血小板裂解液对膝骨关节炎模型大鼠疼痛和软骨损伤的影响及作用机制研究

凌晓宇[1],周康[1],姚黎佳[1],严莉[1],俞光平[2],童培建[1],单乐天[1],肖鲁伟[1]

(1.浙江中医药大学,浙江　杭州　310053;2.浙江省仙居县中医院,浙江　仙居　317300)

摘　要　目的:探讨血小板裂解液(platelet lysate,PL)对膝骨关节炎(knee osteoarthritis,KOA)模型大鼠疼痛和软骨损伤的影响及可能的作用机制。**方法:**取4只SD大鼠从其外周血中分离制备PL,并制成低(1×10^6个·mL^{-1})、中(1×10^7个·mL^{-1})、高(1×10^8个·mL^{-1})3种浓度的PL。取40只SD大鼠,随机分为空白组、模型组、PL低浓度组、PL中浓度组、PL高浓度组,每组8只。空白组不进行造模处理,其余4组大鼠通过向双侧膝关节腔内注射碘乙酸进行KOA造模。造模后第1天开始进行药物干预,PL低、中、高浓度组大鼠双侧膝关节腔分别注射50 μL低、中、高浓度PL,空白组和模型组大鼠双侧膝关节腔分别注射等量生理盐水,每周1次,共注射4次。分别于造模结束后第2周和第4周测定各组大鼠的压痛阈值和热痛阈值。痛阈测定结束后处死大鼠,取双侧膝关节进行组织病理学观察并评定Mankin's评分。另取5只SD大鼠,从大鼠关节软骨中分离获取软骨细胞进行体外培养,将培养的第3代软骨细胞分为空白组、模型组、PL低浓度组、PL中浓度组和PL高浓度组,空白组以含10% FBS的培养基进行培养,模型组以含10% FBS和碘乙酸的培养基进行培养,PL低、中、高浓度组分别以含10% FBS和低、中、高浓度PL的培养基进行培养,以CCK-8法测定细胞增殖情况。**结果:**①压痛阈值测定结果。造模结束后第2周时,5组大鼠的压痛阈值比较,差异有统计学意义[(385.04±116.23)g,(179.23±74.75)g,(257.60±70.97)g,(306.79±56.91)g,(352.13±67.03)g,$F=8.255$,$P=0.000$]。模型组的压痛阈值低于空白组、PL低浓度组、PL中浓度组和PL高浓度组($P=0.001$,$P=0.045$,$P=0.002$,$P=0.000$);PL高浓度组的压痛阈值高于PL低浓度组和PL中浓度组($P=0.000$,$P=0.001$);PL中浓度组的压痛阈值高于PL低浓度组($P=0.001$)。造模结束后第4周时,5组大鼠的压痛阈值比较,差异有统计学意义[(540.58±97.70)g,(352.81±54.41)g,(419.17±44.74)g,(460.43±63.73)g,(493.38±62.53)g,$F=9.137$,$P=0.000$]。模型组的压痛阈值低于空白组、PL低浓度组、PL中浓度组和PL高浓度组($P=0.000$,$P=0.018$,$P=0.003$,$P=0.000$);PL高浓度组的压痛阈值高于PL低浓度组和PL中浓度组($P=0.000$,$P=0.002$);PL中浓度组的压痛阈值高于PL低浓度组($P=0.002$)。②热痛阈值测定结果。造模结束后第2周时,5组大鼠的热痛阈值比较,差异有统计学意义[(8.35±2.17)s,(5.90±1.67)s,(6.77±1.08)s,(7.48±0.91)s,(8.24±1.65)s,$F=4.248$,$P=0.007$]。模型组的热痛阈值低于空白组、PL中浓度组和PL高浓度组($P=0.013$,$P=0.014$,$P=0.007$);模型组与PL低浓度组热痛阈值比较,差异无统计学意义($P=0.118$);PL高浓度组的热痛阈值高于PL低浓度组和PL中浓度组($P=0.000$,$P=0.024$);PL中浓度组的热痛阈值高于PL低浓度组($P=0.002$)。造模结束后第4周时,5组大鼠的热痛阈值比较,差异有统计学意义[(9.75±2.10)s,(6.78±1.46)s,(7.15±1.58)s,(7.91±1.35)s,(8.67±1.55)s,$F=4.310$,$P=0.006$]。模型组的热痛阈值低于空白组、PL中浓度组和PL高浓度组($P=0.005$,$P=0.009$,$P=0.002$);模型组与PL低浓度组热痛阈值比较,差异无统计学意义($P=0.634$);PL高浓度组的热痛阈值高于PL低浓度组和PL中浓度组($P=0.000$,$P=0.019$);PL中浓度组的热痛阈值高于PL低浓度组($P=0.025$)。③膝关节软骨病理学观察结果。5组大鼠膝关节软骨Mankin's评分比较,差异有统计学意义[(1.63±1.11)分,(9.29±1.03)分,(5.14±1.64)分,(3.14±1.73)分,(2.57±1.40)分,$F=37.299$,$P=0.000$]。模型组的Mankin's评分高于空白组、PL低浓度组、PL中浓度组、PL高浓度组($P=0.000$,$P=0.000$,$P=0.000$,$P=0.000$);PL高浓度组的Mankin's评分低于PL低浓度组($P=0.013$);PL中浓度组的Mankin's评分与PL高浓度组、PL低浓度组比较,差异均无统计学意义($P=0.541$,$P=0.062$)。④膝关节软骨细胞增殖测定结果。5组软骨细胞的吸光度比较,差异有统计学意义(0.71±0.06,0.46±0.01,0.58±0.02,0.66±0.11,0.69±0.01,$F=25.644$,$P=0.000$)。模型组的吸光度低于空白组、PL低浓度组、PL中浓度组和PL高浓度组($P=0.001$,$P=0.000$,$P=0.016$,$P=0.000$);PL高浓度组的吸光度高于PL低浓度组($P=0.000$);PL中浓度组的吸光度与PL高浓度组、PL低浓度组比较,差异均无统计学意义($P=0.639$,$P=0.204$)。**结论:**PL可提高KOA模型大鼠疼痛阈值,修复软骨损伤,且其作用效果与PL的剂量有关,其作用机制可能与PL能促进软骨细胞增殖有关。

关键词　骨关节炎,膝;血小板裂解液;疼痛;软骨;细胞增殖;大鼠;动物实验

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通讯作者:童培建　E-mail:tongpeijian@163.com

Effect of platelet lysate on pain and cartilage injury in knee osteoarthritis rat models and its mechanism of action: an experimental study

LING Xiaoyu[1], ZHOU Kang[1], YAO Lijia[1], YAN Li[1], YU Guangping[2], TONG Peijian[1], SHAN Letian[1], XIAO Luwei[1]

1. Zhejiang University of Traditional Chinese Medicine, Hangzhou 310053, Zhejiang, China

2. Traditional Chinese Medical Hospital of Xianju county, Taizhou 317300, Zhejiang, China

ABSTRACT **Objective**: To explore the effect of platelet lysate (PL) on pain and cartilage injury in knee osteoarthritis (KOA) rat models and its mechanism of action. **Methods**: Four SD rats were selected and PL was isolated from their peripheral blood. The PL with 3 different concentration (1×10^6/mL, 1×10^7/mL and 1×10^8/mL) were obtained. Another 40 SD rats were selected and were randomly divided into blank group, model group, PL low-concentration group, PL middle-concentration group and PL high-concentration group, 8 cases in each group. The KOA models were created in rats of model group, PL low-concentration group, PL middle-concentration group and PL high-concentration group by intra-articular injecting iodoacetic acid into bilateral knees, while the rats in blank group were not given any surgical intervention. At the 1st day after the end of modeling, drug intervention were performed on rats in PL low-concentration group, PL middle-concentration group and PL high-concentration group by injecting low-, middle- and high-concentration PL with dose of 50 μL into bilateral knees respectively, while the rats in the other two groups were administrated with intra-articular injection of the same dose of normal saline into bilateral knees respectively, once a week for consecutive 4 times. The pressure pain threshold (PPT) value and heat pain threshold value were detected in each group at 2 and 4 weeks after the end of modeling. After the end of pain threshold measurement, all rats were executed and their bilateral knee joints were fetched out for histopathological observation and the Mankin's scores were evaluated. Another five SD rats were selected and executed, and their knee articular cartilages were fetched out for separating chondrocytes. The third-generation chondrocytes of SD rats cultured in vitro were divided into blank group, model group, PL low-concentration group, PL middle-concentration group and PL high-concentration group. The chondrocytes in blank group and model group were cultured in medium containing 10% FBS and medium containing 10% FBS and iodoacetic acid respectively, and the chondrocytes in PL low-concentration group, PL middle-concentration group and PL high-concentration group were cultured in medium containing 10% FBS and PL with low, middle and high concentration respectively. The cell proliferation were measured by using CCK-8 method. **Results**: At the 2nd week after the end of modeling, there was statistical difference in PPT values between the 5 groups (385.04 +/- 116.23, 179.23 +/- 74.75, 257.60 +/- 70.97, 306.79 +/- 56.91, 352.13 +/- 67.03 g, $F = 8.255$, $P = 0.000$). The PPT value was lower in model group compared to blank group, PL low-concentration group, PL middle-concentration group and PL high-concentration group ($P = 0.001$, $P = 0.045$, $P = 0.002$, $P = 0.000$), and was higher in PL high-concentration group compared to PL low-concentration group and PL middle-concentration group ($P = 0.000$, $P = 0.001$), and was higher in PL middle-concentration group compared to PL low-concentration group ($P = 0.001$). At the 4th week after the end of modeling, there was statistical difference in PPT values between the 5 groups (540.58 +/- 97.70, 352.81 +/- 54.41, 419.17 +/- 44.74, 460.43 +/- 63.73, 493.38 +/- 62.53 g, $F = 9.137$, $P = 0.000$). The PPT value was lower in model group compared to blank group, PL low-concentration group, PL middle-concentration group and PL high-concentration group ($P = 0.000$, $P = 0.018$, $P = 0.003$, $P = 0.000$), and was higher in PL high-concentration group compared to PL low-concentration group and PL middle-concentration group ($P = 0.000$, $P = 0.002$), and was higher in PL middle-concentration group compared to PL low-concentration group ($P = 0.002$). At the 2nd week after the end of modeling, there was statistical difference in the heat pain threshold values between the 5 groups (8.35 +/- 2.17, 5.90 +/- 1.67, 6.77 +/- 1.08, 7.48 +/- 0.91, 8.24 +/- 1.65 s, $F = 4.248$, $P = 0.007$). The heat pain threshold value was lower in model group compared to blank group, PL middle-concentration group and PL high-concentration group ($P = 0.013$, $P = 0.014$, $P = 0.007$). There was no statistical difference in the heat pain threshold value between model group and PL low-concentration group ($P = 0.118$). The heat pain threshold value was higher in PL high-concentration group compared to PL low-concentration group and PL middle-concentration group ($P = 0.000$, $P = 0.024$), and was higher in PL middle-concentration group compared to PL low-concentration group ($P = 0.002$). At the 4th week after the end of modeling, there was statistical difference in the heat pain threshold values between the 5 groups (9.75 +/- 2.10, 6.78 +/- 1.46, 7.15 +/- 1.58, 7.91 +/- 1.35, 8.67 +/- 1.55 s, $F = 4.310$, $P = 0.006$). The heat pain threshold value was lower in model group compared to blank group, PL middle-concentration group and PL high-concentration group ($P = 0.005$, $P = 0.009$, $P = 0.002$). There was no statistical difference in the heat pain threshold value between model group and PL low-concentration group ($P = 0.634$). The heat pain threshold value was higher in PL high-concentration group compared to PL low-concentration group and PL middle-concentration group ($P = 0.000$, $P = 0.019$), and was higher in PL middle-concentration group compared to PL low-concentration group ($P = 0.025$). There was statistical

difference in Mankin's scores of knee articular cartilage between the 5 groups(1.63 +/- 1.11,9.29 +/- 1.03,5.14 +/- 1.64,3.14 +/- 1.73,2.57 +/- 1.40 points,F = 37.299,P = 0.000). The Mankin's score was higher in model group compared to blank group,PL low-concentration group,PL middle-concentration group and PL high-concentration group(P = 0.000,P = 0.000,P = 0.000,P = 0.000),and was lower in PL high-concentration group compared to PL low-concentration group(P = 0.013). There was no statistical difference in Mankin's score between PL middle-concentration group and PL high-concentration group and between PL middle-concentration group and PL low-concentration group(P = 0.541,P = 0.062). There was statistical difference in the absorbance of chondrocytes between the 5 groups(0.71 +/- 0.06,0.46 +/- 0.01,0.58 +/- 0.02,0.66 +/- 0.11,0.69 +/- 0.01,F = 25.644,P = 0.000). The absorbance was lower in model group compared to blank group,PL low-concentration group,PL middle-concentration group and PL high-concentration group(P = 0.001,P = 0.000,P = 0.016,P = 0.000),and was higher in PL high-concentration group compared to PL low-concentration group(P = 0.000). There was no statistical difference in the absorbance between PL middle-concentration group and PL high-concentration group and between PL middle-concentration group and PL low-concentration group(P = 0.639,P = 0.204). **Conclusion**:PL can increase pain threshold value and repair injuried articular cartilage dose - dependently in KOA rat models. The mechanism of action may be related to the promotion of chondrocyte proliferation.

Key words　osteoarthritis,knee;platelet lysate;pain;cartilage;cell proliferation;rats;animal experimentation

膝骨关节炎(knee osteoarthritis,KOA)是临床最常见的慢性退行性骨代谢疾病,也是最常见的老年病之一,与衰老、创伤等因素有关,是成人致残的主要原因[1]。KOA 的典型特征是软骨退变,包括软骨降解、关节滑膜纤维化、关节局部炎症、软骨下骨硬化、骨髓病变、骨赘形成等一系列的退行性病变,最终引起关节疼痛、僵硬、失用等行为功能障碍[2]。而 KOA 关节软骨损伤后无自我修复能力,因而治疗极为困难。

富血小板血浆(platelet-rich plasma,PRP)是全血经分离得到的自体血小板浓缩物,富含各类生长因子,具有促进软骨修复、抑制 KOA 软骨退变的作用[3-5],具有较高的临床应用价值[6-7]。PRP 含有多种生长因子,因存在较强的免疫原性,目前多用于自体移植或干预。血小板裂解液(platelet lysate,PL)是从外周血中制备的血小板浓缩物,作为 PRP 的衍生物,同样具有促进关节软骨修复的作用[8-9],且其修复软骨的生物特性与 PRP 相同[10]。本研究拟通过动物实验探讨 PL 对 KOA 病变中疼痛和软骨损伤的影响及可能的作用机制。

1　材料与仪器

1.1　实验动物　雄性 SPF 级 SD 大鼠 49 只,体质量(200 ± 20)g,购自上海斯莱克实验动物有限公司,实验动物合格证号:SCKK(沪)2007 - 0005。实验在浙江中医药大学动物实验中心进行,实验方案通过医学动物实验伦理委员会批准。

1.2　试剂及仪器　碘乙酸(SIGMA - ALDRICH),戊巴比妥钠、柠檬酸三钠、PBS 磷酸盐缓冲液(赛默飞世尔生物化学制品有限公司),IMDM 液体培养基、FBS、Ⅱ型胶原酶、青链霉素混合液、胰蛋白酶 - EDTA 消化液、CCK - 8、4% 甲醛溶液、EDTA 脱钙液(Solarbio);YLS - 3E 电子压痛仪(安徽省淮北正华生物仪器设备有限公司),37370 足底热辐射测痛仪(UGO BASILE),MDF - J281AT 超低温冰箱(SANYO),DK - S12型恒温水浴锅(上海森信实验仪器有限公司),SW - CJ - 1F 层流超净工作台(苏州安泰空气技术有限公司),AXiovert200 荧光倒置显微镜、全自动多功能酶标仪、CO_2 细胞培养箱(Thermo)。

2　方　法

2.1　PL 制备及质量控制　采用参照 Soffer 等[11]的方法从 SD 大鼠外周血中分离制备 PL。取 4 只 SD 大鼠,以 0.2 % 戊巴比妥钠麻醉,每只大鼠心内采血 7 ~ 8 mL,3.8% 柠檬酸三钠抗凝,室温下 150 r · min^{-1}离心 10 min(离心半径 8 cm)。取上清和血小板层细胞,室温下 1500 r · min^{-1}离心 10 min(离心半径 8 cm),获得血小板沉淀,用 2 mLPBS 缓冲液(pH7.2)重悬。将获取的血小板混悬液连续反复冻融(- 80 ℃/37 ℃)5 次,每次间隔约 10 min,于 4 ℃以 8000 r · min^{-1}离心 30 min(离心半径 8 cm),去除血小板膜和其他细胞残片,取上清液。将收集的 PL 再次离心重悬加工,制成低(1 × 10^6 个 · mL^{-1})、中(1 × 10^7 个 · mL^{-1})、高(1 × 10^8 个 · mL^{-1})3 种浓度的 PL。应用 ELISA 法检测 PL 上清液中细胞因子含量。

2.2　大鼠分组、造模及药物干预　取 40 只 SD 大鼠,随机分为空白组、模型组、PL 低浓度组、PL 中浓度

组、PL 高浓度组,每组 8 只。空白组不进行造模处理,其余 4 组大鼠通过向双侧膝关节腔内各注射 25 mg·mL^{-1}碘乙酸 50 μL 进行 KOA 造模。造模后第 1 天开始进行药物干预,PL 低、中、高浓度组大鼠双侧膝关节腔分别注射 50 μL 低、中、高浓度 PL,空白组和模型组大鼠双侧膝关节腔分别注射等量生理盐水,每周 1 次,共注射 4 次。

2.3 大鼠压痛阈值测定 分别于造模结束后第 2 周和第 4 周采用 YLS-3E 电子压痛仪测定各组大鼠的压痛阈值。测定时将大鼠装入固定桶内,使其处于舒适且被固定的状态,用压痛仪的扁型头对大鼠双侧后足背施压,当大鼠因疼痛出现鸣叫或挣扎时显示的压力值即为其压痛阈值。

2.4 大鼠热痛阈值测定 每次压痛阈值测定后 6 h,采用足底热痛敏测试仪测定各组大鼠双侧后足趾热痛阈值。测定时将大鼠置于透明有机玻璃箱内,室温(25±2)℃。待大鼠安静后(停止梳理毛发和探索性活动),将测试仪上的"十"字形标记置于大鼠左后足底中央并避开足垫。开启仪器,从开始至大鼠出现抬腿回避的时间即为大鼠的热痛阈值。每只大鼠测定 3 次,取平均值,每次间隔 5~6 min。为防止大鼠被热辐射烫伤,将热痛阈值测定的时间上限设定为 20 s,温度上限值设定为 35 ℃[12]。

2.5 大鼠膝关节软骨组织病理学观察 造模结束后第 4 周,最后一次热痛阈值测定结束后,断颈处死所有大鼠。取大鼠双侧膝关节,置于 4% 多聚甲醛中固定 48 h,EDTA 脱钙 4 周,乙醇逐级脱水,浸蜡包埋后在切片机上沿膝关节矢状面将胫骨外侧平台软骨下松质骨和股骨外侧髁软骨下松质骨沿下肢纵轴方向切片,厚度 5 μm,切片常规脱蜡至水,经自来水冲洗后进行番红染色、封片。光镜下观察软骨组织病理学改变,并按照 Mankin's 软骨组织学评分标准[13]进行评分。

2.6 大鼠膝关节软骨细胞分离培养及增殖测定 采用机械-Ⅱ型胶原酶消化法从 SD 大鼠关节软骨中分离获取软骨细胞[14]。另取 5 只 SD 大鼠,抽取腹主动脉血后脱颈处死,在 75% 酒精中浸泡 5 min 后取出膝关节,用手术刀剥离其软骨面,在超净工作台中用含双抗的 PBS 清洗,洗净后剪至 1 mm^3 大小,再用 PBS 清洗 3 次。将软骨组织装入玻璃试管中,先加入 5 倍组织块体积的 0.25% 胰蛋白酶,在 37 ℃震荡消化 30 min,再以 1500 r·min^{-1}离心 5 min(离心半径 8 cm)。弃上清液,加入 0.2% Ⅱ型胶原酶消化 4 h,加入含 10% FBS 的 IMDM 培养液终止消化,用 200 目筛网过滤。将滤液以 1500 r·min^{-1}离心 5 min(离心半径 8 cm),弃上清液,细胞沉淀加入含 10% FBS 的 IMDM 培养液重悬,接种在 6 孔培养板中,置于培养箱中在 37 ℃培养 24 h 后观察贴壁情况。2~3 d 换液 1 次,细胞贴壁长满后传代。

取培养的第 3 代软骨细胞,计数稀释后以每孔 2000~3000 个细胞的密度接种于 96 孔培养板中,分为空白组、模型组、PL 低浓度组、PL 中浓度组和 PL 高浓度组,每组设 5 个复孔。各组细胞均先以无血清 IMDM 培养基进行"饥饿"处理,然后空白组更换为含 10% FBS 的培养基,模型组更换为含 10% FBS 和碘乙酸的培养基,PL 低浓度组、PL 中浓度组和 PL 高浓度组分别更换为含 10% FBS 和低浓度 PL、中浓度 PL、高浓度 PL 的培养基。培养 48 h 后每孔避光加入 20 μL CCK-8 试剂,在培养箱内孵育 2 h 后用全自动酶标仪在 490 nm 波长测定吸光度。

2.7 数据统计分析 采用 DPS9.5 进行数据统计分析。5 组大鼠的压痛阈值、热痛阈值、Mankin's 评分、吸光度的组间总体比较均采用单因素方差分析,组间两两比较均采用 q 检验。检验水准 $\alpha=0.05$。

3 结 果

3.1 PL 细胞因子含量测定结果 经测定,制备的 PL 中主要含有血小板源性生长因子[(61.07±1.13)ng·mL^{-1}]、转化生长因子-β[(6.12±0.11)ng·mL^{-1}]、类胰岛素生长因子-1[(2.23±0.03)ng·mL^{-1}]、成纤维细胞生长因子-b[(7.15±0.55)ng·mL^{-1}]等细胞因子。

3.2 大鼠压痛阈值测定结果 造模结束后第 2 周时,5 组大鼠的压痛阈值比较,差异有统计学意义。模型组的压痛阈值低于空白组、PL 低浓度组、PL 中浓度组和 PL 高浓度组($P=0.001$,$P=0.045$,$P=0.002$,$P=0.000$);PL 高浓度组的压痛阈值高于 PL 低浓度组和 PL 中浓度组($P=0.000$,$P=0.001$);PL 中浓度组的压痛阈值高于 PL 低浓度组($P=0.001$)。见表 1。

造模结束后第 4 周时,5 组大鼠的压痛阈值比较,差异有统计学意义。模型组的压痛阈值低于空白组、PL 低浓度组、PL 中浓度组和 PL 高浓度组($P=0.000$,$P=0.018$,$P=0.003$,$P=0.000$);PL 高浓度组

的压痛阈值高于 PL 低浓度组和 PL 中浓度组($P=0.000$,$P=0.002$);PL 中浓度组的压痛阈值高于 PL 低浓度组($P=0.002$)。见表 1。

表 1　5 组大鼠压痛阈值测定结果　$\bar{x}\pm s$,g

组别	样本量(只)	造模结束后第 2 周	造模结束后第 4 周
空白组	8	385.04±116.23	540.58±97.70
模型组	8	179.23±74.75	352.81±54.41
PL 低浓度组	8	257.60±70.97	419.17±44.74
PL 中浓度组	8	306.79±56.91	460.43±63.73
PL 高浓度组	8	352.13±67.03	493.38±62.53
F 值		8.255	9.137
P 值		0.000	0.000

3.3　大鼠热痛阈值测定结果　造模结束后第 2 周时,5 组大鼠的热痛阈值比较,差异有统计学意义。模型组的热痛阈值低于空白组、PL 中浓度组和 PL 高浓度组($P=0.013$,$P=0.014$,$P=0.007$);模型组与 PL 低浓度组热痛阈值比较,差异无统计学意义($P=0.118$);PL 高浓度组的热痛阈值高于 PL 低浓度组和 PL 中浓度组($P=0.000$,$P=0.024$);PL 中浓度组的热痛阈值高于 PL 低浓度组($P=0.002$)。见表 2。

造模结束后第 4 周时,5 组大鼠的热痛阈值比较,差异有统计学意义。模型组的热痛阈值低于空白组、PL 中浓度组和 PL 高浓度组($P=0.005$,$P=0.009$,$P=0.002$);模型组与 PL 低浓度组热痛阈值比较,差异无统计学意义($P=0.634$);PL 高浓度组的热痛阈值高于 PL 低浓度组和 PL 中浓度组($P=0.000$,$P=0.019$);PL 中浓度组的热痛阈值高于 PL 低浓度组($P=0.025$)。见表 2。

表 2　5 组大鼠热痛阈值测定结果　$\bar{x}\pm s$,s

组别	样本量(只)	造模结束后第 2 周	造模结束后第 4 周
空白组	8	8.35±2.17	9.75±2.10
模型组	8	5.90±1.67	6.78±1.46
PL 低浓度组	8	6.77±1.08	7.15±1.58
PL 中浓度组	8	7.48±0.91	7.91±1.35
PL 高浓度组	8	8.24±1.65	8.67±1.55
F 值		4.248	4.310
P 值		0.007	0.006

3.4　大鼠膝关节软骨组织病理学观察结果　与空白组相比,模型组大鼠膝关节软骨表面有明显缺损,缺损处软骨细胞严重丢失,蛋白聚糖降解,软骨下骨呈现纤维化退变。PL 低、中、高浓度组大鼠膝关节软骨与模型组相比,软骨形态明显改善。PL 低浓度组仍可见软骨表面缺损、软骨细胞缺失和肥大化;PL 中浓度组中软骨细胞大量存活,但仍有表面缺损和细胞肥大化退变;PL 高浓度组软骨基本恢复正常,软骨面增厚,仅有少量肥大软骨细胞。见图 1。5 组大鼠膝关节软骨 Mankin′s 评分比较,差异有统计学意义[(1.63±1.11)分,(9.29±1.03)分,(5.14±1.64)分,(3.14±1.73)分,(2.57±1.40)分,$F=37.299$,$P=0.000$]。模型组的 Mankin′s 评分高于空白组、PL 低浓度组、PL 中浓度组、PL 高浓度组($P=0.000$,$P=0.000$,$P=0.000$,$P=0.000$);PL 高浓度组的 Mankin′s 评分低于 PL 低浓度组($P=0.013$);PL 中浓度组的 Mankin′s 评分与 PL 高浓度组、PL 低浓度组比较,差异均无统计学意义($P=0.541$,$P=0.062$)。

3.5　大鼠膝关节软骨细胞增殖测定结果　培养 48 h 后,5 组软骨细胞的吸光度比较,差异有统计学意义(0.71±0.06,0.46±0.01,0.58±0.02,0.66±0.11,0.69±0.01,$F=25.644$,$P=0.000$)。模型组的吸光度低于空白组、PL 低浓度组、PL 中浓度组和 PL 高浓度组($P=0.001$,$P=0.000$,$P=0.016$,$P=0.000$);PL 高浓度组的吸光度高于 PL 低浓度组($P=0.000$);PL 中浓度组的吸光度与 PL 高浓度组、PL 低浓度组比较,差异均无统计学意义($P=0.639$,$P=0.204$)。

4　讨　论

血小板被激活时,细胞内的 α 颗粒释放大量生长因子,包括血小板源性生长因子、转化生长因子 -β、类胰岛素生长因子 -1、成纤维细胞生长因子 -b 等[15-19]。其中,血小板源性生长因子可促进成骨细胞趋化、增殖,增加胶原蛋白的合成能力;转化生长因子 -β 可刺激成骨前体细胞及成骨细胞趋化和增殖,抑制破骨细胞形成和骨吸收;类胰岛素生长因子 -1 能增加成骨细胞活力、促进软骨与骨基质生成;成纤维细胞生长因子 -b 可以刺激成纤维细胞增殖,吸引多种细胞成分参与组织修复。Lee 等[20]对 26 例 OA 疼痛患者的冷、热、机械刺激的痛觉敏感性测试结果表明,OA 患者血液中的 IL-6 等致炎因子的上调与疼痛出现的敏感点呈正相关。而 T 转化生长因子 -β 等细胞生长因子可有效抑制肿瘤坏死因子 α、白细胞介素 6 等致炎因子的活性,因此可有效改善 OA 关节的疼痛,这些因子的作用使治疗 OA 有了新的方向[21-22]。

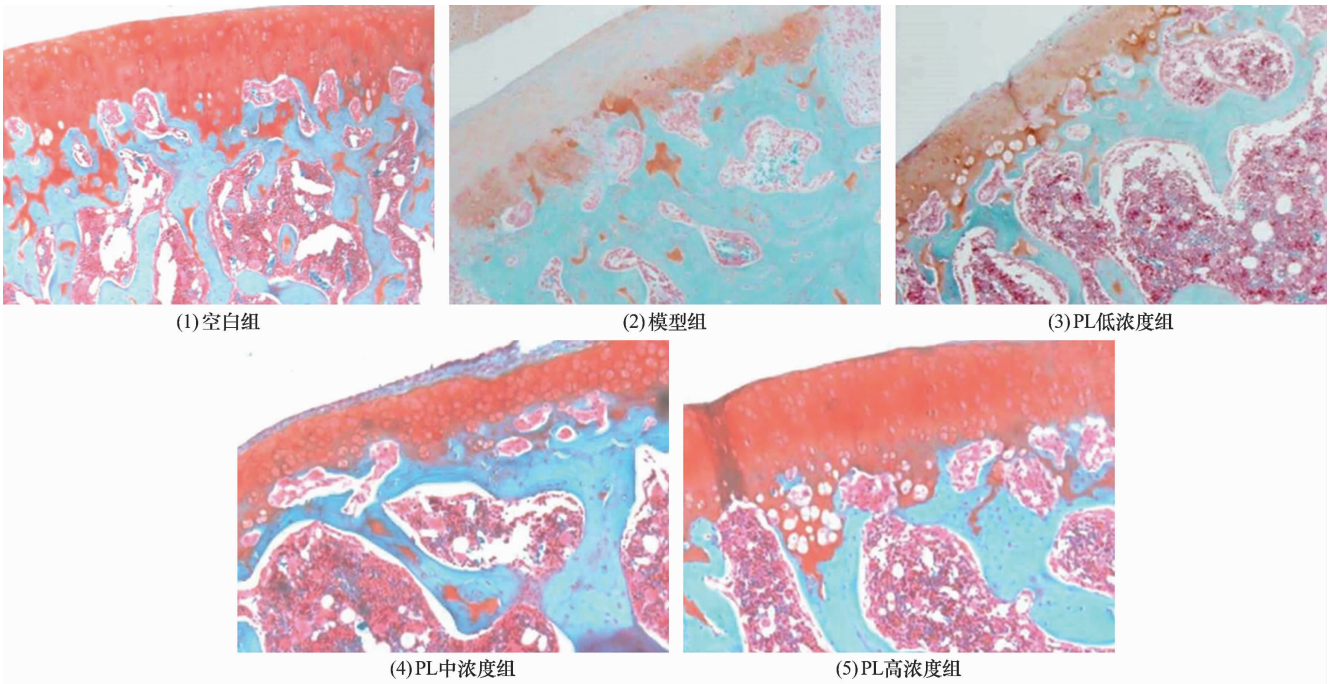

图 1　5 组大鼠膝关节软骨组织切片(番红染色　×100)

本研究中,压痛和热痛阈值的测定结果显示,造模结束后第 2 周、第 4 周 PL 低、中、高浓度组大鼠的疼痛阈值均高于模型组,且 PL 高浓度组的疼痛阈值最高,提示 PL 能提高 KOA 模型大鼠的疼痛阈值,且作用效果与剂量有关。造模结束后第 4 周时,PL 低、中、高浓度组的膝关节软骨 Mankin's 评分均低于模型组,PL 高浓度组和 PL 中浓度组的评分均较低,提示 PL 能修复 KOA 模型大鼠膝关节软骨损伤,作用效果与剂量有关。吸光度测定结果也显示,体外培养 48 h 后,PL 低、中、高浓度组测定的吸光度均高于模型组,PL 高浓度组和 PL 中浓度组的吸光度值均较高,提示 PL 能促进膝关节软骨细胞增殖,作用效果与剂量有关。

本研究的结果提示,PL 可提高 KOA 模型大鼠疼痛阈值,修复软骨损伤,且其作用效果与 PL 的剂量有关,其作用机制可能与 PL 能促进软骨细胞增殖有关。

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