Experimental Study of Periostracum Cicadae and Bombyx Batryticatus in the Treatment of Mesangial Proliferative Glomerulonephritis of Rats*

WANG Hui-hui[1], BAO Hong[1], YU Jun-sheng[2], LU Hong[2], ZHAO Zong-qin[2]

(1The Medical College Of Qingdao University Qingdao 266021;2 TCM Hospital of Qingdao, Qingdao,266033)

ABSTRACT Objective: To investigate the effects of periostracum cicadae and bombyx batryticatus on the treatment of mesangial proliferative glomerulonephritis (MsPGN) of Rats, and to explore its mechanism. Methods: The models of MsPGN rats were established by ameliorated chronic disease serum methods. Rats were randomly divided into model control group, high periostracum cicadae group, low periostracum cicadae group, high bombyx batryticatus group, low bombyx batryticatus group, and normal control group. Five weeks and eight weeks later, urinary protein were detected. Pathological changes were detected by hematoxylin-eosin staining (HE), and the expression levels of TGF-β1 were detected by immunohistological method. Results: Compared with that in the control group, 24h urinary protein decreased significantly in both high groups after five weeks ($P<0.05$), and the levels of expressions of TGF-β1, 24h urinary protein and serum cholesterol decreased significantly in every drugs groups, and serum albumin increased significantly in high and low periostracum cicadae group ($P<0.05$) after eight weeks. There was no significant difference between high and low groups about the levels of expressions of TGF-β1. Conclusions: Periostracum cicadae and bombyx batryticatus may decrease 24h urinary protein and improve lipid metabolism in MsPGN rats, and it may be related with the inhibit of TGF-β1 over-expression.

Key words: Periostracum cicadae; Bombyx batryticatus; Mesangial proliferative glomerulonephritis; 24-hour urine protein; Transforming growth factor β1

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Introduction

Mesangial proliferative glomerulonephritis is a pathological diagnosis, a glomerular nephritis with glomerular mesangial cell proliferation and (or) an increase of mesangial matrix, and the normal capillary wall showed by light microscope. According to our epidemiological survey, the incidence of primary glomerulonephritis was 76.8% to 81.7% in primary glomerular diseases, MsPGN was the most common pathological type in primary glomerular diseases, accounting for 29.7% to 59.6%, and it was one of the main factors leading to renal failure [1-3]. The clinical application of medicines of dispelling wind and dredging channels such as periostracum cicada and bombyx batryticatus to treat MsPGN had gained certain curative effect, but the two drugs in the prevention and control of MsPGN mechanism research report has not been seen. This study was to investigate the effect of periostracum cicada and bombyx batryticatus on the MsPGN rats established by ameliorated chronic disease serum methods on 24h urine protein and kidney tissues TGF-β1.

1 Materials and methods

1.1 Materials

1.1.1 Experimental animal Seventy male wistar rats, with weight ranging from 180 to 200g, were provided by Qingdao Coastal Institute for Drug Control People's Republic of China, certificate of qualified animals: SLXK<lu>20090002.

1.1.2 Drugs and Reagents Periostracum cicadae, Bombyx batryticatus granules were purchased from China Resources Sanjiu Medical & Pharmaceutical Co., Ltd.. Complete Freund's adjuvant was SIGMA company products. Bovine Serum Albumin (BSA) was Roche products.TGF-β1 Immunohistochemistry kit was purchased from Wuhan Boster Biological Technology LTD.

1.2 Experimental methods

1.2.1 The method of establishing model Seventy male wistar rats were adopted and model of MsPGN was made with immunization[4-5], other 10 rats were randomly choosed as normal group. Specific methods were as follows: Surgery: Rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (3mL/kg). Right kidney was removed, and the rats had a rest for 1w before Pre-immune. Pre-immune: Rats were injected subcutaneously with 0.1mL complete Freund's adjuvant plus 3mg BSA, the injection was enhanced at the end of 1w and 2w. At the end of 3w, rats were injected subcutaneously with BSA at the dose of 0.5mg, 1.0mg, 1.5mg, 3.0mg, and the interval time was 1h. The injection was enhanced in next morning (2.0mg). Official immunity: After pre-immune rats were injected subcutaneously each day with BSA, and the dose increased to 5.0mg by 0.5mg, and the dose increased 0.5mg each day. The dose increased to 10.0mg by 5.0mg, and the dose increased 1.0mg each week. Normal control rats were injected with the corresponding dose of physiological saline during the

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Author introduction:WANG Hui-hui(1985-), female, master, mainly engaged in kidney disease,

E-mail:taoyao1900@126.com

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pre-immune and official immune.

1.2.2 Experimental groups The 24h urine protein was detected by turbidimetric method at the end of the fifth week after beginning of establish models. Three rats were negative and excluded, and the remaining rats were randomly divided into model control group(11), high periostracum cicadae group(12), low periostracum cicadae group(11), high bombyx batryticatus group(12), low bombyx batryticatus group(11).

1.2.3 Administration methods Rats were administered with the method of oral gavage in the sixth week after beginning of establishing models once a day, and the gavage continued for 8w. High and low periostracum cicadae groups were administered with periostracum cicadae granule 2mL [crude drug 3.5g / (kg.d) and 1.75g / (kg.d)]. High and low bombyx batryticatus groups were administered with bombyx batryticatus granule 2mL [crude drug 3.5g / (kg.d) and 1.75g / (kg.d)]. Model and normal control group were administered with normal saline.

1.2.4 Targets The 24h urine protein was detected at the end of the fifth and eighth week after administration. All rats were anesthetized by intraperitoneal injection of 10% chloral hydrate after administration of 8w.Bdominal aortic blood was taken from rats, and it was 2ml. Albumin (ALB), urea nitrogen (BUN) and serum total cholesterol (TC) in abdominal aortic blood was detected by AU800 automatic biochemical analyzer. Rats were killed, and the left kidney was cut, capsule was removed, a portion of kidney tissue was taken to microscope slide conventionally for HE staining. Renal morphological changes and histopathological changes were observed by light microscopy. The kidney tissue was fixed by 10% neutral formalin, paraffin sections was done (5μm), sections were dewaxed conventional to water, 1:200 rabbit anti-mouse TGF-β1 was added. Sections were set in 37℃ for 2 hours, two anti-goat anti-rabbit IgG was added. Sections were set for 30min, SABC was added, Sections were set for 20min, DAB stain was added, hematoxylin was added, and sections were mounted by rubber. 0.01M PBS was instead of primary antibody as negative control. The sections were analysised by Image Pro Plus-6.0 color image analysis software. The level of TGF-β1 expression was demonstrated by the average optical density.

1.2.5 Statistical analysis Values are represented as means ± standard deviation. Statistical was analysised by SPSS 17.0.Statistical analysis of differences between the groups were performed by ANOVA. Differences at $P<0.05$ were considered statistically significant and differences at $P<0.01$ were considered prominent and statistically significant.

2 Result

2.1 Result of periostracum cicadae and bombyx batryticatus influenced 24h urinary protein in the MsPGN rats

24h urine protein increased significantly by a big margin in the model control group after the establishing of 5w. After the administration of 5w, compared with that in the model control group, 24h urine protein decreased in both high dose groups, and the differences were different ($P<0.01$); 24h urinary protein continued to increase in the model control group rats after the administration of 8w, and 24h urine protein decreased significantly in each groups (Table 1).

Table1 Result of periostracum cicadae and bombyx batryticatus influenced 24h urinary protein in the MsPGN rats (g/L, $\bar{x}\pm s$)

Groups	n	5w (After establishmodel)	n	5w (After adminis-tration)	n	8w (After adminis-tration)
Normal control group	10	4.86± 1.30	10	4.64± 0.96	10	4.73± 1.29
Model control group	11	10.00± 1.22*	10	23.83± 1.30*	9	32.94± 2.60*
High periostracum cicadae group	12	9.19± 1.41*	11	18.24± 2.16*△	11	20.24± 2.29*△
Low periostracum cicadae group	11	9.78± 2.04*	11	22.91± 1.46*	11	25.28± 2.22*△
High bombyx batryticatus group	12	10.13± 1.79*	12	19.34± 1.41*△	12	20.45± 2.16*△
Low bombyx batryticatus group	11	9.55± 1.99*	10	22.83± 1.80*	10	24.44± 3.20*△

Note:Compared with normal control group, *P<0.01; Compared with model control group, △P<0.01.

2.2 Result of periostracum cicadae and bombyx batryticatus influenced ALB, TC, BUN in the blood of MsPGN rats

Compared with that in the normal control group, ALB decreased significantly, TC and BUN increased significantly in the model control group. Compared with that in the model control group, TC decreased significantly in every dose groups; ALB increased significantly in periostracum cicadae groups; BUN decreased significantly in the high periostracum cicadae group and the differences were significant differences ($P<0.01$) (Table 2).

2.3 Result of periostracum cicadae and bombyx batryticatus influenced pathological changes in the kidney tissue of MsPGN rats

Glomerular structure and size was normal in the normal control group (Fig.1A). Part of the glomerular body capillary dilatated,

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and mesangial cells and matrix had diffuse hyperplasia from moderate to severe(Fig.2A). Glomerular capillary congested mildly, focal had stage expansion, glomerular mesangial cells increased, matrix congested mildly(mesangial width did not exceed the capillary lumen), the capillary wall did not thicken in high periostracum cicadae bombyx batryticatus groups(Fig.1C, Fig.1D).

2.4 Result of periostracum cicadae and bombyx batryticatus influenced the levels of expressions of TGF-β1 in the kidney tissue of MsPGN rats

Positive reaction materia of TGF-β1 in epithelial cells and interstitial cell cytoplasm in uriniferous tubules were yellow particles, and small amounts particles existed in the normal control group (Fig.2A). Strong expression of TGF-β 1 existed in the model control group(Fig.2B). Compared with that in the model control group, the distribution of TGF-β1 in kidney tissue cytoplasm decreased significantly in each dose groups (Fig. 2C, Fig. 2D), the differences were different (P <0.01), and there were no significant differences in periostracum cicadae and bombyx batryticatus groups (Table 3).

Table 2 Result of periostracum cicadae and bombyx batryticatus influenced ALB,TC, BUN in the MsPGN rats (g/L, $\bar{x}\pm s$)

Groups	n	ALB (g/l)	TC (mmol/L)	BUN (mmol/L)
Normal control group	10	25.71± 1.38	1.47± 0.13	8.94± 0.17
Model control group	9	21.71± 1.11*	2.16± 0.19*	11.63± 0.96*
High periostracum cicadae group	11	24.86± 1.35*△	1.84± 0.17*△	10.13± 1.04*△
Low periostracum cicadae group	11	24.57± 1.13*△	1.77± 0.16*△	10.90± 1.04*
High bombyx batryticatusgroup	12	22.29± 0.76*	1.74± 0.10*△	10.83± 0.93*
Low bombyx batryticatus group	10	21.86± 1.07*	1.83± 0.16*△	11.37± 0.79*

Note:Compared with normal control group, *P<0.01; Compared with model control group, △P<0.01.

Table 3 Result of periostracum cicadae and bombyx batryticatus influenced the levels of expressions of TGF-β1 in the kidney tissue of rats ($\bar{x}\pm s$)

Groups	n	TGF-β1
Normal control group	10	0.203± 0.030
Model control group	9	0.354± 0.030*
High periostracum cicadae group	11	0.298± 0.015*△
Low periostracum cicadae group	11	0.301± 0.016*△
High bombyx batryticatusgroup	12	0.299± 0.013*△
Low bombyx batryticatus group	10	0.304± 0.014*△

Note: Compared with normal control group, *P<0.01; Compared with model control group, △P<0.01.

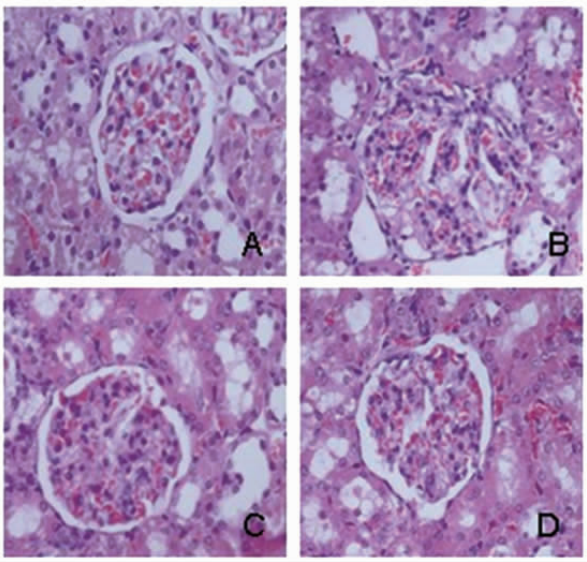

Fig. 1 Pathological changes in kidney in the groups (HE, × 400) :A: normal control group ;B: model control group ;C: high periostracum cicadae group; D: high bombyx batryticatus group

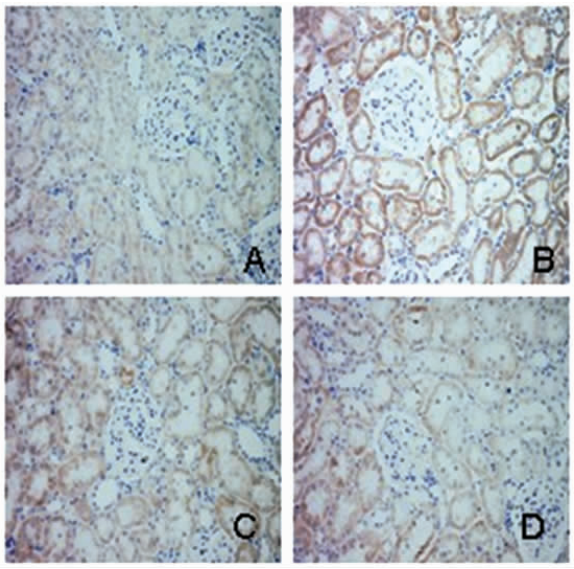

Fig. 2 The expression of TGF-β 1 in the kidney tissue of rats:A:normal control group ;B: model control group; C: high periostracum cicadae group ;D high bombyx batryticatus group

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3 Discussion

MsPGN is the most common pathological type in glomerular diseases. The main pathological characteristics of MsPGN is glomerular mesangial cell proliferation and (or) an increase of mesangial matrix [6]. Patients showed hematuria, proteinuria, nephrotic syndrome, occult nephritis and other clinical manifestations. If you can not control the disease effectively, MsPGN progress will lead to the occurrence of glomerular sclerosis, and ultimately kidney failure[7].

Traditional Chinese medicine deemed that MsPGN pathogenesis is original asthenia and pathogen sthenia, mixture of asthenia and stheniareal. Original asthenia is the asthenia of qi and yin of spleen and kidney, pathogen sthenia including dampness and heat, blood stasis, exogenous etc. The wind pathogen infestation and blood stasis were the most important. Periostracum cicadae and bombyx batryticatus were sentient flesh and blood products. Both of them had the functions of promoting blood, eliminating wind and dredging channels. Therefore, they can get rid of the wind pathogen, blood stasis and other factors in development of MsPGN. Two drugs were clinically proven to have good therapeutic effect for acute and chronic nephritis attack and consolidation [8]. In this study, compared with that in the control group, 24h urine protein decreased significantly after administration of 5w in high-dose groups ($P < 0.01$). Compared with that in the control group, 24h urine protein decreased significantly after administration of 8w in each drug groups. Periostracum cicadae and bombyx batryticatus could effectively reduce 24h urine protein in the MsPGN rats, and high-dose group were better than the low dose group; There were no significant differences between the two drug groups. The two drugs had reduced the lipids, which was reported in the literature [9-10]. In addition, serum albumin increased significantly in periostracum cicadae groups, which indicated periostracum cicadae was better than bombyx batryticatus in increaseing serum albumin.

Many studies indicated that the excessive proliferation and activation of mesangial cells and the excessive expression of extracellular matrix components could lead to glomerular sclerosis[11]. Many growth factors and cytokines involved in the process, and now TGF-β1 play a central role in regulatiing a number of factors. TGF-β1 was a key fibrogenic cytokine invoived in the fibrosis of a number of chronic kidney or other organ diseases [12-15], it had an important biological role in increasing ECM synthesis and inhibiting ECM degradation[16]. Experiments confirmed that, TGF-β1 had chemotactic effects on fibroblast cells and can stimulate fibroblast proliferation, and could gather the monocyte-macrophage cells and lymphocytes and other inflammatory cells, and had important features on promotion of glomerular sclerosis. The results show that compared with that in the control group, the expression of TGF-β1 in renal tubular epithelial cells and interstitial expression of the cytoplasm were significantly reduced in each drug groups.

In summary, periostracum cicadae and bombyx batryticatus improved the pathological changes in rat kidney, ruduce cholesterol and proteinuria symptoms on MsPGN rats, and its mechanism may be related to inhibition of the over-expression of TGF-β1, but its specific roles (cell signaling pathway) have to be confirmed by further studies.

References

[1] Li LS, Guan TG. Pathological characteristics and epidemiologic manifestations in 4298 adult cases with glomerular disease [J]. Chinese Journal of Nephrology, Dialysis & Transplantation, 1997, 6 (2):103-109

[2] Feng XL, Wang RL, Zhang PX. Pathological characteristics and epidemiologic manifestations in 1353 cases with glomerular disease [J]. ShanXi Medial Journal, 2001,30(6) :325-329

[3] Huang PP, Liang D, Tang DS, et al. Epidemiologic analysis of glomerulardisease in 805 cases with renal biopsy [J]. The Journal of Practical Medicine, 2005, 21(13):1421-1422

[4] Jia H, Zou WZ. Improvement of chronic serum sickness of mesangial proliferative glomerulonephritis model [J]. Chinese Journal of Nephrology Dialysis& Transplangtation, 1996,5 (3):21-25

[5] Huang HP, Wang G, Liu L, et al. The experiment study of ShenYanLing on transforming growth factor-β 1 in mesangial proliferative glomerulone phritis rats [J]. Chinese Journal of the Practical With Modern Medicine, 2003,16 (11) :1560-1562

[6] C'howdhury AR, Ehara T, Hiquchi M, et al. Immunohistochemical detection of immunoglobulins and complements in formaldehyde-fixed and paraffin-embedded renal biopsy tissues; all adjunct for diagnosis ofglomerulonephritis [J]. Nephrology(Carlton), 2005,10(3): 298-304

[7] Chen ZQ, Fan HF, Guo DZ, et al. The expression of Circulation meridians of Chinese medicine on thromboxane B2 and 6-keto prostaglandin Flα in MsPGN rats. [J]. Journal of Traditional Chinese Medicine, 2009, 15 (3) :197-199

[8] Li BS, Xie MH, Lu CM. The treatment of insects of the drug in kidney disease [J]. Chinese Journal of Information on Traditional Chinese Medicine, 2007, 14 (10): 71-72

[9] Liu ST, Li JM, Wang LF, et al. The experiment study of periostracum cicadae on hemorheology in rats [J]. Acta Chinese Medicine and Pharm acology, 2004,32 (3) :56-58

[10] Yu J. Clinical applications and modern pharmacological studies of bombyx batryticatus [J]. Journal of China raditional Chinese Medicine Information, 2010,2 (7) :185-186

[11] Yohei M, Naoki K, Tatsuji Y. Inhibition of mesangial cell proliferation by E2F Decoy Oligodeoxynucleotide in vitro and in vivo[J]. JClin Invest, 1998,101(6):2589-2597

[12] Sayin B, Karakayali H, Colak T, et al. Conversion to sirolimus for chronic allograft nephropathy and calcineurin inhibitor toxicity and the adverse effects of sirolimus after conversion [J]. Transplant Proc, 2009,41(7):2789-2793

[13] Inza A, Balda S, Alvarez E, et al. Conversion to everolimus in kidney transplant recipients with decreased renal function [J]. Transplant

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Proc, 2009,41:2134-2136
[14] Caliskan Y, Yazici H, Gorgulu N, et al. Effect of pre-transplant dialysis modality on kidney transplantation outcome[J]. Perit Dial Int, 2009,29:117-122
[15] Weir MR, Wali RK. Minimizing the risk Of chronic allograft nephropathy[J]. Transplantation, 2009, 87:14-18
[16] Yu YF, Dong JX. The role of TGF-β 1 in chronic kidney disease [J], Urology and Nephrology Foreign Medical Sciences,2005,25 (1): 132-134

蝉蜕、僵蚕对大鼠系膜增生性肾炎作用的实验研究*

汪慧惠[1] 包 红[1] 于俊生[2] 吕 洪[2] 赵宗芹[2]
(1 青岛大学医学院 山东 青岛 266021;2 青岛市海慈医疗集团 山东 青岛 266033)

摘要 目的:研究蝉蜕、僵蚕对大鼠系膜增生性肾小球肾炎(MsPGN)的治疗作用。**方法:**采用改良慢性血清病法制备的 MsPGN 模型,随机分为模型对照组、蝉蜕高剂量组、蝉蜕低剂量组、僵蚕高剂量组、僵蚕低剂量组,另设正常对照组。分别在用药 5 周、8 周后检测大鼠 24h 尿蛋白;8 周后处死大鼠,行血液生化指标检测和肾组织 HE 染色观察,免疫组化法检测 TGF-β1 的表达水平。**结果:**与模型对照组比较,用药 5 周后蝉蜕、僵蚕高剂量组显著降低大鼠 24h 蛋白尿(P<0.01);8 周后各治疗组大鼠肾组织 TGF-β1 表达量,24h 蛋白尿和血清胆固醇均显著下降,蝉蜕高、低剂量组血清白蛋白均有所升高,差异具有显著性意义(P<0.01)。肾组织形态学观察显示,蝉蜕、僵蚕高剂量组个别区域肾小球系膜细胞轻度增生,系膜区轻度增宽,管腔无挤压现象,较模型组明显改善。**结论:**蝉蜕、僵蚕均能有效降低 MsPGN 大鼠 24h 尿蛋白,改善脂质代谢,其作用机制可能与抑制 TGF-β1 的过度表达有关。

关键词:蝉蜕;僵蚕;系膜增生性肾小球肾炎;24h 尿蛋白定量;转化生长因子 β1
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作者简介:汪慧惠(1985-),女,硕士,研究方向:中西医结合肾脏病,
E-mail:taoyao1900@126.com,Tel:13156361055
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