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【基础研究】

植物提取物碳酸酐酶对肾结石大鼠的影响

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摘要: 目的 观察碳酸酐酶(CA)对肾结石大鼠的影响。方法 从植物叶片中提取CA并检测活性。将40只大鼠随机分为I、II、III和IV组,每组25只。I组大鼠正常喂养颗粒饲料;II组大鼠每天给予质量分数1%乙二醇+质量分数2%氯化铵2 mL灌胃;III组大鼠每天给予质量分数1%乙二醇+质量分数2%氯化铵2 mL+CA 1 mL灌胃;IV组大鼠每天给予CA 1 mL灌胃。饲养1周后收集4组大鼠24 h尿量,并检测尿液的尿素氮、肌酐、尿酸及钙离子。30 d后处死大鼠,观察肾脏组织结晶形成情况。**结果** 4组大鼠尿液中的尿素氮、肌酐及尿酸水平比较差异均无统计学意义($P > 0.05$);III、IV组大鼠24 h尿量与I、II组比较差异有统计学意义($P < 0.05$);4组大鼠尿钙两两两比较差异均有统计学意义($P < 0.05$)。II组可见明显的肾结石结晶;III、IV组未见明显的肾结石结晶,但可见肾小管结构异常改变。**结论** 植物提取物CA能够抑制大鼠肾结晶的形成,对肾结石的预防和治疗可能有一定的积极作用。

关键词: 碳酸酐酶;肾结石;动物模型;尿排泄

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Effects of carbonic anhydrase extracts from plant on renal calculi rats

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Abstract: Objective To explore the effect of carbonic anhydrase(CA) on the animal model of renal calculi rats.

Methods The CA was extracted from plant and its activity was detected. Forty rats were divided into four groups, with twenty-five rats in each group. The rats in group I were fed pelleted diet; the rats in group II were fed quality concentration 1% ethylene glycol + quality concentration 2% ammonium chloride 2 mL; the rats in group III were fed quality concentration 1% ethylene glycol + quality concentration 2% ammonium chloride 2 mL + CA 1 mL; the rats in group IV were fed CA 1 mL. All rats were oral administration per day for 30 days. A week after feeding, 24 h urine volume was collected, and the urea nitrogen, creatinine, uric acid and calcium was detected. One month later, rats were sacrificed and the kidney tissues crystal were observed.

Results There was no statistic difference of urine urea nitrogen, creatinine and uric acid levels of rats in the four groups ($P > 0.05$); there was statistic difference of 24 h urine volume between group III, group IV and group I, group II ($P < 0.05$); there was statistic difference of calcium oxalate in urine among the four groups ($P < 0.05$). After rats were sacrificed, renal crystal was observed only in group II; no obvious renal crystal was observed in group III and group IV, but abnormal structure of renal tubule was observed. **Conclusion** Plant extraction CA can inhibit the formation of renal crystal, may have certain positive role in prevention and treatment of renal calculi.

Key words: carbonic anhydrase; renal calculi; animal model; urinary excretion

肾结石是泌尿外科常见疾病之一,病因尚不明确,与饮食习惯、生活方式、地域、职业、遗传因素等相关^[1-5]。多数肾结石患者需行体外碎石或经皮肾镜碎石术治疗,治疗后3~5 a复发率较高,可达80%~100%。因此,肾结石的早期预防及治疗成为

重要的研究课题。本研究从新鲜植物叶片中提取碳酸酐酶(carbonic anhydrase, CA)并鉴定,观察其对SD大鼠肾结石的影响,现报道如下。

1 材料与方法

1.1 材料 植物叶片来源于九江彭泽县岩石区; 100只Sprague Dawley雄性大鼠购于南京君科生物工程公司,平均体质量(210.0 ± 20.0)g,大鼠代谢笼由九江学院动物研究所提供。

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1.2 试剂与仪器 台式高速离心机(TGL216G)、电子天平(AG204)及酸碱度(power of hydrogen, pH)计(PHS23C)由九江学院附属医院科研中心提供。尿素氮、肌酐、尿酸、无机磷、尿钙及草酸检测试剂盒购于上海研卉生物科技有限公司,大鼠基础饲料由九江学院动物研究所提供,2-羟乙基-1-哌嗪乙磺酸(2-hydroxyethyl piperazine-1-erhanesulfonic acid-KOH, HEPES-KOH)缓冲液购于上海榕柏生物技术有限公司,二硫苏糖醇、乙二醇、氯化铵、硫酸铵均为分析纯。

1.3 CA液提取及鉴定 摘取新鲜植物叶片,4℃下保存待用。植物提取液为pH=8.2的缓冲液:50 mmol·L⁻¹ HEPES-KOH, 10 mmol·L⁻¹二硫苏糖醇。将新鲜叶片放入预冷的研钵中,加入液氮充分研磨,加入5 mL植物提取液混合均匀,16 000 r·min⁻¹离心15 min,取上清液冷藏待处理。植物CA的纯化:将上清液加入固体硫酸铵至相对饱和度为30%,放置6 h后,4 000 r·min⁻¹离心15 min去沉淀,上清液继续加入固体硫酸铵至相对饱和度为65%,4℃放置过夜,4 000 r·min⁻¹离心15 min,收集沉淀,溶于缓冲液中,透析12 h,每3 h换1次缓冲溶液,透析液定溶至100 mL后4℃保存。CA的活力测定:酶活性单位数(U)=10(T₀/T_e-1),T₀和T_e分别是加入煮沸和未煮沸样品液测得的pH下降1个单位所需时间。酶活性以每毫克蛋白含有的酶活性单位数(U·mg⁻¹)表示。

1.4 实验分组及给药方法 动物饲养1周后,选体质量150~180 g大鼠40只随机分为4组,每组10只。I组大鼠正常喂养颗粒饲料;II组大鼠每天口

服质量分数1%乙二醇+质量分数2%氯化铵2 mL饮用水灌胃^[6];III组大鼠每天给予质量分数1%乙二醇+质量分数2%氯化铵2 mL饮用水灌胃,同时给予CA 1 mL灌胃;IV组大鼠每天给予CA 1 mL灌胃。所有大鼠灌胃30 d。

1.5 观察指标 动物饲养第28天用代谢笼收集并测24 h尿量以及尿素氮、肌酐、尿酸、无机磷、尿钙和草酸的排泄量。动物饲养30 d后脱颈椎处死大鼠,取大鼠的双肾,观察其外观改变,左肾置体积分数10%中性甲醛固定,作常规苏木精-伊红(hematoxylin-eosin, HE)染色,石蜡切片及偏光显微镜下观察肾组织的病理改变以及肾组织草酸钙结晶分布情况。

1.6 统计学处理 应用SPSS 13.0软件进行数据统计和分析,计量资料以均数±标准差($\bar{x} \pm s$)表示,组间比较采用F检验,计数资料比较采用 χ^2 检验, $P < 0.05$ 为差异有统计学意义。

2 结果

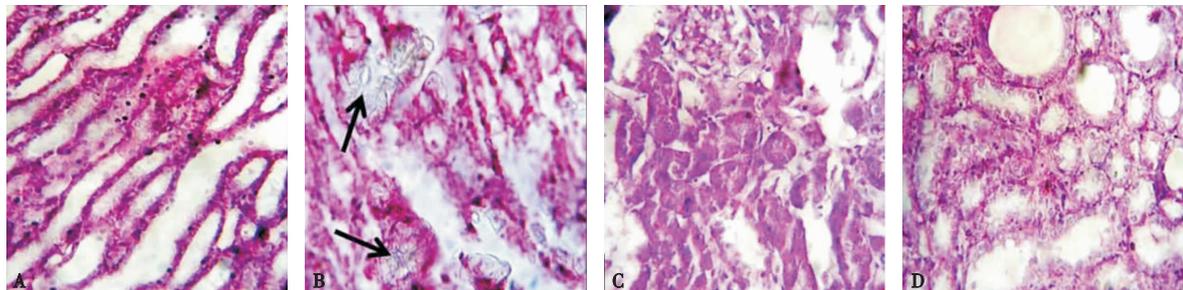
2.1 CA活性及一般指标检测结果 结果见表1。CA活性为(12.63±0.88) U·mg⁻¹。4组大鼠尿液中的尿素氮、肌酐及尿酸水平比较差异均无统计学意义($P > 0.05$);III、IV组大鼠24 h尿量与I、II组比较差异有统计学意义($P < 0.05$);4组大鼠尿钙水平两两比较差异均有统计学意义($P < 0.05$)。

2.2 肾草酸钙结晶形成情况 结果见图1。4组大鼠肾脏组织内只有II组有14例肾结晶形成,可见明显的肾结石结晶及肾小管轻微扩张;III组和IV组的肾脏组织未见明显的肾结石结晶,但可见肾小管结构扭曲及扩张改变。

表1 4组大鼠24 h尿量及相关代谢指标排泄量

| 组别 | n | 24 h 尿量/mL | 尿素氮/($\times 10^{-8}$ g·L ⁻¹) | 肌酐/(μ mol·L ⁻¹) | 尿酸/($\times 10^{-2}$ g·L ⁻¹) | 尿钙/($\times 10^{-5}$ g·L ⁻¹) |
|------|----|-------------------------|--|----------------------------------|---|---|
| I组 | 25 | 17.85±1.58 | 63.44±5.06 | 200.90±9.83 | 17.85±1.58 | 180.10±87.71 |
| II组 | 25 | 16.22±1.54 | 51.68±4.39 | 188.43±10.03 | 16.22±1.54 | 453.24±198.17 ^b |
| III组 | 25 | 20.18±1.48 ^a | 55.16±3.96 | 185.02±10.01 | 18.18±1.48 | 732.30±393.28 ^c |
| IV组 | 25 | 22.75±1.54 ^a | 57.80±5.11 | 199.54±12.95 | 16.75±1.54 | 297.40±126.02 ^a |

注:与I、II组比较^a $P < 0.05$;与I组比较^b $P < 0.05$;与I、II、IV组比较^c $P < 0.05$ 。



A: I组;B: II组;C: III组;D: IV组;箭头所指为肾结晶。

图1 4组大鼠肾脏组织(HE染色,×40)

Fig. 1 Kidney tissue of rats in the four groups(HE staining, ×40)

3 讨论

CA 是一类含锌的金属酶,能够特异催化某些小分子物质的水合反应,从而保持机体稳定的 pH 和体液量。1933 年,CA 第 1 次从动物红细胞提取^[7],在 1940 年鉴定其空间结构并确定蛋白结构中心含有一锌原子。根据氨基酸残基序列中的差异,CA 可分为 α -CA、 β -CA、 γ -CA、 δ -CA 和 ε -CA 5 种亚型。脊椎动物的组织器官多为 α -CA 型,目前至少发现 13 种以上的同工酶,其中肾组织以 α -CA II 为主, α -CA II 还可见于脑组织和眼组织^[8]。 α -CA II 对近端和远端肾小管碳酸氢盐重吸收和维持 pH 具有重要作用,抑制肾 CA 活性会引起近端肾小管酸中毒、远端肾小管酸中毒或混合性肾小管酸中毒^[9]。但目前尚无抑制肾 CA 活性动物模型诱发肾结石的相关研究报道。

本研究发现, I 组大鼠肾脏未发现结晶, II 组大鼠有肾结晶形成, III 组大鼠未发现肾脏结晶形成,提示 CA 可能对肾结晶形成有抑制作用,可能与以下 2 种因素有关:(1)CA 能够促进肾小管碳酸氢根离子的重吸收,促进氢离子排泄,维持酸碱平衡。多项研究也证实了肾小管酸中毒能够降低枸橼酸盐的排泄,是肾结石的独立危险因素^[10-12]。因此,CA 可能是通过促进肾小管的氢离子排泄而降低肾小管酸中毒抑制结晶的形成。(2)血浆中某些因素使血液循环处于偏弱酸环境,从而导致继发性的甲状旁腺功能亢进,引起尿 Ca^{2+} 、磷酸盐排泄增加,增加肾结晶或结石的形成概率^[13-14]。CA 能够作用红细胞的 CA 受体,改善血液循环的弱酸环境,纠正酸碱失衡,从代谢方面抑制肾结晶或肾结石的形成风险。

当 CA 活性受抑制时,患者罹患肾结石的风险增加。非特异性碳酸酐酶抑制剂(carbonic anhydrase inhibitor, CAi)托吡酯临床上用于治疗青光眼,但其可抑制血清中红细胞和肾小管的 CA 活性,导致肾结石风险增加。此外,在非特异性 CAi 乙酰唑胺和磺胺类抑菌药也会增加罹患肾结石的风险^[15-17]。 α -CA II 缺乏症是一种罕见的常染色体隐性遗传病,临床可表现为肾小管性酸中毒、肾结石、肾钙沉着症、脑钙化等^[18-20]。因此,红细胞和肾小管 CA 活性不仅对维持体内酸碱平衡具有重要作用,也能够一定程度上抑制肾结石的形成。

为了进一步评估 CA 治疗肾结石的安全性,本研究增加了阳性对照组即 IV 组,主要目的是为了观察单独使用 CA 是否会引起大鼠肾脏损伤。本实验结果显示,4 组大鼠尿液中尿素氮、肌酐及尿酸的排泄量差异无统计学意义,说明单独使用 CA 对大鼠的肾脏无损伤。但 III、IV 组大鼠尿钙排泄量较 I 组

大鼠升高,说明 CA 能够促进尿钙的排泄,提示 CA 对肾结石具有一定的驱动溶解作用。但尿钙的排泄量升高,可进一步导致肾小管的扩张、扭曲。

本研究中,重新给予外源性 CA 到肾结石大鼠模型中,发现能够降低肾结晶的形成风险,提示肾结晶的形成可能与 CA 活性相关。这与 CA 在其他学科研究^[21-23]是一致的。在地质学和生态学研究,岩石溶解区域中的 CA 活性往往比一般非岩溶区域要高,因为 CA 能够加速岩石的溶解,而这种溶解速度远超过非岩溶区域的岩石溶解^[21]。LI 等^[22]也从岩溶地区分离出能够胞外分泌 CA 的细菌,同时在模拟生态环境下,这种细菌能够快速对石灰岩进行溶解。

综上所述,CA 可能是肾结石治疗的新药物,同时,也为进一步研究 CA 溶解肾结石的机制及用于临床治疗肾结石提供了前期的理论研究基础。

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