

• 基础研究 •

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网络首发 [https://link.cnki.net/urlid/50.1097.r.20240514.1640.010\(2024-05-14\)](https://link.cnki.net/urlid/50.1097.r.20240514.1640.010(2024-05-14))造影剂肾病模型中 GSH-px、SOD、MDA 及
病理组织变化研究*贾高鹏¹,陈秋雨²,皇甫卫忠³,赵子豪³,白金松⁴,杨允诺⁴,张越^{1△}(1. 内蒙古医科大学附属医院老年医学中心,呼和浩特 010030;2. 内蒙古医科大学附属医院血液科,呼和浩特 010030;3. 内蒙古医科大学附属医院全科医学科,呼和浩特 010030;
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[摘要] 目的 研究造影剂肾病(CIN)大鼠模型中血谷胱甘肽过氧化物酶(GSH-px)、超氧化物歧化酶(SOD)、丙二醛(MDA)及病理组织变化,明确氧化应激机制在 CIN 中的作用。方法 选取成年雄性 SD 大鼠 40 只,分为 3 个大组、5 个小组,造模结束后,每组取 6 只状态较好的大鼠进行实验。测定大鼠血清 SOD、GSH-px、MDA 水平;进行肾脏组织活检,比较肾脏细胞形态学变化。结果 空白对照组、对照组、实验组基线资料比较,差异均无统计学意义($P>0.05$)。空白对照组、对照组造模前、造模后 24 h、造模后 48 h 组内血清 GSH-px、SOD 及 MDA 水平比较,差异无统计学意义($P>0.05$)。实验组造模前与造模后 24、48 h 血清 GSH-px、SOD 及 MDA 水平比较,差异有统计学意义($P<0.05$);实验组造模后 24 h 和造模后 48 h 血清 GSH-px、SOD 及 MDA 水平比较,差异无统计学意义($P>0.05$)。3 组在造模后 24 h 的血清 GSH-px、SOD 及 MDA 比较,差异无统计学意义($P>0.05$)。造模后 48 h 3 组及各组两两比较血清 GSH-px、SOD 及 MDA 水平,差异有统计学意义($P<0.05$)。空白对照组及对照组病理切片显示,肾小球、肾小管及肾间质等均未见明显异常变化。实验组造模后 24 h 可见肾间质纤维化及炎症细胞浸润,但肾小管未见明显改变;48 h 后,可见明显肾小管中度灶状萎缩,上皮细胞颗粒变性及空泡样改变。结论 氧化应激机制在 CIN 中存在一定作用,造影剂主要作用于肾小管及肾间质,对于肾小球无明显损伤。

[关键词] 造影剂肾病;氧化应激;谷胱甘肽过氧化物酶;超氧化物歧化酶;丙二醛**[中图分类号]** R692**[文献标识码]** A**[文章编号]** 1671-8348(2024)15-2241-06GSH-px, SOD, MDA and pathological tissue changes
in contrast nephropathy model*JIA Gaopeng¹, CHEN Qiuyu², HUANGFU Weizhong³, ZHAO Zihao³,
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[Abstract] **Objective** To study the changes of blood glutathione peroxidase (GSH-px), superoxide dismutase (SOD), malondialdehyde (MDA) and pathological tissues in the rat contrast-induced nephropathy (CIN) model, and to determine the role of oxidation mechanism in CIN. **Methods** A total of 40 adult male SD rats were selected and divided into three big groups and five small groups. After constructing the model, six rats with good status were taken from each group for conducting the experiment. The serum GSH-px, SOD and MDA levels were measured, the renal tissue biopsy was performed and the morphological changes of kid-

ney cells were compared. **Results** There was no statistically significant difference in the baseline data among the blank control group, the control group and the experimental group ($P > 0.05$). There was no statistically significant difference in serum GSH-px, SOD and MDA levels before model construction, at 24, 48 h after model construction between the blank control group and the control group ($P > 0.05$). There were statistically significant differences in serum GSH-px, SOD and MDA levels of the experimental group between before model construction and after model construction ($P < 0.05$). There was no statistically significant difference in serum GSH-px, SOD and MDA level in the experimental group between at 24 h after modelling and 48 h after modeling ($P > 0.05$). There was no statistically significant difference in serum GSH-px, SOD and MDA levels at 24 h after modeling among the three groups ($P > 0.05$). There were statistically significant differences in serum GSH-px, SOD and MDA levels at 48 h after modeling among the three groups and their pairs ($P < 0.05$). The pathological sections of the blank control group and control group showed no obvious abnormal changes in glomeruli, renal tubule and renal interstitium. Renal interstitial fibrosis and inflammatory cell infiltration were seen after 24 h in the experimental group, but there was no obvious change in the renal tubules. After 48 h, moderate focal-like atrophy of renal tubules, epithelial cell granule degeneration and vacuolar changes were obviously seen. **Conclusion** The oxidative stress mechanism plays a role in CIN. The contrast agent acute renal injury mainly acts on the renal tubules and renal interstitium, and there is no obvious damage to the glomeruli.

[Key words] contrast nephropathy; oxidative stress; glutathione peroxidase; superoxide dismutase; malondialdehyde

造影剂肾病 (contrast induced nephropathy, CIN) 主要指在血管内注射含碘造影剂后出现的急性肾损伤, 是医源性急性肾衰竭的主要原因之一。随着经皮冠状动脉介入治疗的广泛开展, CIN 发生率逐渐升高, 已成为院内急性肾损伤的主要病因之一^[1]。目前, CIN 的发生机制尚不清楚, 可能与肾脏髓质缺血、氧化应激、血管收缩及造影剂直接不良反应有关^[2-4]。研究表明, 造影剂会降低抗氧化酶活性, 生成过多的活性氧 (reactive oxygen species, ROS), 导致氧化应激增加, 肾功能受损。此外, 髓质缺氧还会引起过多 ROS 生成, 导致线粒体过度氧化应激和线粒体功能障碍^[5]。近年来, 氧化应激反应逐渐被研究者发现、认识。造影剂肾损伤中, 氧化应激反应通过损伤肾脏细胞 DNA 片段, 影响 B 淋巴细胞瘤-2 (B-cell lymphoma-2, Bcl-2) 相关蛋白、干扰小 RNA (small interfering RNA, siRNA) 等信号通路, 可导致 ROS 生成减少和细胞凋亡, 致使肾功能下降^[6]。

1 材料与方法

1.1 材料

40 只无特殊病原体 (SPF) 级 SD 大鼠购于北京斯贝福生物技术有限公司 [许可证号: SCXK (京) 2019-0010], 7~8 周, 体重 200 g 左右。谷胱甘肽过氧化物酶 (glutathione peroxidase, GSH-px)、超氧化物歧化酶 (superoxide dismutase, SOD) 试剂盒、丙二醛 (malondialdehyde, MDA) 购于南京建成科技有限公司。

动物实验符合国家实验动物福利相关规定, 已通过内蒙古医科大学医学伦理委员会审查批准 (审批号: YKD202302009)。

1.2 方法

1.2.1 实验对象造模

取 8 只不予注射药物的大鼠纳入空白对照组, 将剩余的 32 只大鼠随机分为对照组 16 只, 实验组 16 只。大鼠自由饮水, 室温 15~25 °C, 自然采光, 实验前适应环境 1 周, 给予常规大鼠饲料喂养。禁水 48 h 后, 实验组大鼠尾静脉注射碘普罗胺 (1 mL/100 g), 建立 CIN 大鼠模型; 对照组处理方法同上, 但用生理盐水替代碘普罗胺。对照组随机分为 B 组和 C 组, 每组 8 只; 实验组随机分为 D 组和 E 组, 每组 8 只。造模结束后, 每组 [空白对照组、对照组 (B 组、C 组)、实验组 (D 组、E 组)] 取 6 只状态较好的大鼠进行实验。分别于造模前、造模后 24 h 及造模后 48 h 检测血清肌酐值, 大鼠造模成功标准: 造影后发生急性肾损伤, 通常以 48 h 内血肌酐比造影前基础水平上升 25%, 或是血肌酐上升 0.5 mg/dL 为标准^[7]。本实验造模 24 h 与 48 h 均达到标准。

1.2.2 氧化应激反应指标测定

分别于造模前经尾静脉对各组 (空白对照组、B 组、C 组、D 组、E 组) 大鼠进行采血, 于造模后 24 h 对空白对照组、对照组 (B 组)、实验组 (D 组) 大鼠进行采血, 于造模后 48 h 对空白对照组、对照组 (C 组)、实验

组(E组)大鼠进行采血。以12 000 r/min离心5 min分离血清,采用水溶性四氮唑-1(water soluble tetrazolium-1,WST-1)法检测血清SOD水平,比色法检测GSH-px水平,硫代巴比妥酸(thiobarbituric acid,TBA)法检测MDA水平。B、C组检测的各项指标水平分别为对照组造模后24、48 h的指标水平,D、E组检测的各项指标水平分别为实验组造模后24、48 h的指标水平。

1.2.3 肾脏组织病理检测

于造模后24 h完成采血后处死B组、D组大鼠,于造模后48 h完成采血后处死空白对照组、C组、E组大鼠。采用麻醉剂过量(10%水合氯醛,5 mL/kg)处死大鼠,每组选取2只标本状态优良的大鼠进行病理切片观察。打开腹腔经腹主动脉冰盐水反复冲洗,取双侧肾脏,去包膜经10%甲醛固定后石蜡包埋切片,用苏木素-伊红(hematoxylin-eosin,HE)染色,在光学显微镜下对比观察大鼠肾脏病理变化。

1.3 统计学处理

采用SPSS26.0统计学软件包建立数据库进行整理及统计学处理,正态分布计量资料以 $\bar{x} \pm s$ 表示,3组及以上比较采用方差分析,组间比较采用独立样本 t 检验,所有统计分析基于双侧假设检验,检验水准 $\alpha=0.05$,以 $P<0.05$ 为差异有统计学意义。

2 结 果

2.1 各组基线资料比较

造模前,空白对照组、对照组(B组和C组)、实验组(D组和E组)血清SOD、GSH-px、MDA水平比较,差异均无统计学意义($P>0.05$),见图1。

2.2 各组不同时间血清GSH-px、SOD及MDA水平比较

空白对照组和对照组造模前,造模后24、48 h组内血清GSH-px、SOD及MDA水平比较,差异均无

统计学意义($P>0.05$),见表1、2。实验组造模前,造模后24、48 h组内血清GSH-px、SOD及MDA水平比较,差异有统计学意义($P<0.05$),但造模后24、48 h各项指标比较差异无统计学意义($P>0.05$),见表3。

2.3 各组造模后24 h血清GSH-px、SOD及MDA水平组间比较

各组造模后24 h血清GSH-px、SOD及MDA水平比较差异均无统计学意义($P>0.05$),见图2。

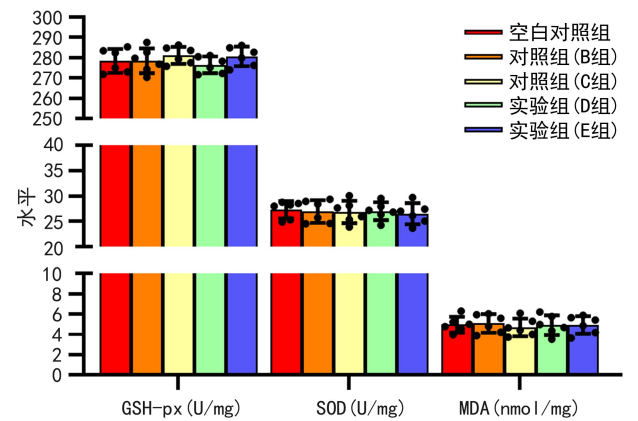


图1 基线资料水平比较

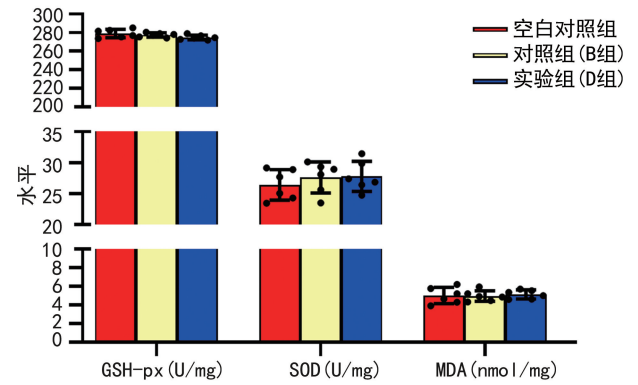


图2 各组造模后24 h血清GSH-px、SOD及MDA水平组间比较

表1 空白对照组不同时间血清GSH-px、SOD及MDA水平比较(n=6, $\bar{x} \pm s$)

项目	造模前	造模后24 h	造模后48 h	F	P
GSH-px(U/mg)	278.52±5.88	279.19±4.55	278.28±5.25	0.049	0.953
SOD(U/mg)	27.31±1.71	26.43±2.47	27.05±1.68	0.313	0.736
MDA(nmol/mg)	4.98±0.78	5.03±0.87	4.81±0.81	0.113	0.894

表2 对照组不同时间血清GSH-px、SOD及MDA水平比较(n=6, $\bar{x} \pm s$)

项目	造模前	造模后24 h	造模后48 h	F	P
GSH-px(U/mg)	279.91±5.19	276.41±2.32	274.75±2.59	3.212	0.061
SOD(U/mg)	26.91±2.11	27.61±2.53	27.79±2.44	0.365	0.698
MDA(nmol/mg)	4.90±0.87	4.97±0.58	5.14±0.18	0.220	0.805

表 3 实验组不同时间血清 GSH-px、SOD 及 MDA 水平比较($n=6, \bar{x} \pm s$)

项目	造模前	造模后 24 h	造模后 48 h	F	P
GSH-px(U/mg)	278.28±5.25	260.96±5.40	260.76±5.03 ^a	22.186	<0.001
SOD(U/mg)	27.05±1.68	20.77±2.06	21.96±1.66 ^a	20.352	<0.001
MDA(nmol/mg)	4.81±0.81	7.73±0.78	7.36±0.63 ^a	27.251	<0.001

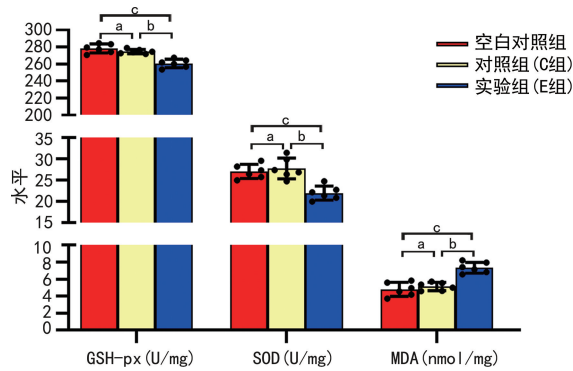
^a: $P>0.05$,与造模后 24 h 比较。

2.4 各组造模后 48 h 血清 GSH-px、SOD 及 MDA 水平组间比较

各组造模后 48 h 血清 GSH-px、SOD 及 MDA 水平比较差异均有统计学意义($P<0.05$),且组间两两比较差异有统计学意义($P<0.05$),见图 3。

2.5 各组大鼠肾脏病理变化

空白对照组和对照组肾小球、肾小管及肾间质等均未见明显异常变化。D 组可见肾间质纤维化及炎症细胞浸润,但肾小管未见明显改变,见图 4;E 组,不仅可见肾间质纤维化及炎性浸润改变,同时可见明显肾小管中度灶状萎缩,上皮细胞颗粒变性及空泡样改变,见图 5。



^a: $P<0.05$; ^b: $P<0.05$; ^c: $P<0.05$ 。

图 3 各组造模后 48 h 血清 GSH-px、SOD 及 MDA 水平组间比较

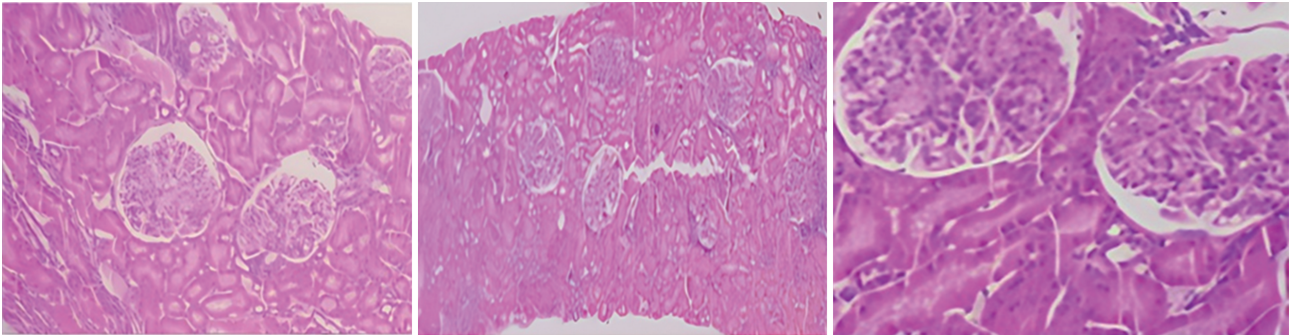


图 4 D 组肾脏病理切片(100×)

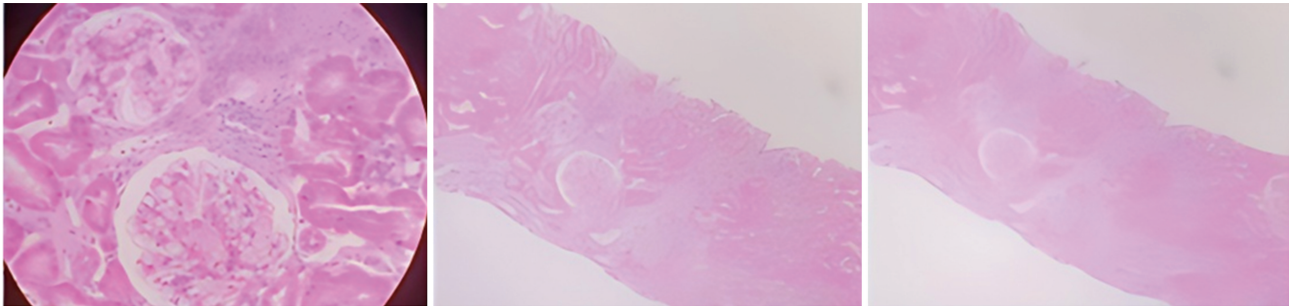


图 5 E 组肾脏病理切片(100×)

3 讨 论

CIN 是医源性急性肾衰竭的主要原因之一^[8],有必要对其进行研究并寻找有效的防治策略。目前,有关 CIN 的发病机制复杂且尚未被完全解析,仅了解其主要特征是由 ROS 形成引起的肾血管收缩、肾小管细胞毒性和髓质缺血^[4,9-10]。研究表明,急性冠状动脉综合征患者发生 CIN 的风险较高,这与冠状动脉造影

术后住院时间延长、发病率和致死率升高有关^[11-12]。CIN 发病率差异较大,对于无明显高危因素患者,其发病率为 0.6%~2.3%;对于高危患者,其发病率可高达 20.0%^[13]。肾损伤检测的新型标志物主要包括中性粒细胞明胶酶相关脂质运载蛋白、胱抑素-C、尿胰岛素样生长因子结合蛋白 7 等^[13-15]。CIN 部分可逆,约有 1%的患者需要进行透析治疗^[16],水化治疗

仍是预防 CIN 最有效的策略^[17-18]。有研究显示,生理盐水联合利尿剂及碳酸氢钠水化预防 CIN 效果优于单纯水化治疗^[19]。扩容治疗可防止肾内血流动力学不稳定,减少血管收缩和髓质缺血,采用更多液体稀释造影剂可降低其浓度和肾毒性^[20-21]。已有研究表明乙酰半胱氨酸、他汀类药物和抗坏血酸可用于 CIN 的预防^[22-25],但仍缺乏一致的数据显示其疗效。

本实验通过尾静脉注射碘普罗胺建立 CIN 模型大鼠,并测定大鼠血清 GSH-px、SOD、MDA 水平反映氧化应激发生程度,最后观察实验组和对照组大鼠肾脏病理变化情况。结果显示,造模前各组大鼠血清 GSH-px、SOD、MDA 水平比较差异无统计学意义($P>0.05$),且空白对照组造模前和造模后 24、48 h 血清 GSH-px、SOD、MDA 水平比较差异无统计学意义($P>0.05$),提示大鼠基线水平一致,注射生理盐水后大鼠状态无影响。实验组造模前和造模后 24、48 h 血清 GSH-px、SOD、MDA 水平比较差异有统计学意义($P<0.05$),且造模后 48 h 实验组与空白对照组、对照组血清 GSH-px、SOD、MDA 水平比较差异均有统计学意义($P<0.05$),提示注射造影剂后可见氧化应激指标明显变化,血清 GSH-px、SOD 水平降低,MDA 水平升高。实验组造模后 24、48 h 血清 GSH-px、SOD、MDA 水平比较差异无统计学意义($P>0.05$),其原因可能有:(1)造影剂联合禁水,导致氧化应激指标 24 h 变化达到高峰,48 h 内无明显变化;(2)造影剂剂量偏大,导致肾损伤进展较快。造模后 48 h,对照组与实验组血清 GSH-px、SOD、MDA 水平比较差异有统计学意义($P<0.05$),表明氧化应激机制与 CIN 发病有关,氧化应激反应可能参与 CIN 疾病的发生。

大鼠肾脏切片结果显示,空白对照组及对照组肾小球、肾小管及肾间质等均未见明显异常改变,而造影剂处理后实验组大鼠肾脏切片可见肾小管中度灶状萎缩,上皮细胞颗粒变性及空泡样改变,以及肾间质可见炎症细胞浸润,肾小球未见明显异常改变,表明造影剂对大鼠肾小管及肾间质有一定损伤作用,但对肾小球无明显损伤。

综上所述,造影剂可能主要作用于肾小管及肾间质细胞,对肾小球细胞无明显损伤,且氧化应激机制在 CIN 发病中发挥了一定作用。

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