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大鼠门静脉分支残端置管模型构建及细胞移植途径的评价 *

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摘要 目的：大鼠肝部分切除模型被广泛的应用于肝脏疾病的研究，随着干细胞治疗肝损伤及护肝药物研究的发展，对大鼠肝损伤模型也提出了很多新的要求。本实验拟在大鼠肝部分切除术的基础上改进以建立大鼠肝断面门静脉分支残端的静脉置管模型，并进行细胞移植实验，对比分析新模型的优劣。**方法：**60 只 F344 大鼠分为三组。A、B 组行行 85 %肝切除术；C 组行 85 %肝切除术 + 肝断面门静脉分支残端置管术。术中 B 组经门静脉注入 4× 10^5 个表达 GFP（green fluorescence protein, GFP）的胎肝干细胞（fetal liver stem/progenitor cells, FLSPCs）。C 组经留置导管注射入同等量的 FLSPCs，A 组注射同等剂量的培养液。72 小时取血清，测定肝功能 ALT、AST，统计死亡率；取肝脏组织切片观察其修复情况。统计学采用方差分析和 LSD-t 检验。**结果：**B、C 组 F344 大鼠 72 小时肝功指标（ALT、AST）均明显优于 A 组；B 组、C 组肝脏组织学的病理损伤的恢复分别较 A 组快。B、C 组间肝功指标无统计学意义。**结论：**经门静脉分支残端置管途径移植 FLSPCs 效果等同于经门静脉穿刺途径，且该模型具有可反复、可选时、减少创伤等优点。

关键词：动物模型；门静脉置管；干细胞移植；胎肝干细胞；动物实验

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Establishing a Model of Acute Hepatic Failure with Catheterization of Portal venous Branch and a Study of the Way of Cell Transplantation*

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ABSTRACT Objective: Rat partial hepatectomy model is widely used in the study of hepatic disease. As the rapid development of research of liver protection drugs and stem cells therapy for liver injury, the model need to be improved. A rat model of acute hepatic failure with catheterization of portal venous branch was established . After a study of the way of cell transplantation was made in our trail, the new model was evaluated. **Methods:** Sixty F344 rats were randomly divided into 3 groups (n=20 in each group). The partial hepatectomy with 85% sizes were performed on the rats of the A and B groups. The same operations and catheterizations of portal venous branch were carried out on the rats of the C group. After the green fluorescent protein (GFP) gene was transferred into the FLSPCs, Every rats of the B group were transplanted into 4× l0^5 cells through the portal veins during the operations. Every rats of the C group were transplanted into the same cells through the ducts. Control rats in the A group received medium of equal volumes. Mortality rates and hepatic functions such as ALT AST were measured after 72 hours. Meanwhile, hepatic pathology was studied. Data were analyzed using variance analysis and LSD-t. **Results:** Compared to control group the hepatic and coagulative functions gradually recovered in stem ce1ls transplant groups(B and C). The hepatic pathology significantly improved in stem ce1ls transplantation groups(B and C). The effects of treatments on the rats of B group are equal with those of C group. **Conclusions:** Stem cells transplantations through ducts of portal venous branch improved the hepatic functions and pathology in acute hepatic injury model of F344 rats as much as those through portal vein directly. The model has advantages in repeated operation, fewer traumas and less complication and can be treated whenever necessary.

Key words: Model of animal; Catheterization of portal venous; Stem cell transp1antation; Fetal liver stem/progenitor cells; Animal experimentation

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前言

由于大鼠肝脏各叶彼此分开，各叶肝蒂游离于肝外，肝叶比重相对固定，方便进行各种比例的肝部分切除。肝部分切除模型主要应用于肝脏肿瘤[1-3]、肝再生[4-7]、急慢性肝衰竭[8-9]以及肝移植[10]等模型的研究。理想的急性肝衰竭动物应具有①可逆性：肝衰竭有潜在的可逆性，适当的方法可以治疗并能长期存活；②可重复性：肝损伤模型的制备可以重复，并应具有相同的

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病死率和较相似的指标；③死于肝衰竭：肝损伤动物模型最终应死于肝衰竭；④治疗窗口期：由发生肝衰竭到死亡应具有足够的时间，以进行适当的治疗；⑤大型动物；⑥对环境和实验人员危害最小。随着干细胞[11]治疗肝损伤及护肝药物研究的发展，对大鼠肝损伤模型也提出了很多新的要求。本实验拟在肝部分切除术的基础上对模型鼠进行改进，并行细胞移植实验，对比分析新模型的优劣。

1 材料与方法

1.1 材料

1.1.1 实验动物 F344 大鼠 由上海 Slack 实验动物中心提供，由第四军医大学动物中心实验动物房饲养。

1.1.2 材料 动物手术台、手术显微镜（OPMI，德国）、高频电刀、无损伤血管夹、小儿硬膜外导管（内径约 0 .7 mm），止血钳等常规手术器械、肝素钠、盐水、全自动生化分析仪（日立 7170 型，日本）。第三代构建 GFP 示踪的胎肝干细胞（本课题组三步分离法制备）[12]，20 % 胎牛血清（Hyclone，USA），胰蛋白酶（Gibco 公司，USA），Williams'E 培养基（Sigma 公司 USA）。

1.2 方法

1.2.1 模型制备 ⑴一次性硬膜外导管制备测孔，并用 50 U/ml 肝素钠注射液肝素化。大鼠术前禁食 12 小时、禁水 4 小时。10%水合氯醛(3 ml/kg)腹腔内注射麻醉，将麻醉后固定于实验台上，备皮、消毒，⑵60 只大鼠分为三组，A 组：对照组；B 组：经门静脉途径注射治疗组；C 组：经肝断面门静脉分支残端置管治疗组。取上腹部剑突下正中切口，长约 5 cm，进入腹腔，A、B 组大鼠依次行 85 %肝切除术（切除肝左外叶、肝中叶、右上叶，保留右下叶、尾叶）[13,14]。C 组大鼠找到进入肝左外叶 Glisson 鞘系统的门静脉，用无损伤血管夹分别夹闭其近心端和远心端，3-0 丝线结扎肝叶静脉后，向肝左外叶方向完全游离长约 1 cm 左右的血管，血管下穿过两根 3-0 丝线。静脉全层剪一"V"形小口，将硬膜外导管沿切口缓慢送入静脉；轻轻放开近心端血管夹，将导管方向向下置入 2 cm，回抽有血后固定，结扎 Glisson 鞘，切断肝左外叶，切除肝中叶、右上叶。导管固定腹壁沿皮下自颈后部穿出，连接接头、肝素帽。

1.2.2 干细胞植入及肝功能检查 术中 B 组即经门静脉穿刺注入 4× 10^5个表达 GFP 的胎肝干细胞（FLSPCs）。C 组经留置管注射入同等量的 FLSPCs，A 组经门静脉注射同等剂量的培养液。72 小时取血测定肝功能 ALT、AST，统计死亡率；取肝脏组织切片观察其修复情况。荧光显微镜下观察胎肝干细胞定植情况。

1.2.3 术后维护 术后三组大鼠饲养于 18-22 ℃ 环境中，饮水使用 5 %葡萄糖溶液，以普通条状块料喂养，腹腔注射青霉素 4 万单位，2 次 / 日。C 组大鼠放入单笼喂养，保温至其苏醒。每日推注 0.5 mL 肝素盐水（25 U /ml）封管，可保持管道通畅。

1.3 统计学方法

应用 SPSS 17.0 统计软件进行分析，数据以（$\bar{x}\pm s$）表示。组间均数比较采用方差分析和 LSD-t 检验。$P<0.05$ 为差异有统计学意义。

2 结果

2.1 大鼠一般情况以及肝功能的影响

三组大鼠均于造模后 12 小时开始出现精神萎靡、拒食，少动，嗜睡、小便失禁、共济失调。12 小时开始出现死亡，48 小时到达高峰。死亡率分别为：A 组 70 %（14/20）、B 组 40 %（8/20）、C 组 45 %（9/20）。术后 C 组 2 只大鼠因导管脱出导致出血死亡。移植干细胞 72 小时后组间肝功能 A 组与 B、C 组 ALT、AST 出现统计学差异，B、C 组肝功恢复较 A 组明显。B、C 组间无明显统计学差异。

表 1 对照组与经门静脉途径注射治疗组于胎肝干细胞移植后 72 小时血清肝功变化（均值± 标准差，IU/L）

Table 1 Comparison of the hepatic function of rats in the group A and group B after the FLSPCs transplantation for 72 hours

Group		Count	ALT	Count	AST
A	n	6	1275.67± 329.59	6	2806.83± 157.01
B	n	12	802.83± 79.22	12	1463.50± 56.22
t value			3.42		19.73
P value			0.007		0.00001

表 2 对照组与经肝断面门静脉残端置管治疗组于胎肝干细胞移植后 72 小时血清肝功变化（均值± 标准差，IU/L）

Table 2 Comparison of the hepatic function of rats in the group A and group C after the FLSPCs transplantation for 72 hours

Group		Count	ALT	Count	AST
A	n	6	1275.67± 329.59	6	2806.83± 157.0
B	n	11	827.83± 63.08	11	1472.83± 52.41
t value			3.23		19.74
P value			0.008		0.00001

表 3 经门静脉途径治疗组与经肝断面门静脉残端置管治疗组于胎肝干细胞移植后 72 小时血清肝功变化（均值± 标准差，IU/L）

Table 3 Comparison of the hepatic function of rats in the group B and group C after the FLSPCs transplantation for 72 hours

Group		Count	ALT	Count	AST
A	n	6	802.83± 79.22	6	1463.50± 56.22
B	n	11	827.83± 63.08	11	1472.83± 52.41
t value			-0.61		-0.3
P value			0.56		0.78

2.2 胚胎肝干细胞移植对肝脏病理的影响

胎肝干细胞移植 72 小时后，取出大鼠肝脏，肉眼观 A 组剩余肝叶代偿性肿大。镜下可见肝窦及中央静脉扩张，伴有炎细胞浸润，肝细胞增生不明显；B、C 组见肝脏体积较对照组有所增大，肝叶代偿，镜下见肝细胞增生明显，肝索结构较完整，肝小叶结构较 A 组有较明显恢复。荧光显微镜下可见 B 组、C 组表达 GFP 的胎肝干细胞大量定植于肝叶组织中的密度大致相同(图 1、2)。

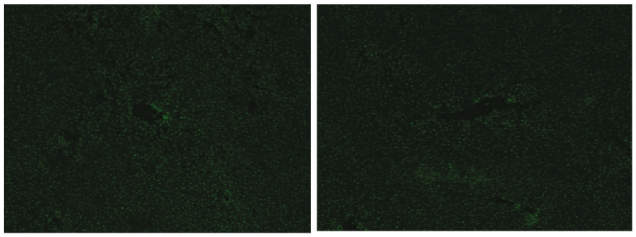

图 1 移植后 72 小时表达 GFP 的 FLSPCs 定植于 B 组肝脏中

Fig.1 FLSPCs expressed GFP colonized in the liver of rats of group B after transplantation for 72 hours

图 2 移植后 72 小时表达 GFP 的 FLSPCs 定植于 C 组肝脏中

Fig.2 FLSPCs expressed GFP colonized in the liver of rats of group C after transplantation for 72 hours

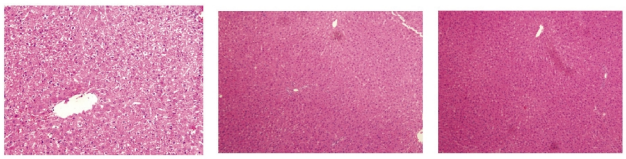

图 3 自左到右分别为移植 FLSPCs 后 72 小时手术对照组肝脏病理切片、经门静脉注射移植手术治疗组肝脏病理切片、经门静脉残端置管移植手术治疗组肝脏病理切片

Fig.3 The figures in order were Liver biopsies of the rats of A,B and C groups after FLSPCs transplantation for 72 hours

3 讨论

大鼠为常用的实验动物，随着肝脏药物及细胞治疗急性肝损伤研究的发展，以其为实验动物时许多研究均需通过静脉持续、反复、不同时间点的给药、测定检验指标。鼠尾静脉便于穿刺，但是易摆动，穿刺针不易固定，且仅适于单次操作。并且在手术制备急性肝损伤模型过程中常常失血量较大，其中仅切除肝叶内储存的血量丢失就足以使循环血量锐减，加之术后动物不能立即恢复饮水进食，常导致其休克、脱水、低血糖引起非肝功能因素死亡[15,16]。因此术中、术后输液尤为重要。基于以上原因，静脉置管成为首选的方法。传统的静脉置管方法均在原有肝脏切除的基础上另取部位进行手术置管，如颈内静脉、腹股沟静脉甚至门静脉属支置管，需在已制备急性肝损伤大鼠手术基础上另附加不同部位的手术创伤、耗时、费力。本实验采用价廉、易得的材料，在大鼠已切除肝叶的肝断面门静脉分支残端置管、固定，建立一可靠、相对经济的实验大鼠静脉通路。

大鼠肝叶分为 6 叶，各叶均有相对独立的 Glisson 鞘系统及肝静脉回流系统，易于绕线阻断血流，Glisson 鞘中均有较粗的门静脉分支，容易分离进行静脉置管等操作。在置管操作过程中应注意一下几点：①保持静脉充盈，血管应先阻断近端，再阻断远端；②用 2 %利多卡因滴于静脉表面浸润，防止因剥离或放线等操作引起血管痉挛；③阻断血流后应往备切除的肝叶方向尽可能的多分离血管，方便插管操作，保证插管深度，不致术后脱出；④插管后不宜过深，防止管道进入门静脉主干或其他肝叶的门脉属支，引起血流障碍或门静脉高压；⑤导管需潜行皮下，自颈背部引出，术后单笼饲养，防止撕咬致导管脱出。

移植部位的不同直接影响到移植细胞的归巢和治疗效果[17-19]。腹腔移植细胞存活率低下，外周静脉注射药物或细胞，最终到达肝脏均有不同程度的剂量减少。门静脉提供 70%以上的肝脏血液供应，到达干细胞血窦后留置时间较长，选择性分布良好，在不改变器官微结构的情况下与肝实质融合。经门静脉移植细胞途径已被认同在细胞移植的途径中具有高浓度、少不良反应、利于细胞迁移定植的优点，其更符合生理学和组织学的特点，且门脉系统内含有高浓度的嗜肝细胞因子，肝脏本身的微环境及门静脉血中的营养成份均对植入的细胞也有益[20]。肝脏的靶向性用药也可提高疗效，减少用药剂量，降低治疗成本以及药物的不良反应。本实验中应用相同量的胎肝干细胞对不同模型的急性肝衰竭大鼠的治疗过程中发现对肝功能、凝血功能的纠正以及对肝脏修复能力，经肝断面门静脉分支残端置管途径效果等同于经门静脉穿刺给药，同时相对于门静脉穿刺给药途径，本模型还具有可反复、多次、选时以及补液治疗等优点，并避免了门脉穿刺过程中出血、血栓形成，同时易于控制给药速度，防止门静脉高压的产生阻碍受体肝脏血流等缺点[21]。

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（上接第 1430 页）

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