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积雪草酸对大鼠神经病理性痛的影响及其相关机制 *

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摘要 目的:探讨积雪草酸对大鼠神经病理性痛的影响及其可能机制。**方法:**选择 32 只体重 220~240 g 的雄性 SD 大鼠,采用随机数字表法将其分为 4 组(n=8):假手术对照组(S 组)、神经病理性痛组(N 组)、积雪草酸 5 mg/kg 组(AA_1 组)及积雪草酸 10 mg/kg 组(AA_2 组)。S 组大鼠只暴露但不结扎坐骨神经;N 组大鼠仅制备神经病理性痛模型;AA_1 组和 AA_2 组大鼠制备神经病理性痛模型,并于模型建立后即刻及术后 1、3、5、7 天分别腹腔注射积雪草酸 5、10、20 mg/kg(溶于 0.1 mL 生理盐水)。于术前 1 天(T0)及术后 1、3、5、7 天(T1-T4)分别测定大鼠机械缩足反应阈位(MWTs)和热缩足潜伏期(TWLs),采用 Western blot 法检测脊髓 HMGB1、RAGE、IL-1β、TNF-α、iNOS 蛋白表达。**结果:** 与 N 组比较,AA_2 组 T1-T4 时间点 MWT 显著增加、TWL 明显缩短,而 AA_1 组仅 MWT 显著增加 (P<0.05),但各组各时间点 TWL 比较差异均无统计学意义 (P>0.05)。与 S 组比较,N 组脊髓浆蛋白 HMGB1 和 RAGE 及总蛋白 IL-1β、TNF-α、iNOS 蛋白表达较高(P<0.05);与 N 组比较,AA_1 和 AA_2 组脊髓浆蛋白 HMGB1 和 RAGE 及总蛋白 IL-1β、TNF-α、iNOS 蛋白表达均明显下降 (P<0.05)。与 AA_1 组比较,AA_2 组脊髓脊髓浆蛋白 HMGB1 和 RAGE 及总蛋白 IL-1β、TNF-α、iNOS 蛋白表达都明显降低(P<0.05)。**结论:**积雪草酸可能通过缓解 HMGB1-RAGE 信号通路介导的脊髓炎症反应减轻神经病理性痛。

关键词:积雪草酸;脊髓;高迁移率族蛋白 B1;炎症反应

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Effects of Asiatic Acid on the Neuropathic Pain and Its Possible Mechanisms*

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ABSTRACT Objective: To investigate the effects of asiatic acid on neuropathic and the possible mechanisms. **Methods:** Thirty-two male sprague dawley rats weighed 220~240 g were randomly divided into 4 groups (n = 8): Sham group (group S), neuropathic pain group (group N), asiatic acid 5 mg/kg group (group AA_1) and asiatic acid 10 mg/kg group (group AA_2). Neuropathic pain was induced with a model of chronic constriction injury (CCI). In group S, the sciatic nerve of rats was exposed without ligation. The rats in group AA_1 and AA_2 received CCI operation, followed by intraperitoneal injection of asiatic acid 10 or 20 mg/kg, respectively immediately after CCI operation 1, 3, 5 day (s) postoperation. The behavioral tests of mechanical paw withdrawal threshold (MWT) and thermal withdrawal latency (TWL) were performed 1 d before operation (T0) and 1, 3, 5, 7 days (T1-T4) postoperation. Western blotting was applied to assess the protein expressions of HMGB1, RAGE, IL-1β, TNF-α and iNOS in the spinal cord. **Results:** Compared with group N, from T1 to T4, the MWTs as well as the TWLs in group AA_2 were higher (P<0.05), whereas the animals in group AA_2 only had an increase in MWTs (P<0.05); Compared with group S, the cytosolic HMGB1 and RAGE protein levels, as well as the protein levels of IL-1β, TNF-α, iNOS from the total cellular extract of spinal cord were higher in group N (P<0.05). Compared with group N, the cytosolic HMGB1 and RAGE protein levels along with the protein levels of IL-1β, TNF-α, iNOS from the total cellular extract were lower in group AA_1 and AA_2 (P<0.05). **Conclusions:** Asiatic acid may alleviate the neuropathic pain induced by CCI via suppression of HMGB1-RAGE signaling-mediated inflammation in the spinal cord.

Key words: Asiatic acid; Spinal cord; High mobility group protein box 1; Inflammation

Chinese Library Classification(CLC): R-33; R338.3; R651.2 Document code: A

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前言

神经病理性痛是指神经损伤或功能失调所导致的慢性疼痛,主要表现为痛觉过敏和触诱发痛[1,2]。虽然脊髓神经元功能异常在神经病理性发挥重要作用,但以抑制神经元过度活动为目的的治疗并不理想。越来越多的研究表明中枢神经系统炎症反应在神经病理性痛的发生和发展中起到重要作用[3-5]。然而,神经病理性痛中神经炎症反应的机制尚不清楚。高迁移率族蛋

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白 B1(HMGB1)是一种中枢神经系统中的关键内源性炎症调节因子[6-8]。最近的研究显示 HMGB1 在神经病理性痛大鼠痛觉过敏中的形成中发挥重要作用[9, 10]。

积雪草酸(又名亚细亚酸)是雷公藤中提取的五环三萜类化合物[11, 12]。研究表明积雪草酸可通过减少线粒体膜电位去极化缓解 H_2O_2[13]诱发的神经损伤。同时,积雪草酸能减轻神经脑缺血再灌注损伤[14]后及代谢综合征大鼠[15]及脊髓损伤[16]中出现的炎症反应。因此,本研究拟探讨积雪草酸对神经病理性痛的影响及 HMGB1 介导的炎症反应在其中的作用。

1 材料与方法

1.1 主要材料及设备

积雪草酸(批号:546712,Sigma 公司,美国),总蛋白提取试剂盒和细胞浆蛋白提取试剂盒购自武汉博士德生物工程有限公司;HMGB1 抗体、RAGE(糖基化终产物受体)抗体、iNOS(诱导型一氧化氮合酶)抗体、IL-1β(白介素 -1β)抗体购自美国 Abcam 公司,批号分别为 ab18256、ab3611、ab49999、ab9722;TNF-α(肿瘤坏死因子 -α)抗体购自美国 Invitrogen 公司(批号 ARC3012),β-actin 抗体购自美国 Santa Cruz Biotechnology 公司(批号为 sc-47778);von Frey 丝购自美国 Stoelting 公司,PL-200 热刺痛仪购自成都泰盟科技有限公司。

1.2 神经病理性痛模型制备

参照文献[17]和[18],采用坐骨神经慢性压迫损伤(chronic constriction injury, CCI)法制备神经病理性痛模型。腹腔注射 40 mg/kg 戊巴比妥钠麻醉大鼠,做一右后肢中段切口,逐渐暴露坐骨神经及其三个主要分支,用 4-0 尼龙线于坐骨神经分支近端结扎 4 道,每道间隔约 1 mm。术毕于缝合伤口并用碘伏消毒。

1.2.1 **实验分组** 体重 220~240 g 雄性 SD 大鼠总计 40 只,购自第四军医大学医学实验中心。采用随机数字表法,将其随机分为 4 组(n = 8),包括假手术对照组(S 组)、神经病理性痛组(N 组)、积雪草酸 5 mg/kg 组(AA_1 组)、积雪草酸 10 mg/kg 组(AA_2 组)。S 组大鼠只暴露但不结扎坐骨神经;N 组大鼠用于制备神经病理性痛模型;AA_1 组和 AA_2 组大鼠遇制备神经病理性痛模型模型建立后即刻及术后 1、3、5 天分别腹腔注射积雪草酸 5 或 10 mg/kg(溶于 0.3 mL 生理盐水)。

1.2.2 **行为学实验** 术前 1 天 (T0) 及模型后 1、3、5、7 天(T1-T4)分别测定大鼠机械缩足反应阈值(mechanical withdrawal thresholds,MWT)和热缩足潜伏期(thermal withdrawal latency,TWL)。a. MWT 实验:参照文献[19],采用 up-down 法测定大鼠 MWTs。用不同压力的 von Frey 丝按照压力从小到大的顺序依次刺激大鼠右后足底部,每次刺激持续 2 秒,以缩足和舔足反应作为阳性反应。von Frey 纤维丝初始刺激力度 2.05 g,每次间隔≥ 30 秒。每次刺激重复 5 次取平均值,即为大鼠 MWT 值;b. TWL 实验:参照文献 [20],使用 PL-200 热刺痛仪测定大鼠 TWL。将热辐射光源照射对准大鼠右侧足底部,测定出现缩足反应的时间,单次照射时间≤ 2 秒,连续测定 3 次每次间隔 3-5 分钟,取平均值为大鼠 TWL 值。

1.2.3 Western blot **检测** 于术后第 7 天最后一次行为学检测后,采用注射过量戊巴比妥钠(80 mg/kg)处死大鼠,取出脊髓 L4-L6 节段,称重后加入裂解液于冰上匀浆。离心后将样本上清平均分为两份,一份用于提取总蛋白,另一份使用细胞浆蛋白抽提试剂盒提取脊髓组织浆蛋白。浆蛋白用于测定胞浆 HMGB1 和 RAGE 蛋白水平,总蛋白用于测定 IL-1β、TNF-α、iNOS 蛋白水平。经 SDS-PAGE 电泳后浆蛋白电转至 PVDF 膜上,5%的脱脂牛奶室温封闭 30 min 后,加入相应稀释比例的一抗:抗 β-actin 抗体(1:500)、抗 HMGB1 抗体(1:1000)、抗 iNOS 抗体(1:1000)、抗 RAGE 抗体(1:1000)、抗 IL-1β 抗体(1:1000)、抗 TNF-α 抗体(1:1000),4 ℃冰箱内孵育过夜。第二天,辣根过氧化物酶标记的二抗室温孵育 1 h,洗膜后加入 ECL 液进行显影反应并于暗室内曝光。采用 Quantity one 软件分析条带光密度值。HMGB1 及 RAGE 蛋白表达水平以目的蛋白 / 浆蛋白 β-actin 光密度值表示,总蛋白 IL-1β、TNF-α、iNOS 蛋白表达水平以目的蛋白 / 总蛋白 β-actin 光密度值表示。

1.3 统计学方法

使用 SPSS 17.0 统计软件进行数据分析,所有数据以均数± 标准差($\bar{x}\pm s$)表示,多组间比较采用单因素方差分析,两组间比较采用 t 检验,以 P<0.05 为差异有统计学意义。

2 结果

2.1 四组大鼠机械缩足反应阈值(MWT)的比较

与 S 组比较,N 组 MWT 于 T1-T4 时间点明显下降(P<0.05);与 N 组比较,T1-T4 时间点 AA_1 和 AA_2 组 MWT 均显著增高(P<0.05);与 AA_1 组比较,AA_2 组 MWT 于 T2 和 T3 时间点较高(P<0.05),见表 1。

2.2 四组大鼠热缩足潜伏期(TWL)的比较

与 S 组比较,N 组 TWL 于 T1-T4 时间点明显缩短(P<0.05);与 N 组比较,T1-T4 时间点 AA_2 组 TWL 延长(P<0.05),而

表 1 四组大鼠机械缩足反应阈值(MWT)的比较(g, n = 8, $\bar{x}\pm s$)

Table 1 Comparison of the mechanical withdrawal thresholds (MWT) among the four experimental groups (g, n = 8, $\bar{x}\pm s$)

Groups	T0	T1	T2	T3	T4
S	14.3 ± 1.7	15.3 ± 1.6	14.2 ± 1.2	15.2 ± 1.7	14.1 ± 1
N	14.6 ± 1.9	9.3 ± 1[a]	7.1 ± 1.2[a]	6.6 ± 1.1[a]	4.2 ± 0.6[a]
AA_1	14.3 ± 1.7	11.2 ± 1.3[b]	9.6 ± 1.7b	8.8 ± 1.4[b]	7.6 ± 1.4[b]
AA_2	14.8 ± 1.7	12.9 ± 1.5[b]	11.9 ± 1.7[bc]	11.2 ± 1.6[bc]	9.2 ± 1.3[b]

Note: S: Sham group, N: neuropathic pain group, AA_1: asiatic acid 5 mg/kg group, AA_2: asiatic acid 10 mg/kg group; Compared with S group, [a]P<0.05; Compared with N group, [b]P<0.05; Compared with AA_1 group, [c]P<0.05.

AA_1 组于各时间点比较差异均无统计学意义(P>0.05)；与 AA_1 组比较，AA_2 组 TWL 于 T3 和 T4 时间点显著延长(P<0.05)，见表 2。

表 2 四组大鼠热缩足潜伏期(TWL)的比较(s, n = 8, $\bar{x}\pm s$)

Table 2 Comparison of the thermal withdrawal latency (TWL) among the four experimental groups (g, n = 8, $\bar{x}\pm s$)

Groups	T0	T1	T2	T3	T4
S	14.4 ± 2.2	13.5 ± 2	13.8 ± 2.3	13.9 ± 2.1	13.8 ± 3.2
N	13.2 ± 3	9.9 ± 1.5[a]	9.5 ± 1.4[a]	7.0 ± 1.2[a]	6.6 ± 1.2[a]
AA_1	13.0 ± 2.4	12.5 ± 2.6	11.5 ± 1.6	9.1 ± 1.5	8.4 ± 1.7
AA_2	14.3 ± 2.5	13.9 ± 2.2[b]	12.6 ± 2.2[b]	12.2 ± 2.3[bc]	11.4 ± 1.7[bc]

Note: S: Sham group, N: neuropathic pain group, AA_1: asiatic acid 5 mg/kg group, AA_2: asiatic acid 10 mg/kg group; Compared with S group, [a]P<0.05; Compared with N group, [b]P<0.05; Compared with AA_1 group, [c]P<0.05.

2.3 四组大鼠脊髓 HMGB1 和 RAGE 蛋白表达的比较

与 S 组比较，N 组脊髓浆蛋白 HMGB1 和 RAGE 蛋白表达更高 (P<0.05)；与 N 组比较，AA_1 和 AA_2 组脊髓浆蛋白 HMGB1 和 RAGE 蛋白表达明显下降(P<0.05)。与 AA_1 组比较，AA_2 组脊髓浆蛋白 HMGB1 和 RAGE 蛋白表达均较低(P<0.05，见图 1)。

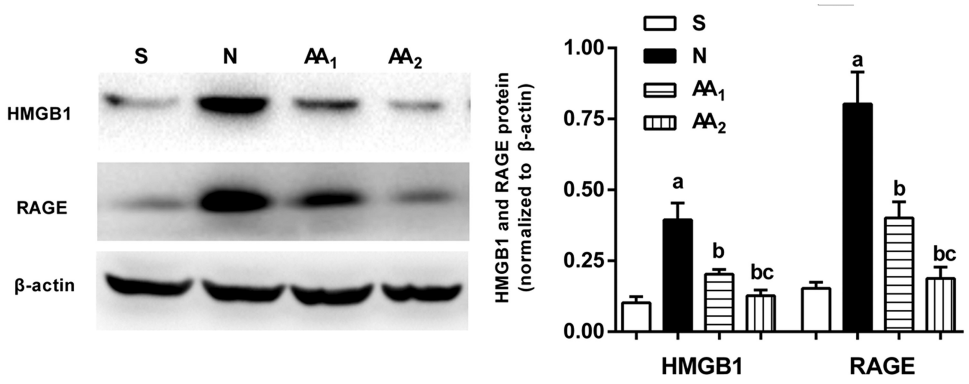

图 1 四组大鼠脊髓浆蛋白 HMGB1 和 RAGE 蛋白表达的比较(n = 8, $\bar{x}\pm s$)

Fig. 1 Comparison of the HMGB1 and RAGE protein expression in the spinal cord among the four experimental groups

Note: S: Sham group, N: neuropathic pain group, AA_1: asiatic acid 5 mg/kg group, AA_2: asiatic acid 10 mg/kg group; Compared with S group, [a]P<0.05; Compared with N group, [b]P<0.05; Compared with AA1 group, [c]P<0.05.

2.4 四组大鼠脊髓 IL-1β、TNF-α 及 iNOS 蛋白水平的比较

与 S 组比较，N 组脊髓 IL-1β、TNF-α、iNOS 蛋白表达较高(P<0.05)；与 N 组比较，脊髓 IL-1β、TNF-α、iNOS 蛋白表达在 AA_1 和 AA_2 组明显较低(P<0.05)；与 AA_1 组比较，AA_2 组脊髓 IL-1β、TNF-α 及 iNOS 蛋白表达均较低(P<0.05，见图 2)。

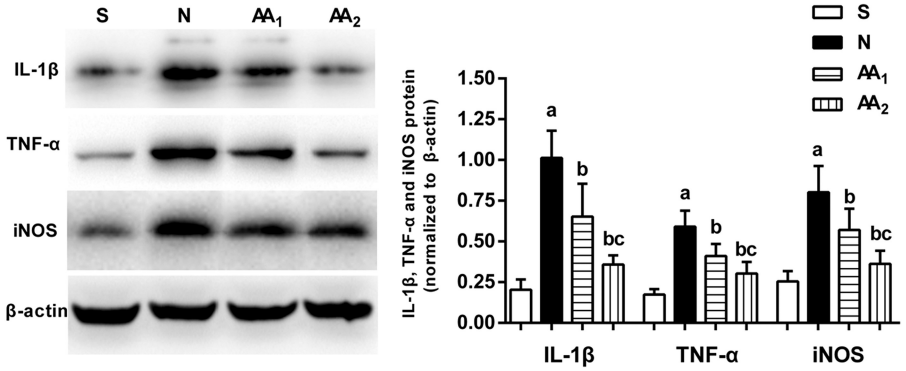

图 2 四组大鼠脊髓浆蛋白 IL-1β、TNF-α 及 iNOS 蛋白表达的比较(n = 8, $\bar{x}\pm s$)

Fig. 2 Comparison of the IL-1β, TNF-α and iNOS protein expression in the spinal cord among the four experimental groups

Note: S: Sham group, N: neuropathic pain group, AA_1: asiatic acid 5 mg/kg group, AA_2: asiatic acid 10 mg/kg group; Compared with S group, [a]P<0.05; Compared with N group, [b]P<0.05; Compared with AA_1 group, [c]P<0.05.

3 讨论

慢性压迫损伤(CCI)模型是研究神经病理性痛机制的经典模型,故本研究采用此法制备大鼠神经病性痛模型。行为学结果结果显示与 S 组(假手术组)比较,其它两组术后机械缩爪阈值(MWTs)和热缩足潜伏期(TWLs)均逐渐下降,并均出现跛行、足外翻等伴随行为学表现,提示神经病理性痛模型制备成功。研究表明腹腔注射 5 和 10 mg/kg 的积雪草酸可缓解小鼠局部炎性水肿所导致的疼痛[21]。因此,本研究给予成年大鼠 5 和 10 mg/kg 的积雪草酸,拟探讨积雪草酸对大鼠神经病理性痛的影响。结果显示与 N 组比较,AA_1 和 AA_2 组大鼠 MWTs 于模型建立后各观察时间点均明显升高,AA_2 组大鼠 TWLs 亦于术后各时间点显著延长,说明积雪草酸 5 和 10 mg/kg 可不同程度的延缓大鼠神经病理性痛的形成。

神经炎症反应在神经病理性痛的发生和发展中发挥重要作用[3-5]。在外周或脊髓损伤后,过量的促炎因子如 IL-1β 和 TNF-α 可由小胶质细胞或反应性星形胶质细胞分泌[3, 22],导致 C 型神经纤维突触长时程增强作用(LTP)[23]并减少抑制性突触递质的活性[24],进而增加神经元及其相关突触电活动。研究表明积雪草酸进而缓解脑缺血再灌注损伤及局部炎性介质注射所导致的炎症反应[14, 21]。与之一致,本研究结果显示积雪草酸可明显减少脊髓促炎因子的释放,提示积雪草酸的抗炎作用可能是其缓解神经病理性痛的重要机制。此外,也有研究表明一氧化氮(NO)相关信号通路的激活在神经病理性痛的形成中发挥重要作用[25-27]。而积雪草酸可与 NO 的关键性诱发酶 iNOS 结合并抑制其活性,并减少代谢综合征大鼠血浆中 NO 及 iNOS 表达水平[15]。因此,本研究检测了积雪草酸对脊髓 iNOS 蛋白表达的影响,结果显示积雪草酸能显著缓解神经病理性痛大鼠脊髓的 iNOS 蛋白表达水平,提示积雪草酸的抑制 NO 作用也可能在是其减轻大鼠神经病理性痛的可能机制。

HMGB1 是管理神经系统炎症反应的重要分子[28, 29]。生理情况下,HMGB1 可与细胞核内组蛋白相结合在维持 DNA 转录的稳定性中发挥重要作用,而在病理性刺激后,HMGB1 可有细胞核转移至胞浆中,诱发 RAGE 分子的产生并与之结合,从而触发小胶质细胞和下游炎症信号通路(NF-κB 和 TLR4 相关信号通路)激活及促炎因子释放[30]。本研究结果显示 5 mg/kg 和 10 mg/kg 积雪草酸均可显著减少神经病理性痛大鼠脊髓浆蛋白 HMGB1 和 RAGE 的蛋白表达,提示积雪草酸可能通过减轻神经病理性痛大鼠脊髓 HMGB1-RAGE 信号通路激活,继而减少促炎因子释放,进而缓解神经病理性痛的发生和发展。

综上所述,积雪草酸可通过缓解 HMGB1-RAGE 信号通路介导的脊髓炎症反应减轻神经病理性痛。

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