的大鼠肝星状细胞增生的影响

马洪德, 蒋明德, 钟显飞, 解方为, 曾维政

马洪德, 蒋明德, 钟显飞, 解方为, 曾维政, 成都军区总医院消化内科 四川省成都市 610083
马洪德, 男, 1967-04-18生, 河南省柘城县人, 汉族. 第三军医大学、成都军区总医院联合培养的在读硕士研究生, 主治医师. 主要从事肝纤维化的基础研究.
全军“十五”医药卫生科研基金资助课题, No.01MB037
项目负责人: 蒋明德, 610083, 四川省成都市天回镇, 成都军区总医院消化内科. jiangmd@mail.sc.cninfo.net
电话: 028-86570346
收稿日期: 2003-01-11 接受日期: 2003-02-19

## Effects of PD98059 on proliferation of rat cultured hepatic stellate cells stimulated by acetaldehyde

Hong-De Ma, Ming-De Jiang, Xian-Fei Zhong, Fang-Wei Xie, Wei-Zheng Zeng

Hong-De Ma, Ming-De Jiang, Xian-Fei Zhong, Fang-Wei Xie, Wei-Zheng Zeng, Department of Gastroenterology, General Hospital of Chengdu Military Command, Chengdu 610083, Sichuan Province, China
Supported by Funds from Military Foundation for Medicine and Health during the 10th 5-year period, No. 01MB037
Correspondence to: Dr. Ming-De Jiang, Department of Gastroenterology, General Hospital of Chengdu Military Command, Chengdu 610083, Sichuan Province, China. jiangmd@mail.sc.cninfo.net
Received: 2003-01-11 Accepted: 2003-02-19

## Abstract

AIM: To study the effects of PD98059, the specific blocking agent of $MEK_1$, on the proliferation of hepatic stellate cells and expression of Proliferating Cell Nuclear Antigen in rat hepatic stellate cells (HSC).

METHODS: HSC stimulated by acetaldehyde were cultured. The cell growth was evaluated by MTT colorimetric assay. Proliferating cell nuclear antigen (PCNA) was examined by immunocytochemical staining.

RESULTS: PD98059 of 20 μmol/L had an inhibitory effect on proliferation of HSC ($P$ <0.05, 0.109±0.020 *vs* 0.146±0.030), which was more obvious when cells exposed to PD98059 at 50 and 100 μmol /L ($P$ <0.05, 0.081±0.010, 0.056±0.020 *vs* 0.146±0.030), and the expression of PCNA also showed a descending tendency with the increase of PD98059 concentration ($P$ <0.05, 0.62±0.09, 0.47±0.04, 0.34±0.04 *vs* 0.74±0.05)

CONCLUSION: PD98059 inhibits proliferation of HSC and expression of PCNA, which is correlated with the decreased activity of PCNA.

Ma HD, Jiang MD, Zhong XF, Xie FW, Zeng WZ. Effects of PD98059 on proliferation of rat cultured hepatic stellate cells stimulated by acetaldehyde. Shijie Huaren Xiaohua Zazhi 2003;11(8):1182-1184

## 摘要

**目的:** 探讨特异性MEK1阻断剂PD98059对乙醛刺激的肝星状细胞(hepatic stellate cell，HSC)增生及其细胞增生核抗原表达的影响.

**方法:** 用PD98059对乙醛刺激的HSC进行处理，分别以MTT比色、免疫细胞化学法检测细胞增生及其细胞增生核抗原表达.

**结果:** PD98059在20 μmol/L时即对HSC增生出现抑制作用($P$ <0.05，C组0.109 ±.020 *vs* B组0.146 ± 0.030)，50、100 μmol/L时抑制作用逐渐增强($P$ <0.05，D、E组0.081 ± 0.010、0.056 ± 0.020 *vs* B组0.146 ± 0.030); HSC中PCNA表达也随PD98059剂量增加而减弱($P$ <0.05，C、D、E组0.62 ± 0.09、0.47 ± 0.04、0.34 ± 0.04 *vs* B组0.74 ± 0.05)

**结论:** PD98059对HSC增生及细胞增生核抗原表达具有抑制作用，提示Erk信号传导通路是调控肝星状细胞增生的重要通道.

马洪德, 蒋明德, 钟显飞, 解方为, 曾维政. PD98059对乙醛刺激的大鼠肝星状细胞增生的影响. 世界华人消化杂志 2003;11(8):1182-1184
http://www.wjgnet.com/1009-3079/11/1182.asp

## 0 引言

肝星状细胞(HSC)是形成肝纤维化的主要细胞，HSC的活化与增生是肝纤维化进展的中心环节[1-5]. 乙醛是激活HSC导致酒精性肝纤维化的关键分子[6,7]. 细胞外信号调节激酶(extracellular signal-regulated kinase，Erk)信号通路参与调控细胞增生与分化，是多种信号交汇点或共同通路[8-14]，但在乙醛刺激的HSC增生中的作用报道较少. 我们用不同剂量的特异性MEK1阻断剂PD98059阻断Erk活性后，观察乙醛刺激的HSC增生及其细胞增生核抗原表达的变化，探讨Erk信号通路调控乙醛刺激的HSC增生的分子机制，为酒精性肝纤维化的防治提供理论依据.

## 1 材料和方法

**1.1 材料** PD98059(NEB公司); 增生细胞核抗原(PCNA)免疫组化法检测试剂盒(北京中山公司); 小牛血清及DMEM/IMDM培养基(美国Gibco公司); CFSC大鼠肝星状细胞株由第三军医大学李小安博士惠赠.

1.2 方法 细胞用含 150 ml/L 小牛血清的 DMEM/IMDM (1∶1)培养基，另添加适量的 HEPES、抗生素(1 × $10^5$ 单位／L 青霉素和 100 mg/L 链霉素)至 37 ℃、50 ml/L $CO_2$ 混合气体的孵箱中培养，细胞换液时间为 2-3 d，传代时间为 3-5 d，传代前用 2.5 g/L 胰酶消化. 实验分为: 空白对照组(A 组)加无血清的 DMEM/IMDM(1∶1)培养液; 乙醛对照组(B 组): 无血清培养液中加乙醛 200 μmol/L; 实验组 1(C 组): 在 B 组基础上培养液中加入 PD98059 (浓度为 20 μmol/L); 实验组 2 (D 组): 在 B 组基础上培养液中加入 PD98059 (浓度为 50 μmol/L); 实验组 3 (E 组): 在 B 组基础上培养液中加入 PD98059 (浓度为 100 μmol/L). 取大鼠 HSC，调整浓度至 1 × $10^8$/L 接种于 96 孔细胞培养板中，每孔 200 μL 细胞悬液，细胞生长至 80 % 以上融合度时，4 ml/L 血清培养基同步化处理 24 h，实验组经 PD98059 预处理 1 h 后，加乙醛 200 μmol/L (终浓度)，$CO_2$ 培养箱中继续孵育 24 h 后(乙醛每 12 h 补充)， 每孔加 5 g/L 的 MTT 20 μL，反应 4 h，用快速翻板法去除培养液，加 DMSO 200 μL，30 min 后用酶标仪(E-Liza Mat-3000)双波长测定其 *A* 值，测定波长为 570 nm，参考波长为 630 nm，酶标仪所示 *A* 值为 *A*570 减去 *A*630，以消除非特异性光吸收效应. 免疫组化: 以 5 × $10^8$/L 接种 12 孔培养板，每孔 1 ml，作细胞爬片. 细胞生长至 80 % 以上融合度行同步化处理后，按上述分组法加药继续培养 24 h. 将培养板孔中的盖玻片取出，PBS 洗涤两次，用 40 g/L 多聚甲醛固定 10 min，按 SP 试剂盒操作方法进行免疫细胞化学 PCNA 检测，DAB 显色，同时用 PBS 代替一抗作阴性对照. 结果判定: 胞核呈棕黄色为阳性，并对染色后的细胞片进行显微镜下观察，摄片，IDA-2000 软件分析平均灰密度，相对定量 PCNA 表达的强度.

统计学处理 所有指标均用($\bar{x} \pm s$)表示，采用 SPSS11.0 软件分析. 成组设计多样本均数的比较用单因素方差分析.

## 2 结果

2.1 MTT 结果 加入乙醛后明显刺激 HSC 增生($P$ <0.05，B 组 0.146 ± 0.030 *vs* A 组 0.030 ± 0.010); 随着 PD98059 用量增加 C (0.109 ± 0.020)、D(0.081 ± 0.010)、E (0.056 ± 0.020) 3 组细胞增生均受到抑制($P$ <0.05，*vs* B 组)，呈明显的剂量效应关系，但 E 组细胞增生仍高于 A 组，表明 100 μmol/L PD98059 仍不能完全抑制乙醛刺激的 HSC 增生.

2. 2 PCNA 免疫组织化学染色 乙醛对照组 HSC 胞核着色最深，呈棕黄色(图 1)，实验组随着 PD98059 用量的增加胞核着色逐渐减弱，PD98059 100 μmol/L (完全抑制Erk活性剂量)胞核呈淡黄色(图2). 经病理分析软件分析显示， 乙醛组 PCNA 平均灰密度较对照组明显增高($P$ <0.05，B 组 0.74 ± 0.05 *vs* A 组 0.24 ± 0.03); 实验组随着 PD98059 用量的增加 HSC 内 PCNA 平均灰密度较乙醛组明显降低($P$ <0.05)，E 组 PCNA 平均灰密度(0.34 ± 0.04)仍高于 A 组($P$ <0.05)，结果表明 PD98059 具有抑制作用，但100 μmol/L PD98059仍不能完全抑制乙醛刺激的 HSC 内 PCNA 表达.

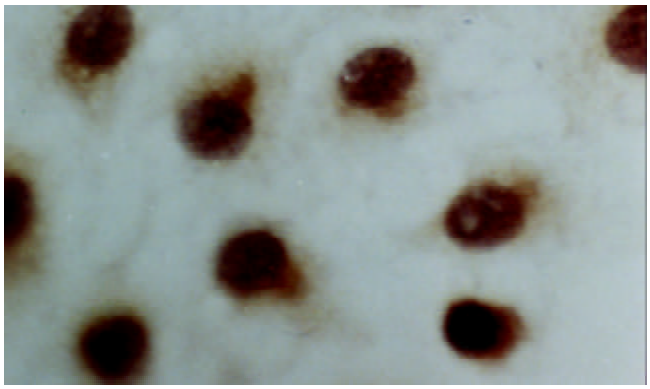

图 1 乙醛组 HSC 免疫细胞化学染色胞核着色呈棕黄色(× 400 SP 法).

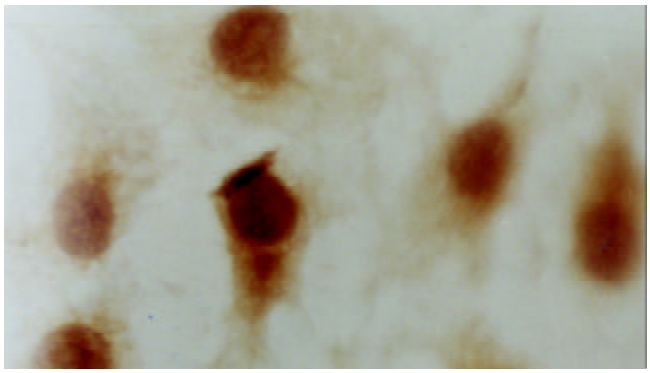

图 2 100 μmmol/LPD98059 予处理乙醛刺激的 HSC 后胞核着色呈谈黄色(× 400 SP 法).

## 3 讨论

肝纤维化的发生机制较复杂[15]，目前关于 ERK 信号传导通路的激活与肝纤维化发生机制的研究甚少. 文献表明: Erk 信号传导通路参与调控 PDGF 等刺激的 HSC 增生[16-19]. 乙醛作为有效刺激信号是激活 HSC 导致酒精性肝纤维化发生的关键分子，但 ERK 信号通路在乙醛刺激的 HSC 增生中的意义报道较少，我们采用 MTT 实验观察 PD98059 对乙醛刺激的 HSC 增生的影响，结果表明阻断 Erk 信号通路后 HSC 增生明显抑制，且有剂量依赖关系，提示Erk信号通路是调控乙醛刺激的HSC增生的重要通道，同时100 μmol/L PD98059(完全抑制 Erk 活性剂量)仍不能完全抑制 HSC 增生，提示 Erk 通路并非是调控乙醛刺激的 HSC 增生的唯一通道.

增生细胞核抗原(proliferating cell nuclear antigen，PCNA)，又称细胞周期蛋白，是真核细胞 DNA 合成时所必需的一种 36 KD 的酸性核蛋白[20]，他在细胞周期调控方面发挥着重要作用[21-26]，其表达亦可反应细胞增生的活跃程度[27-30]，目前 Erk 信号通路调控乙醛刺激的 HSC 增生的分子机制尚不明了，我们用不同浓度 PD98059(20，50，100 μmol/L)分别加入乙醛刺激的 HSC 内 24 h 后，免疫细胞化学染色法检测发现，

PD98059组与乙醛对照组相比，PCNA表达逐渐减弱，100 μmol/L的PD98059组PCNA表达仍弱阳性，提示PD98059抑制乙醛刺激的HSC增生可能与一定程度上抑制细胞周期蛋白PCNA合成有关.

目前对Erk信号通路调控乙醛刺激的HSC增生的分子机制研究刚刚起步，其确切机制尚需进一步研究.

## 4 参考文献


1 Brenner DA, Waterboer T, Choi SK, Lindquist JN, Stefanovic B, Burchardt E, Yamauchi M, Gillan A, Rippe RA. New aspects of hepatic fibrosis. *J Hepatol* 2000;32:32-38

2 Kim KY, Rhim TY, Choi I, Kim SS. N-acetylcysteine induces cell cycle arrest in hepatic stellate cells through its reducing. *Activity J Biol Chem* 2001;276:40591-40598

3 姜慧卿, 张晓岚. 肝纤维化的发生机制. 世界华人消化杂志 2000;8:687-689

4 朱永红, 胡大荣, 聂青和, 刘国栋, 谭朝霞. 人肝脏星形细胞培养激活及其c-fos,c-jun的表达. 世界华人消化杂志 2000;8:299-302

5 王宝恩. 肝星状细胞与肝纤维化. 中华肝脏病杂志 2000;8:197-199

6 Anania FA, Womack L, Jiang MD, Saxena NK. Aldehydes potentiate alpha(2)(I) collagen gene activity by JNK in hepatic stellate cells. *Free Radic Biol Med* 2001;30:846-856

7 Svegliati-Baroni G, Ridolfi F, Di Sario A, Saccomanno S, Bendia E, Bendia E, Benedetti A, Greenwel P. Intracellular signaling pathways involved in acetaldehyde-induced collagen and fibronectin gene expression in human hepatic stellate cells. *Hepatology* 2001;33:1130-1140

8 Peyssonnaux C, Provot S, Felder-Schmittbuhl MP, Calothy G, Eychene A. Induction of postmitotic neuroretina cell proliferation by distinct ras downstream signaling pathways. *Mol Cell Biol* 2000;20:7068-7079

9 Lee JH, Johnson PR, Roth M, Hunt NH, Black JL. ERK activation and mitogenesis in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2001;280:1019-1029

10 Schmidt M, Goebeler M, Posern G, Feller SM, Seitz CS, Brocker EB, Rapp UR, Ludwig S. Ras-independent activation of the Raf/MEK/ERK pathway upon calcium-induced differentiation of keratinocytes. *J Biol Chem* 2000;275:41011-41017

11 Cha H, Shapiro P. Tyrosine-phosphorylated extracellular signal–regulated kinase associates with the golgi complex during G2/M phase of the cell cycle: evidence for regulation of golgi structure. *The J Cell Biol* 2001;153:1355-1368

12 Hoshino R, Tanimura S, Watanabe K, Kataoka T, Kohno M. Blockade of the extracellular signal-regulated kinase pathway induces marked G1 cell cycle arrest and apoptosis in tumor cells in which the pathway is constitutively activated up-regulatton of p27 (kipl). *J Biol Chem* 2001;276:2686-2692

13 Keenan SM, Bellone C, Baldassare JJ. Cyclin-dependent kinase 2 nucleocytoplasmic translocation is regulated by extracellular regulated kinase. *J Biol Chem* 2001; 25: 22404-22409

14 Dolci S, Pellegrini M, Di Agostino S, Geremia R, Rossi P. Signaling through extracellular signal-regulated kinase is required for spermatogonial proliferative response to stem cell factor. *J Biol Chem* 2001;276: 40225-40233

15 Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000; 275: 2247-2250

16 Marra F, Arrighi MC, Fazi M, Caligiuri A, Pinzani M, Romanelli RG, Efsen E, Laffi G, Gentilini P. Extracellular signal-regulated kinase activation differentially regulates platelet-derived growth factors actions in hepatic stellate cells, and is inchuced by in vivo liver injury in the rat. *Hepatology* 1999;30:951-958

17 Lee KS, Cottam HB, Houglum K, Wasson DB, Carson D, Chijkier M. Pentoxifylline blocks hepatic stellate cell activation independently of phosphodiesterase inhibitory activity. *J Physiol* 1997;273:1094-1100

18 Iwamoto H, Nakamuta M, Tada S, Sugimoto R, Enjoji M, Nawata H. Platelet-derived growth factor receptor tyrosine kinase inhibitor AG1295 attenuates rat hepatic stellate cell growth. *J Lab Clin Med* 2000;135:406-412

19 Wang YQ, Ikeda K, Ikebe T, Hirakawa K, Sowa M, Nakatani K, Kawada N, Kaneda K. Inhibition of hepatic stellate cell proliferation and activation by the semisynthetic analogue of fumagillin TNP-470 in rats. *Hepatology* 2000;32:983-989

20 Dervan PA, Magee HM, Buckley C, Carney DN. Proliferating cell nuclear antigen counts in formalin-fixed paraffin-embeded tissue correlate with Ki-67 in fres tissue. *Am J Clin Pathol* 1992;97(5 Suppl 1):S21-28

21 Hida M, Omori S, Awazu M. ERK and p38 MAP kinase are required for rat renal development. *Kidney Int* 2002;61:1252-1262

22 Hirata A, Igarashi M, Yamaguchi H, Suwabe A, Daimon M, Kato T, Tominaga M. Nifedipine suppresses neointimal thickening by its inhibitory effect on vascular smooth muscle cell growth via a MEK-ERK pathway coupling with Pyk2. *Br J Pharmacol* 2000;131:1521-1530

23 Westwood G, Dibling BC, Cuthbert-Heavens D, Burchill SA. Basic fibroblast growth factor (bFGF)-induced cell death is mediated through a caspase-dependent and p53-independent cell death receptor pathway. *Oncogene* 2002; 21:809-824

24 Weber CK, Sommer G, Michl P, Fensterer H, Weimer M, Gansauge F, Leder G, Adler G, Gress TM. Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. *Gastroenterology* 2001;121:657-667

25 Paunesku T, Mittal S, Protic M,Oryhon J,Korolev SV,JoachimiakA, Woloschak GE. Proliferating cell nuclear antigen: (CPCNA) ringmaster of the genome. *Int J Radiat Biol* 2001;77:1007-1120

26 高美霞, 张乃珍, 冀春萱. 胃癌雌激素受体与PCNA的关系. 世界华人消化杂志 2000;8:1117-1120

27 Kitada T, Seki S, Nakatani K, Kawada N, Kuroki T, Monna T. Hepatic expression of c-Myb in chronic human liver disease. *Hepatology* 1997;26:1506-1512

28 Ramakrishna G, Perella C, Birely L, Diwan BA, Fornwald LW, Anderson LM. Decrease in K-ras p21 and increase in Raf1 and activated Erk 1 and 2 in murine lung tumors initiated by N-nitrosodimethylamine and promoted by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol Appl Pharmacol* 2002; 179:21-34

29 Omori S, Fukuzawa R, Hida M, Awazu M. Expression of mitogen-activated protein kinases in human renal dysplasia. *Kidney Int* 2002;61:899-906

30 Ishizuka S, Yano T, Hagiwara K, Sone M, Nihei H, Ozasa H, Horikawa S. Extracellular signal-regulated kinase mediates renal regeneration in rats with myoglobinuric acute renal injury. *Biochem Biophys Res Commun* 1999;254:88-92