DOI:10.16016/j.1000-5404.2003.16.011
第25卷第16期 第三军医大学学报 Vol. 25, No. 16
1428 2003年8月 ACTA ACADEMIAE MEDICINAE MILITARIS TERTIAE Aug. 2003

论著

文章编号:1000-5404(2003)16-1428-03

应用脂变供肝行减体肝移植的实验研究

叶 晟,韩本立,董家鸿 (第三军医大学附属西南医院全军肝胆外科研究所、西南肝胆外科医院,重庆 400038)

提 要:目的 探讨大鼠脂变供肝用于减体肝移植的适宜植肝量及病理类型。方法 采用70%、60%、50%大鼠减体肝移植(ROLT)模型,观察术后存活率、肝质量/体质量、移植肝/受体原肝及病理的改变。结果 大鼠Ⅰ级脂变的全肝移植和70%、60%、50% ROLT的1周存活率分别为91.67%,75%,75%,25%;2周存活率分别为83.33%,75%,58.33%,0。Ⅱ级脂变的全肝及70%ROLT的1周存活率为83.33%和25%。小泡性脂变为主的供肝,其全肝及70%、60%、50%ROLT移植的1周存活率分别为83.33%,75%,75%,33.33%;2周存活率分别为75%,66.67%,66.67%,0。Ⅰ级或以小泡性脂变为主的供肝50%ROLT的生存率与其它组相比均有显著性差异。术后病理可见肝再生及脂变减轻或消失,中央静脉及肝窦扩张。结论 要获得较高的术后存活率,大鼠Ⅰ级或以小泡性脂变为主的供肝行减体肝移植其移植肝/受体体质量应大于(2.28±0.12)%(即移植肝/受体原肝>60%);而Ⅱ级脂变供肝不宜用于减体肝移植;术后脂变可减轻或逆转。

关键词:大鼠;脂变供肝;减体肝移植
中图法分类号:R575.5;R617;R657.3 文献标识码:A

Experimental study of the reduced-size orthotopic liver transplantation in different steatotic rats

YE Sheng, HAN Ben-li, DONG Jia-hong (Institute of Hepatobiliary Surgery of PLA, Southwest Hospital, Third Military Medical University, Chongqing 400038, China)

Abstract: Objective To observe the survival time, pathological change and liver regeneration in different kinds of reduced-size liver transplantation in steatotic rats. Methods Rat models of different kinds of reduced-size orthotopic liver transplantation were performed by modified two-cuff vascular anastomoses and end-to-end suture for bile duct to observe the recipient body weight, graft weight, recipient original liver weight, histological and pathological and electron-microscopic findings in comparison with those of the whole rat liver transplantation. Results One-week survival rate of the whole liver transplantation, 70% reduced-size liver transplantation (ROLT), 60% ROLT and 50% ROLT group (grade Ⅰ steatotic donor) was 91.67%, 75%, 75% and 25%, respectively, and 2-week survival rate was 83.33%, 75%, 58.33% and 0, respectively. In grade Ⅱ steatotic donor, 1-week survival rate of the whole liver transplantation and 70% ROLT was 83.33% and 25%. As to donor livers with microvesicular steatosis, 1-week survival rate of the whole liver, 70% ROLT, 60%ROLT and 50% ROLT was 83.33%, 75%, 75% and 33.33% and the 2-week survival rate was 75%, 66.67% 66.67% and 0. The survival rates of 50% ROLT in grade Ⅰ steatotic donor and livers mainly with microv
groups. The 1-week survival rate of 70% ROLT was very poor in steatotic donors in grade Ⅱ. Pathological findings after operation included liver regeneration and mild lymphocyte infiltration in portal space, the amelioration of the steatosis in some cases and dilation of the central vein and sinusoids. Conclusion To obtain long term survival of reduced-size liver transplantation using steatotic donors, the GRBW should be over (2.28±0.12)(the ratio of graft to recipient liver weight over 60%). Steatotic livers in grade Ⅱ should not be used as donors in ROLT. The steatosis can be ameliorated after operation.

Key words: rat; steatotic donors; reduced-size liver transplantation

减体肝移植(Reduced-size orthotopic liver transplantation, ROLT,包括活体和劈裂式肝移植)近年在国内逐渐开展起来,成为临床上全肝移植的必要补充。对于正常供肝,要求移植肝体积或质量(Graft volume/weight, GV/W)与标准肝体积或质量(Standard liver volume/weight, SLV/W)之比>30%~40%,或移植肝质量与受体体质量之比(Graft recipient body weight, GRBW)>1%[1,2]。但对于有脂变的供肝此比值必须加以修正。本实验通过大鼠脂变供肝的不同减体肝移植模型

作者简介:叶 晟(1968-),男,浙江省台州市人,博士,主治医师,主要从事肝移植的实验和临床方面的研究,发表论文10篇。电话:(023)68754000-73079
通信作者:董家鸿,电话:(023)68754168;E-mail:jhdong@hbsky.com.cn
收稿日期:2002-08-03;修回日期:2003-03-25

?1994-2016 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

来探讨此类供肝用于减体肝移植的合适的GV/SLV 和GRBW 比值及病理类型。

1　材料与方法

1.1　实验动物及分组

同批同月龄雄性Wistar 大鼠 120 只, 体质量 180～220 g, 第三军医大学实验动物中心提供, 二级动物。编号后用随机数字表法选取供肝鼠, 喂饲标准饲料 79%加猪油 20%加胆固醇 1%的高脂高胆固醇饲料, 同时 50%乙醇灌胃(1 ml·100 g^{-1}·d^{-1}), 分别诱导成不同的脂变肝脏[3], A 组(Ⅰ级大泡脂变, n=48)、B 组(Ⅱ级大泡脂变, n=24)、D 组(小泡脂变为主, n=48)。根据减体肝移植的不同再细分为: A1 组(全肝, n=12)、A2 组(70% ROLT, n=12)、A3 组(60% ROLT, n=12)、A4 组(50% ROLT, n=12); B1 组(全肝, n=12)、B2 组(70% ROLT, n=12); D1 组(全肝, n=12)、D2 组(70% ROLT, n=12)、D3 组(60% ROLT, n=12)、D4 组(50% ROLT, n=12)。受体鼠手术前后均给予标准饲料。每对供、受体体质量相差≤10 g。

1.2　减体肝移植模型

参照 Kamada[4] 法采用二袖套、胆管端端缝合行大鼠肝移植[5]。①70%减体肝移植方法: 提起左侧叶, 向光看清中肝叶肝静脉与左侧叶肝静脉的汇合处, 在其外侧细针穿过 1 号丝线, 分别向上、向下两缘结扎, 再剪去左侧叶, 残端缝扎; ②60%减体肝移植方法: 同法切除供肝的左叶和双尾叶; ③50%减体肝移植方法: 参考 Omura 的方法加以修改[6]: 供肝切除左叶、双尾叶和右叶前部。

1.3　标本采集及统计学方法

大鼠术后存活时间(超过 24 h 视为手术成功)。统计数据以 $\bar{x}\pm s$ 表示, 各组间均数比较采用 SPSS10.0 独立样本 t 检验或 Oneway Anova 检验。生存时间采用 SPSS10.0 软件包绘制成 Kaplan-Meier 曲线, 比较采用对数秩检验(log-rank)。计数资料用 χ^2 检验。

2　结果

2.1　受体移植前、后肝质量的变化情况, 见表 1

表 1　受体移植前、后肝质量的变化(g, n=12, $\bar{x}\pm s$)

Tab 1　Changes of recipient liver weight before and after transplantation(g, n=12, $\bar{x}\pm s$)

Group	Recipient body weight	Recipient original liver weight	Graft weight	Graft/ recipient body weight(%)	Graft/ recipient original liver weight(%)
A1	276.43±14.71	10.29±0.28 *	10.45±0.16	3.56±0.36	101.34±4.18
A2	280.58± 9.95	10.36±0.32	7.10±0.41	2.53±0.15 #	68.60±3.80
A3	277.33±12.23	10.23±0.26	6.32±0.29	2.28±0.12 ▲	61.81±2.32
A4	284.75±16.21	10.43±0.36	5.19±0.25	1.83±0.16 ◆	49.82±3.29
B1	273.97±15.32	10.11±0.21	10.31±0.27	3.61±0.29	101.79±3.07
B2	279.91±13.07	10.25±0.35	7.33±0.55	2.58±0.16	70.63±4.91
D1	275.76±11.34	10.65±0.24	10.44±0.37	3.58±0.25	97.74±2.31
D2	281.91±17.30	10.41±0.46	7.41±0.64	2.62±0.18	71.61±5.27
D3	274.33±9.69	10.28±0.21	6.50±0.26	2.29±0.20	61.18±4.89
D4	284.66±8.18	10.25±0.36	5.21±0.24	1.82±0.08	50.86±3.17

*: Comparison of liver weight in the groups(F=0.96, P<0.05); #: A2, B2 *vs* D2(F=0.985, P=0.348); ▲: A3 *vs* D3(t=0.199, P=0.844); ◆: A4 *vs* D4(t=0.077, P=0.94)

2.2　各组生存率比较, 见表 2

表 2　各组生存率情况(n=12)

Tab 2　Comparison of survival rates of different groups(n=12)

Group	One week survival(%)	Two week survival(%)
A1	91.67	83.33
A2	75.00	75.00
A3	75.00	58.33
A4	25.00	0.00
B1	83.33	0
B2	25.00	0
D1	83.33	75.00
D2	75.00	66.67
D3	75.00	66.67
D4	33.33	0

A1 *vs* A4(χ^2=19.09, P<0.05); A2 *vs* A4(χ^2=14.04, P<0.05); B1 *vs* B2(χ^2=7.34, P<0.05)D1 *vs* D4(χ^2=17.39, P<0.05); D2 *vs* D4(χ^2=10.16, P<0.05); D3 *vs* D4(χ^2=9.40, P<0.05)

2.3　组织学和超微结构

术后存活较长时间的大鼠可见脂变减轻或消失, 同时伴有肝再生、肝窦扩张, 部分大鼠还可见窦状隙内胶原纤维增生, 周围可见大小不等的脂滴空泡, 见图 1～3。

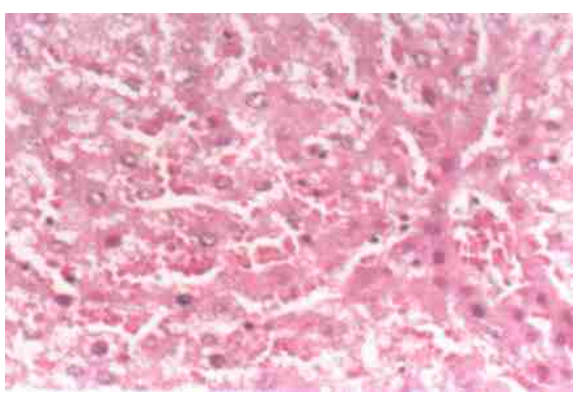


图 1　小泡性脂变供肝减体术后 14 d, 脂变减轻伴肝细胞再生　(HE×400)

Fig 1　Amelioration of steatosis accompanied by liver regeneration at 14 d after operation　(HE×400)

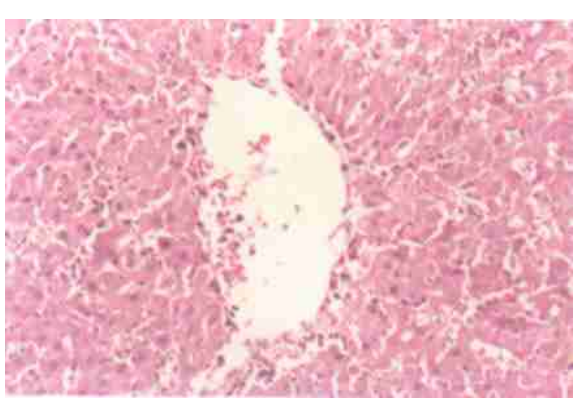


图 2　脂肪变性供肝减体术后 26 d 脂变基本消失, 窦状隙扩大, 少数脂变细胞　(HE×200)

Fig 2　Disappearance of steatosis and enlarged sinusoidal space at 16 d after operation　(HE×200)

?1994-2016 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net

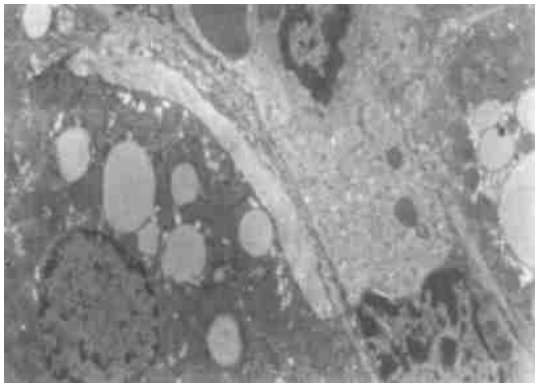


图3 脂变供肝减体移植术后窦状隙少量胶原增生 (TEM×4 200)

Fig 3 Mild fibrosis in sinusoidal space of macrosteatosis after ROLT(TEM×4 200)

3 讨论

大鼠减体肝移植是一难度较高的动物手术模型,因减体积操作时易引起残端出血,肝上下腔静脉因肝切除后残端的影响缝合较困难。减体积完成的时相点有3种:一是在供肝灌注前的在体切除,但此时易出血,还可即刻启动供肝再生,对实验结果有干扰;二是在离体的冰浴容器内进行,操作简便易行,但要到受体植上供肝后才可能发现出血;三是在受体植上供肝、血流再通后进行,易出血并增加了受体的创伤、降低存活率。本实验全部采用离体冰浴容器中减体积的方法,结扎加缝扎确保残端不出血。

Xu 等[7] 采用正常的、供受体大小不等的大鼠行减体肝移植,要获得较高的存活率其供、受体肝质量比应在(0.53~1.26)∶1之间。本实验结果显示大鼠Ⅰ级脂变供肝(大泡为主)的全肝移植(A1)及70%(A2)、60%(A3)、50% ROLT(A4)的1周存活率分别为91.67%,75%,75%,25%,2周存活率分别为83.33%,75%,58.33%,0;而Ⅱ级脂变供肝(大泡为主)的全肝(B1)及70%ROLT(B2)的1周存活率分别为83.33%和25%;小泡性脂变为主的供肝其全肝(D1)及70%(D2)、60%(D3)、50% ROLT(D4)移植的1周存活率分别为83.33%,75%,75%,33.33%,2周存活率分别为75%,66.67%,66.67%,0。A1和A2、A3组生存率曲线相比无显著性差异($\chi^2=0.26$, 1.57, $P>0.05$),而A4和A1、A2和A3组相比均有显著性差异($\chi^2=19.09$, 14.04, 11.85, $P<0.01$)。说明大鼠Ⅰ级脂变供肝行减体肝移植的最低限度应不小于60%的受体原肝质量,即移植肝与受体体质量之比(GRBW)应大于(2.28±0.12)%。Ⅱ级脂变供肝的全肝(B1)与70%ROLT(B2)相比存活率有显著性差异($\chi^2=7.34$, $P=0.0067$),此类供肝不宜用于减体肝移植。而对于D组即小泡为主的脂变供肝,术后的存活率反而较高,术后其脂肪变清除快,似乎是一暂时的、可逆性的病变,此组的全肝移植和70%、60%ROLT生存率无显著性差异($\chi^2=0.24$, 1.41, $P>0.05$),而D4(50%ROLT)的存活明显下降,与D1相比有显著性差异($\chi^2=17.39$, $P<0.001$),因此,此类供肝的GRBW应至少大于(2.29±0.20)%才可获得较高的1周存活率。临床上此种病变常见于急性肝损伤或脂肪变的初期或恢复期,临床肝移植也发现小泡脂变供肝的预后较好[8]。我们在供肝诱导中发现8周时大鼠ALT、TBIL水平达到最高,大鼠一般情况较差,移植此供肝存活率最低,而10周或以上的大鼠一般情况反而好转,其血清ALT、TBIL水平呈下降的趋势,病理主要为小泡性脂变,移植此供肝的存活率明显好于B组。大鼠这种可逆性改变的机制仍不清楚,可能是大鼠对乙醇和高脂、高胆固醇饲料产生了耐受,中止了脂肪变的发展、导致病变的改善。大鼠胆碱-蛋氨酸缺乏饲料诱导的脂变肝脏也发现有类似的结果[9]。

术后病理观察可见轻度汇管区淋巴细胞浸润,常见有中央静脉、窦状隙扩张,这可能与肝减体积后的门静脉压力升高、导致再生的肝脏血管扩张的原因[10]。术后长期存活的大鼠其脂变有不同程度减轻甚至消失,并可见较多的肝细胞再生。超微结构示窦状隙有少量的胶原增生,可能与脂变的刺激有关。因此,大鼠脂变供肝减体肝移植术后由于消除了致病因素,脂变可以减轻或逆转,肝细胞仍可再生。

参考文献:

[1] Kiuchi T, Kacahara M, Uryuhara K, *et al*. Impact of graft size mismatching on graft prognosis in liver transplantaion from living donors[J]. Transplantation, 1999, 67(2): 321-327.

[2] Nishizaki T, Ikegami T, Hiroshige S, *et al*. Small graft for living donor liver transplantation[J]. Ann Surg, 2

[3] 叶 晟, 韩本立, 董家鸿. 大鼠脂肪变性供肝移植的可行性研究[J]. 第三军医大学学报, 2003, 25(10): 880-883.

[4] Kamada N, Calne R Y. Orthotopic liver transplantation in the rat: technique using cuff for portal vein anastomosis and biliary drainage[J]. Transplantation, 1979, 28(1): 47-52.

[5] 叶 晟, 韩本立, 董家鸿. 大鼠原位肝移植的显微外科技巧[J]. 中华显微外科杂志, 2003, 26(1): 49-50

[6] Omura T, Ascher N L, Emond J C. Fifty-percent partial liver transplantation in the rat[J]. Transplantation, 1996, 62(2): 292-293.

[7] Xu H S, Pruett T L, Jones R S. Study of donor-recipient liver size match for transplantation[J]. Ann Surg, 1994, 219(1): 46-50

[8] Fishbein T M, Fiel I M, Emre S, *et al*. Use of livers with microvescicular fat safely expands the donor pool[J]. Transplantation, 1997, 64(2): 248-251.

[9] Cheng Y F, Chen C L, Lai C Y, *et al*. Assessment of donor fatty livers for liver transplantation[J]. Transplantation, 2001, 71(9): 1221-1225.

[10] Sato Y, Tsukada K, Hatakeyama K, *et al*. Role of shear stress and immune responses in liver regeneration after a partial hepatectomy[J]. Surg Today, 1999, 29(1): 1-9

(编辑5 龚国文)

?1994-2016 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net