替米沙坦对阿霉素肾损伤大鼠血浆中血管紧张素(1~7)水平的影响

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摘 要 目的 观察替米沙坦对阿霉素(adriamycin, ADR)诱导的肾损伤大鼠血浆中血管紧张素(1~7)[Ang(1~7)]水平的影响。**方法** 50 只健康、雄性 SD 大鼠,随机分为对照组($n=10$)、ADR 模型组($n=20$)、替米沙坦组($n=20$)。对照组腹腔注射同等剂量的生理盐水;另两组均腹腔注射 ADR 2.5mg/kg,每周 3 次,共 2 周,累积量 15mg/kg,同时替米沙坦组给予替米沙坦 10mg/(kg·d)(累积量 420mg/kg)灌胃干预共 6 周。实验过程中观察大鼠精神、活动、饮食等一般情况。于末次注射停药后 4 周,检测大鼠终末体重、血浆 Ang(1~7)水平,处死大鼠后制作肾脏病理切片并观察组织学变化。**结果** ADR 模型组及替米沙坦组大鼠体重均较对照组下降(P 均 <0.01),但替米沙坦组体重较 ADR 模型组有所增加($P<0.01$),ADR 模型组与替米沙坦组大鼠血浆 Ang(1~7)的水平较对照组亦下降(4.27±2.79 vs 10.26±2.39ng/ml, $P<0.01$;7.16±2.13 vs 10.26±2.39ng/ml, $P<0.05$),但替米沙坦组血浆 Ang(1~7)水平较 ADR 模型组升高($P<0.05$),组织病理学变化:与对照组相比,ADR 模型组大鼠肾脏炎性细胞成簇聚集、浸润明显增多,且肾小管上皮细胞水肿;替米沙坦组炎性细胞浸润则较模型组明显减少,肾小管上皮细胞水肿程度亦减轻。**结论** 替米沙坦能够提高阿霉素肾损伤大鼠血浆中 Ang(1~7)的水平,且该变化水平可能与肾脏炎性反应的严重程度有一定的相关性。

关键词 血管紧张素(1~7) 替米沙坦 阿霉素 肾损伤模型

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Effects of Telmisartan on the Plasma Angiotensin-(1-7) Levels in Adriamycin Induced Renal Injury in Rats. *Zong Wenna, Qu Feng, Dong Wei, et al. Department of Critical Care Medicine, The First People's Hospital of Jining, Shandong 272011, China*

Abstract Objective To investigate effects of Telmisartan on plasma angiotensin(1-7) [Ang(1-7)] levels in Adriamycin (ADR)-induced renal injury in rats. **Method** Male Sprague-Dawley rats were randomly divided into controls ($n=10$), ADR-model group ($n=20$) and Telmisartan group ($n=20$). Control rats were intraperitoneally injected the same volume of normal saline, and the other two groups were all intraperitoneally injected a cumulative dose of 15 mg/kg of ADR (each dose of 2.5mg/kg×6) for 2 weeks, and Telmisartan group was simultaneously treated with Telmisartan (10mg/kg daily, total 420mg/kg) orally for 6 weeks. During the test, rats were observed for spirit, activity, diet and other general living conditions. Four weeks after drug withdrawal, terminal weight of rats were detected by scale, plasma Ang (1-7) levels was detected by ELISA, and the histological changes of renal were observed by HE staining. **Results** The weight of rats in ADR-model group and Telmisartan group were both decreased ($P<0.01$), but in Telmisartan group, rats weight were increased than in ADR-model group ($P<0.01$), plasma Ang (1-7) levels in both of ADR-model group and Telmisartan group were also decreased (4.27±2.79 vs 10.26±2.39ng/ml, $P<0.01$; 7.16±2.13 vs 10.26±2.39ng/ml, $P<0.05$). However, in Telmisartan group, it was higher than in ADR-model group ($P<0.05$). Histological changes of rat kidney indicated that inflammatory cells were clustered and increased significantly, and the renal tubular epithelial cells edema in model group. In Telmisartan group, inflammatory cells were obviously reduced and the degree of dropsy in renal tubular epithelial cells were also alleviated. **Conclusion** Plasma Ang (1-7) levels were improved in ADR-induced renal injury in rats after the intervention of Telmisartan, and there may be some correlation between the plasma Ang (1-7) levels and the severity of inflammation in Kidney.

Key words Angiotensin(1-7); Telmisartan; Adriamycin; Renal injury model

血管紧张素(1~7)[Ang(1~7)]及其 Mas 受体在心血管方面的保护作用已被广泛阐述,近年研究发现,Ang(1~7)的生物学效应亦涉及其他脏器。例如 Ang(1~7)抑制大鼠脑胶质瘤 C6 细胞的增殖有减轻糖尿病大鼠视网膜炎性细胞浸润与氧化损伤阻断肺

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成纤维细胞迁移并抑制肺纤维化通过抑制 NF－κB 及细胞外信号调节激酶 1/2(ERK1/2)信号通路减轻过敏性哮喘炎性反应等[1~4]。在糖尿病肾病或单克隆抗 Thy－1 抗体 ox－7 诱导的肾炎模型中,输注外源性 Ang(1～7),可通过不同信号转导途径减轻肾脏纤维化及炎性反应[5,6]。另有报道,经高盐高脂饮食喂养的小鼠,其体内 Ang(1～7)水平降低,且肾脏纤维化较明显,而经缬沙坦干预后,其通过增加 Ang(1～7)的表达缓解肾脏纤维化[7]。本研究中采用阿霉素(adriamycin, ADR)诱导大鼠发生肾损伤,经替米沙坦干预后,观察大鼠血浆中 Ang(1～7)水平的变化,探讨该变化水平与肾脏炎性反应程度之间可能存在的相关性。

材料与方法

1. 材料:实验动物:健康雄性 SD 大鼠 50 只,体重 217±17.7g,由上海斯莱克实验动物有限公司提供,予标准无菌固体饲料分笼正常喂养,自由饮清洁水,实验过程中对动物处置符合动物伦理学标准。主要药物和试剂:阿霉素购自浙江海正药业,替米沙坦购自上海诺华公司。

2. 方法:(1)动物分组及模型制作:50 只大鼠饲养 1 周后随机分为对照组($n=10$)、模型组($n=20$)、替米沙坦组($n=20$)。对照组腹腔注射生理盐水,后两组均腹腔注射 ADR 2.5mg/kg,每周 3 次,共 2 周,累积量 15mg/kg;替米沙坦组给予替米沙坦 10mg/(kg·d)(累积量 420mg/kg)灌胃干预 6 周。实验过程中,每天观察动物的精神、活动、饮食、皮毛、有无腹腔积液及死亡情况等。每 3 天测体重 1 次,并以此调整阿霉素、替米沙坦的给药剂量。于末次注射停药后 4 周后,检测大鼠终末体重、血浆 Ang(1～7)水平,并制肾脏病理切片观察其组织学变化。(2)血浆 Ang(1～7)水平测定:将大鼠以 10% 水合氯醛 0.3ml/100g 腹腔麻醉,仰卧固定,备皮消毒,铺无菌孔巾,剑突下沿腹正中线切开,剪开腹膜入腹腔,暴露腹主动脉,插入一次性采血针取血,一般 5～10ml,存放于 EDTA 抗凝管中,2500r/min,离心 15min,取上清分装后－20℃保存备用。血浆 Ang(1～7)水平测定:采用酶联免疫吸附法(ELISA)测定血浆 Ang(1～7)水平,试剂盒购自上海西塘生物科技有限公司,酶标仪型号为 Clinibio－128C。按说明书操作,加样、孵育、洗涤、显色、终止反应。以空白孔调零,于酶标仪在 450 nm 波长上读出各孔吸光度 A 值,并根据标准品制作标准曲线,计算各标本血浆 Ang(1～7)水平。(3)肾脏病理学切片制作:处死大鼠后,立即取出肾脏置于冷生理盐水中洗去血液,滤纸吸干水分,即取部分组织置于 4% 多聚甲醛固定液内,常规石蜡包埋切片,切片厚度 5μm,HE 染色,光镜观察。

3. 统计学方法:以 SPSS 11.5 统计软件和 Excel 软件对结果进行分析,计量资料采用均数±标准差($\bar{x}\pm s$)表示,两组间均数比较采用 t 检验,以 $P<0.05$ 为差异有统计学意义。

结　　果

1. 大鼠存活情况:第 3 次给药后,模型组、替米沙坦组大鼠与对照组相比,体重增加缓慢、精神萎靡、反应迟钝,毛发欠光泽;第 4 次给药后,模型组 4 只、替米沙坦组 1 只大鼠死亡;第 6 次给药后,模型组共 7 只、替米沙坦组共 4 只大鼠死亡;6 周后,模型组共 11 只、替米沙坦组共 8 只大鼠死亡。对照组大鼠生存状态良好,无死亡现象。

2. 大鼠体重检测:与对照组(408.6±12.8g)相比,模型组(304.4±19.8g)及替米沙坦组(343.0±22.6g)大鼠体重均下降(P 均 <0.01),但替米沙坦组大鼠体重较模型组有所增加,差异均有统计学意义($P<0.01$)。

3. 血浆 Ang(1～7)水平:模型组与对照组相比,血浆 Ang(1～7)水平明显下降(4.27±2.79 vs 10.26±2.39ng/ml, $P<0.01$),替米沙坦组与对照组相比,血浆 Ang(1～7)水平亦下降(7.16±2.13 vs 10.26±2.39ng/ml, $P<0.05$),组间差异均有统计学意义。而与模型组相比,其血浆 Ang(1～7)水平升高(7.16±2.13 vs 4.27±2.79 ng/ml, $P<0.05$),差异有统计学意义。

4. 肾脏组织学变化:HE 染色结果显示,对照组大鼠肾脏无炎性细胞浸润,肾小管上皮细胞无水肿,细胞间隙正常;模型组肾脏炎性细胞成簇聚集、浸润明显增多,且肾小管上皮细胞水肿,细胞间隙消失;替米沙坦组炎性细胞浸润则较模型组明显减少,肾小管上皮细胞水肿程度亦减轻,细胞间隙基本正常,与正常大鼠肾脏组织相比,差异无统计学意义($P>0.05$,图 1)。

讨　　论

肾脏是肾素－血管紧张素(RAS)作用的主要靶器官之一,除受循环中 RAS 影响外,肾脏局部组织中血管紧张素原、肾素、血管紧张素Ⅱ(AngⅡ)、AngⅡ1 型受体(AT_1R)、血管紧张素转化酶/血管紧张素转化酶 2(ACE/ACE2)及 Ang(1～7)的表达水平,对肾脏

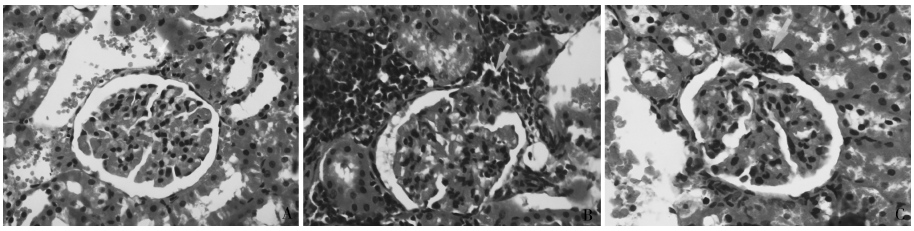

图 1　各组大鼠肾脏组织学改变(HE, ×400)

A. 对照组;B. 模型组;C. 替米沙坦组

疾病的发生、发展起到重要作用。其中,ACE2 水解 Ang Ⅱ 是生成 Ang(1 ~ 7)的主要途径,另外,Ang(1 ~ 7)也可由位于肾脏近端肾小管刷状缘膜或细胞质中的脑啡肽酶、脯氨酰寡肽酶及其他寡肽酶水解血管紧张素 Ⅰ(Ang Ⅰ)生成[8]。而 Ang(1 ~ 7)又可通过减轻糖尿病小鼠肾脏的氧化应激反应及氧化应激诱导蛋白(NADPH 氧化酶 4,核因子 NF - E2 相关因子 2,血红素氧合酶 1)的表达,抑制肾小管上皮细胞凋亡,对肾小管上皮细胞起到部分保护作用[9]。在生理条件下,血浆 Ang(1 ~ 7)与 Ang Ⅱ、ACE 与 ACE2 水平保持平衡,而在病理条件下,Ang(1 ~ 7)及 ACE2 表达均低于正常水平[10]。本研究中阿霉素模型组大鼠血浆中 Ang(1 ~ 7)的水平明显低于正常对照组($P < 0.01$),且该组大鼠肾脏病理切片显示肾小管上皮细胞水肿、炎性细胞聚集。笔者推测这可能与阿霉素的肾脏毒性作用有关,阿霉素在体内蓄积后,可通过 ERK1/2、丝裂原活化蛋白激酶(p38 MAPK)磷酸化等信号转导途径启动内在凋亡通路及通过 NF - κB 启动外在凋亡通路引起细胞凋亡,或通过其他机制引起细胞坏死,肾小管损伤后,位于该部位的脑啡肽酶、脯氨酰寡肽酶及其他寡肽酶功能减退或失活,水解 Ang Ⅰ的能力下降,进而引起 Ang(1 ~ 7)水平降低,致使其在一定程度上对炎性反应的抑制及抗纤维化作用减弱,炎性细胞浸润增多,形成恶性循环,加剧肾功能损害[11 ~ 13]。

本研究结果显示,替米沙坦组 Ang(1 ~ 7)水平虽低于正常对照组($P < 0.05$),但高于模型组($P < 0.05$),病理切片提示替米沙坦组大鼠肾小管上皮细胞水肿程度较模型组明显减轻,细胞间隙基本正常,与正常大鼠肾脏组织相比,差异无统计学意义($P > 0.05$)。其可能的机制包括:①替米沙坦对肾脏的直接保护作用,该类药物可阻断 Ang Ⅱ 与 AT_1R 结合,使肾小球出球小动脉扩张,降低肾小球毛细血管压力,减低蛋白尿而延缓肾脏病进展。替米沙坦还可激活过氧化物酶体增殖物激活受体(PPAP - γ)起到抗炎、抗氧化作用[14];②替米沙坦间接升高血浆 Ang(1 ~ 7)的水平,早期研究证实,ARB 类药可使 ACE2 表达增加[15],而后者也是降解 Ang Ⅱ 或 Ang Ⅰ 生成 Ang(1 ~ 7)的主要酶,通过该途径,使得血浆 Ang(1 ~ 7)水平升高,经由前面所述各种机制发挥其肾脏保护效应;③Ang(1 ~ 7)与替米沙坦的协同作用;④Ang(1 ~ 7)除直接与其受体 Mas 结合外,还可能通过 AT_1R、AT_2R 及缓激肽 β_2 受体减轻 Ang Ⅱ 对机体的影响[8]。

本研究通过 ADR 的肾脏毒性作用制作大鼠肾损伤模型,经替米沙坦干预后,大鼠血浆 Ang(1 ~ 7)的水平有所升高,且肾脏炎性反应程度明显减轻,提示 Ang(1 ~ 7)与肾脏炎性反应之间可能存在某种程度的相关性,其具体机制有待于进一步研究。

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