

Study on the biomechanical property and histomorphological changes of fractured vertebrae after percutaneous vertebroplasty Quan Renfu*, Ni Yueming, Zheng Xuan, Xie Shangju, Li Changming. * Xiaoshan Hospital of Traditional Chinese Medicine, Hangzhou 311201, Zhejiang, China

ABSTRACT Objective: To observe the biomechanical property and histomorphological changes of fractured vertebrae after percutaneous vertebroplasty (PVP). **Methods:** One hundred and five New Zealand female rabbits were randomly divided into 3 groups, 35 cases in each group. The rabbits in group B and group C were administrated with ovariectomy and Dexamethasone muscle injection to build models of osteoporosis. Then, bone defects were created through surgery in the vertebral body of L₄ and L₅ for all rabbits. The vertebral body with bone defects of rabbits in group C were administrated with polymethylmethacrylate III (PMMA III) injection to simulate PVP. At 2, 4, 8, 12, 16, 24 and 48 weeks after the simulated PVP, 5 rabbits were selected from each group and were executed, and their vertebrae of L₄ and L₅ were fetched out for determining the biomechanical strength. Meanwhile, the samples of group C were fluorescent labeled with tetracycline hydrochloride, and the histomorphological changes of vertebrae were observed after toluidine blue staining. **Results:** The axial compression test showed that there were statistical differences in axial compressional displacement of vertebral specimens between the 3 groups at different post-operative time points (2 weeks: 0.62 ± 0.10, 0.92 ± 0.22, 0.43 ± 0.09 mm, $F = 13.489$, $P = 0.001$; 4 weeks: 0.65 ± 0.17, 1.01 ± 0.16, 0.44 ± 0.08 mm, $F = 24.843$, $P = 0.000$; 8 weeks: 0.61 ± 0.12, 1.27 ± 0.23, 0.50 ± 0.11 mm, $F = 32.262$, $P = 0.000$; 12 weeks: 0.61 ± 0.15, 1.10 ± 0.10, 0.49 ± 0.13 mm, $F = 25.488$, $P = 0.000$; 16 weeks: 0.58 ± 0.19, 1.17 ± 0.16, 0.54 ± 0.10 mm, $F = 24.730$, $P = 0.000$; 24 weeks: 0.55 ± 0.17, 1.10 ± 0.28, 0.53 ± 0.15 mm, $F = 11.998$, $P = 0.001$), except at 48 weeks (0.54 ± 0.14, 0.83 ± 0.26, 0.54 ± 0.16 mm, $F = 3.744$, $P = 0.054$). Further pairwise comparison showed that the axial compressional displacement at each time point in group A and group C were all less than those of group B (group A vs group B: $P = 0.009$, $P = 0.001$, $P = 0.000$, $P = 0.000$, $P = 0.001$; group C vs group B: $P = 0.000$, $P = 0.001$), and there were no statistical differences in axial compressional displacement at each time point between group A and group C ($P = 0.062$, $P = 0.328$, $P = 0.208$, $P = 0.648$, $P = 0.894$) except at 4 weeks ($P = 0.038$). The three-point bend test showed that there were statistical differences in maximum load on vertebral specimens between the 3 groups at different post-operative time points (2 weeks: 178.0 ± 7.7, 130.3 ± 6.2, 232.0 ± 1.7 N, $F = 385.253$, $P = 0.000$; 4 weeks: 178.3 ± 4.4, 127.7 ± 7.1, 226.0 ± 5.4 N, $F = 371.286$, $P = 0.000$; 8 weeks: 182.4 ± 4.4, 131.8 ± 5.2, 221.0 ± 3.1 N, $F = 536.544$, $P = 0.000$; 12 weeks: 184.0 ± 0.8, 137.0 ± 6.6, 215.0 ± 3.2 N, $F = 422.579$, $P = 0.000$; 16 weeks: 182.9 ± 0.9, 140.2 ± 1.5, 217.0 ± 4.3 N, $F = 1006.122$, $P = 0.000$; 24 weeks: 189.0 ± 3.2, 140.6 ± 1.7, 194.0 ± 4.9 N, $F = 351.372$, $P = 0.000$; 48 weeks: 191.9 ± 3.9, 142.4 ± 2.1, 191.0 ± 8.1 N, $F = 139.682$, $P = 0.000$). Further pairwise comparison showed that the maximum load at each time point in group A and group C were all greater than those of group B (group A vs group B: $P = 0.000$, $P = 0.000$; group B vs group C: $P = 0.000$, $P = 0.000$), and the maximum load at each time point in group C were all greater than that of group A ($P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.000$) except at 24 and 48 weeks ($P = 0.054$, $P = 0.724$). The resist-torsion test showed that there were statistical differences in the torsional angle of vertebral specimens between the 3 groups at different post-operative time points (2 weeks: 3.8° ± 0.6°, 5.4° ± 0.5°, 2.4° ± 0.6°, $F = 37.977$, $P = 0.000$; 4 weeks: 4.0° ± 1.3°, 5.8° ± 1.6°, 2.4° ± 0.7°, $F = 9.408$, $P = 0.003$; 8 weeks: 3.7° ± 0.8°, 5.7° ± 0.4°, 2.3° ± 0.7°, $F = 32.229$, $P = 0.000$; 12 weeks: 3.5° ± 0.8°, 5.8° ± 0.4°, 2.4° ± 0.5°, $F = 38.685$, $P = 0.000$; 16 weeks: 3.5° ± 0.8°, 5.7° ± 0.4°, 2.4° ± 0.4°, $F = 41.931$, $P = 0.000$; 24 weeks: 3.4° ± 0.8°, 5.2° ± 1.2°, 2.5° ± 0.8°, $F = 10.072$, $P = 0.003$; 48 weeks: 3.0° ± 0.3°, 5.1° ± 0.4°, 2.7° ± 0.4°, $F = 53.166$, $P = 0.000$). Further pairwise comparison showed that the torsional angle at each time point in group A and group C were all less than those of group B (group A vs group B: $P = 0.001$, $P = 0.045$, $P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.012$, $P = 0.000$; group B vs group C: $P = 0.000$, $P = 0.001$, $P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.000$, $P = 0.001$, $P = 0.000$), and the torsional angle at each time point in group C were all less than those of group A ($P = 0.001$, $P = 0.008$, $P = 0.014$, $P = 0.017$) except at 4, 24 and 48 weeks ($P = 0.057$, $P = 0.171$, $P = 0.347$). A great number of chondrocytes and osteoblasts were found at the junction of PMMA III and host bone at 2 and 4 weeks after PVP, while no fibrous tissues were found. PMMA III were integrated tightly with the host bones 8 weeks after the surgery, and the cartilage tissues were replaced by the neogenetic osteoid. Meanwhile, the neogenetic bone tissues increased significantly and no fibrous tissues were found at the conjunctive area. At 12 and 16 weeks after the surgery, the fibrous tissues appeared and the PMMA III were integrated more tightly with the host bones. Meanwhile the osteoid decreased and the mineralization bony callus increased, and the woven bone was replaced by the lamellar bone. At 24 and 48 weeks after

the surgery, a small number of osteoclasts and bone units appeared and the neogenetic bone tissues were integrated tightly with the PMMA III at most of the interfaces. **Conclusion:** After PVP, the solidified PMMA III can be osseointegrated with bone tissues, as a result, it can provide good short-term and long-term biomechanical property for the fractured vertebrae.

Key words Spinal fractures; Vertebroplasty; Biomechanics; Histology; Animal experimentation

经皮椎体成形术 (percutaneous vertebroplasty, PVP) 目前已成为微创治疗骨质疏松性椎体压缩骨折的主要手段, 该手术能增强骨折椎体强度和稳定性, 部分恢复椎体高度, 防止塌陷, 有效缓解患者的腰背疼痛。我们通过动物实验研究了 PVP 术后骨折椎体的生物力学强度, 观察了其组织形态变化, 以期研究 PVP 的疗效提供基本参数。

1 材料与仪器

1.1 实验动物 新西兰雌兔 105 只, 体质量 2.3 ~ 2.8 kg, 中位数 2.5 kg。购自浙江中医药大学, 实验动物合格证号: SYXK(浙)2008-0116。

1.2 实验试剂 聚甲基丙烯酸甲酯骨水泥 III (polymethylmethacrylate III, PMMA III) (天津市合成材料产业研究所), 地塞米松注射液 (天津金耀氨基酸有限公司), 甲苯胺蓝试剂 (美国 Sigma 公司)。

1.3 实验仪器 岛津材料力学实验机 (日本岛津公司), EXAKT310 硬组织切片机 (德国 EXAKT 公司), 光学显微镜 (上海光学仪器厂)。

2 方法

2.1 分组及骨质疏松造模 将 105 只新西兰雌兔随机分为 3 组, 每组 35 只。B 组和 C 组采用去势加地塞米松肌肉注射法^[1]进行骨质疏松造模, 并采用双能骨密度仪检测骨密度, 确定模型达到骨质疏松标准^[2]。

2.2 模拟 PVP 手术 按照赵刚等^[3-4]的方法在所有实验动物 L₄ 和 L₅ 椎体上通过手术造成骨缺损, 在 C 组实验动物形成骨缺损的椎体中模拟 PVP 手术注射调制好的 PMMA III, 单个椎体注射量约 0.6 mL。术后所有实验动物肌肉注射青霉素, 每次 100 万单位, 每天 1 次, 连续 7 d。

2.3 椎体生物力学强度测定和组织学观察 分别于模拟 PVP 手术完成后 2 周、4 周、8 周、12 周、16 周、24 周、48 周从各组中随机选取 5 只实验动物处死, 取出进行过手术的 2 个椎体进行实验。其中 C 组所有实验动物均在处死前 14 d、13 d、4 d 和 3 d 分别按 25 mg·kg⁻¹ 肌肉注射 20 mg·mL⁻¹ 盐酸四环素作荧光标记, 所取得的 2 个椎体标本分别进行生物力学强

度测定和组织学观察。

2.3.1 椎体生物力学强度测定 去除椎体上的所有附件仅保留椎体, 然后使用牙托粉将椎体两端托平, 用双层塑料袋密闭后冷藏在 -20 °C 条件下保存备用。室温下自然解冻后, 将腰椎标本置于岛津材料力学实验机上进行轴向压缩、三点弯曲及抗扭转力学测试。轴向压缩力学测试, 载荷 200 N、加载速度为 0.1 mm·min⁻¹; 三点弯曲力学测试, 跨距 15 mm、加载速度 3 mm·min⁻¹; 抗扭转力学测试, 载荷 50 N、扭矩 5 Nm。

2.3.2 椎体组织学观察 将新鲜椎体进行乙醇梯度脱水后, 用树脂包埋剂进行包埋, 待包埋块凝固后, 用 EXAKT310 硬组织切片机切片, 进行甲苯胺蓝染色后分别对骨、骨水泥和界面 3 部分的细胞进行组织学观察。

2.4 数据统计分析 采用 SPSS17.0 统计软件对所得数据进行统计分析, 3 组实验动物椎体标本 PVP 术后不同时间点轴向压缩位移、三点弯曲实验中最大载荷及抗扭转实验中扭转角度的比较采用单因素方差分析, 组间两两比较采用 *q* 检验, 检验水准 $\alpha = 0.05$ 。

3 结果

3.1 椎体生物力学强度测定结果

3.1.1 椎体轴向压缩实验结果 除 48 周外, 3 组实验动物椎体标本术后 2 周、4 周、8 周、12 周、16 周、24 周时的轴向压缩位移比较, 组间差异均有统计学意义。进一步两两比较, A 组和 C 组各时点轴向压缩位移均小于 B 组 ($P = 0.009, P = 0.001, P = 0.000, P = 0.000, P = 0.000, P = 0.001; P = 0.000, P = 0.000, P = 0.000, P = 0.001$); 除 4 周时外 ($P = 0.038$), A 组各时点轴向压缩位移与 C 组比较, 差异均无统计学意义 ($P = 0.062, P = 0.328, P = 0.208, P = 0.648, P = 0.894$)。见表 1。

3.1.2 椎体三点弯曲实验结果 3 组实验动物椎体标本术后 2 周、4 周、8 周、12 周、16 周、24 周和 48 周时的最大载荷比较, 组间差异均有统计学意义。进一步两两比较, A 组和 C 组各时点的最大载荷均大于 B

组 ($P = 0.000, P = 0.000$; $P = 0.000, P = 0.000$); 除 24 周和 48 周 ($P = 0.054, P = 0.724$), C 组各时点的最大载荷均大于 A 组 ($P = 0.000, P = 0.000, P = 0.000, P = 0.000, P = 0.000$)。见表 2。

3.1.3 椎体抗扭转实验结果 3 组实验动物椎体标本术后 2 周、4 周、8 周、12 周、16 周、24 周和 48 周时

的扭转角度比较,组间差异均有统计学意义。进一步两两比较,A 组和 C 组各时点的扭转角度均小于 B 组 ($P = 0.001, P = 0.045, P = 0.000, P = 0.000, P = 0.000, P = 0.012, P = 0.000$; $P = 0.000, P = 0.001, P = 0.000, P = 0.000, P = 0.001, P = 0.000$); 除 4 周、24 周和 48 周外 ($P = 0.057, P = 0.171, P = 0.347$), C 组各时点的扭转角度均小于 A 组 ($P = 0.001, P = 0.008, P = 0.014, P = 0.017$)。见表 3。

表 1 轴向压缩实验中 3 组椎体标本的轴向压缩位移 mm

组别	不同时间点椎体标本的轴向压缩位移						
	2 周	4 周	8 周	12 周	16 周	24 周	48 周
A 组	0.62 ± 0.10	0.65 ± 0.17	0.61 ± 0.12	0.61 ± 0.15	0.58 ± 0.19	0.55 ± 0.17	0.54 ± 0.14
B 组	0.92 ± 0.22	1.01 ± 0.16	1.27 ± 0.23	1.10 ± 0.10	1.17 ± 0.16	1.10 ± 0.28	0.83 ± 0.26
C 组	0.43 ± 0.09	0.44 ± 0.08	0.50 ± 0.11	0.49 ± 0.13	0.54 ± 0.10	0.53 ± 0.15	0.54 ± 0.16
F 值	13.489	24.843	32.262	25.488	24.730	11.998	3.744
P 值	0.001	0.000	0.000	0.000	0.000	0.001	0.054

表 2 三点弯曲实验中 3 组椎体标本的最大载荷 N

组别	不同时间点椎体标本的最大载荷						
	2 周	4 周	8 周	12 周	16 周	24 周	48 周
A 组	178.0 ± 7.7	178.3 ± 4.4	182.4 ± 4.4	184.0 ± 0.8	182.9 ± 0.9	189.0 ± 3.2	191.9 ± 3.9
B 组	130.3 ± 6.2	127.7 ± 7.1	131.8 ± 5.2	137.0 ± 6.6	140.2 ± 1.5	140.6 ± 1.7	142.4 ± 2.1
C 组	232.0 ± 1.7	226.0 ± 5.4	221.0 ± 3.1	215.0 ± 3.2	217.0 ± 4.3	194.0 ± 4.9	191.0 ± 8.1
F 值	385.253	371.286	536.544	422.579	1 006.122	351.372	139.682
P 值	0.000	0.000	0.000	0.000	0.000	0.000	0.000

表 3 抗扭转实验中 3 组椎体标本的扭转角度 °

组别	不同时间点椎体标本的扭转角度						
	2 周	4 周	8 周	12 周	16 周	24 周	48 周
A 组	3.8 ± 0.6	4.0 ± 1.3	3.7 ± 0.8	3.5 ± 0.8	3.5 ± 0.8	3.4 ± 0.8	3.0 ± 0.3
B 组	5.4 ± 0.5	5.8 ± 1.6	5.7 ± 0.4	5.8 ± 0.4	5.7 ± 0.4	5.2 ± 1.2	5.1 ± 0.4
C 组	2.4 ± 0.6	2.4 ± 0.7	2.3 ± 0.7	2.4 ± 0.5	2.4 ± 0.4	2.5 ± 0.8	2.7 ± 0.4
F 值	37.977	9.408	32.229	38.685	41.931	10.072	53.166
P 值	0.000	0.003	0.000	0.000	0.000	0.003	0.000

3.2 椎体组织学观察结果 PVP 术后 2 周和 4 周时,PMMA Ⅲ与宿主骨交界处有大量软骨细胞和成骨细胞,越靠近 PMMA Ⅲ边缘,软骨细胞越多,交界处未见纤维组织[图 1(1),图 1(2)]。8 周时 PMMA Ⅲ与宿主骨结合紧密,软骨组织被新生的类骨质替代,部分类骨质已钙化,形成矿化骨痂,新生骨组织明显增加,且密度逐步提高,排列不规则,与 PMMA Ⅲ边缘结合紧密,交界处未见纤维组织[图 1(3)]。12 周和 16 周时可见纤维组织,PMMA Ⅲ与宿主骨的结合较之前更加紧密,宿主骨与植入材料界面融为一体,界面处的类骨质减少,矿化骨痂增多,骨质钙化更成熟,排列紊乱的编织骨被排列规则的板层骨取代,骨组织改建

趋于成熟[图 1(4),图 1(5)]。24 周和 48 周时骨组织改建进一步成熟,可见少量破骨细胞和骨单位,大部分界面结合处新生骨组织与 PMMA Ⅲ结合紧密[图 1(6),图 1(7)]。

4 讨论

PVP 是目前临床治疗骨质疏松性椎体压缩骨折的主要手段^[5-6]。该手术通过微创方式向骨折椎体内注射骨水泥,注入椎体的骨水泥可沿骨小梁间隙分布至整个椎体,凝固后对椎体有明显的支撑作用,可有效缓解患者的腰背疼痛^[7],部分恢复病变椎体高度和纠正后凸畸形^[8]。PMMA 作为最常用的骨缺损修复的人工植入材料,具有组织相容性好、毒性小及价

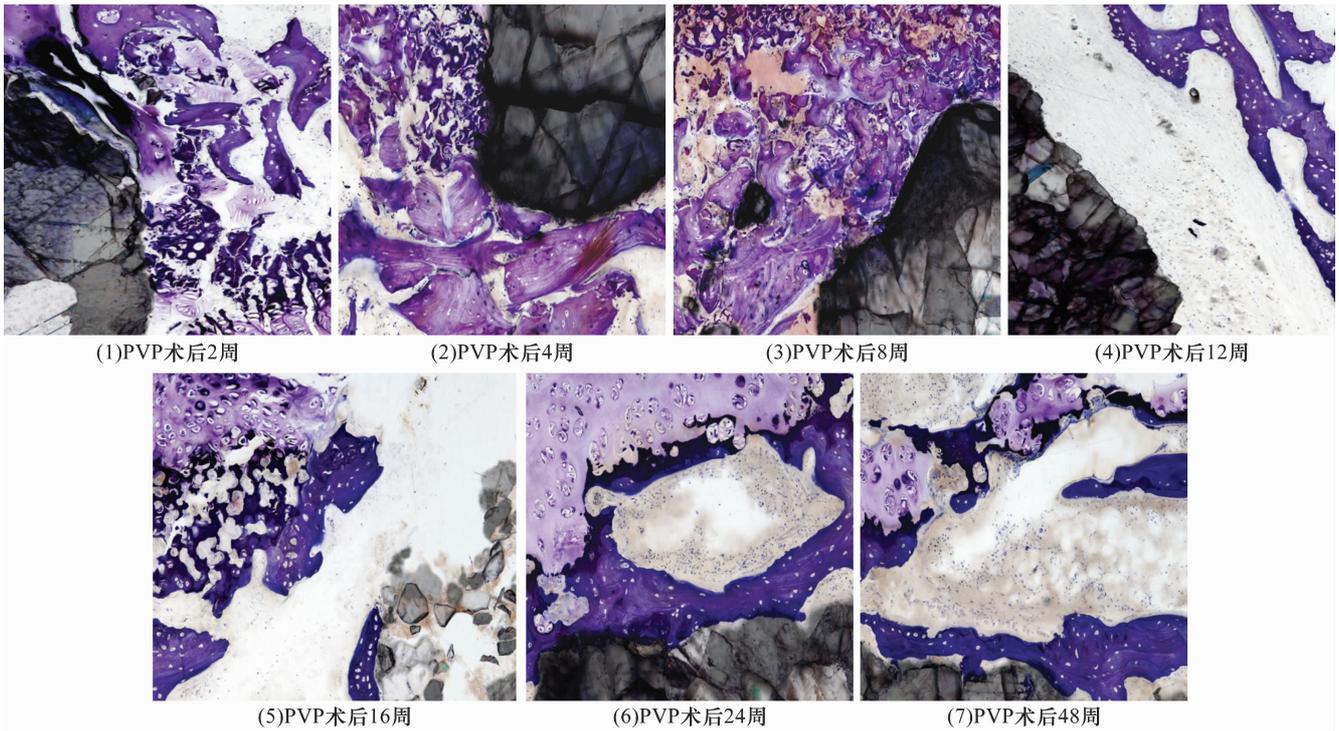


图 1 C 组 PVP 术后骨水泥与骨界面光镜观察结果(甲苯胺蓝染色 ×100)

格低等优势。

从本研究的结果可以看出,C 组椎体在轴向压缩实验中的轴向压缩位移(除 48 周外)和抗扭转实验中的扭转角度均小于 B 组,三点弯曲实验中的最大载荷大于 B 组;C 组椎体在轴向压缩实验中的轴向压缩位移(除 4 周时外)、24 周和 48 周时三点弯曲实验中的最大载荷及 4 周、24 周和 48 周时抗扭转实验中的扭转角度与 A 组相当;其余时点三点弯曲实验中的最大载荷大于 A 组,抗扭转实验中的扭转角度小于 A 组。同时,椎体组织学观察结果显示,注入 PMMA Ⅲ后,短期内在 PMMA Ⅲ和宿主骨的交界处就重新开始骨形成,界面结合紧密。这说明通过椎体内注射 PMMA Ⅲ能明显增强骨折椎体的力学性能。

PVP 术后早期即可显著提升骨折椎体的力学性能,这对于骨质疏松性椎体压缩骨折患者至关重要。由于这些患者多为老年人,常合并多种基础疾病,如果不能及时恢复骨折椎体高度和力学性能,长期卧床不仅会加重患者的骨质疏松,由此引起的并发症更是导致患者术后死亡的主要原因。从椎体组织学观察结果来看,虽然 C 组骨折椎体术后 24 周和 48 周时有破骨细胞出现,但生物力学指标仍与 A 组相当,优于 B 组。这说明 PVP 术后远期 PMMA Ⅲ仍能保证骨折椎体有较好的力学性能。

本研究的结果提示,PVP 术后凝固的 PMMA 能

与骨组织形成骨性结合,为骨折椎体提供较好的近期和远期生物力学性能。

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