

doi: 10.13241/j.cnki.pmb.2015.17.015

芪卫颗粒对 2 型糖尿病大鼠肾脏氧化应激和病理的影响

曹广超¹ 张凤梅² 张兰举³ 邢立志³ 李晓云⁴

(1 山东省临沂市妇幼保健院药剂科 山东 临沂 276000; 2 山东省临沂市肿瘤医院头颈外科 山东 临沂 276000;

3 山东省临沂市人民医院内分泌科 山东 临沂 276000; 4 山东省临沂市妇幼保健院产科 山东 临沂 276000)

摘要 目的:研究芪卫颗粒对 2 型糖尿病大鼠肾脏氧化应激和病理的影响。**方法:**先诱导 2 型糖尿病大鼠模型 50 只,按照随机数字表法将大鼠分为 A 组和 B 组,每组 25 只,另选取 25 只正常鼠为对照组;A 组给予芪卫颗粒,B 组给予 α -硫辛酸,均治疗 3 个月,检测 3 组血糖(BG)、糖化血红蛋白(HbA1c)、血脂、肾功能指标、超氧化物歧化酶(SOD)、丙二醛(MDA)、谷胱甘肽氧化酶(GSH-Px),并观察肾脏的病理变化。**结果:**A 组 BG、HbA1c 和血脂水平均显著优于 B 组,差异有统计学意义($P < 0.05$);A 组肾功能指标与对照组比较无统计学意义($P > 0.05$),A 组肾功能指标显著优于 B 组,差异有统计学意义($P < 0.05$);A 组 SOD、MDA 和 GSH-Px 均优于 B 组,差异有统计学意义($P < 0.05$);A 组肾小球病理变化显著优于 B 组,差异有统计学意义($P < 0.05$)。**结论:**芪卫颗粒对 2 型糖尿病大鼠肾脏氧化应激有一定抑制作用,能改善肾功能和病理。

关键词:糖尿病;芪卫颗粒;氧化应激;病理

中图分类号:Q95-3;R587.1;R285 文献标识码:A 文章编号:1673-6273(2015)17-3256-03

Influence of Qi Wei Particles on Kidney Oxidative Stress and Pathology in Type 2 Diabetic Rats

CAO Guang-chao¹, ZHANG Feng-mei², ZHANG Lan-ju³, XING Li-zhi³, LI Xiao-yun⁴

(1 Department of Pharmacy, Linyi Maternal and Child Health Hospital of Shandong Province, Linyi, Shandong, 276000, China;

2 Department of Head and Neck Surgery, Linyi Tumour Hospital of Shandong Province, Linyi, Shandong, 276000, China;

3 Department of Endocrinology, Linyi People's Hospital of Shandong Province, Linyi, Shandong, 276000, China;

4 Department of Obstetrics, Linyi Maternal and Child Health Hospital of Shandong Province, Linyi, Shandong, 276000, China)

ABSTRACT Objective: To study the influence of Qi Wei particles on kidney oxidative stress and pathology in type 2 diabetic rats.

Methods: 50 type 2 diabetic rats' models were first induced and then randomly divided into group A (n=25) and group B (n=25); 25 normal rats were selected as control group. Group A was given Qi Wei particles; group B were received α -lipoic acid. Both of the two groups were treated for 3 months. Blood glucose (BG), glycated hemoglobin (HbA1c), blood lipids, renal function, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) of the three groups were detected, and the pathological changes in kidney were observed. **Results:** BG, HbA1c and blood lipid levels of group A were significantly better than those of group B, with statistical significance ($P < 0.05$); Compared with control group, renal function of group A had no statistical significance ($P > 0.05$), but much better than that of group B, with statistical significance ($P < 0.05$); SOD, MDA, GSH-Px and glomerular pathological changes of group A were all significantly better than those of group B, with statistical significance ($P < 0.05$) ($P < 0.05$). **Conclusion:** Qi Wei particles has a certain inhibition to renal oxidative stress in type 2 diabetic rats, which can improve their renal function and pathology.

Key words: Diabetes; Qi Wei particles; Oxidative stress; Pathology

Chinese Library Classification(CLC): Q95-3; R587.1; R285 **Document code:** A

Article ID: 1673-6273(2015)17-3256-03

前言

糖尿病肾病是糖尿病常见的并发症,是导致糖尿病死亡的常见原因。据统计,糖尿病肾病是临床肾病的主要原因,该病的发病机制尚不明确,临床上也无显著的防治药物^[1-3]。所有研究糖尿病肾病的发病机制,选择有效的防治药物具有重要的意义。氧化应激对糖尿病肾病的发生和发展具有重要的意义,改

善氧化应激来预防糖尿病肾病具有显著作用^[4]。本研究旨在分析芪卫颗粒对 2 型糖尿病大鼠肾脏氧化应激和病理的影响,现将结果报告如下。

1 资料与方法

1.1 一般资料

先诱导 2 型糖尿病大鼠模型 50 只,按照随机数字表法将大鼠分为 A 组和 B 组,每组 25 只,A 组雌鼠 13 只,雄鼠 12 只,体重 180-230 g,平均体重(201 ± 21.3) g,月龄为 3-10 个月,平均月龄(5.2 ± 1.2)个月;B 组雌鼠 12 只,雄鼠 13 只,体重 180-230 g,平均体重(202 ± 19.4) g,月龄为 3-10 个月,平均月

作者简介:曹广超(1978-),男,本科,主管药师,从事药学与临床药学方面的研究

(收稿日期:2015-01-06 接受日期:2015-01-23)

龄(5.3± 1.1)个月,另选取 25 只正常鼠为对照组,A 组雌鼠 12 只,雄鼠 13 只,体重 180-230 g,平均体重(201± 18.9) g,月龄为 3-10 个月,平均月龄(5.3± 0.9)个月,三组雌雄、体重以及月龄比较均无显著差异($P>0.05$),具有可比性。

1.2 方法

A 组:每日给予芪卫颗粒 20 g/(kg·d)灌胃;B 组:每日给予 a- 硫辛酸 20 mg/(kg·d)灌胃;对照组:每日给予 20 g/(kg·d)的蒸馏水灌胃。连续处理 3 个月。处理结束前将老鼠放入禁食笼中留取 24 小时尿液,然后将大鼠麻醉下取腹主动脉血液,然后处理留取血清,并保存在 -70 ℃ 环境中。采用离子交换层析法测量糖化血红蛋白(HbA1c),采用全自动生化分析仪测定三酰甘油(TG)、总胆固醇(TC)、尿素氮(BUN)、血肌酐(Scr)。其中肌酐清除率(C_{cr})= 尿肌酐 / 血肌酐 × 每分钟尿量^[9]。病理检测:在麻醉下将所有入选大鼠剖腹将其左肾取出,将包膜去掉,应用戊二醛固定,然后制成薄片。剩余的肾组织放置于甲醛中固定,然后染色观察微关病理的改变,并测量肾小球平均截面积

(AG)、直接(DG),肾小球体积 $VG=1.25 \times AG^2/2^{[10]}$ 。应用 PASM 将肾组织染色并且将肾组织切片,每个标本选择 5 个肾小球,并且在每个肾小球中取出 5-8 个基底膜(GBM),然后测量取平均值。取肾组织,在冰的 PBS 液中将残留的血液洗净,并取 4.5 ml 生理盐水将其制成 10%的组织匀浆,然后离心并取上清液,应用化学比色法来测量超氧化物歧化酶(SOD)、丙二醛(MDA)、谷胱甘肽氧化物酶(GSH-Px)。

1.3 统计学方法

全部数据均在 SPSS17.0 软件上统计,其中计量资料用($\bar{x} \pm s$)表示,应用 t 检验,计数资料应用 χ^2 检验,检验标准以 $P<0.05$ 表示有统计学意义。

2 结果

2.1 三组血糖和血脂比较

由表 1 可知,A 组 BG、HbA1c 和血脂水平均显著优于 B 组,两组比较差异具有统计学意义($P<0.05$)。

表 1 三组血糖和血脂水平比较($\bar{x} \pm s$)

Table 1 Comparison of blood glucose and blood lipid levels of the three groups($\bar{x} \pm s$)

组别 Groups	例数 n	BG(mmol/L)	HbA1c(%)	TC (mmol/L)	TG (mmol/L)
对照组 Control group	25	5.03± 0.02	5.19± 0.23	1.42± 0.14	0.81± 0.13
A 组 Group A	25	13.02± 0.32 ^a	9.03± 0.01 ^a	1.64± 0.32 ^a	1.39± 0.35 ^a
B 组 Group B	25	19.32± 0.42 ^b	13.23± 1.03 ^b	2.01± 0.42 ^b	1.62± 1.01 ^b

注:A 组与对照组比较, $t=10.324, 11.042, 9.895, 9.968$, $^aP=0.019, 0.013, 0.019, 0.025$; B 组与对照组比较, $t=11.542, 12.073, 10.982, 9.073$, $^bP=0.012, 0.011, 0.015, 0.021$; A 组与 B 组比较, $t=9.708, 9.987, 8.096, 8.964$, $^aP=0.018, 0.021, 0.026, 0.028$ 。

Note: Group A compared with the control group, $t=10.324, 11.042, 9.895, 9.968$, $^aP=0.019, 0.013, 0.019, 0.025$; Group B compared with the control group, $t=11.542, 12.073, 10.982, 9.073$, $^bP=0.012, 0.011, 0.015, 0.021$; Group A compared with Group B, $t=9.708, 9.987, 8.096, 8.964$, $^aP=0.018, 0.021, 0.026, 0.028$ 。

2.2 三组肾功能指标比较

由表 2 可知,A 组肾功能指标与对照组比较无统计学意义

($P>0.05$),A 组肾功能指标显著优于 B 组,两组比较差异具有统计学意义($P<0.05$)。

表 2 三组肾功能指标比较($\bar{x} \pm s$)

Table 2 Comparison of renal function of the three groups($\bar{x} \pm s$)

组别 Groups	例数 n	BUN (mmol/L)	Scr (mmol/L)	Ccr (ml/min)
对照组 Control group	25	8.04± 0.21	68.34± 0.13	1.03± 0.01
A 组 Group A	25	8.19± 0.14 ^a	70.23± 0.12 ^a	0.99± 0.13 ^a
B 组 Group B	25	9.32± 0.21	80.32± 0.42	0.79± 0.21

注:A 组与 B 组比较, $t=9.032, 8.982, 9.327$, $^aP=0.017, 0.023, 0.021$ 。

Note: Group A compared with Group B, $t=9.032, 8.982, 9.327$, $^aP=0.017, 0.023, 0.021$ 。

2.3 三组氧化应激比较

由表 3 可知,A 组 SOD、MDA 和 GSH-Px 均优于 B 组,两

组比较差异具有统计学意义($P<0.05$)。

表 3 三组氧化应激比较($\bar{x} \pm s$)
Table 3 Comparison of oxidative stress of the three groups($\bar{x} \pm s$)

组别 Groups	例数 n	SOD($U \cdot mg^{-1} \cdot Pr^{-1}$)	GSR-Px($U \cdot mg^{-1} \cdot Pr^{-1}$)	MDA($nmol \cdot mg^{-1} \cdot Pr^{-1}$)
对照组 Control group	25	32.93 $\pm$ 1.01	2.38 $\pm$ 0.05	1.84 $\pm$ 0.01
A 组 Group A	25	30.24 $\pm$ 0.89 ^a	2.03 $\pm$ 0.13 ^a	2.16 $\pm$ 0.24 ^a
B 组 Group B	25	23.09 $\pm$ 0.03	1.34 $\pm$ 0.22	3.49 $\pm$ 1.04

注:A 组与 B 组比较, $t=10.345, 11.052, 9.697, ^aP=0.018, 0.023, 0.021$ 。

Note: Group A compared with Group B, $t=10.345, 11.052, 9.697, ^aP=0.018, 0.023, 0.021$.

2.4 三组病理检测比较

较差异具有统计学意义($P < 0.05$)。

由表 4 可知,A 组肾小球病理变化显著优于 B 组, 两组比

表 4 三组病理检测结果比较($\bar{x} \pm s$)
Table 4 Comparison of pathology test results of the three groups($\bar{x} \pm s$)

组别 Groups	例数 n	DG(μm)	VG	GBM(mm)
对照组 Control group	25	75.83 $\pm$ 2.03	450.42 $\pm$ 2.04	149.89 $\pm$ 0.29
A 组 Group A	25	79.03 $\pm$ 1.13 ^a	512.45 $\pm$ 8.02 ^a	178.98 $\pm$ 2.98 ^a
B 组 Group B	25	89.43 $\pm$ 0.42	672.4 $\pm$ 2.32	198.89 $\pm$ 3.86

注:A 组与 B 组比较, $t=11.529, 12.839, 11.067, ^aP=0.013, 0.011, 0.017$ 。

Notes: Group A was compared with Group B, $t=11.529, 12.839, 11.067, ^aP=0.013, 0.011, 0.017$.

3 讨论

许多研究均显示较高的血糖以及氧化应激和糖尿病肾病直接存在较大的相关关系^[7,9]。其中氧化应激状态是引起糖尿病肾病发生和发展的重要因素,是糖尿病肾病研究的一个新的突破,为临床了解糖尿病肾病提供了基础,也为预防提供一定的依据^[10]。糖尿病肾病的患者和糖尿病的试验动物模型均具有较强烈的氧化应激反应,当机体出现氧化和抗氧化系统紊乱时,其氧化的能力会显著升高,甚至超过抗氧化的能力,进而引起肾脏组织的氧化损伤^[11-13]。所以,应用抗氧化剂治疗糖尿病患者能改善机体的氧化应激状态,进而减轻其氧化损伤,起到保护肾脏功能的作用。可以缓解糖尿病患者肾脏功能的衰减作用,可以作为临床治疗糖尿病肾病的新方法^[14,15]。

本研究发现,A 组和 B 组大鼠的血糖相比对照组显著升高,Scr 和 Ccr 均高于对照组,提示大鼠出现肾脏功能损伤,同时研究发现,A 组和 B 组大鼠肾脏的抗氧化酶作用显著高于对照组,提示大鼠肾脏组织中存在较明显的氧化应激反应,处于较高的氧化应激状态。且病理结果证实,A 组和 B 组肾小球出现肥大,肾小球出现基底膜增厚等,提示肾脏存在较明显的病理改变。芪卫颗粒是由黄芪、地黄、大黄以及卫矛等等药物组成,具备益气养阴和化痰通络的作用。临床上可以用来早期或者临床期的糖尿病肾病,且安全有效^[16]。现代药理研究发现,黄

芪可以抗氧化,降低血糖和减轻肾脏损伤的作用,可以修复损伤的肾脏组织,进而改善肾脏功能^[17]。地黄可以降低血脂和血糖作用,具有抗氧化的作用。卫矛可以降低血脂和血糖的作用,且具有抗氧化的作用,能抑制自由基的作用。大黄还可以改善脂质代谢,能够抑制氧自由基,进而抑制系膜细胞的增殖作用,使肾脏的血流变学改善。各种药物组合能显著改善脂质代谢情况,抑制氧自由基和系膜细胞增生,改善肾脏功能,修复病理损伤组织^[18,19]。本研究发现,A 组 BG、HbA1c 和血脂水平均显著优于 B 组,提示芪卫颗粒对 2 型糖尿病大鼠的血糖和血脂有较好的调节作用,A 组肾功能指标显著优于 B 组,且 A 组 SOD、MDA 和 GSH-Px 均优于 B 组,A 组肾小球病理变化显著优于 B 组,和其他研究结果具有一致性^[20],提示芪卫颗粒治疗 2 型糖尿病大鼠能显著改善大鼠的肾脏功能和病理改变,改善大鼠的氧化应激状态。

综上所述,芪卫颗粒治疗 2 型糖尿病大鼠具有较好的效果,能显著改善大鼠的肾脏氧化应激状态,改善大鼠病理改变。为临床治疗糖尿病或者糖尿病肾病提供新疗法,进而延缓对患者肾脏损伤作用。

参考文献(References)

- [1] Ruiz SI, Pergola PE, Zager RA, et al. Targeting the Transcription Factor Nrf2 to Ameliorate Oxidative Stress and Inflammation in Chronic Kidney Disease[J]. Kidney Int, 2013, 83(6): 1029-1041

- 病理学变化的影响[J]. 解放军医学杂志, 2009, 34(5): 633-635
- Huang Chang-lin, Zhu Lv-gang. Influence of the percutaneous impulsive current stimulation of hepatic region on the hepatic tissue of exercise-induced fatigue rats [J]. Medical Journal of Chinese People's Liberation Army, 2009, 34(5): 633-635
- [13] Dishman RK, Renner KJ, White-Welkey JE, et al. Treadmill exercise training augments brain norepinephrine response familiar and novel stress[J]. Brain Res Bull, 2000, 52(5): 337-342
- [14] Blomstrand, E, F, CelsingandE, A. Newsholme Changes in plasma concentrations of aromatic and branch-chain amino acids during sustained exercise in man and their possible role in fatigue [J]. Acta Physiol Scand, 1988, 133(1): 115-121
- [15] Reeves PC, Nielsen FH. ALN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet [J]. Nutr, 1993, 123 (11): 1939-1951
- [16] DavisJM, Alderson NL, Welsh RS. Serotonin and central nervous system fatigue: nutritional considerations [J]. AM J Clin Nutr, 2000, 72(2): 573-578
- [17] Wisnik P, Chmura J, Ziemba A W, et al. The effect of branched chain amino acids on psychomotor performance during treadmill exercise of changing intensity simulating a soccer game [J]. Appl Physiol Nutr Metab, 2011, 36(6): 856-862
- [18] Hsu M C, Chien K Y, 'Hsu C C, et al. Effects of BCAA arginine and carbohydrate combined drink on post-exercise biochemical response and psychological condition[J]. Chin J Physiol, 2011, 54(2): 71-78
- [19] Wagenmakers AJM, Brookes JH, Coakley JH, et al. Exercise-induced activation of the branched chain 2-oxoacid dehydrogenase in human muscle[J]. Eur J Physiol, 1989, 59(3): 159-167
- [20] Richard Green A. Neuropharmacology of 5-hydroxytryptamine [J]. Br J Pharmacol, 2006, 47(1): 145-152
- [21] Bailey SP, Davis JM, Ahlborn EN. Neuroendocrine and substrate response to alter brain 5-HT activity during exercise to fatigue [J]. Appl Physiol, 1993, 74(6): 3006-3012
- [22] 刘蓓蓓, 张蕴琨. 不同周期训练对大鼠纹状体 5-HT 含量及代谢的影响[J]. 中国运动医学杂志, 2010, 29(5): 547-549
- Liu Bei-bei, Zhang Yun-kun. Effect of Different Training Durations on the Concentration of 5-HT and Its Metabolism in Striatum of Rats [J]. China Sports Med, 2010, 29(5): 547-549
- (上接第 3258 页)
- [2] Mo C, Dai Y, Kang N, et al. Ectopic Expression of Human MutS Homologue 2 on Renal Carcinoma Cells Is Induced by Oxidative Stress with Interleukin-18 Promotion via p38 Mitogen-activated Protein Kinase (MAPK) and c-Jun N-terminal Kinase (JNK) Signaling Pathways[J]. J Biol Chem, 2012, 287(23): 19242-19254
- [3] Perl A. Oxidative stress in the pathology and treatment of systemic lupus erythematosus[J]. Nat Rev Rheumatol, 2013, 9(11): 674-686
- [4] Daffu G, del Pozo CH, O'Shea KM, et al. Radical Roles for RAGE in the Pathogenesis of Oxidative Stress in Cardiovascular Diseases and Beyond[J]. Int J Mol Sci, 2013, 14(10): 19891-19910
- [5] Styskal J, Van Remmen H, Richardson A, et al. Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models?[J]. Free Radic Biol Med, 2012, 52(1): 46-58
- [6] Bhatia K, Elmarakby AA, El-Remessy AB, et al. Oxidative stress contributes to sex differences in angiotensin II-mediated hypertension in spontaneously hypertensive rats [J]. Am J Physiol Regul Integr Comp Physiol, 2012, 302(2): R274-282
- [7] Jaime González, Nicolás Valls, Roberto Brito, et al. Essential hypertension and oxidative stress: New insights [J]. World J Cardiol, 2014, 6(6): 353-366
- [8] Reis GS, Augusto VS, Silveira AP, et al. Oxidative-stress biomarkers in patients with pulmonary hypertension [J]. Pulm Circ, 2013, 3(4): 856-861
- [9] Erdélyi K, Pacher P, Virág L, et al. Role of poly (ADP-ribosylation) in a 'two-hit' model of hypoxia and oxidative stress in human A549 epithelial cells in vitro[J]. Int J Mol Med, 2013, 32(2): 339-346
- [10] Tapia E, Cristóbal M, García-Arroyo FE, et al. Synergistic effect of uricase blockade plus physiological amounts of fructose-glucose on glomerular hypertension and oxidative stress in rats [J]. Am J Physiol Renal Physiol, 2013, 304(6): F727-736
- [11] Mathis KW, Venegas-Pont M, Masterson CW, et al. Oxidative stress promotes hypertension and albuminuria during the autoimmune disease systemic lupus erythematosus [J]. Hypertension, 2012, 59(3): 673-679
- [12] Rubattu S, Mennuni S, Testa M, et al. Pathogenesis of Chronic Cardiorenal Syndrome: Is There a Role for Oxidative Stress?[J]. Int J Mol Sci, 2013, 14(11): 23011-23032
- [13] Pitocco D, Tesaro M, Alessandro R, et al. Oxidative Stress in Diabetes: Implications for Vascular and Other Complications [J]. Int J Mol Sci, 2013, 14(11): 21525-21550
- [14] Borrego S, Vazquez A, Dasí F, et al. Oxidative Stress and DNA Damage in Human Gastric Carcinoma: 8-Oxo-7'-8-dihydro-2'-deoxyguanosine (8-oxo-dG) as a Possible Tumor Marker[J]. Int J Mol Sci, 2013, 14(2): 3467-3486
- [15] Radak Z, Zhao Z, Koltai E, et al. Oxygen Consumption and Usage During Physical Exercise: The Balance Between Oxidative Stress and ROS-Dependent Adaptive Signaling[J]. Antioxid Redox Signal, 2013, 18(10): 1208-1246
- [16] Magenta A, Greco S, Gaetano C, et al. Oxidative Stress and MicroRNAs in Vascular Diseases [J]. Int J Mol Sci, 2013, 14(9): 17319-17346
- [17] Aroor AR, Mandavia C, Ren J, et al. Mitochondria and Oxidative Stress in the Cardiorenal Metabolic Syndrome [J]. Cardiorenal Med, 2012, 2(2): 87-109
- [18] Starke RM, Chalouhi N, Ali MS, et al. The Role of Oxidative Stress in Cerebral Aneurysm Formation and Rupture [J]. Curr Neurovasc Res, 2013, 10(3): 247-255
- [19] Chhunchha B, Fatma N, Kubo E, et al. Curcumin abates hypoxia-induced oxidative stress based-ER stress-mediated cell death in mouse hippocampal cells (HT22) by controlling Prdx6 and NF-κB regulation[J]. Am J Physiol Cell Physiol, 2013, 304(7): C636-655
- [20] Rogers NM, Stephenson MD, Kitching AR, et al. Amelioration of renal ischaemia-reperfusion injury by liposomal delivery of curcumin to renal tubular epithelial and antigen-presenting cells [J]. Br J Pharmacol, 2012, 166(1): 194-209