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· 基础研究 ·

单人直视下改良建立大鼠原位肝移植模型的体会*

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摘要 目的:建立稳定大鼠原位肝移植模型,缩短术中无肝期时间,提高手术成功率及受体存活率。**方法:**在 Kamada "二袖套法"的基础上改进,单人直视下建立大鼠原位肝移植模型,行 60 例 SD 大鼠原位肝移植手术。本研究简化供受体麻醉方式,供肝采用经门静脉(必要时配合腹主动脉补救方式)进行冷灌注,缩短修肝时间,提前预置牵引线,固定进针位置,改进植入肝脏肝上下腔静脉吻合、肝下下腔静脉及门静脉套管。观察并记录各组大鼠供体手术、修肝套管、无肝期、受体手术及肝移植手术总时间。术后检测 1, 7, 30 天受体大鼠肝功能(血清丙氨酸转氨酶(ALT), 天冬氨酸转氨酶(AST)及总胆红素(TB))并分析生存情况。**结果:**无肝期结束后,供体肝脏灌注良好,受体麻醉移除后较快苏醒。供体手术、修肝套管、无肝期、受体手术及肝移植手术总时间分别为(32.5±1.58)、(7.3±1.43)、(15.6±2.62)、(53.2±3.74)、(108.5±2.34)min。大鼠术后 24 h(手术成功率)为 95%, 1 周生存率分别为 90%, 1 月生存率分别为 86.7%。大鼠术后短时间内肝功能水平增高, 24 h 时 ALT(228.5±54.5 IU/L), AST(439.3±86.3 IU/L), TB(6.2±0.7 μM), 1 周后逐渐恢复正常。**结论:**改良后的方法可以简易麻醉流程,缩短无肝期,提高手术成功率及受体的生存率。

关键词:肝移植;大鼠肝移植;无肝期;移植模型

中图分类号:Q95-3;R657.3 **文献标识码:**A **文章编号:**1673-6273(2018)14-2601-05

An Improved Method to Establish Rat Orthotopic Liver Transplantation Model under Single Operator Direct Vision*

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ABSTRACT Objective: To establish a stable rat orthotopic liver transplantation model, reduce the time of anhepatic phase, improve the operation success and receptor survival rate. **Methods:** We make improvement of the Kamada's "two cuff method", the rat orthotopic liver transplantation model was established under the direct vision of single operator, and 60 cases were performed in the SD rats. We simplified the method of anesthesia for the donor and recipient, the donor liver was subjected to cold perfusion via the portal vein (if necessary with the abdominal aorta), reduced the donor liver repair time, an improved anastomosis of the suprahepatic and inferior vena cava was performed, the inferior hepatic vena cava and portal vein were cannulated by improving technique. Observe and record the time of donor surgery, mend liver and cannula, anhepatic phase, recipient surgery and the total time of liver transplantation. Liver function of recipient rats (including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TB)) at 1, 7, and 30 days were detected and analyzed, their survival was also analyzed. **Results:** When the anhepatic period was over, the donor liver perfusion was perfused well and the receptor recovered quickly after the anesthesia removed. The time of donor operation, liver repair cannula, anhepatic phase, recipient operation and total time of liver transplantation were (32.5±1.58), (7.3±1.43), (15.6±2.62), (53.2±3.74), and (108.5±2.34) min respectively. After operation, the success rate of operation was 95%, the one-week survival rate was 90%, and the one-month survival rate was 86.7%. The level of liver function increased in a short time after operation, at 24h after operation the ALT/AST/TB in serum were detected and the values were (228.5±54.5 IU/L) / (439.3±86.3 IU/L) / (6.2±0.7 μM), then the liver function gradually returned to normal after one week. **Conclusions:** The modified method could simplify the anesthesia progress, shorten the period of anhepatic, improve the success rate of the operation and the survival rate of the receptor.

Key words: Liver Transplantation; Rat liver transplantation; Anhepatic phase; Transplantation model

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前言

肝脏移植首次由 Welch 1955 年提出^[1], 1963 年 Starzl^[2]行世界首例人体肝移植, 历经半个世纪发展, 肝脏移植已成为治疗终末期肝病的有效手段。动物实验在肝脏移植领域发挥重要作用, 动物模型的建立应用于移植免疫排斥、免疫耐受及器官保存等相关问题的研究。1973 年 Lee^[3]首次建立大鼠原位肝移植(rat orthotopic liver transplantation, ROLT)模型, 后经 Kamada 改进, 确立了“二袖套法”建立 ROLT 模型, 即用套管连接肝下下腔静脉(IHVC)和门静脉(PV)替代缝合的连接方式, 此法大大缩短无肝期, 降低手术难度, 提高手术成功率和受体存活率^[4]。ROLT 模型的建立手术难度较大, 国内外研究者基于“二袖套法”提出各种改良方法, 如何熟练操作, 缩短无肝期是初学者成功建立此模型的关键步骤^[5-8]。本研究在“二袖套法”的基础上, 结合相关文献报道, 进行操作及套管方法的改进, 有效缩短了无肝期, 提高大鼠生存率。

1 材料和方法

1.1 材料

1.1.1 动物 供受体均采用 SPF 级健康雄性 SD 大鼠 120 只(上海斯莱克实验动物有限责任公司提供), 质量 220-260 g, 保证供体质量小于受体 10-20 g, 实验及大鼠饲养皆在上海交通大学附属上海市第一人民医院动物实验中心, 动物房为 SPF 级。实验过程中对大鼠的处理符合动物伦理学标准。

1.1.2 器械耗材 显微手术器械, 无损伤缝合线(8-0, 6-0, prolene), 丝线(1-0, 5-0)(图 1), PV, IHVC 管套由心脏介入导管外鞘制作, 胆总管支架管由硬膜外导管制作。腹部拉钩由克氏针制作, 牵引皮条采用小号橡皮筋制作, 注射器、纱布、输液皮条等均由动物实验中心提供。

1.1.3 麻醉及其他试剂 供体麻醉采用戊巴比妥钠(4%)腹腔注射, 受体采用水合氯醛(10%)腹腔注射, 术中辅助异氟烷吸入麻醉(麻醉剂由上海市第一人民医院动物实验中心提供)。肝素钠注射液、青霉素、生理盐水、碳酸氢钠注射液等均购于上海市第一人民医院。

1.2 方法

1.2.1 术前准备 ① 套管的制作: IHVC 袖套用 6 F、7 F 心脏

介入导管鞘制作, PV 袖套用 5、6 F 导管鞘制作, 袖套管体长 3.0-3.5 mm, 留长约 2 mm 的袖套柄以利夹持, 套管体用蚊氏钳钳夹环绕一周, 以便环线结扎、固定^[9]。胆管支架由硬膜外导管制作, 两端削成斜形平行切面, 长 4-6 mm(图 1B)。

② 供受体准备: 供受体术前禁食 12 h。

③ 其他: 动脉快速肝素化注射液(含肝素钠 50 U/mL), 0-4℃冲肝生理盐水(含肝素钠 25 U/mL)^[10]。

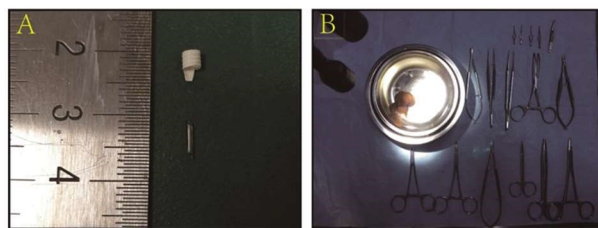


图 1 门静脉管套、胆管支架及手术器械

Fig.1 (A) A cuff for the portal vein (PV) and bile duct (BD); (B) Surgical instruments

1.2.2 手术过程 (1) 供体手术: ① 麻醉成功后, 十字大切口入腹, 暴露肝区, 游离胆总管, 于胆总管前壁距肝管汇合 5 mm 处作一 V 形切口, 用显微钳置入胆管支架, 5-0 丝线环扎, 远端离断(图 2A)。② 游离右三角及冠状韧带、IHVC、右肾静脉(RV)及右肾上腺静脉, 8-0 缝线缝扎右 RV 及右肾上腺静脉, 右 RV 和右肾动脉(RA)一起结扎。分离左三角韧带、左冠状韧带, 紧贴肝上下腔静脉(SHVC), 分离左膈下静脉(IPV)以 5-0 丝线两端结扎中间离断, 肝脏食管静脉丛同样两端结扎中间离断(图 2B)。③ 穿刺髂静脉分叉处, 注入含 50 U 肝素生理盐水 2 mL, 完成供体全身肝素化, 湿棉球按压穿刺点。④ 紧贴 PV 以 8-0 血管缝线穿过幽门静脉及脾静脉, 打结但不拉紧。随即用 8 号头皮针穿刺 PV 并固定针头, 用 0-4℃肝素钠生理盐水(25 U/mL)以 1 滴/秒的速度开始稳定灌洗, 弯剪离断左 RV 水平的 I-HVC(切口呈喇叭状), 同时剪开膈肌, 离断上腔静脉。灌洗同时以 0-4℃生理盐水不时浇注供肝表面, 可使供肝温度迅速下降, 继续灌注至肝脏变为土黄色(图 2C)。⑤ 于结扎线外离断右 RV 及右肾上腺静脉, 拉紧结扎幽门静脉及脾静脉, 远端离断。于脾静脉结扎线下 2 mm 处离断 PV(切口呈喇叭状), 将牵引皮筋略往下拉, 紧贴膈肌环离断 IHVC, 取出供肝置于 0-4℃生理盐水中浸没保存(图 2D)。

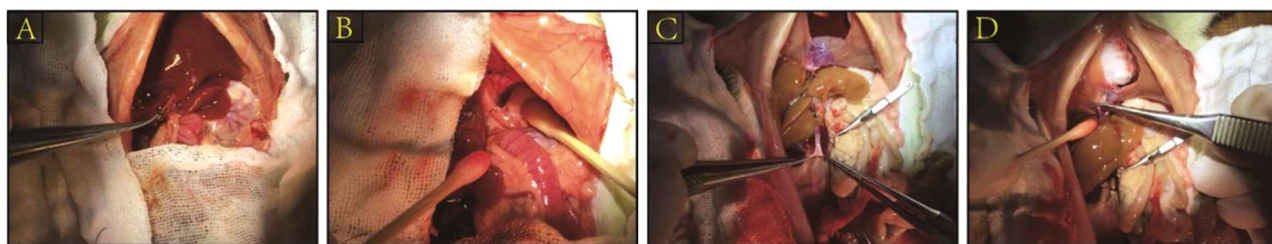


图 2 供体手术

(A) 胆管插管; (B) 左膈下静脉及肝食管静脉丛处理; (C) 肝脏冷灌注; (D) 肝上下腔静脉离断

Fig.2 Donor surgery

(A) Bile duct intubation; (B) The treatment of the left inferior phrenic vein and the liver and esophagus vein;

(C) Cold perfusion of liver; (D) suprahepatic vena cava disconnection

(2)供肝准备:操作均在 0-4℃冰生理盐水中进行。血管钳夹持 PV 袖套柄,橡皮泥固定于盆壁上。以两把显微镊配合,将 PV 断端外翻于套管体上,5-0 丝线环扎固定,相同方法行 I-HVC 套管(图 3A)。SHVC 两侧角各吊一根 8-0 显微外科缝线,预留 3 cm 长的尾线(图 3B)。

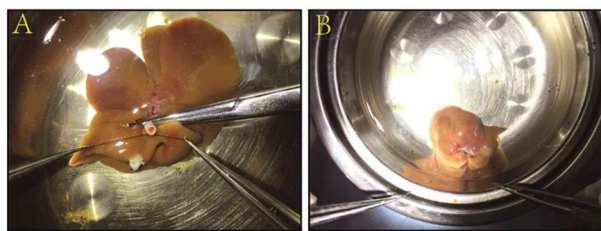


图 3 套管和置牵引线

Fig.3 (A) Portal vein cannula; (B) Preset traction line of suprahepatic vena cava

(3)受体手术:①受体麻醉后,取正中上腹部直切口入腹,以自制小拉钩将腹壁向两侧牵开并固定,蚊式钳夹住剑突,向头侧翻起,充分暴露。生理盐水纱布覆盖肠管并推向左下腹。②游离左三角韧带、左冠状韧带,分离左 IPV、游离肝脏食管静脉丛(二者皆两侧结扎、中间离断)。游离尾状叶,将肝脏向头侧翻起,用湿纱布覆盖,第一肝门肝管汇合处以 5-0 丝线结扎胆总管两端中间离断,游离干净,充分暴露 PV。8-0 缝线缝扎肝固有动脉(图 4A)。③游离右 RV 水平以上的 IHVC,离断右三角韧带、右冠状韧带,远离 IHVC 缝扎右肾上腺静脉丛(稍偏离肝脏面)。游离右下叶与后腹膜间的联系及 SHVC 后方组织,穿过一细橡皮筋备用(图 4B)。④分离第一肝门 PV 左右分支间的结缔组织,于 PV 分支下方 5 mm 处套过 5-0 丝线,暂不结扎,I-HVC 进行同样操作。⑤2 根 8-0 prolene 带针缝合线于 PV 两侧贴近肝脏处进行穿刺打结,进针位置约 3、9 点钟处,减去尾线留置牵引线,小心操作防止撕破血管出血,同样方法处理 PV (图 4C)。⑥右 RV 水平以上以 3 mm 微血管夹阻断 IHVC,于

幽门静脉水平阻断 PV,开始无肝期,穿刺 PV 分叉处,向肝内缓慢注入 2 mL 常温生理盐水直至肝脏变黄。于下腔静脉入肝处以一把 Satinsky 钳夹阻断(带一部分膈肌),用橡皮条将肝脏下拉,贴近肝脏离断 SHVC,在牵引线上方离断 IHVC 及 PV,移去受者自身肝(图 4D)。⑦将供肝小心从冰水浴中移出,原位置入受者右上腹腔,下面垫一生理盐水湿纱布。供肝 SHVC 两侧角的留置线分别与受体对应位置吻合后打结,两侧尾线用血管钳夹持向两侧牵拉。先从右角开始在腔内连续缝合 SHVC 后壁,至左角与该处的牵引线打结,再以另一根留置线连续缝合前壁至右侧角,用冲肝水冲洗出腔内气泡及血凝块后,与牵引线的短头打结,完成 SHVC 吻合(图 4E)。⑧将供肝向头侧翻起,用湿纱布轻轻覆盖,暴露 PV,将 PV 两侧的牵引线分别用蚊式钳夹住,向头侧两边约 30° 角牵拉(注意带针线压在纱布上,不接触肝组织),PV 阻断夹由幽门静脉水平下移至脾静脉汇入 PV 处,放出淤血后用冲肝水冲洗干净,用显微持针器夹住供肝 PV 套管柄,在 PV 约 12 点钟处用显微镊适度提拉管壁,让受体 PV 管腔撑开,置入供体 PV 套管,事先预置的 5-0 丝线环扎固定(图 4F)。放开 SHVC 的 Satinsky 钳及 PV 阻断夹,结束无肝期,肉眼可见供肝迅速变红,然后相同方法进行 I-HVC 套管(图 4G)。剪除多余留置线,移除纱布,解剖位摆正供肝。若有部分花斑色,从大鼠阴经背静脉注射 0.5 mL 的碳酸氢钠注射液+0.5 mL 的葡萄糖注射液,供肝灌注更加充分,色泽鲜红,胆汁不断流出,受体肠系膜动脉搏动有力。于受体胆总管前壁作一 V 形切口,将供肝胆总管支架管外 1/2 部分通过 V 形口插入受体胆总管内,5-0 丝线环扎固定,后将供受体胆总管结扎线拉拢打结固定(图 4H)。用温生理盐水清洗腹腔,棉球擦净,检查有无出血点,后剪掉套管柄,80 万 U 青霉素的液 1 mL 滴入腹腔,逐层关腹,大鼠很快苏醒、翻身、觅食。

(4)移植后处理:术后大鼠进行复温 1 h,术后两小时补充 2 mL 的浓糖水,不禁饮,术后 12 h 喂食,然后进行单笼饲养 2 d。每次采血后,进行补液。

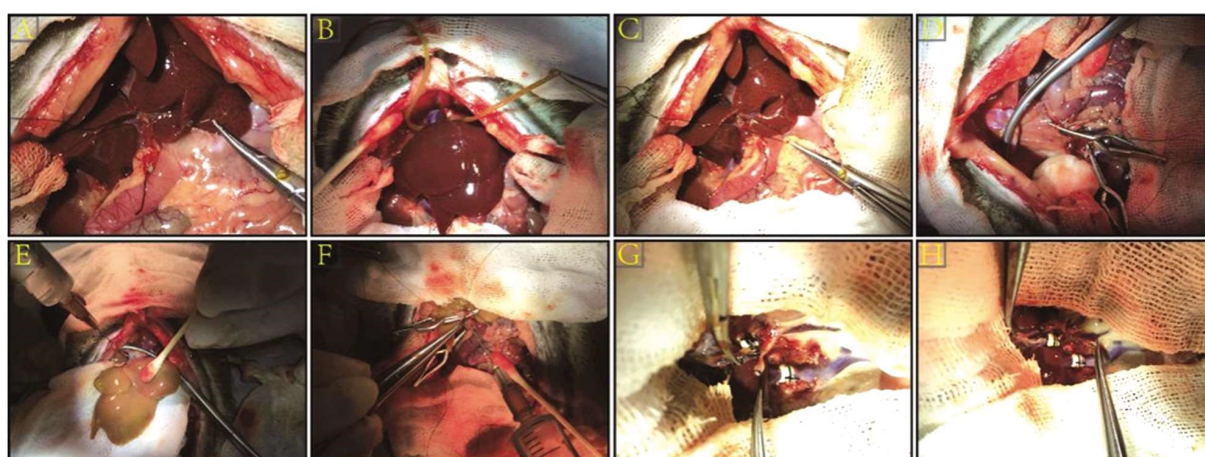


图 4 受体手术

(A)胆管的处理;(B)牵拉皮条置入;(C)肝固有动脉的结扎、门静脉预置牵引线;(D)受体肝脏移除(E)肝上下腔静脉的吻合;(F)门静脉套管置入;(G)胆管插管;(H)血管开放

Fig.4 Recipient surgery

(A)Bile duct ligation and disconnection; (B) Distraction strip placement; (C) Ligation of the liver intrinsic artery and the prepositioned tractive line of the portal vein; (D) Receptor liver removal; (E) Anastomosis of suprahepatic vena cava; (F) Placement of the portal vein cuff; (G) Bile duct intubation; (H) Vascular opening

1.2.3 主要观测指标 (1)围手术期情况:记录大鼠原位肝移植各阶段手术时间(供体手术、修肝套管、无肝期、受体手术及肝移植手术总时间);对术后各时间点(1 d,7 d,30 d)的血清检测指标血清丙氨酸转氨酶(ALT),天冬氨酸转氨酶(AST)及总胆红素(TB)进行分析。(2)对术后死亡受体大鼠进行死亡分析。(3)生存情况:观察大鼠术后生存时间,观察期为1个月,存活超过1个月的大鼠麻醉后处死。

1.3 统计学方法

采用SPSS 20.0统计软件进行数据分析,手术过程中各阶段时间和肝功能指标采用采用 $\bar{x} \pm s$ 表示。

2 结果

采用改良"二袖套法"行大鼠原位肝移植60例,移植术后大鼠精神状态良好,食欲正常,活动量随着术后切口恢复逐

渐恢复正常,无巩膜黄染,尿色清。

供体手术、修肝套管、无肝期、受体手术及肝移植手术总时间分别为(32.5 $\pm$ 1.58)、(7.3 $\pm$ 1.43)、(15.6 $\pm$ 2.62)、(53.2 $\pm$ 3.74)、(108.5 $\pm$ 2.34)min。其中SHVC吻合时间约(8.6 $\pm$ 1.41)min,PV吻合时间(3.3 $\pm$ 1.03)min,IHVC吻合时间(2.3 $\pm$ 0.92)min。

移植后手术成功率(存活时间达24 h手术即成功)95%(57/60),术后24 h内因PV血栓1例,SHVC后壁破裂1例,套管环扎线滑脱1例。1周内死亡3例,胆道梗阻1例,IHVC血栓1例,肠梗阻1例,1周存活率90%。1月内死亡2例,胆道梗阻1例,感染1例,1月存活率86.7%。

血清ALT,AST,TB在术后24 h达到较高水平,分别为228.5 $\pm$ 54.5 IU/L、439.3 $\pm$ 86.3 IU/L、6.2 $\pm$ 0.7 μ M,1周后逐渐下降到正常范围。(表1)。

表1 术后肝功能检测($\bar{x} \pm s$)
Table 1 Detection of liver function after operation($\bar{x} \pm s$)

术后存活时间(天) Survival time of Recipients(d)	大鼠例数(只) Number of rats(n)	肝功能指标 Liver function indexes		
		ALT(U/L)	AST(U/L)	TB(μ M)
1 d	57	228.5 $\pm$ 54.5	439.3 $\pm$ 86.3	6.2 $\pm$ 0.7
7 d	54	137.4 $\pm$ 23.7	259.8 $\pm$ 45.2	5.1 $\pm$ 0.3
30 d	52	87.4 $\pm$ 10.7	129.3 $\pm$ 14.2	2.8 $\pm$ 0.4

3 讨论

ROLT手术难度大,操作流程繁琐,围手术期处理复杂,成功建模须经历长时间艰苦训练。我们基于Kamada"二袖套"法,参照相关文献总结并改进相关环节,获得了稳定的肝移植模型,下面就改进之处作出讨论。

(1)术前对供受体大鼠进行禁食,空腹时肝脏与周围器官组织之间可获得较好的操作空间,减少对肝脏的触碰;笔者最开始对供体不禁食^[11,12],发现大鼠胃内容物较多,尾状叶及PV游离时有妨碍。

(2)本实验结合相关文献及前期摸索^[13,14],供体的大鼠麻醉采用4%戊巴比妥钠5 mL/kg腹腔注射,受体大鼠采用10%水合氯醛2.5 mL/kg腹腔注射,以0.5 mL/kg 10%水合氯醛注射于腹部皮下,术中观察大鼠有苏醒迹象辅助异氟烷吸入麻醉15-20 s^[15,16],如此可以防止受体大鼠麻醉过深,对麻醉设备要求也较低,而乙醚具有较强的刺激气味,呼吸道分泌物会增加,需术前肌注阿托品,而本实验采取上述麻醉方式取得较好效果。

(3)供体采用腹部十字大切口,供肝游离常规多采用顺时针方向游离,但肝脏组织脆弱,在灌注前棉签过度接触挤压会导致冲肝过程中局部组织成花斑色^[17,18],故本实验先进行胆管插管,后游离IHVC、右RV及右肾上腺静脉,使用两个弯血管钳配合分离,可有效分离血管周围脂肪结缔组织,结扎右肾上腺静脉可经其后方绕过线包绕的方法,无需翻动肝脏。对幽门静脉及脾静脉作保留置线不打结处理,剑突拉向头侧可较好暴露左IPV及SHVC,处理左IPV时,0-4℃湿纱布轻覆盖肝脏表面,避免血管钳分离左IPV时直接接触肝组织,右肾动静脉可不分离一起结扎。稳定均匀的灌注流速及适当的灌注压力可获

得色泽均匀、质地良好的供肝,而不在于灌注液的总量^[19,20]。冲肝时经PV使用8号头皮针用哈巴狗钳固定,可以避免滑动损伤血管壁;冲肝水放置操作台上60 cm处,流出速度约1滴/s(约3-4 mL/min)。文献有提出胸主动脉及PV冲肝^[21,22],我们前期实验中发现,SD/Wistar大鼠采用PV冲肝可获较好效果,DA大鼠采用胸主动脉冲肝会取得较好效果,若使用PV冲肝,发现肝组织不能很快变色时,可经胸主动脉进行补救,反之亦然。取肝时IHVC紧贴着膈肌环剪断,可避免修肝时修剪腔静脉。脾静脉与幽门静脉提前置线,可以迅速结扎,减少操作时间。

(4)修肝时基于供体手术,无需剔除血管多余组织和脂肪,可直接进行套管,SHVC两侧角各预留一根8-0的牵引线,尾线留置3-5 cm,注意两侧角的进针位置要稍偏后壁。如此在供肝植入时两侧带针牵引线可直接与供肝的SHVC相对应位置进行吻合打结^[23],用蚊氏钳夹住预留的尾线向受体两侧牵拉,撑开SHVC血管后壁,方便后壁的吻合。

(5)相关文献指出无肝期在26 min以内是安全时间,无肝期越短对肝脏的损伤越小,术后效果也更佳^[4,24]。在实验过程中,尤其在受体肝脏切除及供肝植入过程中,速度要快。笔者认为受体手术过程中,首先保证出血少,在结扎左IPV,食管静脉丛,右肾上腺静脉等相关操作时,要操作轻柔,动作准确到位,一般血管游离开后两端结扎,中间剪断。胆总管尽力贴近肝脏处及下方1 mm处双侧结扎,后中间离断,下端游离到幽门静脉附近,此举可较好暴露PV及预留足够长的胆总管与受体胆管支架吻合^[25]。在供肝植入前,准备好所有需要的物品在自己操作附近区域,方便拿取,尽可能节约无肝期的时间。首先游离干净待移除肝脏周围血管组织和韧带,受体供肝的SHVC、IHVC及PV提前的预置线可以大大节约腔静脉、PV吻合和套

管的时间^[20]。防止套合后血管血栓,可放出待套管血管内的淤血,冲肝水冲洗干净管腔内血凝块;IHVC 内会有残留气泡,可用棉签一边轻轻挤压血管壁下部部分肝组织,用冲肝水冲洗出来,同时能预防防止血栓形成。IHVC 吻合时,很容易用力拉紧丝线导致管壁撕裂,建议 8-0 prolene 线针距 2 mm,进针 3 次收 1 次线,血管内翻缝合,两侧角多缝扎两次然后和尾线打结,可以防止边角出血。胆道支架置入前有新鲜胆汁流出,直接与受体胆管吻合^[27,28]。

(6)不同大鼠对手术的耐受力也是有差异的,为保证术后长期存活,术前应挑选健康的大鼠。术后大鼠及时补液及复温,单笼饲养可保证大鼠不被同伴踩踏啃噬,笼内敷料应该保证两天更换一次^[29]。

单人直视下大鼠肝移植是一项复杂的手术,建立在显微操作相对熟练的基础上,最好前期在显微镜下进行过相对数量大鼠原位肝移植操作,如此更容易掌握。较之显微操作,单人直视下建模操作会更快,节约无肝期及其他阶段时间,但是相对于血管游离、胆管套管及视野清楚方面考虑,显微镜下更具有优势。笔者结合文献及本课题组早期建模的基础上进行改良^[30],简化了麻醉过程,提升操作体验;简化修肝,降低套管及腔静脉吻合时间,缩短无肝期,本实验的无肝期平均时间为 15.6 min,大鼠术后生存率同其他研究者报道相比相似,或有所提升。同时大鼠原位肝移植的建模更需要操作者的细心、耐心及刻苦的训练,经过一定时间操作,能够成功建模。

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