

[文章编号] 1671-587X(2024)03-0587-09

DOI:10.13481/j.1671-587X.20240301

NLRP3炎症小体在大鼠单侧输尿管梗阻引起肾间质纤维化中的作用及其机制

阮颖新¹, 贾俊亚¹, 武占飞¹, 商文雅¹, 张鹏宇²

(1. 天津医科大学总医院肾内科, 天津 300052; 2. 国家恶性肿瘤临床医学研究中心 天津医科大学肿瘤医院输血科, 天津 300060)

[摘要] **目的:** 探讨核苷酸结合寡聚化结构域样受体蛋白3 (NLRP3) 炎症小体在大鼠单侧输尿管梗阻 (UUO) 模型肾间质纤维化中的作用, 并阐明其可能的作用机制。**方法:** 健康雄性 Wistar 大鼠 30 只随机分为假手术组 ($n=6$) 和 UUO 组 ($n=24$), 假手术组大鼠仅分离输尿管不结扎, UUO 组分别于术后 3、7 和 14 d 处死大鼠, 并按照处理时间分为 UUO 3 d 组 ($n=8$)、UUO 7 d 组 ($n=8$) 和 UUO 14 d 组 ($n=8$)。HE 染色和 Masson 染色观察各组大鼠肾组织病理形态表现, 试剂盒检测各组大鼠肾组织中丙二醛 (MDA) 水平、超氧化物歧化酶 (SOD) 活性和羟脯氨酸 (HYP) 水平, 免疫组织化学法检测各组大鼠肾组织中 α -平滑肌肌动蛋白 (α -SMA) 和转化生长因子 β 1 (TGF- β 1) 蛋白表达水平, Western blotting 法检测各组大鼠肾组织中 NLRP3 蛋白表达水平。**结果:** HE 染色, UUO 组大鼠出现明显肾小管扩张, 肾间质水肿和增宽, 可见较多炎症细胞浸润, 部分肾小管腔内可见脱落的上皮细胞。与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠 HE 染色肾间质纤维化评分均明显升高 ($P<0.05$); 与 UUO 3 d 组和 UUO 7 d 组比较, UUO 14 d 组大鼠 HE 染色肾间质纤维化评分明显升高 ($P<0.05$)。Masson 染色, UUO 组大鼠肾间质炎症细胞浸润明显, 可见明显纤维组织增生; 随 UUO 作用时间增加, 大鼠部分肾小管消失, 肾间质明显增宽, 胶原沉积逐渐增多, 皮髓交界处胶原沉积程度更加明显。与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠 Masson 染色肾间质纤维化评分均明显升高 ($P<0.05$); 与 UUO 3 d 组和 UUO 7 d 组比较, UUO 14 d 组大鼠 Masson 染色肾间质纤维化评分明显升高 ($P<0.05$)。与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠梗阻侧肾组织中 MDA 水平均明显升高 ($P<0.05$), SOD 活性明显降低 ($P<0.05$)。与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠梗阻侧肾组织中 HYP 水平均明显升高 ($P<0.05$); 与 UUO 3 d 组比较, UUO 14 d 组大鼠梗阻侧肾组织中 HYP 水平明显升高 ($P<0.05$)。免疫组织化学法, 与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠肾组织中 α -SMA 蛋白表达水平明显升高 ($P<0.05$); 与 UUO 3 d 组和 UUO 7 d 组比较, UUO 14 d 组大鼠肾组织中 α -SMA 蛋白表达水平均明显升高 ($P<0.05$); 与假手术组比较, UUO 3 d 组、UUO 7 d 组和 UUO 14 d 组大鼠肾小管上皮细胞和肾小管间质组织中 TGF- β 1 蛋白表达水平明显升高 ($P<0.05$); 与 UUO 3 d 组比较, UUO 14 d 组大鼠肾小管上皮细胞和肾小管间质组织中 TGF- β 1 蛋白表达水平明显升高 ($P<0.05$)。Western blotting 法, 与假手术组比较, UUO 7 d 组和 UUO 14 d 组大鼠肾组织中 NLRP3 蛋白表达水平均明显升高 ($P<0.05$)。**结论:** NLRP3 炎症小体在 UUO 大鼠肾纤维化过程中发挥重要作用, 其作用机制与氧化应激增加和 TGF- β 1 蛋白表达水平升高有关。

[关键词] 核苷酸结合寡聚化结构域样受体蛋白3; 氧化应激; 转化生长因子 β 1; 单侧输尿管梗阻; 肾间质纤维化

[中图分类号] R692

[文献标志码] A

[收稿日期] 2023-07-14

[基金项目] 国家重点研发计划项目 (2019YFF0216502); 天津市卫健委医学重点学科 (专科) 建设项目 (TJYXZDXK-071C)

[作者简介] 阮颖新 (1977-), 女, 黑龙江省哈尔滨市人, 主治医师, 医学博士, 主要从事肾脏病诊治方面的研究。

[通信作者] 张鹏宇, 主任技师 (E-mail: pzhang@tmu.edu.cn)

Effect of NLRP3 inflammatome in renal interstitial fibrosis induced by unilateral ureteral obstruction in rats and its mechanism

RUAN Yingxin¹, JIA Junya¹, WU Zhanfei¹, SHANG Wenya¹, ZHANG Pengyu²

(1. Department of Nephrology, General Hospital, Tianjin Medical University, Tianjin 300052, China;

2. Department of Blood Transfusion, Tumor Hospital, Tianjin Medical University, National Clinical Research Center for Cancer, Tianjin 300060, China)

ABSTRACT Objective: To discuss the effect of NOD-like receptor protein 3 (NLRP3) inflammasome on the renal interstitial fibrosis in the unilateral ureteral obstruction (UUO) model rats, and to clarify its potential mechanism. **Methods:** Thirty healthy male Wistar rats were randomly divided into sham operation group ($n=6$) and UUO group ($n=24$). The rats in sham operation group underwent the dissection of the ureter without ligation, while the rats in UUO group were sacrificed on the 3rd, 7th, and 14th days after operation, and based on the treatment duration, the rats were divided into UUO 3 d group ($n=8$), UUO 7 d group ($n=8$), and UUO 14 d group ($n=8$). HE staining and Masson staining were used to observe the pathomorphology of kidney tissue of the rats in various groups; reagent kits were used to detect the levels of malondialdehyde (MDA), activities of superoxide dismutase (SOD), and levels of hydroxyproline (HYP) in kidney tissue of the rats in various groups; immunohistochemistry method was used to detect the expression levels of α -smooth muscle actin (α -SMA) and transforming growth factor- β 1 (TGF- β 1) proteins in kidney tissue of the rats in various groups; Western blotting method was used to detect the expression levels of NLRP3 protein in kidney tissue of the rats in various groups. **Results:** The HE staining results showed significant tubular dilation, interstitial edema, and widening, with increased infiltration of inflammatory cells, and shedding of epithelial cells was seen in parts of the tubular lumina of the rats in UUO group. Compared with sham operation group, the interstitial fibrosis scores of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were significantly increased ($P<0.05$); compared with UUO 3 d group and UUO 7 d group, the interstitial fibrosis score of the rats in UUO 14 d group was significantly decreased ($P<0.05$). The Masson staining results showed that in UUO group, there was evident infiltration of inflammatory cells in the renal interstitium and a noticeable increase in fibrotic tissue proliferation; with the increasing of duration of UUO, some tubular structures disappeared, and the interstitial widened further with gradually increasing collagen deposition, particularly at the corticomedullary junction. Compared with sham operation group, the interstitial fibrosis scores of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were significantly increased ($P<0.05$); and compared with UUO 3 d and UUO 7 d groups, the interstitial fibrosis score of the rats in UUO 14 d group was significantly decreased ($P<0.05$). Compared with sham operation group, the levels of MDA in obstructed kidney tissue of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were significantly increased ($P<0.05$), and the SOD activities were significantly decreased ($P<0.05$). Compared with sham operation group, the levels of HYP in obstructed kidney tissue of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were also significantly increased ($P<0.05$); compared with UUO 3 d group, the level of HYP in obstructed kidney tissue of the rats in UUO 14 d group was significantly increased ($P<0.05$). The immunohistochemistry results showed that compared with sham operation group, the expression levels of α -SMA protein in kidney tissue of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were significant increased ($P<0.05$); compared with UUO 3 d and UUO 7 d groups, the expression levels of α -SMA protein in kidney

tissue of the rats in UUO 14 d group was significantly increased ($P < 0.05$); compared with sham operation group, the expression levels of TGF- β 1 protein in renal tubular epithelial cells and renal tubule interstitial tissue of the rats in UUO 3 d, UUO 7 d, and UUO 14 d groups were also significantly increased ($P < 0.05$); compared with UUO 3 d group, the expression levels of TGF- β 1 protein in the tubular epithelial cells and renal tubule interstitial tissue of the rats in UUO 14 d group were significantly decreased ($P < 0.05$). The Western blotting results showed that compared with sham operation group, the expression levels of NLRP3 protein in kidney tissue of the rats in UUO 7 d and UUO 14 d groups were significantly increased ($P < 0.05$). **Conclusion:** The NLRP3 inflammasome plays a critical role in renal fibrosis of the UUO rats, and its mechanism may be related to the increasing of oxidative stress and the increasing of expression level of TGF- β 1 protein.

KEYWORDS Nucleotide combined with structure of oligomerization domain receptor protein 3; Oxidative stress; Transforming growth factor β 1; Unilateral ureteral obstruction; Renal interstitial fibrosis

肾纤维化是一种常见的病理过程, 部分原发性或继发性肾脏疾病可以继续进展至终末期肾脏疾病(end stage renal disease, ESRD)。肾纤维化的发病机制尚未完全阐明, 目前有效的治疗方法较少, 且仅可缓解患者的症状^[1-4]。有多种因素可以促进肾纤维化的发展, 如炎症、细胞凋亡、氧化应激、上皮-间质转化、细胞外基质沉积和免疫反应等。核苷酸结合寡聚化结构域样受体蛋白3(nucleotide combined with structure of oligomerization domain receptor protein 3, NLRP3)炎症小体在肾脏疾病的发生发展过程中发挥重要作用。研究^[5-6]显示: 在慢性肾脏病(chronic kidney disease, CKD)或肾纤维化患者肾脏中NLRP3和含半胱氨酸的天冬氨酸蛋白水解酶(cysteiny aspartate specific proteinase, Caspase)-1蛋白表达水平明显升高, 提示NLRP3炎症小体可能被激活并参与肾纤维化的调节。在NLRP3激活过程中, 其经典或非经典信号通路均可在机体对外界或内部有害信号的免疫应答中发挥作用。本研究建立单侧输尿管梗阻(unilateral ureteral obstruction, UUO)大鼠模型, 检测NLRP3蛋白表达水平, 探讨肾组织氧化应激和转化生长因子 β 1(transforming growth factor- β 1, TGF- β 1)在NLRP3炎症小体促进大鼠肾间质纤维化中的作用, 以期为肾间质纤维化的临床研究提供参考。

1 材料与方法

1.1 实验动物、主要试剂和仪器 30只雄性成年Wistar大鼠购自北京斯贝福生物有限公司, 动物生产许可证号: SCXK(京)2019-0010, 体质量为

180~220 g。羟脯氨酸(hydroxyproline, HYP)试剂盒、丙二醛(malondialdehyde, MDA)试剂盒、超氧化物歧化酶(superoxide dismutase, SOD)试剂盒和考马斯亮蓝蛋白测定试剂盒均购自南京建成生物工程研究所, α -平滑肌肌动蛋白(α -smooth muscle actin, α -SMA)单克隆抗体购自美国Sigma公司, TGF- β 1多克隆抗体和NLRP3单克隆抗体购自上海艾比玛特生物医药有限公司, 碱性磷酸酶标记的山羊抗兔抗体、辣根过氧化物酶标记的山羊抗鼠IgM抗体、SP免疫组织化学试剂盒和DAB显色试剂盒购自北京中杉金桥生物技术有限公司, 5-溴-4-氯-3-吲哚-磷酸盐(5-bromo-4-chloro-3-indolyl-phosphate, BCIP)/硝基蓝四唑(nitrotetrazolium blue chloride, NBT)底物显色试剂盒购自北京天为时代科技有限公司。SDS-聚丙烯酰胺凝胶电泳(SDS-polyacrylamide gel electrophoresis, SDS-PAGE)仪购自美国Bio-Rad公司。

1.2 实验动物造模和分组 30只大鼠随机分为假手术组($n=6$)和UUO组($n=24$)。假手术组大鼠仅分离输尿管不结扎, UUO组分别于术后3、7和14 d处死大鼠, 并按照处理时间不同分为UUO 3 d组($n=8$)、UUO 7 d组($n=8$)和UUO 14 d组($n=8$)。按照参考文献[7]中的方法制备各组大鼠模型。腹腔注射麻醉大鼠后, 行左侧耻骨上切口, 沿左肾下极寻找左输尿管, 用4-0号丝线上下结扎两处, 然后从中剪断输尿管以防逆行性感染, 分层缝合关闭腹腔。假手术组除结扎和剪断输尿管外, 完成其他手术过程。各组大鼠分别于术后第3、7和14天处死, 留取肾组织及血液标本。

取大鼠梗阻侧肾脏,部分置于4%多聚甲醛中固定,其余置于液氮中速冻后 -80°C 冰箱保存。

1.3 HE染色和Masson染色观察各组大鼠肾组织病理形态表现 将所有大鼠标本按实验室常规方法固定和包埋,肾组织切片厚度为 $2\mu\text{m}$,分别行HE染色和Masson染色。光学显微镜下观察各组大鼠肾小球和肾小管间质组织病理形态表现。HE染色和Masson染色分别进行半定量分析并计算各组大鼠肾间质纤维化评分。随机选取20个互不重叠高倍($\times 200$)视野对大鼠肾间质纤维化程度进行半定量评估,分值为0~4分,评分标准:①肾小管无明显改变,肾间质中无或极少见炎症细胞,无纤维化组织增生计为0分;②肾小管上皮细胞轻度萎缩和变性,坏死较轻,呈灶性分布,肾间质少量炎症细胞浸润,纤维化组织增生,病变范围 $<25\%$ 计为1分;③肾小管上皮细胞中度萎缩和变性,坏死较轻,炎症细胞浸润,纤维化组织中度增生,病变范围 $25\%\sim 49\%$ 计为2分;④肾小管上皮细胞萎缩和变性,坏死较重,呈片状分布,大量弥散或聚集成灶的炎症细胞浸润,纤维化组织增生呈束状、多灶或网状成片,病变范围 $50\%\sim 75\%$ 计为3分;⑤大面积病变,病变范围 $>75\%$ 计为4分。计算10个肾组织高倍($\times 200$)视野HE染色肾间质纤维化评分,取平均值。随机选取20个互不重叠的高倍($\times 200$)视野,根据肾间质蓝色着色占全片的范围分为5级,阴性为0分,微量着色 $<25\%$ 为1分,轻度着色 $25\%\sim 49\%$ (2分),中度着色 $50\%\sim 75\%$ (3分),广泛着色 $>75\%$ (4分)。计算10个肾组织高倍($\times 200$)视野Masson染色肾间质纤维化评分,取平均值。

1.4 试剂盒检测各组大鼠肾组织中MDA水平和SOD活性 取适量大鼠肾组织称质量,剪碎并匀浆,离心后取上清液,制成 10% 和 1% 匀浆,分别按MDA和SOD试剂盒说明书方法进行操作,采用比色法检测各组大鼠肾组织中MDA水平和SOD活性。

1.5 试剂盒检测各组大鼠肾组织中HYP水平 称取大鼠肾组织 $100\sim 150\text{mg}$,置入试管中,加水解液混匀,沸水浴水解,调节pH值,取适量水解液加活性炭混匀、离心,取 1mL 上清,按照试剂盒说明书进行操作。采用分光光度计检测波长 560nm 处吸光度(A)值,并绘制标准曲线,计算各组大鼠肾组织中HYP水平。

1.6 免疫组织化学法检测各组大鼠肾组织中 α -SMA和TGF- $\beta 1$ 蛋白表达水平 石蜡包埋切片,常规脱蜡至水, $3\%\text{H}_2\text{O}_2$ 处理后抗原热修复,常规山羊血清封闭后,分别滴加一抗 α -SMA($1:100$)和TGF- $\beta 1$ ($1:100$), 4°C 孵育过夜。滴加二抗孵育,磷酸盐缓冲液(phosphate buffered saline, PBS)清洗,显色,并采用PBS缓冲液代替一抗作为阴性对照。采用半定量分析计算各组大鼠肾组织中 α -SMA和TGF- $\beta 1$ 蛋白表达免疫组织化学评分。随机选取20个互不重叠高倍($\times 200$)视野,根据肾间质阳性着色的范围评分:阴性计为0分,微量着色计为0.5分,轻度着色计为1分,中度着色计为2分,广泛着色计为3分。计算10个高倍($\times 200$)视野免疫组织化学评分,取平均值,以其代表 α -SMA和TGF- $\beta 1$ 蛋白表达水平。

1.7 Western blotting法检测各组大鼠肾组织中NLRP3蛋白表达水平 称取等量大鼠肾组织,加入裂解液裂解组织蛋白,组织匀浆,离心收集上清液。取 $100\mu\text{g}$ 蛋白与上样缓冲液混合后, 100°C 变性 5min ,取变性后NLRP3蛋白进行蛋白上样,SDS-PAGE电泳,转移至PVDF膜, 4°C 封闭过夜,分别与NLRP3单克隆抗体($1:1000$)稀释,山羊抗兔二抗孵育,碱性磷酸酶试剂盒显色。采用Image J软件分析蛋白条带灰度值,以 β -tubulin为内参,计算目的蛋白表达水平。目的蛋白表达水平=目的蛋白条带灰度值/内参蛋白条带灰度值。

1.8 统计学分析 采用SPSS 19.0统计软件进行统计学分析和图像绘制。各组大鼠肾组织HE染色和Masson染色肾间质纤维化评分,各组大鼠肾组织中MDA和HYP水平及SOD活性,TGF- $\beta 1$ 、 α -SMA和NLRP3蛋白表达水平均符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用LSD- t 检验,采用Pearson相关分析法分析各组大鼠肾组织中HYP水平与NLRP3蛋白表达水平的相关性。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 各组大鼠肾组织病理形态表现 HE染色结果显示:UUO组大鼠出现明显肾小管扩张,肾间质水肿和增宽,可见较多炎症细胞浸润,部分肾小管腔内可见脱落的上皮细胞。与假手术组比较,UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠HE染色肾间质纤维化评分均明显升高($P<0.05$);

与UUO 3 d组和UUO 7 d组比较, UUO 14 d组大鼠HE染色肾间质纤维化评分明显升高 ($P < 0.05$)。Masson染色结果显示: UUO组大鼠肾间质炎症细胞浸润明显, 可见明显纤维组织增生; 随着UUO作用时间增加, 部分肾小管消失, 肾间质明显增宽, 胶原沉积逐渐增多, 皮髓交界处胶原沉积程度更加

明显。与假手术组比较, UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠Masson染色肾间质纤维化评分均明显升高 ($P < 0.05$); 与UUO 3 d组和UUO 7 d组比较, UUO 14 d组大鼠Masson染色肾间质纤维化评分明显升高 ($P < 0.05$)。见图1和表1。

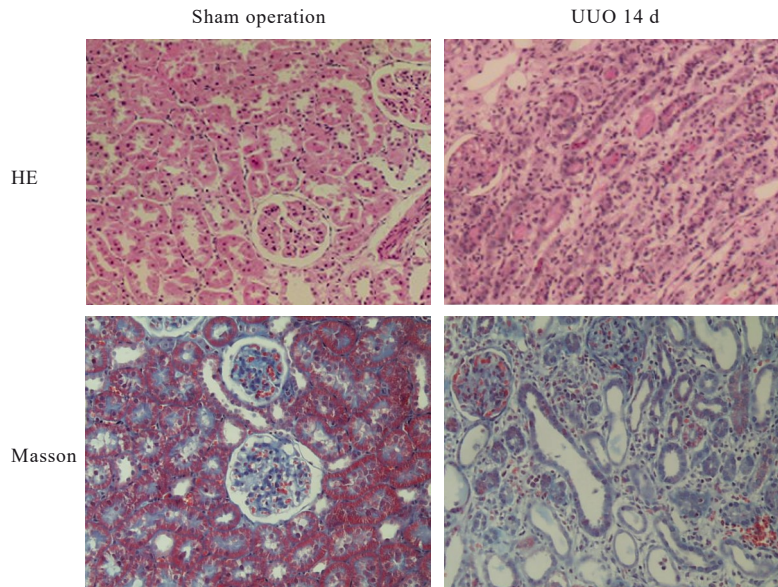


图1 HE染色和Masson染色观察2组大鼠肾组织病理形态表现($\times 400$)

Fig. 1 Pathomorphology of kidney tissue of rats in various groups observed by HE staining and Masson staining($\times 400$)

表1 各组大鼠肾间质纤维化评分

Tab. 1 Scores of renal interstitial fibrosis of rats in various groups

Group	n	Score of renal interstitial fibrosis	
		HE staining	Masson staining
Sham operation	6	0.41±0.14	0.44±0.13
UUO 3 d	8	1.42±0.40*	1.57±0.37*
UUO 7 d	8	2.01±0.45*	2.01±0.44*
UUO 14 d	8	2.68±0.53* ^{△#}	2.64±0.57* ^{△#}

* $P < 0.05$ compared with sham operation group; [△] $P < 0.05$ compared with UUO 3 d group; [#] $P < 0.05$ compared with UUO 7 d group.

2.2 各组大鼠肾组织中MDA水平和SOD活性

与假手术组比较, UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠梗阻侧肾组织中MDA水平均明显升高 ($P < 0.05$), SOD活性明显降低 ($P < 0.05$)。UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠肾组织中MDA水平和SOD活性组间比较差异均无统计学意义 ($P > 0.05$)。见表2。

表2 各组大鼠肾组织中MDA水平和SOD活性

Tab. 2 Levels of MDA and activities of SOD in kidney tissue of rats in various groups

Group	n	Level of MDA	Activity of SOD
		[$m_B/(\mu\text{mol}\cdot\text{g}^{-1})$]	[$\lambda_B/(\text{U}\cdot\text{mg}^{-1})$]
Sham operation	6	1.42±0.60	106.00±25.97
UUO 3 d	8	4.14±1.61*	74.88±21.82*
UUO 7 d	8	3.89±1.44*	55.38±18.60*
UUO 14 d	8	3.58±1.38*	49.13±15.83*

* $P < 0.05$ compared with sham operation group.

2.3 各组大鼠肾组织中HYP水平 与假手术组

[(0.19±0.07) $\text{mg}\cdot\text{g}^{-1}$] 比较, UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠梗阻侧肾组织中HYP水平 [(0.43±0.15) $\text{mg}\cdot\text{g}^{-1}$ 、(0.56±0.19) $\text{mg}\cdot\text{g}^{-1}$ 和 (0.63±0.23) $\text{mg}\cdot\text{g}^{-1}$] 均明显升高 ($P < 0.05$)。与UUO 3 d组比较, UUO 14 d组大鼠梗阻侧肾组织中HYP水平明显升高 ($P < 0.05$)。其余各组比较差异均无统计学意义 ($P > 0.05$)。

2.4 各组大鼠肾组织中 α -SMA和TGF- β 1蛋白表达水平 α -SMA蛋白主要表达于大鼠肾小管上皮细胞。假手术组大鼠肾组织中 α -SMA蛋白仅表达于肾血管平滑肌肌层, UUO 3 d组大鼠肾组织中可见 α -SMA蛋白于肾间质少量表达, UUO 7 d组和UUO 14 d组大鼠肾组织中 α -SMA蛋白表达逐渐增加, 并广泛分布于肾间质和肾小管上皮细胞, 呈广泛弥漫分布。与假手术组比较, UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠肾组织中 α -SMA蛋白表达水平明显升高 ($P<0.05$); 与UUO 3 d组和

UUO 7 d组比较, UUO 14 d组大鼠肾组织中 α -SMA蛋白表达水平均明显升高 ($P<0.05$)。假手术组大鼠肾小管间质有少量TGF- β 1蛋白表达; 与假手术组比较, UUO 3 d组、UUO 7 d组和UUO 14 d组大鼠肾小管上皮细胞和肾小管间质组织中TGF- β 1蛋白表达水平明显升高 ($P<0.05$); 与UUO 3 d组比较, UUO 14 d组大鼠肾小管上皮细胞和肾小管间质组织中TGF- β 1蛋白表达水平明显升高 ($P<0.05$)。见图2和表3。

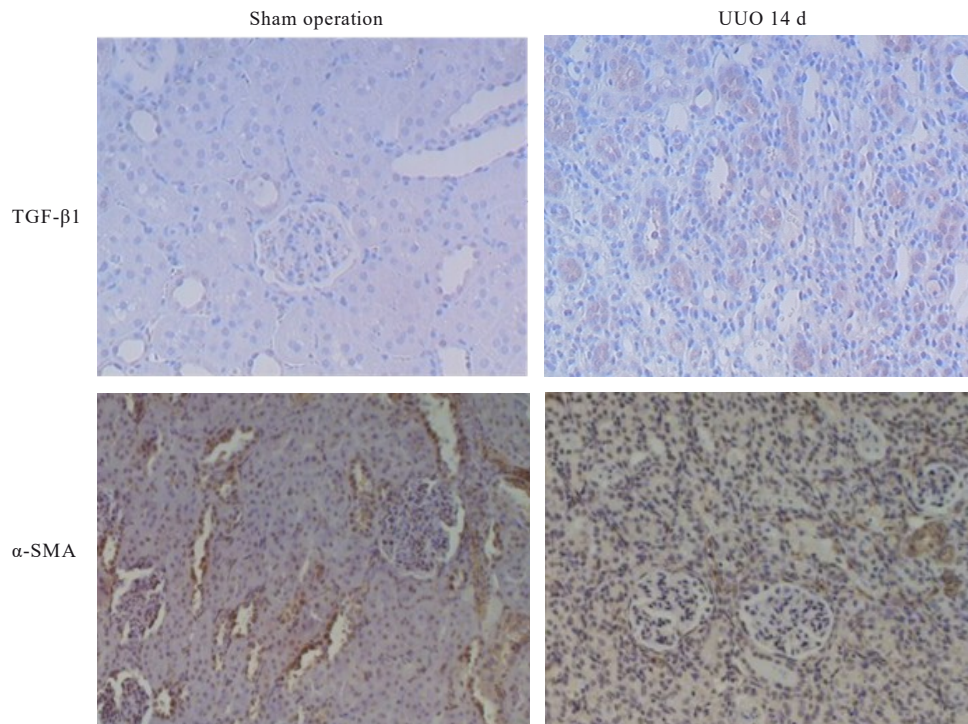


图2 免疫组织化学法检测2组大鼠肾组织中TGF- β 1和 α -SMA蛋白表达情况($\times 400$)

Fig. 2 Expressions of TGF- β and α -SMA proteins in kidney tissue of rats in two groups detected by immunohistochemistry method ($\times 400$)

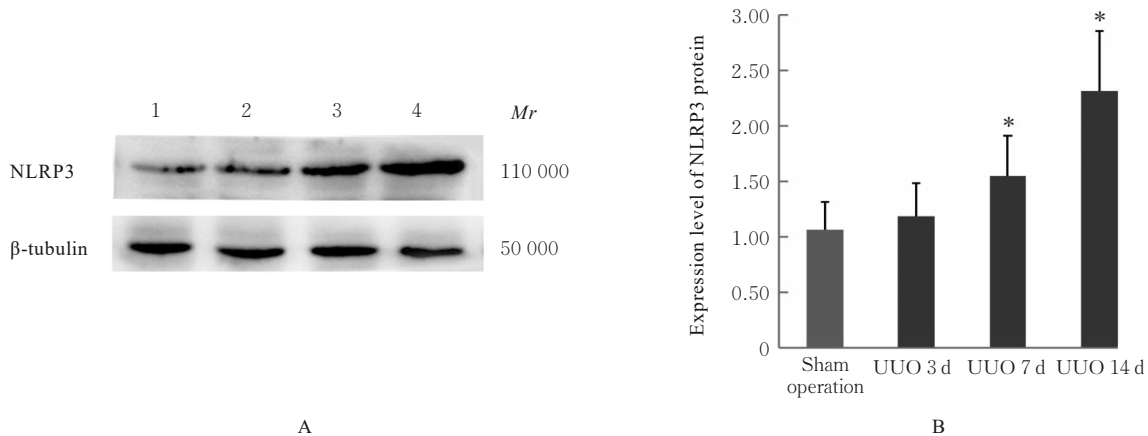
表3 各组大鼠肾组织中 α -SMA和TGF- β 1蛋白表达水平
Tab. 3 Expression levels of α -SMA and TGF- β 1 proteins in kidney tissue of rats in various groups

Group	n	α -SMA protein	TGF- β 1 protein
Sham operation	6	0.32 \pm 0.11	0.31 \pm 0.12
UUO 3 d	8	0.99 \pm 0.39*	1.42 \pm 0.40*
UUO 7 d	8	1.65 \pm 0.50*	1.84 \pm 0.57*
UUO 14 d	8	2.47 \pm 0.72* Δ #	2.20 \pm 0.68* Δ

* $P<0.05$ compared with sham operation group; $\Delta P<0.05$ compared with UUO 3 d group; # $P<0.05$ compared with UUO 7 d group.

2.5 各组大鼠肾组织中NLRP3蛋白表达水平 与假手术组比较, UUO 3 d组大鼠肾组织中NLRP3蛋白表达水平差异无统计学意义 ($P>0.05$), UUO 7 d组和UUO 14 d组大鼠肾组织中NLRP3蛋白表达水平均明显升高 ($P<0.05$)。见图3。

2.6 各组大鼠肾组织中HYP水平与NLRP3蛋白表达水平的相关性分析 Pearson相关分析结果显示: 大鼠肾组织中HYP水平与NLRP3蛋白表达水平呈正相关关系 ($r=0.453$, $P<0.05$)。



Lane 1: Sham operation group; Lane 2: UUO 3 d group; Lane 3: UUO 7 d group; Lane 4: UUO 14 d group. * $P < 0.05$ compared with sham operation group.

图3 Western blotting法检测各组大鼠肾组织中NLRP3蛋白表达电泳图(A)和直条图(B)

Fig. 3 Electrophoregram(A) and histogram(B) of expressions of NLRP3 protein in kidney tissue of rats in various groups detected by Western blotting method

3 讨论

炎症小体是由细胞质传感器、含有Caspase激活和招募结构域的凋亡相关斑点样蛋白(apoptosis-associated speck-like protein containing a CARD, ASC)或人细胞凋亡相关斑点样蛋白及pro-Caspase-1组成的多聚体复合物,在调节Caspase依赖的炎症和细胞死亡中发挥重要作用^[8-9]。研究^[10-12]发现:炎症小体在多种疾病中发挥重要作用,包括自身免疫性疾病、感染和非感染性疾病。同样在肾脏疾病中,炎症小体参与肾脏的炎症反应,导致病理改变和肾损伤,其在肾脏疾病的发生发展中起重要作用^[13]。研究^[14-15]证实:NLRP3是肾脏中最典型的炎症小体,在多种肾脏疾病中起着重要的调节作用,并影响疾病的进展。

UUO模型是研究CKD肾纤维化的常用模型,为分析肾纤维化分子和细胞作用机制提供了研究基础^[16]。UUO模型中,Caspase-1、白细胞介素(interleukin, IL)-1 β 和IL-18蛋白表达水平升高,NLRP3蛋白被激活;NLRP3型基因敲除小鼠在UUO术后肾小管损伤及纤维化程度较轻^[5]。本研究结果显示:UUO组大鼠术后3d即可见梗阻侧肾脏发生早期间质纤维化损害的表现,且随着梗阻时间延长,纤维化程度逐渐加重;HYP水平明显升高,肾间质纤维化程度更加明显;肾小管上皮细胞中 α -SMA蛋白表达水平明显升高,提示肾小管上皮细胞转化为肌成纤维细胞增加;同时NLRP3蛋白表达水平明显升高,提示NLRP3炎症小体可

能被激活,并参与肾间质纤维化的调节。

肾小管间质损伤小鼠中NLRP3炎症小体表达水平升高,而肾小管上皮细胞缺氧时,细胞中NLRP3蛋白表达水平与Caspase-1和ASC蛋白表达水平无相关性^[17-18]。与高尿酸血症大鼠肾上皮细胞中NLRP3/ASC蛋白表达水平升高和肾上皮-间质转化的研究^[19-21]结果一致。高糖、脂肪多糖和氧化应激可促进NLRP3炎症小体的组装及激活^[22-23]。氧化应激是由细胞内活性氧(reactive oxygen species, ROS)和其他自由基水平升高而引起的,部分氧化应激反应会损伤线粒体或损害其功能,如NADPH氧化酶,诱导ROS产生并激活NLRP3炎症小体^[24]。过量ROS刺激炎症小体,引起肾小管间质严重损伤和细胞凋亡^[19]。高尿酸水平通过线粒体损伤诱导NLRP3炎症小体激活,促进ROS水平升高^[20]。尿酸激活的NLRP3-ASC-Caspase-1轴局部触发炎症级联,导致高尿酸血症性肾病和肾小管损伤^[21]。本研究结果显示:UUO组大鼠术后细胞膜受到自由基损伤,脂质过氧化物标志物MDA水平明显升高。机体重要的抗氧化酶SOD活性在UUO组大鼠术后3、7和14d明显降低,提示UUO组大鼠术后有大量ROS产生。UUO组大鼠术后7和14d NLRP3蛋白表达水平明显升高,UUO模型大鼠肾小管间质损伤和肾间质纤维化程度与大鼠梗阻术后持续时间有关。上述结果提示NLRP3蛋白表达水平与氧化应激程度和肾纤维化进展有密切关联。

研究^[25]发现:肾成纤维细胞中的NLRP3蛋白

可能通过TGF- β 信号通路诱导肾小管间质纤维化。NLRP3蛋白与TGF- β 诱导的上皮-间质转化及UUO后肾纤维化有关^[5, 26]。NLRP3炎症小体可以影响TGF- β 信号通路,并独立于Caspase-1、IL-1 β 和IL-18信号通路,进而激活受体调节型Smads(receptor-regulated Smads, R-Smads)。NLRP3还可通过TGF- β 1/Smad信号在心脏成纤维细胞中调控线粒体ROS水平,TGF- β 诱导的纤维化信号在NLRP3缺陷成纤维细胞中明显减弱^[27]。本研究结果显示:UUO组大鼠术后3、7和14d肾小管上皮细胞及肾间质中TGF- β 1蛋白表达水平明显升高,提示TGF- β 1蛋白表达水平升高与NLRP3炎症小体激活并促进UUO致肾间质纤维化作用有关。

综上所述,NLRP3炎症小体在UUO大鼠肾纤维化过程中发挥重要作用,氧化应激增加和TGF- β 1蛋白表达水平升高与NLRP3炎症小体的激活密切相关,抑制或阻断NLRP3炎症小体激活对于预防和治疗肾脏疾病具有重要的临床意义。

利益冲突声明:

所有作者声明不存在利益冲突。

作者贡献声明:

阮颖新参与实验设计、实验操作、数据整理和论文撰写,贾俊亚参与实验设计和病理阅片分析,武占飞参与动物模型制作,商文雅参与动物组织标本处理和病理制片,张鹏宇参与实验设计、数据分析和论文审校。

[参考文献]

- [1] BLACK L M, LEVER J M, AGARWAL A. Renal inflammation and fibrosis: a double-edged sword[J]. *J Histochem Cytochem*, 2019, 67(9): 663-681.
- [2] HUMPHREYS B D. Mechanisms of renal fibrosis[J]. *Annu Rev Physiol*, 2018, 80: 309-326.
- [3] ZHOU T, LUO M C, CAI W, et al. Runt-related transcription factor 1 (RUNX1) promotes TGF- β -induced renal tubular epithelial-to-mesenchymal transition (EMT) and renal fibrosis through the PI3K subunit p110 δ [J]. *EBioMedicine*, 2018, 31: 217-225.
- [4] HUANG R S, FU P, MA L. Kidney fibrosis: from mechanisms to therapeutic medicines [J]. *Signal Transduct Target Ther*, 2023, 8(1): 129.
- [5] VILAYSANE A, CHUN J, SEAMONE M E, et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD[J]. *J Am Soc Nephrol*, 2010, 21(10): 1732-1744.
- [6] KE B, SHEN W, FANG X D, et al. The NLRP3 inflammasome and obesity-related kidney disease[J]. *J Cell Mol Med*, 2018, 22(1): 16-24.
- [7] 阮颖新,张鹏宇,林珊,等.内质网应激相关凋亡途径参与单侧输尿管梗阻大鼠肾间质纤维化[J]. *中华肾脏病杂志*, 2011, 27(5): 357-362.
- [8] HORTELANO S, GONZÁLEZ-COFRADÉ L, CUADRADO I, et al. Current status of terpenoids as inflammasome inhibitors [J]. *Biochem Pharmacol*, 2020, 172: 113739.
- [9] ZHANG W J, LI K Y, LAN Y, et al. NLRP3 Inflammasome: a key contributor to the inflammation formation[J]. *Food Chem Toxicol*, 2023, 174: 113683.
- [10] LIN C, JIANG Z X, CAO L, et al. Role of NLRP3 inflammasome in systemic sclerosis [J]. *Arthritis Res Ther*, 2022, 24(1): 196.
- [11] POTERE N, DEL BUONO M G, CARICCHIO R, et al. Interleukin-1 and the NLRP3 inflammasome in COVID-19: Pathogenetic and therapeutic implications[J]. *EBioMedicine*, 2022, 85: 104299.
- [12] ALLOATTI G, PENNA C, COMITÀ S, et al. Aging, sex and NLRP3 inflammasome in cardiac ischaemic disease [J]. *Vascul Pharmacol*, 2022, 145: 107001.
- [13] BRAGA T T, FORESTO-NETO O, CAMARA N O S. The role of uric acid in inflammasome-mediated kidney injury[J]. *Curr Opin Nephrol Hypertens*, 2020, 29(4): 423-431.
- [14] ARANDA-RIVERA A K, SRIVASTAVA A, CRUZ-GREGORIO A, et al. Involvement of inflammasome components in kidney disease [J]. *Antioxidants*, 2022, 11(2): 246.
- [15] HUANG G Z, ZHANG Y D, ZHANG Y Y, et al. Chronic kidney disease and NLRP3 inflammasome: Pathogenesis, development and targeted therapeutic strategies[J]. *Biochem Biophys Rep*, 2023, 33: 101417.
- [16] ARANDA-RIVERA A K, CRUZ-GREGORIO A, APARICIO-TREJO O E, et al. Redox signaling pathways in unilateral ureteral obstruction (UUO)-induced renal fibrosis[J]. *Free Radic Biol Med*, 2021, 172: 65-81.
- [17] WU M, HAN W X, SONG S, et al. NLRP3 deficiency ameliorates renal inflammation and fibrosis in diabetic mice[J]. *Mol Cell Endocrinol*, 2018, 478: 115-125.
- [18] KIM Y G, KIM S M, KIM K P, et al. The role of inflammasome-dependent and inflammasome-independent NLRP3 in the kidney[J]. *Cells*, 2019, 8(11): 1389.
- [19] MISHRA S R, MAHAPATRA K K, BEHERA B P,

- et al. Mitochondrial dysfunction as a driver of NLRP3 inflammasome activation and its modulation through mitophagy for potential therapeutics [J]. *Int J Biochem Cell Biol*, 2021, 136: 106013.
- [20] ZHUANG Y B, YASINTA M, HU C Y, et al. Mitochondrial dysfunction confers albumin-induced NLRP3 inflammasome activation and renal tubular injury [J]. *Am J Physiol Renal Physiol*, 2015, 308(8): F857-F866.
- [21] WU Y S, HE F, LI Y Q, et al. Effects of Shizhifang on NLRP3 inflammasome activation and renal tubular injury in hyperuricemic rats [J]. *Evid Based Complement Alternat Med*, 2017, 2017: 7674240.
- [22] FENG H, GU J L, GOU F, et al. High glucose and lipopolysaccharide prime NLRP3 inflammasome via ROS/TXNIP pathway in mesangial cells [J]. *J Diabetes Res*, 2016, 2016: 6973175.
- [23] LIU H X, ZHAO L, YUE L, et al. Pterostilbene attenuates early brain injury following subarachnoid hemorrhage via inhibition of the NLRP3 inflammasome and Nox2-related oxidative stress [J]. *Mol Neurobiol*, 2017, 54(8): 5928-5940.
- [24] 梁文杰, 毕建成, 许庆友. NLRP3炎症小体在慢性肾脏病的表达及中药的干预机制 [J]. *中药药理与临床*, 2016, 32(3): 208-211.
- [25] ANDERS H J, SUAREZ-ALVAREZ B, GRIGORESCU M, et al. The macrophage phenotype and inflammasome component NLRP3 contributes to nephrocalcinosis-related chronic kidney disease independent from IL-1-mediated tissue injury [J]. *Kidney Int*, 2018, 93(3): 656-669.
- [26] WANG W J, WANG X Y, CHUN J, et al. Inflammasome-independent NLRP3 augments TGF- β signaling in kidney epithelium [J]. *J Immunol*, 2013, 190(3): 1239-1249.
- [27] BRACEY N A, GERSHKOVICH B, CHUN J, et al. Mitochondrial NLRP3 protein induces reactive oxygen species to promote Smad protein signaling and fibrosis independent from the inflammasome [J]. *J Biol Chem*, 2014, 289(28): 19571-19584.