Flk1$^+$ 间充质干细胞减轻四氯化碳导致的肝纤维化的研究

Flk1$^+$ Mesenchymal Stem Cells Ameliorate Carbon Tetrachloride-induced Liver Fibrosis in Mice

史明霞[1]，房佰俊[1]，廖联明[1]，杨少光[2]，刘煜昊[1]，赵春华[1*]

SHI Ming-Xia[1], FANG Bai-Jun[1], LIAO Lian-Ming[1], YANG Shao-Guang[2], LIU Yu-Hao[1] and ZHAO Chun-Hua[1*]

1. 中国医学科学院 中国协和医科大学 基础医学研究所 组织工程中心，北京 100005

2. 中国医学科学院 中国协和医科大学 血液学研究所 实验血液学国家重点实验室，天津 300020

1. *Tissue Engineering Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing* 100005, *China*

2. *State Key Lab of Experimental Haematology, Institute of Haematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin* 300020, *China*

摘 要 许多慢性肝脏疾病都会发生肝纤维化，但是目前尚缺乏对肝纤维化切实有效的治疗手段。实验发现，Flk1(fetal liver kinase)阳性间充质干细胞(MSC)能够减轻四氯化碳(CCl_4)所致小鼠肝纤维化。取雄性 BALB/c 小鼠骨髓，分离培养 Flk1$^+$ MSC，用 CCl_4 制作雌性小鼠肝纤维化模型，在 CCl_4 损伤后立即或 1 周后经尾静脉注射 Flk1$^+$ MSC，2 或 5 周后检测受体小鼠肝脏的纤维化程度和供体细胞的植入。结果发现，CCl_4 损伤后立即注射 Flk1$^+$ MSC，可以使肝脏损伤程度明显减轻，减少胶原沉积，使肝脏羟脯氨酸含量及血清纤维化指标显著下降；而损伤 1 周后注射细胞则无明显变化。免疫荧光、PCR 和荧光原位杂交方法证实，在受体肝脏中有供体细胞植入，呈上皮细胞形态，并表达白蛋白，但是数量很少。因此，Flk1$^+$ MSC 具有潜在的植入肝组织的能力，并可能启动肝组织的内源性修复，减轻 CCl_4 导致的肝纤维化。

关键词 间充质干细胞，肝纤维化，移植

中图分类号 R575 **文献标识码** A **文章编号** 1000-3061(2005)03-0396-06

Abstract Fibrosis is the common end stage of most liver diseases. Unfortunately, there is no effective treatment available currently. This study was designed to evaluate the effect of Flk1$^+$ mesenchymal stem cells (MSC) from murine bone marrow (Flk1$^+$ MSC) on fibrosis formation induced by carbon tetrachloride (CCl_4). In this study Flk1$^+$ MSC were isolated from bone marrow of male BALB/c mice. A CCl_4 induced hepatic fibrosis model was used. Flk1$^+$ MSC were systemically infused immediately or one week after the female mice were challenged with CCl_4. Fibrosis index and donor cell engraftment were assessed two or five weeks after CCl_4 challenge. We found that Flk1$^+$ MSC transplantation immediately, but not one week after exposure to CCl_4, significantly reduced CCl_4-induced liver damage and collagen deposition. In addition, levels of hepatic hydroxyproline and serum fibrosis markers (HA, P-III-P) in mice receiving immediate Flk1$^+$ MSC transplantation after CCl_4 challenge were significantly lower compared to those of control mice. More importantly, histological examination suggested that

Received: November 1, 2004; Accepted: January 20, 2005.

This study was supported by "863 projects" of Ministry of Science and Technology of P.R. China (No. 2002AA205061) and the National Science Fund for Distinguish Scholars (No.30125018).

* Corresponding author. Tel: 86-10-65125311; E-mail: chunhuaz@public.tpt.tj.cn

国家高技术研究发展计划 863 资助项目(No.2002AA205061)，国家杰出青年基金资助项目(No.30125018)。

hepatic damage recovery was much better in these immediately Flk1$^+$ MSC-treated mice. Immunofluorescence, PCR, and fluorescence *in situ* hybridization (FISH) analysis revealed that donor cells engrafted into host liver, had epithelium-like morphology and expressed albumin (ALB), although at low frequency. In conclusion Flk1$^+$ MSC might initiate endogenous hepatic tissue regeneration, engraft into host liver in response to CCl_4 injury, and ameliorate its fibrogenic effects.

Key words mesenchymal stem cells, liver fibrosis, transplantation

肝纤维化及其终末阶段的肝硬化,是威胁人类健康的严重疾患。目前对于肝硬化的治疗仅限于去除潜在的有害刺激,用干扰素、病毒唑和拉米呋定(lamivudine)等对病毒性肝炎进行抗病毒治疗,以及最后的治疗手段——肝移植。肝移植是治疗末期肝硬化的有效手段,5 年生存率达 75%。但是由于移植器官的来源有限,需要移植的患者数量众多,以及由于一些因素的限制并非每个患者都适于移植,因此迫切需要发展有效的抗纤维化治疗手段。

近来我们和其他一些学者的研究证明,来自其他组织的干细胞能够归巢到肝脏,并可能参与肝组织的再生[1-3],从而为发展干细胞治疗肝脏疾病提供了新的希望。

本文采用 CCl_4 导致的小鼠肝纤维化模型来研究 Flk1 (fetal liver kinase) 阳性间充质干细胞 (mesenchymal stem cells, MSC) 对肝纤维化的影响。结果显示 Flk1$^+$ MSC 能够启动肝组织的内源性修复并植入受损肝脏,植入的供体细胞表现为上皮细胞形态,并表达白蛋白(albumin, ALB)。而且,只有在损伤早期输入 MSC 才能减轻肝纤维化的形成。

1 材料和方法

1.1 实验动物

6 周龄 BALB/c 小鼠,购自北京维通利华实验动物技术有限公司。

1.2 主要试剂和材料

CD45、GlyA、CD34 磁珠购于 Miltenyi Biotec 公司,DF12、MCDB 购于 Sigma 公司,胎牛血清为 Gibco 公司产品,Flk1 抗体购于 Santa Cruz 公司,CD34、CD45、CD31、GlyA、vWF、CD11a、CD11b 抗体购于晶美生物工程有限公司,羊抗鼠 ALB 抗体购于 Bethyl Laboratories 公司,Cy5 标记的兔抗羊二抗购于 Jackson Immuno Research 公司,小鼠 FITC 标记 Y 染色体探针及原位杂交试剂盒为 Innogenex 公司产品。

1.3 Flk1$^+$ MSC 的分离和培养

无菌状态下取雄性 BALB/c 小鼠股骨和胫骨,冲出骨髓(bone marrow, BM),经 4 号针头反复冲洗,制成单细胞悬液。用 Ficoll-Paque (1.077g/mL)分离出单个核细胞,用 MACS CD45,GlyA 和 CD34 磁珠去除造血细胞,以 10^7 个/mL 密度接种在 25cm^2 的培养瓶内。培养液成分为 40% MCDB、55% DF12、4% 胎牛血清、100u/mL 青霉素和 1000u/mL 链霉素,置于 37℃、5% CO_2 培养箱培养。24h 后去掉悬浮细胞,细胞融合达 70% 时,用 0.125% 胰酶消化并以 1∶2 比例传代。这些细胞的免疫表型传代 30 次以上始终保持 CD34, CD45, CD31, vWF, GlyA, CD11a, CD11b 阴性,Flk1 阳性(资料未显示)。因而我们称之为 Flk1$^+$ MSC。

1.4 CCl_4 肝损伤和 Flk1$^+$ MSC 的输入

将雌性 BALB/c 鼠随机分为 4 组(20 只/组)。1~3 组均给予 CCl_4 灌胃(1mL/kg 体重,溶解在 150μL 玉米油中),每周 2 次,直到在接受首剂 CCl_4 2 或 5 周后处死。来自雄性 BALB/c 鼠的 Flk1$^+$ MSC 经尾静脉注射输入受体鼠(10^6/只),其中第 2 组(G2)在接受 CCl_4 后立即进行细胞注射,第 3 组(G3)则在首剂 CCl_4 一周后接受供体细胞移植。第 1 组(G1)仅接受等体积的生理盐水注射。第 4 组(G4)只给予玉米油,作为正常对照。在接受 CCl_4 2 或 5 周后,处死小鼠。用 4% 多聚甲醛固定肝左叶,石蜡包埋,切片。肝脏的其他部分则用液氮冷冻,保存在 -70℃,用于提取基因组 DNA 和总 RNA。收集血液,评价肝功能和肝纤维化指标。

1.5 组织学检测和免疫荧光染色

肝脏切片用苏木素-伊红常规染色或 Masson 三色染色法来检测肝脏变性、坏死的范围以及纤维化的程度。用山羊抗鼠 ALB 的多克隆抗体和 Cy5 标记的兔抗山羊二抗进行 ALB 的免疫荧光染色,用小鼠 Y 染色体特异性探针行 Y 染色体荧光原位杂交(FISH)检测。

1.6 肝脏的羟脯氨酸含量和血清学检测

参考 Yoshiji 等[4]的方法,用 200mg 冰冻标本测定肝脏的羟脯氨酸含量。谷丙转氨酶和总胆红素用常规方法测定。血清透明质酸(HA)和Ⅲ型前胶原蛋白(P-Ⅲ-P)的测定方法与 Yoshiji 等人[5]相同。

1.7 PCR

用 PCR 分析雄性特异性 Sry 基因，5′-CAG CTA ACA CTG ATC TTT TC-3′，5′-TTA CTG AGC CAG AAT CAT AG-3′，反应条件为：94℃ 3min，94℃ 30s，50℃ 30s，72℃ 2.5min，循环 35 次，最后 72℃ 延伸 5min。另外，用半定量 PCR 检测纤维化标志物转化生长因子-β1（transforming growth factor-β1，TGF-β1）和 α-平滑肌肌动蛋白（α-smooth muscle actin，α-SMA）的表达，以 β-肌动蛋白（β-actin）为管家基因。引物如下：TGF-β1（498bp），5′-AAT ACG TCA GAC ATT CGG GAA GCA-3′，5′-GTC AAT GTA CAG CTG CCG CAC ACA-3′；α-SMA（368bp），5′-CTG GAG AAG AGC TAC GAA CTG C-3′，5′-CTG ATC CAC ATC TGC TGG AAG G-3′；β-肌动蛋白（319bp），5′-TGT ACG TAG CCA TCC AGG C-3′，5′-TTC TCC AGG GAG GAA GAG GA-3′。反应条件为：95℃ 5min，95℃ 30s，60℃（TGF-β1）或 62℃（α-SMA）30s，72℃ 90s，循环 30 次，最后 72℃ 延伸 10min。

1.8 统计学方法

统计量以 mean ± sd 表示，两组间均数差异性检验以 Mann-Whitney U 检验，两组以上各组间均数差异性检验以 Kruskal-Wallis 检验，$P < 0.05$ 为差异有显著意义，$P < 0.01$ 为差异有极显著意义。

2 结果

2.1 Flk1$^+$ MSC 在 CCl_4 损伤小鼠体内的植入及分化

过去，我们发现来源于人骨髓的 MSC 在移植到经照射的 NOD/SCID 鼠（non-obese diabetic/severe combined immunodeficient mice）体内后，能够分化为有功能的肝细胞[1]。本研究中，CCl_4 损伤 5 周后处死的第 2 和第 3 组雌性受体小鼠的肝脏里，用 Y 染色体 FISH 的方法检测到来自雄性供体的细胞（绿色信号），同时免疫组化证实它们具有上皮细胞表型，

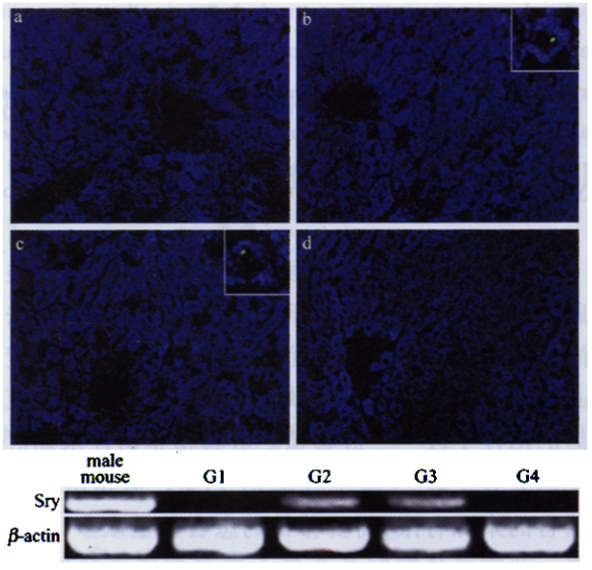

图 1 雌性受体肝脏中的 Sry 基因和 ALB$^+$/Y 染色体$^+$细胞

Fig.1 Presence of *Sry* gene and ALB/Y chromosome-positive cells in the female recipients

After five weeks to CCl_4, recipient mice were killed and livers were processed as described in Materials and Methods. (A) Engraftment of epithelial cells was detected by staining with antibodies to mouse ALB and Y chromosome FISH in the liver of G2 mice (b) and G3 mice (c). In G1 mice (a) and G4 mice (d), no donor cell was detected. Mouse Y chromosome painting probe was labeled with FITC. Green signals indicate Y chromosome positive hepatocytes. (B) PCR analysis of *Sry* gene in the liver of experimental mice five weeks after CCl_4 exposure. The 800-bp band is shown in normal male liver. No signal was detected in normal female liver. A distinct band was observed in G2 and G3 mice. Flk1$^+$ MSCs were injected in G2 immediately after exposure to CCl_4. Mice in G3 received Flk1$^+$ MSCs one week after the first dose of CCl_4. Animals in G1 received the same volume of saline and served as control. Mice in G4 received only corn oil and served as normal control.

能够表达 ALB（蓝色信号）（图 1A）。另外，Sry 基因的 PCR 也证实在这两组受体的肝脏中均有来自雄性供体的细胞（图 1B）。但是，在 CCl_4 损伤 2 周后处死的实验组小鼠肝脏中，未检测到 ALB 和 Y-染色体双阳性细胞（表 1）。提示，供体来源的 Flk1$^+$ MSC 在

表 1 Flk1$^+$ MSC 对 CCl_4 损伤 2 或 5 周后受体肝功能的影响

Table 1 Effect of Flk1$^+$ MSC on murine liver function after exposure to CCl_4 for two or five weeks (W)

Mice	HA (ng/mL)		P-Ⅲ-P (ng/mL)		ALT (u/mL)		Total bilirubin μg/L)		ALB$^+$/Y-chromosome$^+$ cells	
	2W	5W	2W	5W	2W	5W	2W	5W	2W	5W
G1	145.2 ± 18.4	181.3 ± 23.4	29.8 ± 4.0	39.7 ± 5.2	189.2 ± 37.2	216.3 ± 47.6	120.1 ± 20.5	124.8 ± 21.2	−	−
G2	29.8 ± 11.6 *	39.1 ± 5.1*	8.9 ± 2.7*	13.1 ± 2.9*	136.8 ± 28.7*	79.6 ± 6.4*	117.4 ± 23.1 †	121.4 ± 20.1 †	−	+
G3	134.8 ± 19.8 †	183.1 ± 27.6†	30.1 ± 4.1†	37.9 ± 4.9†	181.8 ± 24.9†	213.7 ± 37.6†	122.6 ± 22.5† †	120.2 ± 23.1 †	−	+
G4	9.6 ± 2.0	9.8 ± 2.1	1.8 ± 0.7	1.9 ± 0.7	41.9 ± 7.1	37.7 ± 6.9	50.4 ± 20.7	51.1 ± 20.4	−	−

* $p < 0.01$ compared with the G1 mice; † $p > 0.05$ compared with the G1 mice.

CCl_4 损伤的受体小鼠肝脏中能够分化为产生 ALB 的细胞,但这一向组织特异性细胞分化的过程是逐渐发生的,可能需要 2 周以上。

2.2 Flk1$^+$ MSC 减轻 CCl_4 导致的肝纤维化

CCl_4 处理 2 周后,各实验组肝脏均可见广泛的变性坏死,但 1 ~ 3 组之间无显著差异(图 2A)。而在 5 周以后,第 2 组肝脏变性坏死范围较 1、3 组明显缩小(图 2B)。

Masson 三色染色法显示用 CCl_4 处理 5 周可以导致显著的肝纤维化形成。第一组小鼠的肝脏显示有桥状连接形成(图 3A),第 2 组纤维化程度明显减轻(图 3B),但是在首剂 CCl_4 后 1 周再接受 Flk1$^+$ MSC 移植的第 3 组与第 1 组相比无显著差异(图 C)。正常对照组小鼠的肝脏无纤维化发生(图 3D)。

CCl_4 刺激 5 周后,肝脏羟脯氨酸含量第 1 组为 516 ± 84μg/g,明显高于正常对照组(48 ± 7μg/g)。而在接受首剂 CCl_4 后就立即进行 Flk1$^+$ MSC 移植的第 2 组为 128 ± 21μg/g,尽管明显高于正常对照,但较第 1 组明显降低。第 3 组肝脏羟脯氨酸含量有所降低(482 ± 69μg/g),但与第 1 组相比无统计学差异。第 2 组小鼠血清中的纤维化标志物 HA、P-Ⅲ-P 以及 ALT 与第 1 组相比明显降低,而第 3 组无明显差异。1 ~ 3 组血清中总胆红素水平无明显差异(表 1)。

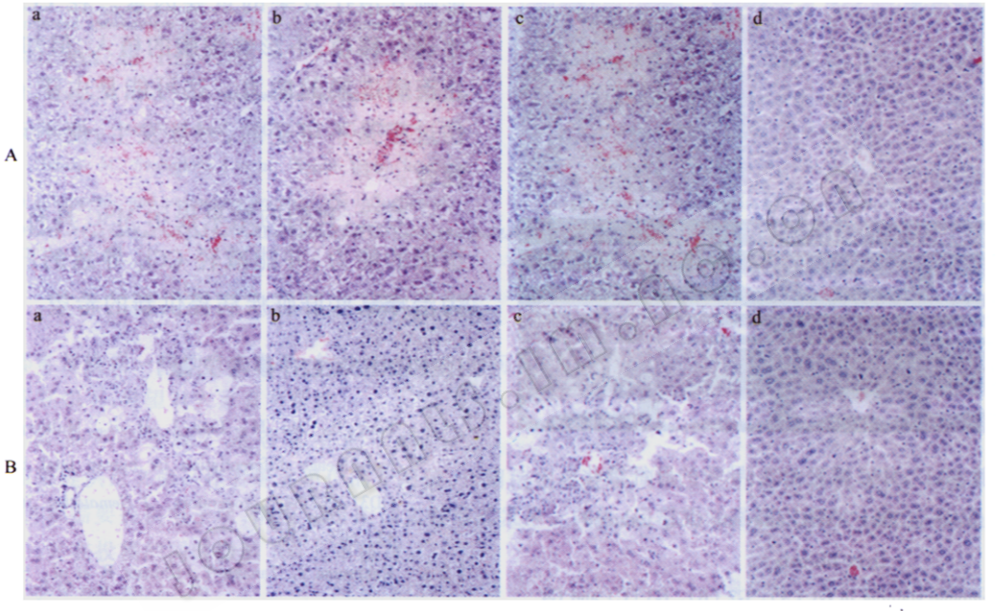

图 2 CCl_4 处理 2 或 5 周后各组小鼠肝脏常规 HE 染色

Fig.2 Representative photomicrographs of the liver 2 (A) or 5 (B) weeks after CCl_4 exposure in G1 (a), G2 (b), G3 (c), and G4 (d) mice

(A) after administration of CCl_4 for 2 weeks, the extent of liver degeneration/necrosis was not significantly different among mice from G1, G2, and G3. (B) five weeks later, however, areas of liver degeneration/necrosis were smaller in G2 mice compared with those in both G1 and G3 mice. Paraffin-embedded sections were processed for H E staining.

2.3 TGF-β1 和 α-SMA mRNA 的表达

第 1 和第 3 组的 TGF-β1 和 α-SMA mRNA 水平都明显高于第 2 组(图 3E)。

3 讨论

近年来,干细胞因为其在心脑血管疾病、恶性肿瘤和组织损伤修复等多种疾病的治疗方面有着广阔的应用前景而倍受人们的重视。成体干细胞具有“可塑性”,能在一定外界环境下转变成其它组织系统的细胞,实现跨系统甚至跨胚层分化[6-9]。骨髓干细胞移植能够使实验动物的某些生理功能得以恢复[10-12]。

然而,在再生医学的概念中,成体干细胞的作用恐怕并不仅仅是简单的直接替代被破坏的细胞。最近,有报道神经干细胞移植能够挽救脑组织中由于缺氧-缺血损伤导致功能丧失的宿主神经元细胞[13,14],骨髓来源的干细胞能够启动胰腺的再生[15]。本研究中,Flk1$^+$ MSC 挽救受损肝脏的一个重要机制可能是它们启动了肝组织的内源性再生,从而使其功能得以恢复。结果显示,CCl_4 刺激 5 周后,第 2 组肝组织修复明显好于其它实验组,尽管能够检测到 ALB$^+$ 的供体来源细胞,但其比率很低,说

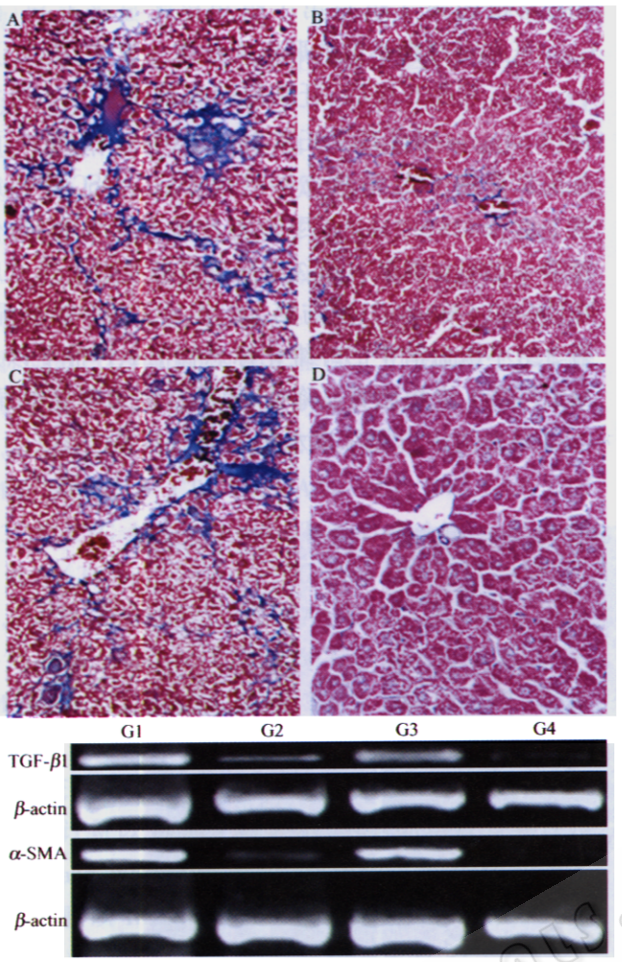

图 3 注射 Flk1$^+$ MSC 减轻 CCl_4 导致的肝纤维化

Fig.3 Infusion of Flk1$^+$ MSC alleviate CCl_4-induced liver fibrosis

Liver Masson trichromic staining in G1 (A), G2 (B), G3 (C) and G4 (D) mice. (E) Steady-state hepatic TGF-β1 and α-SMA mRNA level in G1, G2, G3, and G4 mice 5 weeks after CCl_4 exposure. Ethidium bromide-stained 2% agarose gels of the RT-PCR products were shown. Gels were scanned with a digital image analysis system, the products were quantified, and results were adjusted to housekeeping gene β-actin. Representative images are shown.

明这些骨髓来源细胞本身并不能通过替代被破坏细胞而使肝功能得以改善。因此,可以提出这样的假设:Flk1$^+$ MSC 移植能够促进宿主体内有功能的肝细胞增殖,从而降低 ALT、HA 和 P-Ⅲ-P 的水平。在蜥蜴和线虫已经发现类似的再生过程。尽管骨髓来源的干细胞诱导内源性肝组织修复的机制还不清楚,但是快速的修复过程和肝细胞的增殖,提示内源性的肝脏干细胞可能参与了这一过程。

骨髓移植有可能成为控制患者肝损伤的有效手段,并扩展用于促进其它组织或器官的再生[15-17]。研究表明,MSC 能够分泌多种生长因子和细胞因子,通过复杂的旁分泌等方式,改变植入部位的微环境,从而达到减轻炎症、促进自身修复和保护植入器官的作用[18-20]。在 CCl_4 损伤后立即给予 Flk1$^+$ MSC 输注,可以保护肝组织,减少胶原沉积,其机制可能是多因素的。首先,Flk1$^+$ MSC 可能在植入部位通过产生白介素 10(interleukin-10, IL-10)来改变局部的微环境,已有很多证据表明 IL-10 可作为一种抗纤维化的细胞因子发挥作用,或与肿瘤坏死因子竞争,打乱纤维化的信号途径[21-23];第二,血清 TGF-β1 水平降低,减少肝星状细胞的活化;第三,Flk1$^+$ MSC 在一定程度上可能抑制激活的肝星状细胞分泌 TGF-β1。有趣的是 Flk1$^+$ MSC 的输注时间是影响其治疗效果的重要因素。在 CCl_4 损伤 1 周后才进行移植,不影响细胞的植入,但却阻碍了它们防止疾病进展的能力。这可能是由于 CCl_4 导致的损伤过程是不可逆的,一旦发生,便不可能被 MSC 所挽救。

总之,我们的结果提示 MSC 具有潜在的植入肝组织的能力,并能减轻 CCl_4 导致的肝纤维化,可能为治疗某些肝脏疾病提供新的希望。

REFERENCES(参考文献)

[1] Fang B, Shi M, Liao L *et al*. Multiorgan engraftment and multilineage differentiation by human fetal bone marrow Flk1$^+$/CD31$^-$/CD34$^-$ progenitors. *J Hematother Stem Cell Res*, 2003, **12**: 603 - 613

[2] Kakinuma S, Tanaka Y, Chinzei R *et al*. Human umbilical cord blood as a source of transplantable hepatic progenitor cells. *Stem Cells*, 2003, **21**: 217 - 227

[3] Mallet VO, Mitchell C, Mezey E *et al*. Bone marrow transplantation in mice leads to a minor population of hepatocytes that can be selectively amplified *in vivo*. *Hepatology*, 2002, **35**: 799 - 804

[4] Yoshiji H, Kuriyama S, Miyamoto Y *et al*. Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. *Hepatology*, 2000, **32**: 1248 - 1254

[5] Yoshiji H, Kuriyama S, Yoshii J *et al*. Angiotensin-Ⅱ type 1 receptor interaction is a major regulator for liver fibrosis development in rats. *Hepatology*, 2001, **34**: 745 - 750

[6] Ferrari G, Cusella-De Angelis G, Coletta M *et al*. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*, 1998, **279**: 1528 - 1530

[7] Mezey E, Chandross KJ, Harta G *et al*. Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science*, 2000, **290**: 1779 - 1782

[8] Orlic D, Kajstura J, Chimenti S *et al*. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci*, 2001, **938**: 221 - 229

[9] Krause DS, Theise ND, Collector MI *et al*. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, 2001, **105**:369 - 377

[10] Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature*, 2003, **422**: 901 - 904

[11] Orlic D, Kajstura J, Chimenti S *et al*. Bone marrow cells regenerate infarcted myocardium. *Nature*, 2001, **410**: 701 - 705

[12] Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med*, 2003, **9**: 702 - 712

[13] Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat Biotechnol*, 2002; **20**: 1111 - 1117

[14] Ourednik J, Ourednik V, Lynch WP *et al*. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nat Biotechnol*, 2002, **20**: 1103 - 1110

[15] Hess D, Li L, Martin M *et al*. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol*, 2003, **21**: 763 - 770

[16] Markova M, Haluzik M, Svobodova J *et al*. Serum leptin levels in patients with sideropenic and pernicious anemia: the influence of anemia treatment. *Physiol Res*, 2000, **49**: 679 - 684

[17] Ortiz LA, Gambelli F, McBride C *et al*. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 2003, **100**: 8407 - 8411

[18] Deans RJ and Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol*, 2000, **28**(8):875 884

[19] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*, 2005, **105**(4): 1815 - 1822

[20] Togel F, Hu Z, Weiss K *et al*. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol*, 2005, Epub ahead of print.

[21] Dai WJ, Jiang HC. Advances in gene therapy of liver cirrhosis: a review. *World J Gastroenterol*, 2001, **7**:1 - 8

[22] Grove J, Daly AK, Bassendine MF *et al*. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut*, 2000, **46**:540 - 545

[23] Louis H, Le Moine O, Goldman M, Deviere J. Modulation of liver injury by interleukin-10. *Acta Gastroenterol Belg*, 2003, **66**(1):7 - 14