·基础研究·

淫羊藿苷干预大鼠椎间盘源性腰痛的实验研究

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摘　要　目的:探讨淫羊藿苷(icariin,ICA)干预大鼠椎间盘源性腰痛的效果及可能的作用机制。**方法:**将 45 只 8 周龄 SPF 级雄性 SD 大鼠随机分为 5 组,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组各 10 只,塞来昔布组 5 只。生理盐水组、ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}及塞来昔布组大鼠通过 $L_{4\sim5}$和 $L_{5\sim6}$椎间盘穿刺建立大鼠腰痛模型;假手术组仅显露椎间盘,不进行穿刺。自造模后第 7 天开始进行药物干预,至造模后第 21 天结束。ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组以 ICA 溶液进行灌胃(ICA 溶于去离子水中),每天剂量分别为 50 mg·kg^{-1}和 100 mg·kg^{-1};塞来昔布组以塞来昔布胶囊进行灌胃(塞来昔布胶囊粉剂溶于去离子水中),每天 100 mg·kg^{-1};生理盐水组以生理盐水灌胃,每天 100 mg·kg^{-1};假手术组常规饲养,不进行干预。从各组随机选取 5 只大鼠,分别于造模前、造模后第 7 天、第 14 天、第 21 天、第 28 天、第 35 天进行足底机械性疼痛阈值检测。造模后第 35 天,足底疼痛阈值测定结束后处死假手术组、生理盐水组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组的 5 只大鼠,以 qRT-PCR 法测定椎间盘组织中 P 物质(substance P,SP)mRNA 和降钙素基因相关肽(calcitonin gene related peptide,CGRP)mRNA 的含量。造模后第 21 天时,处死假手术组、生理盐水组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组其余的 5 只大鼠,以 ELISA 法测定大鼠椎间盘组织中细胞因子诱导的中性粒细胞趋化剂-1(cytokine-induced neutrophil chemoattractant-1,CINC-1)含量。**结果:**①足底机械性疼痛阈值。时间因素和分组因素存在交互效应($F=17.971$,$P=0.001$)。5 组大鼠的足底机械性疼痛阈值总体比较,差异有统计学意义,即存在分组效应($F=146.660$,$P=0.000$)。造模前后不同时点间足底机械性疼痛阈值的差异有统计学意义,即存在时间效应($F=118.057$,$P=0.000$)。假手术组足底机械性疼痛阈值随时间未见明显变化,一直处于较高水平[(14.60±0.89)g,(14.79±0.47)g,(15.00±0.00)g,(15.00±0.00)g,(15.00±0.00)g,(15.00±0.00)g,$F=0.836$,$P=0.537$]。生理盐水组足底机械性疼痛阈值造模后明显降低,后期一直维持在较低水平[(14.67±0.74)g,(3.44±2.22)g,(3.19±1.37)g,(2.84±0.96)g,(3.92±1.36)g,(3.02±0.98)g,$F=58.882$,$P=0.000$]。ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组造模前后的足底机械性疼痛阈值变化趋势基本一致,造模后均先降低,药物干预开始后逐渐回升,药物干预结束后又逐渐降低[(15.00±0.00)g,(2.78±0.81)g,(4.64±1.67)g,(5.59±1.25)g,(8.52±1.63)g,(6.11±1.49)g,$F=56.088$,$P=0.000$;(14.66±0.76)g,(2.53±1.37)g,(10.65±2.69)g,(9.67±2.41)g,(10.67±2.04)g,(8.59±2.95)g,$F=16.684$,$P=0.000$;(15.00±0.00)g,(2.28±1.03)g,(12.09±1.28)g,(12.37±1.34)g,(6.71±2.89)g,(5.18±1.88)g,$F=44.668$,$P=0.000$]。造模后第 7 天时,ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值两两比较,组间差异均无统计学意义。造模后第 14 天时,ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值均高于 ICA 50 mg·kg^{-1}组($P=0.001$;$P=0.000$);ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值比较,差异无统计学意义。造模后第 21 天时,ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值均高于 ICA 50 mg·kg^{-1}组($P=0.009$;$P=0.000$);ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值比较,差异无统计学意义。造模后第 28 天时,ICA 100 mg·kg^{-1}组的足底机械性疼痛阈值高于塞来昔布组($P=0.049$);ICA 50 mg·kg^{-1}组与 ICA 100 mg·kg^{-1}组、塞来昔布组比较,组间差异均无统计学意义。造模后第 35 天时,ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值两两比较,组间差异均无统计学意义。②椎间盘组织 CINC-1 含量。造模后第 21 天,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组及 ICA 100 mg·kg^{-1}组大鼠椎间盘组织中 CINC-1 含量比较,差异有统计学意义[(534.2±142.6)pg·mL^{-1},(28 376.0±976.7)pg·mL^{-1},(21 866.0±1 536.0)pg·mL^{-1},(9 956.0±1 010.0)pg·mL^{-1},$F=423.000$,$P=0.000$]。生理盐水组的 CINC-1 含量高于假手术组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组($P=0.000$;$P=0.003$;$P=0.000$);ICA 50 mg·kg^{-1}组的 CINC-1 含量高于 ICA 100 mg·kg^{-1}组($P=0.000$)。③椎间盘组织 SP mRNA 和 CGRP mRNA 含量。造模后第 35 天,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组及 ICA 100 mg·kg^{-1}组大鼠椎间盘组织中 SP mRNA 和 CGRP mRNA 含量比较,组间差异均有统计学意义(1.04±0.49,183.50±118.60,55.50±10.26,19.53±8.05,$F=9.499$,$P=0.000$;0.74±0.21,6.29±2.06,1.55±0.69,1.19±0.37,$F=27.180$,$P=0.000$)。生理盐水组的 SP mRNA 含量高于假手术组、ICA 50 mg·kg^{-1}组

基金项目:河南省中医药科学研究专项课题(2019ZYZD02)

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和 ICA 100 mg·kg^{-1}组($P=0.008$;$P=0.042$;$P=0.015$);ICA 50 mg·kg^{-1}组的 SP mRNA 含量高于 ICA 100 mg·kg^{-1}组($P=0.000$)。生理盐水组的 CGRP mRNA 含量高于假手术组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组($P=0.000$;$P=0.001$;$P=0.000$);ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组的 CGRP mRNA 含量比较,差异无统计学意义。**结论**:ICA 可有效缓解大鼠椎间盘源性腰痛,其镇痛效果与剂量有关,与同剂量的塞来昔布相比其镇痛效果持续时间更长,降低大鼠椎间盘组织中 CINC-1 的水平可能是其镇痛的作用机制之一。

关键词　腰痛;椎间盘退行性变;淫羊藿甙;大鼠,Sprague-Dawley;动物实验

An experimental study of icariin for intervention of discogenic low back pain in rats

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ABSTRACT　**Objective**: To explore the effects of icariin (ICA) on discogenic low back pain (DLBP) in rats and its possible mechanism of action. **Methods**: Forty-five 8-week-old SPF-grade male SD rats were selected and were randomly divided into sham-operated group (10), normal saline group (10), ICA 50 mg/kg group (10), ICA 100 mg/kg group (10) and celecoxib group (5). The lumbago models were built in rats of normal saline group, ICA 50 mg/kg group, ICA 100 mg/kg group and celecoxib group by puncturing L_4/L_5 and L_5/L_6 intervertebral disc, while the surgeries were performed on rats in sham-operated group to expose their intervertebral discs and no intervertebral disc puncture was performed. The drug intervention were performed on rats from day 7 to day 21 after modeling. The rats in ICA 50 mg/kg group were intragastric administrated with ICA solution (ICA was dissolved into deionized water) in daily dosages of 50 mg/kg. The rats in ICA 100 mg/kg group, celecoxib group and normal saline group were intragastric administrated with ICA solution, celecoxib solution (celecoxib capsule powders were dissolved into deionized water) and normal saline respectively in daily dosage of 100 mg/kg, while the rats in sham-operated group were bred normally and were not given any drug interventions. Five rats were randomly selected out from each group, and plantar mechanical pain threshold values were measured before modeling and at the 7th, 14th, 21st, 28th and 35th day after the modeling respectively. At the 35th day after the modeling, the five rats in sham-operated group, normal saline group, ICA 50 mg/kg group and ICA 100 mg/kg group were executed after the last measurement of pelma pain threshold value and the contents of substance P (SP) mRNA and calcitonin gene related peptide (CGRP) mRNA in intervertebral disc tissues were measured by using qRT-PCR assays. At the 21st day after the modeling, the other 5 rats in sham-operated group, normal saline group, ICA 50 mg/kg group and ICA 100 mg/kg group were executed, and the content of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in intervertebral disc tissues of rats were measured by using ELISA. **Results**: There was interaction between time factor and group factor in plantar mechanical pain threshold values ($F=17.971$, $P=0.001$). There was statistical difference in plantar mechanical pain threshold values between the 5 groups in general, in other words, there was group effect ($F=146.660$, $P=0.000$). There was statistical difference in plantar mechanical pain threshold values between different timepoints before and after the modeling, in other words, there was time effect ($F=118.057$, $P=0.000$). No significant time-dependent change of plantar mechanical pain threshold values were found in sham-operated group, and the pain threshold values remained at a high level (14.60 +/- 0.89, 14.79 +/- 0.47, 15.00 +/- 0.00, 15.00 +/- 0.00, 15.00 +/- 0.00, 15.00 +/- 0.00 g, $F=0.836$, $P=0.537$). The plantar mechanical pain threshold values decreased significantly in normal saline group after modeling, and then remained at a low level (14.67 +/- 0.74, 3.44 +/- 2.22, 3.19 +/- 1.37, 2.84 +/- 0.96, 3.92 +/- 1.36, 3.02 +/- 0.98 g, $F=58.882$, $P=0.000$). The plantar mechanical pain threshold values were basically consistent with each other in the variation tendency in ICA 50 mg/kg group, ICA 100 mg/kg group and celecoxib group before and after the modeling, and they all decreased firstly after the modeling and increased gradually after the beginning of drug intervention and then decreased gradually after the end of drug intervention (15.00 +/- 0.00, 2.78 +/- 0.81, 4.64 +/- 1.67, 5.59 +/- 1.25, 8.52 +/- 1.63, 6.11 +/- 1.49 g, $F=56.088$, $P=0.000$; 14.66 +/- 0.76, 2.53 +/- 1.37, 10.65 +/- 2.69, 9.67 +/- 2.41, 10.67 +/- 2.04, 8.59 +/- 2.95 g, $F=16.684$, $P=0.000$; 15.00 +/- 0.00, 2.28 +/- 1.03, 12.09 +/- 1.28, 12.37 +/- 1.34, 6.71 +/- 2.89, 5.18 +/- 1.88 g, $F=44.668$, $P=0.000$). At the 7th day after the modeling, further pairwise comparison showed that there was no statistical difference in the plantar mechanical pain threshold values between ICA 50 mg/kg group, ICA 100 mg/kg group and celecoxib group. At the 14th day after the modeling, the plantar mechanical pain threshold values were higher in ICA 100 mg/kg group and celecoxib group compared to ICA 50 mg/kg group ($P=0.001$;$P=0.000$), and there was no statistical difference between ICA 100 mg/kg group and celecoxib group. At the 21st day after the modeling, the plantar mechanical pain threshold values were higher in ICA 100 mg/kg group and celecoxib group compared to ICA 50 mg/kg group ($P=0.009$;$P=0.000$), and there was no statistical

difference between ICA 100 mg/kg group and celecoxib group. At the 28th day after the modeling, the plantar mechanical pain threshold values were higher in ICA 100 mg/kg group compared to celecoxib group($P=0.049$), and there was no statistical difference between ICA 50 mg/kg group and ICA 100 mg/kg group and between ICA 50 mg/kg group and celecoxib group. At the 35th day after the modeling, further pairwise comparison showed that there was no statistical difference in the plantar mechanical pain threshold values between ICA 50 mg/kg group, ICA 100 mg/kg group and celecoxib group. At the 21st day after the modeling, there was statistical difference in the content of CINC-1 in intervertebral disc tissues of rats between sham-operated group, normal saline group, ICA 50 mg/kg group and ICA 100 mg/kg group(534.2 +/- 142.6, 28 376.0 +/- 976.7, 21 866.0 +/- 1 536.0, 9 956.0 +/- 1 010.0 pg/mL, $F=423.000$, $P=0.000$). The content of CINC-1 was higher in normal saline group compared to sham-operated group, ICA 50 mg/kg group and ICA 100 mg/kg group($P=0.000$; $P=0.003$; $P=0.000$), and was higher in ICA 50 mg/kg group compared to ICA 100 mg/kg group($P=0.000$). At the 35th day after the modeling, there was statistical difference in the contents of SP mRNA and CGRP mRNA in intervertebral disc tissues of rats between sham-operated group, normal saline group, ICA 50 mg/kg group and ICA 100 mg/kg group(1.04 +/- 0.49, 183.50 +/- 118.60, 55.50 +/- 10.26, 19.53 +/- 8.05, $F=9.499$, $P=0.000$; 0.74 +/- 0.21, 6.29 +/- 2.06, 1.55 +/- 0.69, 1.19 +/- 0.37, $F=27.180$, $P=0.000$). The content of SP mRNA in intervertebral disc tissues was higher in normal saline group compared to sham-operated group, ICA 50 mg/kg group and ICA 100 mg/kg group($P=0.008$; $P=0.042$; $P=0.015$), and was higher in ICA 50 mg/kg group compared to ICA 100 mg/kg group($P=0.000$). The content of CGRP mRNA in intervertebral disc tissues was higher in normal saline group compared to sham-operated group, ICA 50 mg/kg group and ICA 100 mg/kg group($P=0.000$; $P=0.001$; $P=0.000$), and there was no statistical difference between ICA 50 mg/kg group and ICA 100 mg/kg group. **Conclusion**: ICA can effectively relieve DLBP in rats, and its analgesic effect is associated with the dose and lasted longer than that of same dose of celecoxib. It can decrease the level of CINC-1 in intervertebral disc tissues, which may be one of its analgesic mechanisms.

Keywords low back pain; intervertebral disc degeneration; icariin; rats, Sprague-Dawley; animal experimentation

流行病学调查表明,18.3%的成年人曾有过腰痛病史,而最近1个月内有过腰痛的患者比例高达30.8%[1]。与椎间盘退变密切相关的腰痛和颈痛已成为一个非常严峻的公共卫生问题,严重影响患者的生活质量和身心健康[2-3]。虽然非甾体类抗炎药或吗啡类镇痛药能缓解腰痛,但存在的一系列不良反应使其无法成为腰痛治疗的最佳药物,因而寻找新的腰痛治疗药物十分必要[4-6]。

中医学理论认为,腰腿痛的发生与肝肾不足有关[7-8]。淫羊藿主归肾经,性温,在治疗骨质疏松、腰腿痛和椎间盘疾病方面具有良好的效果[9-11]。淫羊藿苷(icariin,ICA)是中药淫羊藿的主要成分,来源于淫羊藿的叶和茎,已被证明能缓解一系列椎间盘相关疾病[11-13]。为进一步探讨ICA对椎间盘源性腰痛的治疗效果及可能的作用机制,我们应用大鼠椎间盘源性腰痛模型进行了实验研究,现总结报告如下。

1 材料与仪器

1.1 实验动物 8周龄SPF级雄性SD大鼠45只,体质量250~300 g,购买自上海吉辉实验动物饲养有限公司,实验动物许可证号:SCXK(沪)2017-0012。所有大鼠在实验开始前适应性饲养1周,饲养环境维持恒温恒湿,每笼2只。实验方案通过医学动物实验伦理委员会批准。

1.2 实验药物与试剂 ICA(纯度≥94%,Sigma-Aldrich公司,批准文号:I1286),塞来昔布胶囊(每粒200 mg,辉瑞制药有限公司,国药准字J20140072),RNA Trizol提取试剂(Invitrogen公司,批准文号:15596018),RNA逆转录酶(TakaRa公司,批准文号:639522),RNA引物探针试剂盒(TakaRa公司,批准文号:RR420Q),细胞因子诱导的中性粒细胞趋化剂-1(cytokine-induced neutrophil chemoattractant-1,CINC-1)ELISA试剂盒(R&D Systems公司,批准文号:RCN100),BCA总蛋白检测试剂盒(上海碧云天生物技术有限公司,批准文号:P0010),2.5%戊巴比妥钠(CHEMMART公司,批准文号:096956-001)。

1.3 实验仪器 Von Frey Filament足底机械性疼痛阈值检测系统(Stoelting公司),ABI 7500核酸序列检测仪(Applied Biosystems公司)。

2 方 法

2.1 动物分组及造模 将45只大鼠分为5组,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组各10只,塞来昔布组5只。按2 mL·kg^{-1}用2.5%戊巴比妥钠对所有大鼠进行麻醉后,经腹部正中切口暴露$L_{4\sim5}$和$L_{5\sim6}$椎间盘,除假手术组外均利用18号

针头垂直穿刺髓核建立大鼠腰痛模型[14],穿刺深度根据术前 X 线片确定。术前术后所有动物均不使用抗生素和实验用药以外的其他镇痛药。

2.2　药物干预　自造模后第 7 天开始进行药物干预,至造模后第 21 天结束。ICA 50 mg · kg^{-1}组和 ICA 100 mg · kg^{-1}组以 ICA 进行灌胃(ICA 溶于去离子水中),每天的剂量分别为 50 mg · kg^{-1}和 100 mg · kg^{-1};塞来昔布组以塞来昔布胶囊进行灌胃(塞来昔布胶囊粉剂溶于去离子水中),每天 100 mg · kg^{-1};生理盐水组以生理盐水灌胃,每天 100 mg · kg^{-1};假手术组常规饲养,不进行干预。

2.3　实验指标检测

2.3.1　足底机械性疼痛阈值　从各组随机选取 5 只大鼠,分别于造模前、造模后第 7 天、第 14 天、第 21 天、第 28 天、第 35 天进行足底机械性疼痛阈值检测。检测时将大鼠放置于测试箱内,用 Von Frey Filament 探针通过铁丝网的孔洞垂直作用于大鼠脚掌后 1/3 至 1/2 处,待探针完全弯曲后继续压迫 2 ~ 3 s,观察在该直径探针刺激下大鼠的下肢反应。若大鼠出现下肢快速回缩伴或不伴舔、咬脚掌等快速收缩动作判定为阳性反应,未出现上述动作则判定为阴性反应。若大鼠在该直径探针下出现阳性反应,则选择直径小一号的探针继续测试;若出现阴性反应,则选择直径大一号的探针继续测试。每 2 次测试间隔时间≥1 min,以消除上次探针刺激的影响。双侧后足分开测试,每侧均测试 6 次,取平均值作为最终结果。

2.3.2　椎间盘组织 CINC - 1 含量　造模后第 21 天时,过量注射 2.5% 戊巴比妥钠(4 mL · kg^{-1})处死假手术组、生理盐水组、ICA 50 mg · kg^{-1}组和 ICA 100 mg · kg^{-1}组的另外 5 只大鼠。收集大鼠 $L_{4\sim5}$和 $L_{5\sim6}$椎间盘组织,液氮速冻并加入 0.01 mol · L^{-1}的 PBS 匀浆,于 4 ℃以 10 000 r · min^{-1}离心 15 min(离心半径10 cm),收集上清液。上清液继续在4 ℃以 10 000 r · min^{-1}离心 5 min(离心半径 10 cm)后再次收集上清液。使用 CINC - 1 ELISA 检测试剂盒测定 CINC - 1 的含量,最终结果为 CINC - 1 浓度除以总蛋白浓度。

2.3.3　椎间盘组织 P 物质 mRNA 和降钙素基因相关肽 mRNA 含量　造模后第 35 天,足底疼痛阈值测定结束后,过量注射 2.5% 戊巴比妥钠(4 mL · kg^{-1})处死假手术组、生理盐水组、ICA 50 mg · kg^{-1}组和 ICA 100 mg · kg^{-1}组用于检测足底机械性疼痛阈值的 5 只大鼠。获取大鼠 $L_{4\sim5}$和 $L_{5\sim6}$椎间盘组织,液氮速冻并加入 0.01 mol · L^{-1}的 PBS 匀浆,于 4 ℃以 10 000 r · min^{-1}离心 15 min(离心半径 10 cm),收集上清液。用 ABI 7500 核酸序列检测仪,以 qRT - PCR 法测定椎间盘组织中 P 物质(substance P,SP)mRNA 和降钙素基因相关肽(calcitonin gene related peptide,CGRP)mRNA 含量。循环条件为:95 ℃变性 5 s,60 ℃扩增 24 s,40 个循环。以甘油醛 - 3 - 磷酸脱氢酶(glyceraldehyde - 3 - phosphate dehydrogenase,GAPDH)为内参基因,引物由生工生物工程(上海)股份有限公司合成,引物序列见表 1。目标基因的 mRNA 表达量采用 $2^{-\Delta\Delta C_T}$法与 GAPDH 的表达水平进行校正。

2.4　数据统计　采用 GraphPad prism 6.0 软件进行数据统计分析。各组大鼠足底机械性疼痛阈值的总体比较采用重复测量资料的方差分析,同一时点组间两两比较采用 Tukey 多重检验;各组大鼠椎间盘组织中 CINC - 1 含量、SP mRNA 含量及 CGRP mRNA 含量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 Tukey 多重检验。检验水准 $\alpha = 0.05$。

3　结　果

3.1　足底机械性疼痛阈值测定结果　时间因素和分组因素存在交互效应。5 组大鼠的足底机械性疼痛阈值总体比较,差异有统计学意义,即存在分组效应。造模前后不同时点间足底机械性疼痛阈值的差异有

表 1　qRT - PCR 检测所用引物序列

基因名称		引物序列
Rat GAPDH	forward	5' - ATGACTCTACCCACGGCAAG - 3'
	reverse	5' - TACTCAGCACCAGCATCACC - 3'
Rat SP	forward	5' - TGGTCAGATCTCTCACAAAGG - 3'
	reverse	5' - TGCATTGCGCTTCTTTCATA - 3'
Rat CGRP	forward	5' - TCTAGTGTCACTGCCCAGAAGAGA - 3'
	reverse	5' - GGCACAAAGTTGTCCTTCACCACA - 3'

GAPDH:甘油醛 - 3 - 磷酸脱氢酶;SP:P 物质;CGRP:降钙素基因相关肽

统计学意义,即存在时间效应。假手术组足底机械性疼痛阈值随时间未见明显变化,一直处于较高水平。生理盐水组足底机械性疼痛阈值造模后明显降低,后期一直维持在较低水平。ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组造模前后的足底机械性疼痛阈值变化趋势基本一致,造模后均先降低,药物干预开始后逐渐回升,药物干预结束后又逐渐降低。造模后第 7 天时,ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值两两比较,组间差异均无统计学意义(ICA 50 mg·kg^{-1}组与 ICA 100 mg·kg^{-1}组:P=0.979;ICA 50 mg·kg^{-1}组与塞来昔布组:P=0.868;ICA 100 mg·kg^{-1}组与塞来昔布组:P=0.981)。造模后第 14 天时,ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值均高于 ICA 50 mg·kg^{-1}组(P=0.001;P=0.000);ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值比较,差异无统计学意义(P=0.614)。造模后第 21 天时,ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值均高于 ICA 50 mg·kg^{-1}组(P=0.009;P=0.000);ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值比较,差异无统计学意义(P=0.091)。造模后第 28 天时,ICA 100 mg·kg^{-1}组的足底机械性疼痛阈值高于塞来昔布组(P=0.049);ICA 50 mg·kg^{-1}组与 ICA 100 mg·kg^{-1}组、塞来昔布组比较,组间差异均无统计学意义(P=0.401;P=0.539)。造模后第 35 天时,ICA 50 mg·kg^{-1}组、ICA 100 mg·kg^{-1}组和塞来昔布组的足底机械性疼痛阈值两两比较,组间差异均无统计学意义(ICA 50 mg·kg^{-1}组与 ICA 100 mg·kg^{-1}组:P=0.269;ICA 50 mg·kg^{-1}组与塞来昔布组:P=0.888;ICA 100 mg·kg^{-1}组与塞来昔布组:P=0.088)。见表 2、图 1。

表 2　5 组大鼠造模前后足底机械性疼痛阈值

组别	样本量（只）	足底机械性疼痛阈值($\bar{x}\pm s$,g)							F 值	P 值
		造模前	造模后第 7 天	造模后第 14 天	造模后第 21 天	造模后第 28 天	造模后第 35 天	合计		
假手术组	5	14.60±0.89	14.79±0.47	15.00±0.00	15.00±0.00	15.00±0.00	15.00±0.00	14.90±0.40	0.836	0.537
生理盐水组	5	14.67±0.74	3.44±2.22	3.19±1.37	2.84±0.96	3.92±1.36	3.02±0.98	5.18±4.50	58.882	0.000
ICA 50 mg·kg^{-1}组	5	15.00±0.00	2.78±0.81	4.64±1.67	5.59±1.25	8.52±1.63	6.11±1.49	7.11±4.16	56.088	0.000
ICA 100 mg·kg^{-1}组	5	14.66±0.76	2.53±1.37	10.65±2.69	9.67±2.41	10.67±2.04	8.59±2.95	9.46±4.18	16.684	0.000
塞来昔布组	5	15.00±0.00	2.28±1.03	12.09±1.28	12.37±1.34	6.71±2.89	5.18±1.88	8.94±4.82	44.668	0.000
合计	25	14.79±0.59	5.16±5.07	9.11±4.83	9.09±4.69	8.97±4.17	7.58±4.50	9.12±5.10	118.057[1)]	0.000[1)]
F 值		0.505	83.415	46.622	60.347	25.715	34.415	146.660[1)]	F=17.791[2)],	
P 值		0.732	0.000	0.000	0.000	0.000	0.000	0.000[1)]	P=0.001[2)]	

1)主效应的 F 值和 P 值;2)交互效应的 F 值和 P 值

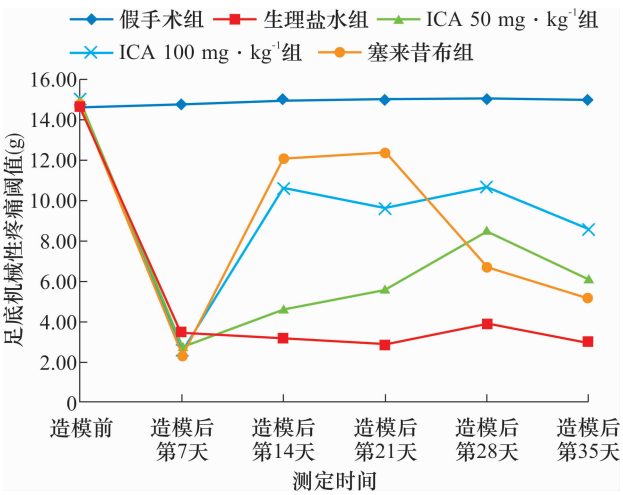

图 1　5 组大鼠造模前后足底机械性疼痛阈值变化趋势图

3.2　椎间盘组织 CINC－1 含量测定结果　造模后第21 天,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组及 ICA 100 mg·kg^{-1}组大鼠椎间盘组织中 CINC－1 含量比较,差异有统计学意义[(534.2±142.6)pg·mL^{-1},(28 376.0±976.7)pg·mL^{-1},(21 866.0±1 536.0)pg·mL^{-1},(9 956.0±1 010.0)pg·mL^{-1},F=423.000,P=0.000]。生理盐水组的 CINC－1 含量高于假手术组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组(P=0.000;P=0.003;P=0.000);ICA 50 mg·kg^{-1}组的 CINC－1 含量高于 ICA 100 mg·kg^{-1}组(P=0.000)。

3.3　椎间盘组织 SP mRNA 和 CGRP mRNA 含量测定结果　造模后第 35 天,假手术组、生理盐水组、ICA 50 mg·kg^{-1}组及 ICA 100 mg·kg^{-1}组大鼠椎间

盘组织中 SP mRNA 和 CGRP mRNA 含量比较,组间差异均有统计学意义。生理盐水组的 SP mRNA 含量高于假手术组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组($P=0.008$;$P=0.042$;$P=0.015$);ICA 50 mg·kg^{-1}组的 SP mRNA 含量高于 ICA 100 mg·kg^{-1}组($P=0.000$)。生理盐水组的 CGRP mRNA 含量高于假手术组、ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组($P=0.000$;$P=0.001$;$P=0.000$);ICA 50 mg·kg^{-1}组和 ICA 100 mg·kg^{-1}组的 CGRP mRNA 含量比较,差异无统计学意义($P=0.334$)。见表 3。

表 3　4 组大鼠椎间盘组织中 P 物质 mRNA 和降钙素基因相关肽 mRNA 含量

组别	样本量(只)	P 物质 mRNA($\bar{x}\pm s$)	降钙素基因相关肽 mRNA($\bar{x}\pm s$)
假手术组	5	1.04±0.49	0.74±0.21
生理盐水组	5	183.50±118.60	6.29±2.06
ICA 50 mg·kg^{-1}组	5	55.50±10.26	1.55±0.69
ICA 100 mg·kg^{-1}组	5	19.53±8.05	1.19±0.37
F 值		9.499	27.180
P 值		0.000	0.000

4　讨　论

本研究根据既往文献[14]报道的方法,在大鼠 $L_{4\sim5}$ 和 $L_{5\sim6}$ 椎间盘用 18 号针头进行穿刺,从而建立了大鼠腰痛模型。穿刺能直接改变椎间盘的机械性能,导致纤维环损伤和细胞因子增加,最终导致椎间盘变性和椎间盘源性腰痛[15-17]。大鼠足底机械性疼痛阈值是量化腰痛的可靠方法[14,18]。非甾体类抗炎药虽然能够有效镇痛,但其镇痛持续时间较短。在本研究中,塞来昔布组在药物干预结束后疼痛阈值快速降低,也证实了这一点。对于大鼠足底机械性疼痛阈值的测定结果显示,ICA 每天按照 50 mg·kg^{-1}和 100 mg·kg^{-1}使用,均能提高大鼠疼痛阈值,而且 ICA 100 mg·kg^{-1}组镇痛效果更好。与同剂量的塞来昔布相比,ICA 具有相似的镇痛效果,而且镇痛效果更持久。

SP 和 CGRP 是两种与疼痛直接相关的肽,可作为疼痛的"生物标记物",与足底机械性痛觉阈值这种行为学指标相结合,可全面反映大鼠的腰痛程度[14,19-20]。实验结果显示,ICA 可有效降低大鼠椎间盘组织中 SP mRNA 和 CGRP mRNA 的表达,ICA 100 mg·kg^{-1}组的效果更好,这与大鼠足底机械性痛觉阈值测定结果一致。

CINC-1 是大鼠体内白细胞介素-8(interleukin-8,IL-8)的同源物,是一种重要的促疼痛炎症因子。IL-8 的表达水平与人椎间盘源性疼痛呈正相关[21-22],相关动物实验也证实了 CINC-1 与疼痛的关联性[23-24]。本研究的结果显示,ICA 能降低大鼠椎间盘组织中 CINC-1 水平。结合 ICA 的镇痛效果,提示降低椎间盘组织内 CINC-1/IL-8 水平是 ICA 缓解腰痛的重要药理学机制。

本研究的结果提示,ICA 可有效缓解大鼠椎间盘源性腰痛,其镇痛效果与剂量有关,与同剂量的塞来昔布相比其镇痛效果持续时间更长,降低大鼠椎间盘组织中 CINC-1 的水平可能是其镇痛的作用机制之一。

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(收稿日期:2020-02-06 本文编辑:李晓乐)