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脾切除对肝硬化门静脉高压症大鼠免疫功能的影响及意义*

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摘要 目的:对肝硬化门静脉高压症大鼠行脾切除术,观察术后大鼠免疫系统功能的变化情况,探讨脾切除术对免疫系统的影响,为临床治疗提供理论基础。**方法:**选取 SD 大鼠 45 只建立 PHT 模型,随机分为 PHT 组、脾全切组及部分脾切除组,每组各 15 只。另选取 10 只健康大鼠为对照组。分别对四组大鼠行不同的脾切除方案,术后检测四组大鼠的血常规、免疫球蛋白含量、T 淋巴细胞亚群的变化及组织病理学染色结果等,分析各指标的意义。**结果:**四组大鼠血常规检测结果显示,PHT 组大鼠 WBC 高于对照组,PHTTS 组高于 PHT 组,PHTSS 组高于 PHTTS 组,差异显著且具有统计学意义($P<0.05$)。四组大鼠血浆中 IgG 含量差异显著($P<0.05$);IgA、IgM 含量无显著性差异($P>0.05$)。四组大鼠 CD4⁺、CD8⁺ 含量差异显著($P<0.05$);CD29⁺ 含量无显著性差异($P>0.05$)。**结论:**脾脏直接参与细胞介导免疫调节,脾切除可降低门脉压力,消除脾亢,有利于肝硬化门脉高压症的缓解。

关键词:肝硬化门静脉高压;脾切除术;免疫功能

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Effects and Significance of Splenectomy on the Immunity of Rats with Portal Hypertension*

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ABSTRACT Objective: To discuss the effects and significance of splenectomy on the immunity of rats with portal hypertension so as to make a reference for the clinical surgery. **Methods:** Forty-five SD rats were selected to make the PHT model and randomly divided into the three groups which referred to the PHT group, the PHTTS group that taking the total splenectomy surgery and the PHTSS group that undergoing the partial splenectomy surgery. Another fifteen healthy rats were chosen as the control group. Then the detection of the blood, the immunity, the T lymphocyte subsets and the pathological changes of the rats were observed and analyzed. **Results:** There was statistically significant difference of blood routine of rats between the four groups ($P<0.05$); There was statistically significant difference of IgG in the plasma of rats between the four groups ($P<0.05$); There was no statistically significant difference about the IgA and IgM ($P>0.05$); There were statistically significant differences of CD4⁺ and CD8⁺ of rats between the four groups ($P<0.05$); There was no statistically significant difference about CD29⁺ of rats between the four groups ($P>0.05$). **Conclusion:** It is indicated that the splenectomy could reduce the portal pressure, eliminate the port and relieve the cirrhotic portal hypertension.

Key words: Cirrhotic portal hypertension; Splenectomy; Immunity

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前言

脾脏是机体最活跃、最大的免疫器官,占全身淋巴组织总量的 25%,含有大量的淋巴细胞和巨嗜细胞,是机体细胞免疫和体液免疫的中心,是血循环中重要的过滤器。它直接参与细胞介导免疫调节,通过 T 细胞和可溶性脾因子对脾内及外周血内的免疫活性细胞的量和比例及其它淋巴组织细胞群的变化进行调控^[1-3]。肝硬化患者由于门脉压力升高而导致脾脏肿大,

而脾脏肿大又增加了门静脉的血流量,促使门脉系统高动力灌注,导致门脉压力进一步升高^[4]。目前,脾切断流术是肝硬化门静脉高压脾功能亢进患者的首选治疗方法,不仅能够矫正脾功能亢进,还可减少门静脉血流量,从而降低门静脉压力^[5]。有研究表明,脾脏切除术对门脉高压症患者的机体淋巴细胞再生具有促进作用,可改善或调节其免疫系统的功能^[6]。为了深入研究脾切除对免疫系统的影响,我们建立了 PHT 大鼠模型,采取不同的脾切除术,分析术后大鼠免疫系统的变化情况,旨在为临

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床研究提供实验基础和理论依据。

1 材料与方法

1.1 实验动物及试剂

70 只健康雄性成年 SD 大鼠,体重 180-220 g(由解放军医学院动物实验中心提供)、全自动血球分析仪(CELL-DYN1700 型,美国)、一次性自动穿刺针(MAXCORE,16 G,巴德机械公司,美国)、兔抗人 IgM、IgA、IgG 多克隆抗体(中杉金桥公司,北京)、BCA 蛋白定量试剂盒、免疫组织化学染色试剂盒(Sigma, USA)、全自动生化分析仪(Gibco,USA)、离心机(TaKaRa, 日本)、流式细胞仪 (Gene,USA)、倒置相差显微镜(Olympus, Japan)。

1.2 实验方法

1.2.1 模型制备 取 60 只清洁级雄性大鼠,给予足量的饲料。按 0.3 ml/100g 体重,对大鼠皮下注射 40%四氯化碳橄榄油溶液,给予 5%乙醇溶液为饮用水,持续两周;第三周改为注射 50%四氯化碳橄榄油溶液,给予 10%乙醇溶液为饮用水,持续两周;第五周改为注射 60%四氯化碳橄榄油溶液,给予 20%乙醇溶液为饮用水,持续四周^[7]。待肉眼观察肝脏表面生成大小不等的弥漫性结节、肝脏组织病理切片显示有假小叶形成,认定为造模成功。

1.2.2 实验分组及手术方法 将造模成功的 45 只肝硬化门静脉高压大鼠随机分为三组,每组 15 只。各组大鼠于手术前 8 h 禁食水,乙醚吸入麻醉配合 5%利多卡因注射液局部浸润麻醉。取左上腹部纵切口,逐层切开腹壁进入腹腔。①肝硬化门静脉高压组(PHT 组):切除部分大网膜后逐层关腹^[8]。②肝硬化门静脉高压全脾切除组(PHTTS 组):游离脾脏周围韧带,双结扎切断脾蒂血管,切除全部脾脏,检查无活动性出血后逐层关腹。③肝硬化门静脉高压部分脾切除组(PHTSS 组):游离脾上极血管,双结扎切断脾蒂血管,保留脾结肠韧带及脾下极脾隔韧带,切除约 75%脾脏,彻底止血,逐层关腹。④另选取 10 只健康大鼠为对照组。

1.3 观察指标及检测方法

1.3.1 血常规检测 各组大鼠于术后第 3 周穿刺心脏采血 21.5

mL,置于含 EDTA 的抗凝管中,利用全自动血球分析仪检测红细胞计数(RBC)、白细胞计数(WBC)及血小板计数(PLT)^[9]。

1.3.2 免疫球蛋白测定 术后第 4 周,取大鼠血清 0.1 mL,与生理盐水 0.3 mL 混合摇匀,分别注入 G、A、M 板孔中,置于 37℃ 培养箱中。48 h 后常规染色,0.01M(pH7.2)磷酸盐缓冲液冲洗 5 min;利用全自动生化分析仪(Gibco, USA)检测免疫球蛋白的含量,其中 IgM 抗原检测采用热修复法,即常规染色,0.01 M (pH6.0)柠檬酸液冲洗,置于微波炉中加热^[10]。

1.3.3 T 淋巴细胞亚群检测 将已分离的各组小鼠脾淋巴细胞置于 96 孔板中,清洗两次,离心 2000rpm(2 min,4℃),甩掉上清,加入 CD4、CD8、CD29 抗体,4℃下避光孵育 30 min;每孔加入 100 μ L wash buffer 洗两次,离心 2000 rpm;每孔再加入 100 μ L 2%的多聚甲醛固定(4℃,15 min),避光。利用流式细胞仪检测 CD4⁺、CD8⁺、CD29⁺。

1.3.4 免疫组化染色 用一次性自动穿刺针穿刺获取脾脏组织切片标本,获取脾组织长度 >1.5 cm,石蜡切片 4-5 μ m,脱水,以 4%甲醇双氧水固定,8 min 后包埋,连续切片。倒置相差显微镜下观察各组大鼠脾脏组织的病理变化。

1.4 统计学方法

采用 SPSS 13.0 软件进行数据分析,计量资料以均数 $\pm$ 标准差表示,组间均数比较采用单因素方差分析,以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 血常规检测结果

如表 1 所示,健康组大鼠的红细胞数为 41.34 ± 16.46 ,白细胞数为 43.86 ± 11.73 ,血小板数为 48.76 ± 18.64 ;肝硬化门静脉高压组大鼠的红细胞数为 61.96 ± 18.43 ,白细胞数为 69.64 ± 16.45 ,血小板数为 68.54 ± 21.97 ;肝硬化门静脉高压全脾切除组大鼠的红细胞数为 75.34 ± 13.46 ,白细胞数为 74.35 ± 21.57 ,血小板数为 76.35 ± 21.57 ;肝硬化门静脉高压部分脾切除组大鼠的红细胞数为 78.63 ± 12.12 ,白细胞数为 79.88 ± 10.26 ,血小板数为 77.12 ± 12.45 。组间比较差异显著,具有统计学意义($P<0.05$)。

表 1 各组大鼠血常规检测结果

Table 1 Results of the blood routine examination of rats in different groups

	Number	Control Group	PHT Group	PHTTS Group	PHTSS Group	P
RBC	15	41.34 ± 16.46	61.96 ± 18.43	75.34 ± 13.46	78.63 ± 12.12	<0.05
WBC	15	43.86 ± 11.73	69.64 ± 16.45	74.35 ± 21.57	79.88 ± 10.26	<0.05
PLT	15	48.76 ± 18.64	68.54 ± 21.97	76.35 ± 21.57	77.12 ± 12.45	<0.05

2.2 各组大鼠免疫球蛋白检测结果

如表 2 所示,四组大鼠 IgA、IgM 含量无显著差异($P>0.05$)。健康大鼠 IgG 含量为 49.40 ± 11.66 ,肝硬化门静脉高压组大鼠 IgG 含量为 62.93 ± 12.82 ,肝硬化门静脉高压全脾切除组大鼠

IgG 含量为 72.66 ± 11.21 ,肝硬化门静脉高压部分脾切除组大鼠 IgG 含量为 92.43 ± 12.42 。四组大鼠 IgG 含量差异显著,具有统计学意义($P<0.05$)。

表 2 各组大鼠免疫球蛋白检测结果(g/dl)

Table 2 Results of the detection of immune globulin of rats in different groups

	Number	Control Group	PHT Group	PHTTS Group	PHTSS Group	P
IgA	15	46.54 ± 17.67	42.54 ± 21.34	49.62 ± 18.65	44.32 ± 27.45	>0.05
IgM	15	52.55 ± 21.23	58.43 ± 23.43	55.39 ± 13.00	56.87 ± 12.09	>0.05
IgG	15	49.40 ± 11.66	62.93 ± 12.82	72.66 ± 11.21	92.43 ± 12.42	<0.05

2.3 各组大鼠 T 淋巴细胞亚群检测结果

如表 3 所示,健康大鼠 CD4⁺ 含量为 68.65± 7.55, CD8⁺ 含量为 38.09± 7.80; 肝硬化门静脉高压组大鼠 CD4⁺ 含量为 71.15± 8.61, CD8⁺ 含量为 39.95± 6.76; 肝硬化门静脉高压全脾切除组大鼠 CD4⁺ 含量为 74.95± 7.75, CD8⁺ 含量为 35.81±

6.59; 肝硬化门静脉高压部分脾切除组大鼠 CD4⁺ 含量为 76.79± 4.04, CD8⁺ 含量为 37.65± 5.44。四组大鼠 CD4⁺, CD8⁺ 含量差异显著,具有统计学意义(P<0.05), CD29⁺ 含量无显著性差异(P>0.05)。

表 3 各组大鼠 T 淋巴细胞亚群检测结果(%)

Table 3 Results of the detection of T lymphocyte subsets of rats in different groups

	Number	Control Group	PHT Group	PHTTS Group	PHTSS Group	T	P
CD4 ⁺	15	68.65± 7.55	71.15± 8.61	74.95± 7.75	76.79± 4.04	5.10	<0.01
CD8 ⁺	15	38.09± 7.80	39.95± 6.76	35.81± 6.59	37.65± 5.44	3.20	<0.05
CD29 ⁺	15	31.55± 9.81	29.15± 6.34	28.85± 6.81	30.05± 4.91	2.65	>0.05

2.4 四组大鼠组织形态学观察结果

通过手术大体观察及淋巴细胞检测发现, PHT 大鼠脾增大, 被膜增厚, 脾小梁增宽, 脾小体周围淋巴鞘数量减少、结构疏松, 淋巴细胞密度降低, 脾窦扩张瘀血, 脾索增厚, 纤维组织增多, 排列紊乱。PHT 全脾切除组大鼠淋巴细胞增多, 纤维组织减少, 脾窦扩张程度与 PHT 组无显著差别。PHT 部分脾切除组大鼠脾窦扩张程度减轻, 脾索纤维组织较多, 淋巴细胞密度偏低, 数量偏少。

3 讨论

肝硬化是一种以肝组织弥漫性纤维化, 假小叶和再生结节形成为特征的慢性肝病, 临床以肝功能损害和门静脉高压为主要表现, 脾脏对人体的体液免疫和细胞免疫具有重要的调控作用和强大的抗感染功能^[1-13]。大量研究表明, 肝硬化患者的免疫功能均低于正常值, 而脾脏的免疫功能紊乱会促进肝硬化的发展^[14]。因此, 对肝硬化门静脉高压症患者的免疫功能进行评估是临床检验脾切除术是否有利于改善脾脏功能的方法之一。肝硬化患者发生脾功能亢进时会出现全血细胞减少的现象, 以白细胞和血小板减少为主^[15]。本研究中, 行脾切除术的大鼠血小板明显升高, 说明切除肿大的脾脏对肝硬化门静脉高压患者的血循环具有一定的改善作用。脾脏在机体免疫系统中占重要地位, 是血源性抗原发生免疫应答的主要场所, 也是产生抗体的主要基地。免疫球蛋白是免疫系统体液调节的主要抗体, 其中 IgG 可以激活补体、促进细胞的吞噬功能, IgA 具有抑制病菌吸附和侵入粘膜的局部抗感染作用, IgM 具有杀菌、中和毒素的功能^[16,17]。据相关研究证实, 部分脾切除术能够使保留的脾脏保持生理功能, 从而维持机体的免疫功能^[18]。本研究中, 肝硬化门脉高压大鼠的 IgG 为 62.93± 12.82, 全脾切除组大鼠的 IgG 为 72.66± 11.21, 部分脾切除组大鼠的 IgG 为 92.43± 12.42。结果说明保留部分脾切除不仅能够消除脾亢、降低门静脉压力, 而且可以使余脾继续发挥自身的免疫功能, 有利于肝硬化门静脉高压患者的免疫系统功能得到改善。T 淋巴细胞在机体特异性免疫应答及免疫调节中起重要作用, T 细胞亚群的构成是反映宿主免疫功能的重要指标。CD4⁺ 细胞为辅助性 T 淋巴细胞, CD8⁺ 细胞为抑制性或杀伤性 T 淋巴细胞, 具有使 T 细胞稳定的作用^[19]。肝硬化门脉高压患者的脾脏组织中 CD4⁺ 淋巴细胞减少, CD8⁺ 淋巴细胞增加, 说明脾肿大导致免疫功能紊乱^[20]。本研究中, 肝硬化门脉高压大鼠的 CD4⁺ 淋巴细胞为 71.15± 8.61, CD8⁺ 淋巴细胞为 39.95± 6.76; 全脾切除组大鼠的

CD4⁺ 淋巴细胞为 74.95± 7.75, CD8⁺ 淋巴细胞为 35.81± 6.59; 部分脾切除组大鼠的 CD4⁺ 淋巴细胞为 76.79± 4.04, CD8⁺ 淋巴细胞为 37.65± 5.44。结果说明, 脾切除后 CD4⁺ 淋巴细胞增多, CD8⁺ 淋巴细胞减少, T 淋巴细胞亚群的免疫功能得到改善。

综上所述, 肝硬化门静脉高压症患者肿大的脾脏会导致机体免疫功能紊乱, 而脾切除术后机体免疫功能可获的改善, 说明脾切除有利于降低门静脉压力、消除脾亢, 有利于肝硬化门脉高压症的缓解。临床治疗中应根据患者自身情况采取相应的脾切除术, 以改善其免疫功能、提高手术疗效。

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