脂氧素A_4受体激动剂BML-111对大鼠肝纤维化模型氧化/抗氧化平衡的影响

余忠建[1, 3#] 胡泉东[1#] 徐方云[2] 黄永红[2] 朱 俊[2] 周晓燕[2*]

([1]南昌大学药学院药理学教研室, 南昌 330006; [2]南昌大学基础医学院病理生理系, 南昌 330006; [3]江西省肿瘤医院, 南昌 330006)

摘要 该文探讨了脂氧素A_4受体激动剂BML-111[5(S),6(R),7-trihydroxyheptanoic acid methyl ester]对四氯化碳(carbon tetrachloride, CCl_4)诱导的大鼠肝纤维化模型氧化和抗氧化平衡的影响。40只SD(Sprague-Dawley)大鼠皮下注射40% CCl_4以建立肝纤维化模型, 干预处理为在不同时间段皮下注射1 mg/kg BML-111。经不同的方式处理过的40只SD大鼠被分为4组, 分别为对照组、CCl_4组、治疗组和预防组。通过观察肝脏表面的颜色、光滑度及结节确定大鼠肝纤维化程度; 通过病理组织切片HE(hematoxylin-eosin)染色对大鼠肝脏损伤和炎症细胞浸润情况进行评估; 通过检测肝组织匀浆中丙氨酸转氨酶(alanine transaminase, ALT)和天冬氨酸转氨酶(aspartate transaminase, AST)活性进行评价大鼠肝功能情况; 通过检测肝组织中脂质氧化产物丙二醛(malondialdehyde, MDA)含量、抗氧化酶如谷胱甘肽过氧化物酶(glutathione peroxidase, GSH-Px)、超氧化物歧化酶(superoxide dismutase, SOD)和过氧化氢酶(catalase, CAT)活性以及肝组织中总抗氧化能力(total antioxidative capacity, T-AOC)对大鼠体内氧化/抗氧化平衡状态进行评价。结果显示, CCl_4组大鼠肝脏颜色明显偏黄, 表面失去光滑, 结节化程度最严重; BML-111预防或治疗后, 其症状明显改善。BML-111可以抑制CCl4诱导的大鼠肝脏损伤和炎症细胞浸润。CCl_4组肝组织匀浆液中ALT和AST活性明显降低; BML-111能够提高ALT和AST的活性(P<0.05)。CCl_4组MDA含量明显升高, 而GSH-Px、SOD和CAT的活性降低, 总抗氧化能力T-AOC下降。BML-111预防或治疗处理后, MDA含量明显下降(P<0.05), T-AOC增强(P<0.05), GSH-Px、CAT和SOD的活性均明显回升(P<0.05)。由此可知, BML-111可以抑制CCl_4诱导的大鼠肝纤维化, 此效应与其调节肝组织的氧化/抗氧化平衡相关。

关键词 脂氧素; 肝纤维化; 肝功能; 氧化; 抗氧化

BML-111, A Lipoxin A_4 Receptor Agonist, Regulated the Balance of Oxidation and Antioxidation in Rat Model with Hepatic Fibrosis

Yu Zhongjian[1,3#], Hu Quandong[1#], Xu Fangyun[2], Huang Yonghong[2], Zhu Jun[2], Zhou Xiaoyan[2*]

([1]*Department of Pharmacology, Medical College of Nanchang University, Nanchang 330006, China*; [2]*Department of Pathophysiology, Medical College of Nanchang University, Nanchang 330006, China*; [3]*Jiangxi Province Cancer Hospital, Nanchang 330006, China*)

Abstract In order to confirm the effect of BML-111, a lipoxin A_4 receptor agonist, in regulating the balance of oxidation and antioxidation in CCl_4-induced experimental hepatic fibrosis, forty Sprague-Dawley (SD) rats were used to build the model of hepatic fibrosis through hypodermic injection of 40% CCl_4. And 1 mg/kg BML-111 was

收稿日期: 2016-04-07 接受日期: 2016-08-26

国家自然科学基金(批准号: 81100299、81460126)资助的课题

#共同第一作者

*通讯作者。Tel: 0791-86360556, E-mail: zhouxiaoyan@ncu.edu.cn

Received: April 7, 2016 Accepted: August 26, 2016

This work was supported by the National Natural Science Foundation of China (Grant No.81100299, 81460126)

#These authors contributed equally to this work

*Corresponding author. Tel: +86-791-86360556, E-mail: zhouxiaoyan@ncu.edu.cn

网络出版时间: 2016-10-28 17:37:08 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20161028.1737.010.html

administrated via subcutaneous for prevention and treatment. These SD rats were respectively divided into 4 groups via treating with different ways: control group, CCl_4 group, treatment group and prevention group. The severity of hepatic fibrosis was defined by observing the change in color, surface glossy and the extent of nodule in rat liver. The degree of hepatic injury and inflammatory infiltration was assessed by hematoxylin-eosin (HE) staining for the hepatic tissue sections. The liver function was evaluated by detecting the activities of alanine transaminase (ALT) and aspartate transaminase (AST) in hepatic homogenate. The balance of oxidation and antioxidation was evaluated by detecting the content of malondialdehyde (MDA), the activities of antioxidant enzyme, including GSH-Px, superoxide dismutase (SOD) and catalase (CAT), and the total antioxidative capacity (T-AOC). Through the above experiments, several results were found. When compared to the control group, the livers of CCl_4 treated rats looked obviously slant yellow, lost surface glossy, and performed lots of nodules, which could be improved by BML-111, both in the prevention and the treatment groups. BML-111 could inhibit CCl_4-induced hepatic injury and inflammatory cell infiltration in rats. CCl_4 treated rats performed obviously lower activity of AST and ALT in hepatic homogenate, which was reversed when BML-111 was injected (P<0.05). BML-111, both in the prevention and the treatment groups, the content of CCl_4-induced MDA was decreased, but the level of T-AOC and the activities of SOD, GSH-Px and CAT were all increased. The results showed that BML-111 could inhibit CCl_4-induced experimental hepatic fibrogenesis by regulating redox homeostasis in rat liver tissue.

Keywords lipoxin; hepatic fibrosis; oxidation and antioxidation balance

脂氧素(lipoxins, LXs)是体内重要的内源性抗炎物质, 具有强大的抗炎和促炎症消退作用[1-2]。由于LXs极不稳定, 其受体激动剂BML-111常被当作LXs体内研究的等效替代物。本课题组已证实BML-111可通过下调转化生长因子-β(transforming growth factor-β, TGF-β)和血小板源性生长因子(platelet derived growth factor, PDGF)来抑制肝纤维化[3]。肝纤维化时, 肝组织活性氧(reactive oxygen species, ROS)含量异常升高[4], ROS不仅可以直接损伤肝细胞, 诱导肝星状细胞(hepatic stellate cell, HSC)活化[5-7], 还可以刺激最重要的促纤维化因子TGF-β和PDGF大量合成, 从而促进细胞外基质(extracellular matrixc, ECM)的合成, 加速肝纤维化进展[8-9]。由此可见, 氧化/抗氧化平衡是影响肝纤维化发生发展的关键因素之一。为此, 本文研究了BML-111对肝纤维化大鼠模型氧化/抗氧化平衡的调节作用。

1 材料与方法

1.1 材料

BML-111购自美国Cayman公司, CCl_4购自Sigma公司, AST、ALT、MDA、GSH-Px、SOD、CAT及T-AOC等检测试剂盒均购自南京建成生物有限公司。

1.2 方法

1.2.1 大鼠肝纤维化模型建立 SD大鼠购自南昌大学动物科学实验中心, 体重180~220 g, 雌雄不限, 全部动物实验相关操作通过南昌大学药学院伦理审查委员会批准。大鼠在适宜条件下(温度23±2 ℃, 湿度55%±10%)饲养, 自由进食进饮。40只大鼠随机分为4组, 每组10只。对照组: 每周皮下注射2次3 mL/kg PBS, 共10周。CCl_4组: 每周皮下注射2次3 mL/kg 40% CCl_4/橄榄油(4:6, V:V), 共10周。预防组: 第1~4周皮下注射1 mg/kg BML-111(浓度1 mg/mL), 其他处理同CCl_4组。治疗组: 第5~10周皮下注射浓度1 mL/kg BML-111(浓度1 mg/mL), 其他处理同CCl_4组。

1.2.2 大鼠肝纤维化严重程度的评价 摘取完整大鼠肝脏组织, 观察大鼠肝脏组织表面的颜色以及结节化程度。

1.2.3 大鼠肝脏损伤和炎症细胞浸润情况评估 取大鼠肝脏组织切片进行苏木精–伊红染色, 观察肝小叶结构及炎症细胞浸润情况。

1.2.4 大鼠肝组织丙氨酸转氨酶(alanine transaminase, ALT)和天冬氨酸转氨酶(aspartate transaminase, AST)活性检测 取大鼠肝组织匀浆, 严格按照AST与ALT试剂盒说明书检测肝组织匀浆液中AST和ALT的活性。

1.2.5 大鼠肝组织匀浆中MDA含量, GSH-Px、SOD和CAT活性以及总抗氧化能力T-AOC的检测 取大鼠肝组织制备10%匀浆液, BCA试剂盒测定匀浆液中蛋白质浓度, 之后, 严格按照试剂盒说明书的要求和流程完成上述指标的检测。

1.3 统计学方法

采用SPSS 19.0软件对实验数据进行统计学处理, 独立实验的数据采用Mean±S.D.表示, 组间差异比较采用One-Way ANOVA, $P<0.05$为差异具有统计学意义。

2 结果

2.1 BML-111抑制CCl_4诱导的大鼠肝纤维化

在实验过程中, CCl_4组3只大鼠死亡, 其他组别无大鼠死亡。肝脏大体外观显示, CCl_4组大鼠肝脏颜色偏黄、表面粗糙及结节程度非常严重; BML-111干预大鼠的肝脏外观明显改善, 表面粗糙度减弱, 结节化程度明显减轻, 颜色也恢复暗红, 且预防组效果优于治疗组(图1)。

2.2 BML-111抑制CCl_4诱导的大鼠肝脏损伤和炎症细胞浸润

CCl_4组大鼠肝脏组织破坏严重, 基本无法观察到肝小叶结构, 肝细胞大量坏死, 炎症细胞大量浸润。预防组与治疗组大鼠肝脏损伤相对较轻, 部份肝细胞虽发生脂肪变性, 但同时伴随明显的肝细胞再生; 肝小叶结构破坏和炎症细胞浸润程度较CCl_4组都有所减轻。以上提示, BML-111抑制CCl_4诱导肝组织损伤, 减少炎症细胞浸润(图2)。

2.3 BML-111具有保护肝纤维化大鼠肝功能的作用

肝细胞损伤时, AST和ALT被释放入血, 血清中

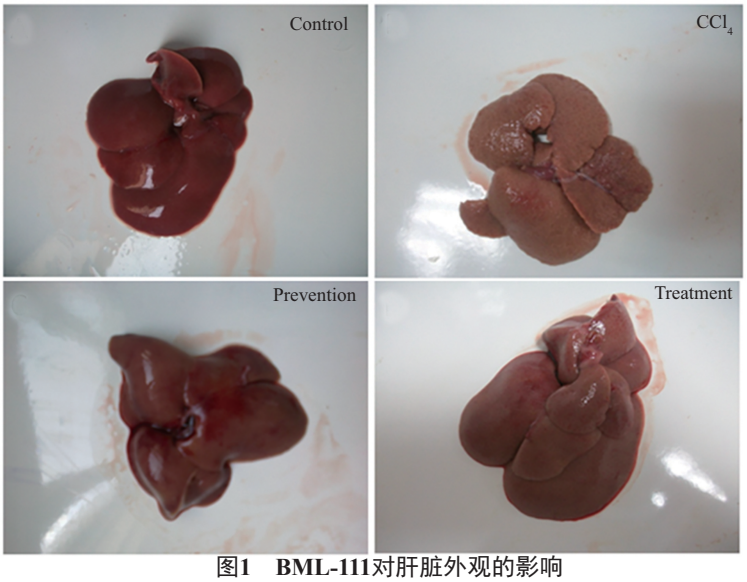


图1 BML-111对肝脏外观的影响

Fig.1 Effects of BML-111 on the appearance of liver

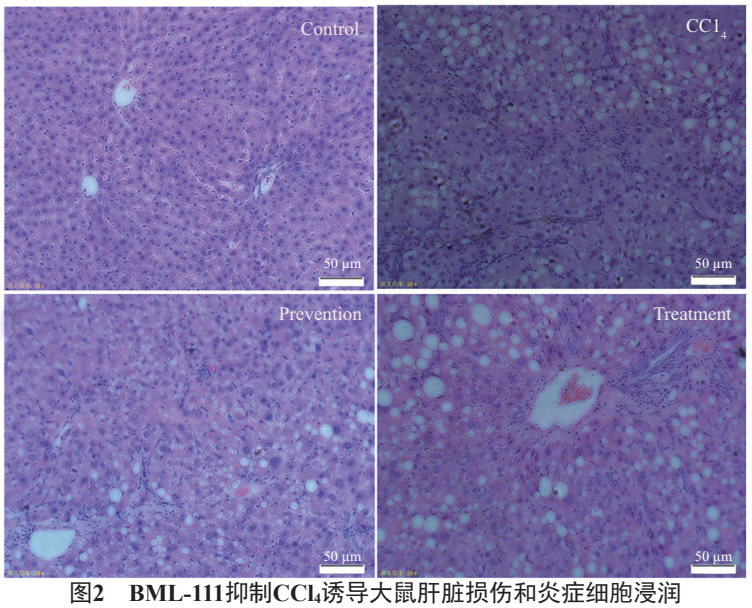


图2 BML-111抑制CCl_4诱导大鼠肝脏损伤和炎症细胞浸润

Fig.2 BML-111 inhibit CCl_4-induced hepatic injury and inflammatory cell infiltration in rats

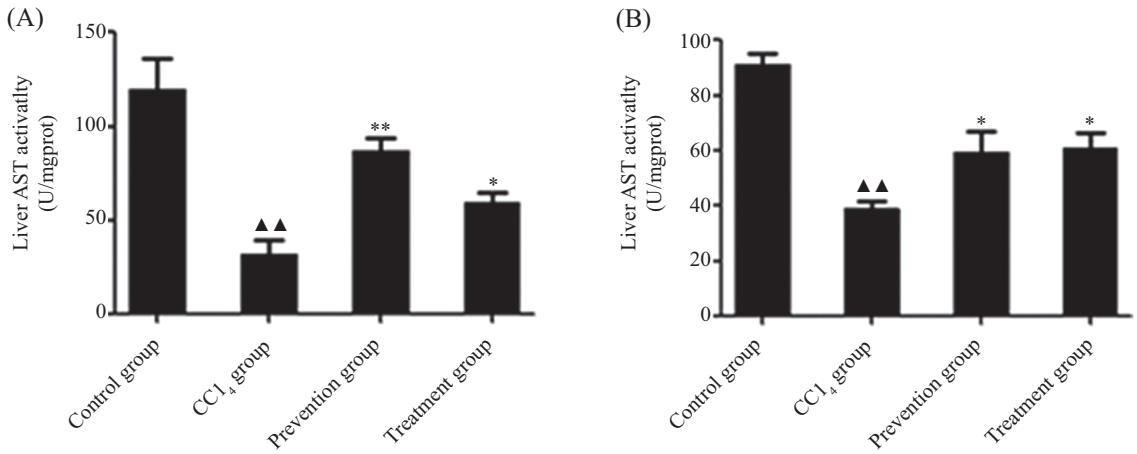
AST和ALT的含量升高, 同时肝组织AST和ALT的含量下降。为评估BML-111对肝功能的影响, 本文检测了大鼠肝组织匀浆液中AST和ALT的活性。结果显示, CCl_4组大鼠肝组织匀浆液中AST和ALT活性明显降低, 预防组和治疗组大鼠肝组织匀浆液的AST和ALT活性均得到一定程度回升(图3)。

2.4 BML-111对肝纤维化大鼠氧化/抗氧化平衡的调节

表1和表2结果显示, (1)CCl_4诱导MDA大量生成, 治疗组和预防组大鼠肝组织MDA含量降低; (2)CCl_4降低抗氧化酶SOD、CAT和GSH-Px活性, 而BML-111可以明显回升SOD、CAT和GSH-Px的活性; (3)CCl_4抑制大鼠肝组织的总抗氧化能力, 但该现象可被BML-111逆转。

3 讨论

在纤维化肝组织匀浆液中可以检测到大量ROS[4], 过量ROS不仅可以损伤肝细胞, 还可以刺激HSC活化, ROS在肝纤维化发生发展过程中扮演重要作用[5-7]。另一方面, HSC活化和肝纤维化也可以促进ROS大量释放, 增强肝组织内的氧化应激能力, 从而加速肝纤维进程[10-12]。最重要的促纤维化细胞



n=6, ▲▲*P*<0.01, 与对照组比较; **P*<0.05, ***P*<0.01, 与CCl_4组比较。

n=6, ▲▲*P*<0.01 *vs* control group; **P*<0.05, ***P*<0.01 *vs* CCl_4 group.

图3 BML-111对肝组织AST和ALT活性的影响

Fig.3 Effects of BML-111 on the activities of AST and ALT in hepatic tissue

表1 抗氧化酶CAT、GSH-Px和SOD活性

Table 1 The activities of antioxidant enzyme CAT、GSH-Px and SOD

组别 Goups	过氧化氢酶活性(U/mgprot) CAT (U/mgprot)	谷胱甘肽过氧化物酶活性(U/mgprot) GSH-Px (U/mgprot)	超氧化物歧化酶活性(U/mgprot) SOD (U/mgprot)
Control group	629.163±141.578	180.976±53.884	221.099±32.162
CCl_4 group	352.614±96.851▲▲	94.231±15.876▲▲	120.145±36.901▲▲
Prevention group	433.029±70.094*	173.857±36.194**	196.401±14.152*
Treatment group	425.724±247.415*	145.268±13.073*	169.346±42.325*

n=6, ▲▲*P*<0.01, 与对照组比较; **P*<0.05, ***P*<0.01, 与CCl_4组比较。

n=6, ▲▲*P*<0.01 *vs* control group; **P*<0.05, ***P*<0.01 *vs* CCl_4 group.

表2 MDA含量及总抗氧化能力T-AOC的变化

Table 2 The change of MDA content and total antioxidant capacity T-AOC

组别 Goups	丙二醛含量(μg/gprot) MDA (μg/gprot)	总抗氧化能力(U/mgprot) T-AOC (U/mgprot)
Control group	1.166±0.237	0.885 7±0.224 7
CCl_4 group	4.406±1.784▲▲	0.301 5±0.273 6▲▲
Prevention group	2.687±0.859*	0.531 2±0.187 9*
Treatment group	3.154±1.594*	0.487 5±0.236 6*

n=6, ▲▲*P*<0.01, 与对照组比较; **P*<0.05, 与CCl_4组比较。

n=6, ▲▲*P*<0.01 *vs* control group; **P*<0.05 *vs* CCl_4 group.

因子TGF-β和PDGF正是通过ROS激活其下游信号通路从而活化HSC, 促进纤维化发展。Adachi等[13]和Borkham等[14]的研究指出, PDGF通过诱导ROS生成而使AKT(protein kinase B)与p38 MAPK(p38 mitogen activated protein kinases)磷酸化而促进HSC细胞大量增殖。Javeluad等[15]和Vayalil等[16]也发现, TGF-β可通过增加ROS生成, 激活MAPK途径, 诱导氧化还原敏感型转录因子AP-1(activator protein-1)和SP1(specificity protein 1)活化发挥其激活HSC细胞的效应。此外, ROS也能通过二次损伤肝细胞, 诱导TGF-β合成, 上调*Smad*(drosophila mothers against decapentaplegic protein)活性, 促进*ECM*基因的表达, 增加ECM合成, 从而加速肝纤维化进展[8-9]。不难看出, 氧化/抗氧化平衡是影响肝纤维化发生、发展的关键因素之一。

抗氧化物质能够清除体内过多的活性氧自由基, 抗氧化剂的应用在许多慢性肝脏损伤的动物模型或患者中都得了较好的效果[17-19]。Ning等[20]研究指出, 抗氧化剂α-硫辛酸可以通过降低体内ROS水平, 抑制PI3K(phosphatidylinositol 3 kinase)/AKT以及MAPK磷酸化, 阻断TGF-β/PDGF诱导HSC细胞激活和增殖, 有效地抑制小鼠肝纤维化发生。抗氧化物质, 如丹参素、甘草甜素、粉防己碱、氧化苦参碱、姜黄色素等也被广泛用于治疗实验性动物肝纤维并取得良好效果[21-23]。因此, 调节氧化/抗氧化平衡被认为是抑制肝纤维化的重要手段之一。

在前期工作中我们也发现, BML-111能抑制CCl_4引起的肝脏组织中胶原沉积及促纤维化因子如TGF-β等的表达, 抑制CCl_4引起的大鼠纤维化进程[3]。本实验的研究结果提示, BML-111可以抑制肝纤维化, 对CCl_4诱导的肝纤维化大鼠分别利用BML-111进行预防或治疗, 可以明显改善大鼠肝脏的颜色、光滑度和结节化程度, 从而表现出抑制肝纤维化的效应, 与前期结果相一致。BML-111还具有保护大鼠肝功能的作用, HE染色结果图显示, BML-111抑制CCl_4诱导的大鼠肝脏损伤和炎症细胞浸润, CCl_4能够明显导致肝小叶的破坏, 并能看到组织中大量存在的炎症细胞, 而经过BML-111预防或治疗处理后该现象明显减轻。此外, 通过对肝组织中ALT与AST的活性检测显示, BML-111可以明显提高大鼠肝组织中被CCl_4下调的ALT和AST活性, 也提示了BML-111的肝脏保护作用。在氧化/抗氧化平衡的调节方面, BML-111可以明显抑制CCl_4诱导的MDA, 增加肝组织总抗氧化能力T-AOC, 还能够明显恢复SOD、CAT和GSH-Px活性。

综上所述, BML-111可以抑制实验性大鼠肝纤维化, 此效应可能通过调节肝组织氧化/抗氧化平衡实现。本研究结果提示, 脂氧素及其类似物可能是治疗肝纤维化的潜在药物。

参考文献 (References)

1 Romano M. Lipoxin and aspirin-triggered lipoxins. Sci World J 2010; 10(1): 1048-64.

2 Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol 2005; 6(12): 1191-7.

3 Zhou XY, Yu ZJ, Yan D,Wang HM, Huang YH, Sha J, *et al*. BML-11, a lipoxin receptor agonist, protected carbon tetrachloride-induced hepatic fibrosis in rats. Inflammation 2013; 36(5): 1101-6.

4 Maurizio P, Gaia R. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001; 35(2): 297-306.

5 Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. Toxicol Sci 2002; 65(2): 166-76.

6 Kaplowitz N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis 2002; 22(2): 137-44.

7 Poli G. Pathogenesis of liver fibrosis: Role of oxidative stress. Mol Aspects Med 2000; 21(3): 49-98.

8 Massagué J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. EMBO J 2000; 19(8):1745-54.

9 Foo NP, Lin SH, Lee YH, Wu MJ, Wang YJ. α-Lipoic acid inhibits liver fibrosis through the attenuation of ROS-triggered signaling in hepatic stellate cells activated by PDGF and TGF-β. Toxicology 2011; 282(1/2): 39-46.

10 Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115(2): 209-18.

11 Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000; 275(4): 2247-50.

12 Lotersztajn S, Julien B, Teixeira-Clerc F, Grenard P, Mallat A. Hepatic fibrosis: Molecular mechanisms and drug targets. Annu Rev Pharmacol Toxicol 2005, 45(1):605-28.

13 Adachi T, Togashi H, Suzuki A, Kasai S, Ito J, Sugahara K, *et al*. NAD(P)H oxidase plays a crucial role in PDGF-induced proliferation of hepatic stellate cells. Hepatology 2005; 41(6): 1272-81.

14 Borkham-Kamphorst E, Meurer SK, Gressner AM, Weiskirchen R. Disruption of intermolecular disulfide bonds in PDGF-BB dimers by N-acetyl-L-cysteine does not prevent PDGF signaling in cultured hepatic stellate cells. Biochem Biophys Res Commun 2005; 338(4): 1711-8.

15 Javelaud D, Mauviel A. Crosstalk mechanisms between the mitogenactivated protein kinase pathways and Smad signaling downstream of TGF-beta: Implications for carcinogenesis. Oncogene 2005; 24(37): 5742-50.

16 Vayalil PK, Iles KE, Choi J, Yi AK, Postlethwait EM, Liu RM.

Glutathione suppresses TGF-beta-induced PAI-1 expression by inhibiting p38 and JNK MAPK and the binding of AP-1, SP-1, and Smad to the PAI-1 promoter. Am J Physiol Lung Cell Mol Physiol 2007; 293(5): L1281-92.

17 Boigk G, Stroedter L, Herbst H, Waldschmidt, Riechen EO, Schuppan D. Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. Hepatology 1997; 26(3): 643-39.

18 Brown KE, Poulos JE, Li L, Soweid AM, Ramm GA, O'Neil R, *et al*. Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload. Am J Physiol 1997; 272(1 pt 1): G116-23.

19 Houglum K, Venkataramani A, Lyche K, Chojkier M. A pilot study of the effects of d-alpha-tocopherol on hepatic stellate cell activation in chronic hepatitis C. Gastroenterology 1997; 113(4): 1069-73.

20 Feng Y, Cheung KF, Wang N, Liu P, Nagamatsu T, Tong Y. Chinese medicines as a resource for liver fibrosis treatment. Chin Med 2009; 4(1): 16.

21 Tsai MK, Lin YL, Huang YT. Effects of salvianolic acids on oxidative stress and hepatic fibrosis in rats. Toxicol Appl Pharmacol 2010; 242(2): 155-64.

22 Tao YY, Liu CH. Progress of research on mechanism of salvia miltiorrhiza and its chemical ingredients against liver fibrosis. J Chin Integr Med 2004; 2(2): 145-8.

23 Liu P, Hu YY, Liu C, Zhu DY, Xue HM, Xu ZQ, *et al*. Clinical observation of salvianolic acid B in treatment of liver fibrosis in chronic hepatitis B. World J Gastroenterol 2002; 8(4): 679-85.