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The effect of cordycepin on renal damage by correcting hypothyroidism

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Abstract: The paper investigated the effect of cordycepin on renal damage induced by hypothyroidism, and studied the effect of thyroid function recovery on renal damage. The hypothyroid rat model was established by continuous intragastric administration of propylthiouracil (PTU). The general state, thyroid function, renal function, blood lipid, pathological changes and damage of kidney tissues of rats in each group. The expressions of MCP-1 and Desmin proteins, which is a marker of podocyte damage, in the kidney were all detected by immunohistochemistry. The result of thyroid function examination was consistent with the characteristics of hypothyroidism. The renal damage and lipid metabolism disorder appeared in the hypothyroidism rats along with the progression of the disease, with the blood lipid increased and the expression of MCP-1 increased. Using this model to study the pathogenesis of hypothyroidism renal damage is more clinically practical, and the administration of Cordycepin can improve the symptoms. Cordycepin can improve thyroid function, and can significantly alleviate renal damage in rats.By correcting thyroid function, Cordycepin can reduce renal damage and blood lipid, reduce the expressions of MCP-1 and Desmin proteins, thus delaying the progress of renal damage and protecting the kidney.

Keywords: Cordycepin, hypothyroidism, renal damage.

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INTRODUCTION

Hypothyroidism is a kind of pathological state in which the thyroid hormone in tissues is insufficient. The continuous decrease of thyroid hormone level in the body during hypothyroidism will cause damage to target tissues and organs, among which the damage to renal structure and function has been recognized by most scholars, and has increasingly attracted academic attention. The lack of thyroid hormone in the body during hypothyroidism can cause damage to the kidney in many ways, leading to changes in the structure and function of the kidney. With the aggravation of the degree of hypothyroidism, it will eventually lead to renal fibrosis. The levels of total cholesterol, low-density lipoprotein, triglyceride and apolipoprotein B in patients with renal failure and hypothyroidism will increase significantly. At the same time, the incidence of atherosclerosis is also significantly increased. Renal vascular stenosis caused by atherosclerosis and hyperlipidemia synergistically promote renal damage, among which the damage of glomerular basement membrane, mesangial cells and podocytes are the most obvious (Udovcic *et al*., 2017; Shan *et al*., 2016; Lehotsky *et al*., 2015; Park *et al*., 2015; Pan *et al*., 2013).

In our study, PTU was taken orally to establish the hypothyroid rat model, and immunohistochemistry was used to detect the kidney conditions, so as to further understand the characteristics and mechanism of hypothyroid renal damage. In the model, part of the rats were given PTU only and part of them were given PTU cessation plus cordycepin, thus to explore the effect of thyroid function recovery on hypothyroid renal damage and the recovery of renal damage.

Cordycepin plays an effective role in neuroprotection, anti-tumor, antidepressant, anti-inflammatory and other aspects, but the role of cordycepin itself in improving kidney damage due to hypothyroidism has not been reported, so our research is of some innovation.

MATERIALS AND METHODS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and has been approved by the Ethics Committee of Affiliated Hospital of Hebei University (SYXK[JI]2022-009).

Animal grouping and establishment of hypothyroidism rat model

80 SD rats were obtained and placed at room temperature of 20°C-25°C with consecutive light and darkness for 12 h every day. The rats were fed with thrice-distilled water and common fodder. The groups are as follows:

Group A (the normal Control group): rats were given the same dose of normal saline as in the hypothyroidism group by gavage once a day. They were randomly divided into 5 subgroups with 4 rats in each subgroup. Blood was

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taken from the heart at 3, 6, 8, 10 and 12 weeks respectively, then the kidney was obtained.

Group B (the hypothyroidism group): The hypothyroidism animal model was prepared by continuous intragastric administration of PTU. Each rat was given 10mg PTU of every 100 g bodyweight, every day. Blood was taken from the heart at 3, 6, 8, 10 and 12 weeks of every 8 rats, respectively. The kidneys were obtained.

Group C (the treatment control group): The rats were treated the same as in Group B. But At 8th week, PTU was changed to the same amount of normal saline for gavage until the 12th week. Blood was taken from the heart, and the kidney was obtained.

Group D (the cordycepin treatment group): The rats were treated the same as in Group B. But at the 8th week, PTU was stopped and changed to cordycepin. Each rat was given 10ug cordycepin per 100 g bodyweight per day by gavage. Blood and kidney were taken at the 12th week.

Detecting various indexes of thyroid function

The blood of rats in each group was taken from the heart to detect the thyroid function. Indexes such as TSH, FT_3, FT_4, and blood lipids were measured. For renal function test, the kidneys were quickly taken out, and the filter paper was used to absorb water to prepare pathological samples.

HE staining

The kidney tissues containing cortex and medulla in each group were fixed in 4% paraformaldehyde. The fixed tissue blocks were dehydrated with regular gradient ethanol, vitrificated with xylene and embedded with paraffin. The slices were routinely dewaxed to water. After hematoxylin staining for 1 minute, the slices were rinsed with tap water. Following 1% hydrochloric acid differentiation, tap water washing, Eosin staining for 2 minutes, conventional gradient alcohol dehydration and xylene vitrification, the slices were mounted with neutral gum.

Immunohistochemical determination of MCP-1 and Desmin proteins

Slices of each group was obtained for routine dewaxing to water for antigen repair, then added with primary antibody and secondary antibody. The tissues were put in horseradish peroxidase labeled chain enzyme ovalbumin working solution for DAB color development. After gradient alcohol dehydration and xylene vitrification, the slices were sealed with neutral gum.

Determination of serum lipids by automatic biochemical analyzer

TC, TG, LDL, HDL, urea nitrogen and creatinine were determined according to the instructions.

Ethical approval

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and has been approved by the Ethics Committee of Affiliated Hospital of Hebei University vide reference No. No.SYXK2022-009.

STATISTICAL ANALYSIS

SPSS 11.5 statistical software was used for analysis. All data were expressed as $x \pm s$. Analysis of variance (one-way ANOVA) was used for comparison between multiple groups, and q test was used for comparison between two groups. Linear correlation analysis method was applied. $\alpha=0.05$, and $p<0.05$ denoted statistical significance.

RESULTS

Thyroid function

At 3, 6, 8, 10 and 12 weeks, FT_3 and FT_4 of rats in group B were significantly lower than those in group A. With the prolongation and aggravation of the course of disease, FT_3 and FT_4 showed a downward trend. At 3, 6 and 8 weeks, TSH was significantly higher than that of Group A at the same time point, and significantly higher than that of the Control group at 10 and 12 weeks. At 10 weeks, FT_4 of rats in group C was significantly higher than that of group B, FT_3 was higher than that of group B, but TSH was lower than that of group B. FT_3 and FT_4 of rats in group D were significantly higher than those in group B, while TSH was lower than those in group B. At 12 weeks, FT_3 and FT_4 of rats in group D and C were significantly higher than those in group B, while TSH was lower than that in group B. There was no significant difference of thyroid function between group D and the Control group, as shown in table 1 and table 2.

HE results

No obvious abnormality was found in the glomerulus and renal interstitium of rats in group A. The connective tissues between renal tubules increased in rats in group B. Inflammatory cells increased. Cells in the proximal renal tubule have cytoplasmic autolysis and vasoconstriction, which tend to aggravate with the progression of the disease. Compared with group B, the pathological changes in group D were obviously alleviated, as shown in fig. 1.

Immunohistochemical results

MCP-1: In normal renal tissue, MCP-1 expressed extremely weak in only a small part of glomerular cells and tubular epithelial cells. However, in hypothyroid rats, the expression of MCP-1 in glomerular cells and tubular epithelial cells increased significantly. With the progression of hypothyroidism, the expression of MCP-1 increased gradually.

Table 1: Thyroid function

Time	Thyroid function	A Group	B Group
3W	FT3 (pmol/l)	6.43±1.01	3.74 ±0.71**
	FT4(pmol/l)	8.73±0.32	2.39 ±0.34**
	TSH(ug/ml)	2.80±0.54	3.51 ± 0.82*
6W	FT3 (pmol/l)	6.29±0.83	3.24 ±0.80**
	FT4(pmol/l)	8.63 ±0.92	1.92 ±0.73**
	TSH(ug/ml)	2.69 ± 0.77	3.73 ± 0.57*
8W	FT3 (pmol/l)	6.31 ± 0.38	3.03 ± 0.93**
	FT4(pmol/l)	8.51 ± 0.93	1.77 ± 0.69**
	TSH(ug/ml)	2.37 ± 0.81	3.98 ± 0.65*
10W	FT3 (pmol/l)	6.25 ± 0.42	2.73 ± 0.51**
	FT4(pmol/l)	8.49 ± 0.39	1.65 ± 0.66**
	TSH(ug/ml)	2.65 ± 0.36	4.21 ± 0.44**
12W	FT3 (pmol/l)	6.22 ± 0.32	2.31 ± 0.36**
	FT4(pmol/l)	8.45 ± 0.27	1.47 ± 0.69**
	TSH(ug/ml)	2.69 ± 0.32	4.81 ± 0.65**

Table 2: The effect of cordycepin on thyroid function.

Group	FT3 (pmol/l)	FT4 (pmol/l)	TSH(ug/ml)
A	6.31 ± 1.01**	8.53 ± 0.81**	2.71 ± 0.31**
B	2.79 ± 0.83	1.54 ± 0.78	4.42 ± 0.82
C	5.62 ± 0.72*	6.53 ± 1.11**	3.39 ± 0.76
D	6.12 ± 0.21**	8.32 ± 0.39**	2.84 ± 0.78*

Table 3: The Effect of cordycepin on various biochemical indexes.

Blood Fat	A	B	C	D
TC (mmol/L)	1.20 ± 0.51**	2.89 ± 0.42	2.01 ± 0.26	1.70 ± 0.14*
TG (mmol/L)	0.13 ± 0.09**	0.37 ± 0.21	0.27 ± 0.23	0.18 ± 0.02**
HDL (mmol/L)	1.12 ± 0.32**	0.55 ± 0.22	0.68 ± 0.03	1.02 ± 0.13**
LDL (mmol/L)	0.83 ± 0.27*	1.27 ± 0.21	1.11 ± 0.54	1.02 ± 0.16*
BUN (mmol/L)	5.22 ± 0.54**	11.89 ± 1.22	11.51 ± 1.78	9.08 ± 1.21*
Crea (μmol/L)	33.11 ± 1.03**	55.92 ± 2.08	43.21 ± 2.22*	38.11 ±1.87**

**p<0.01 vs B Group *p<0.05 vs B Group

The staining of group C and group D was lighter than that of group B, indicating that the expression of MCP-1 was significantly reduced, as shown in fig. 2.

Desmin: Desmin occasionally expressed in the glomerulus of normal rats, while Desmin expression in the glomerulus of hypothyroid rats was significantly increased. With the progression of hypothyroidism, Desmin expression showed an increasing trend, especially in group B. The staining of group C and group D was lighter than that of group B, indicating that the expression of Desmin was significantly reduced, as shown in fig. 3.

The results of various biochemical indexes

TC, LDL and TG in the hypothyroidism group were significantly higher than those in the Control group. TC, TG and LDL were progressively higher and HDL was progressively lower with the course of the disease. Compared with group B, TC, TG and LDL in C and D groups were lower and HDL was higher, as shown in table 3.

DISCUSSIONS

Hypothyroidism refers to a pathological state of insufficient or absent thyroid hormones in tissues. The common clinical manifestations are chilliness, weakness, apathy, slow reaction, slow movement, edema, weight gain and sparse hair. The continuous decrease of thyroid hormone level during hypothyroidism will cause damage to target tissues and organs, among which the damage to renal structure and function has been recognized by most scholars. The lack of thyroid hormone in the body during hypothyroidism can cause damage to kidney in many ways and affect the functional state of its cell membrane and dopamine system, leading to changes in the structure and function of the kidney. With the aggravation of hypothyroidism, the damage will eventually lead to renal fibrosis and renal failure (Berta *et al*., 2019; Razvi *et al*., 2018; Udovcic *et al*., 2017).

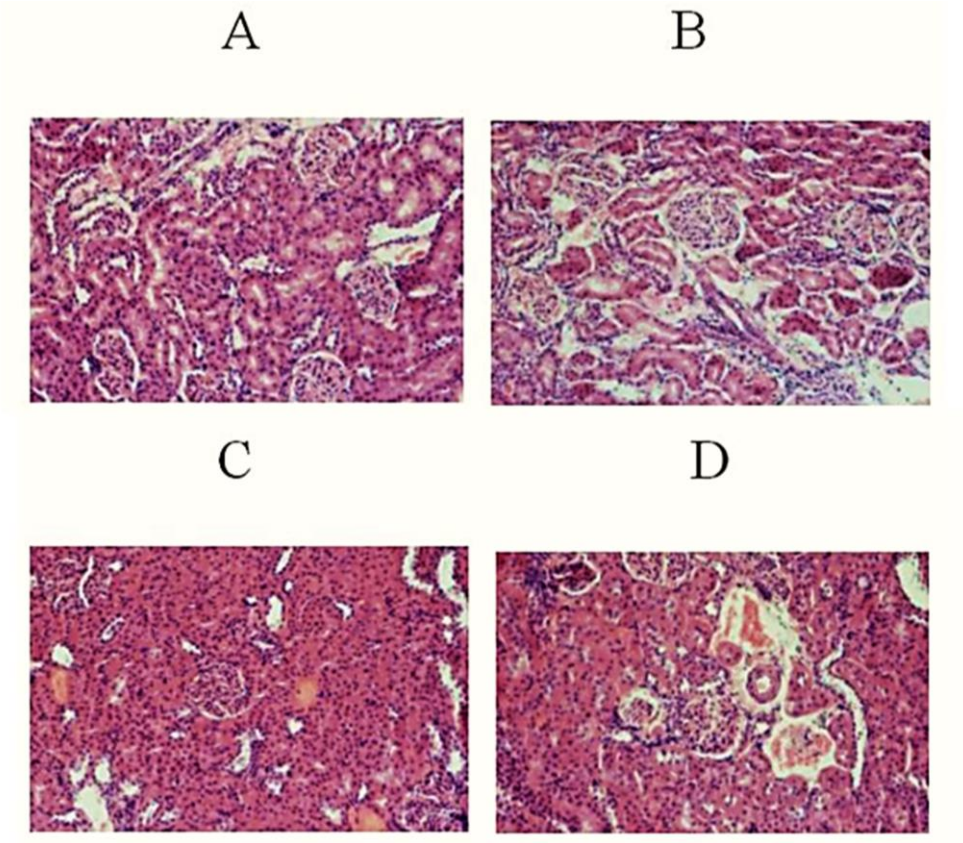

Fig. 1: Effect of cordycepin on HE results.

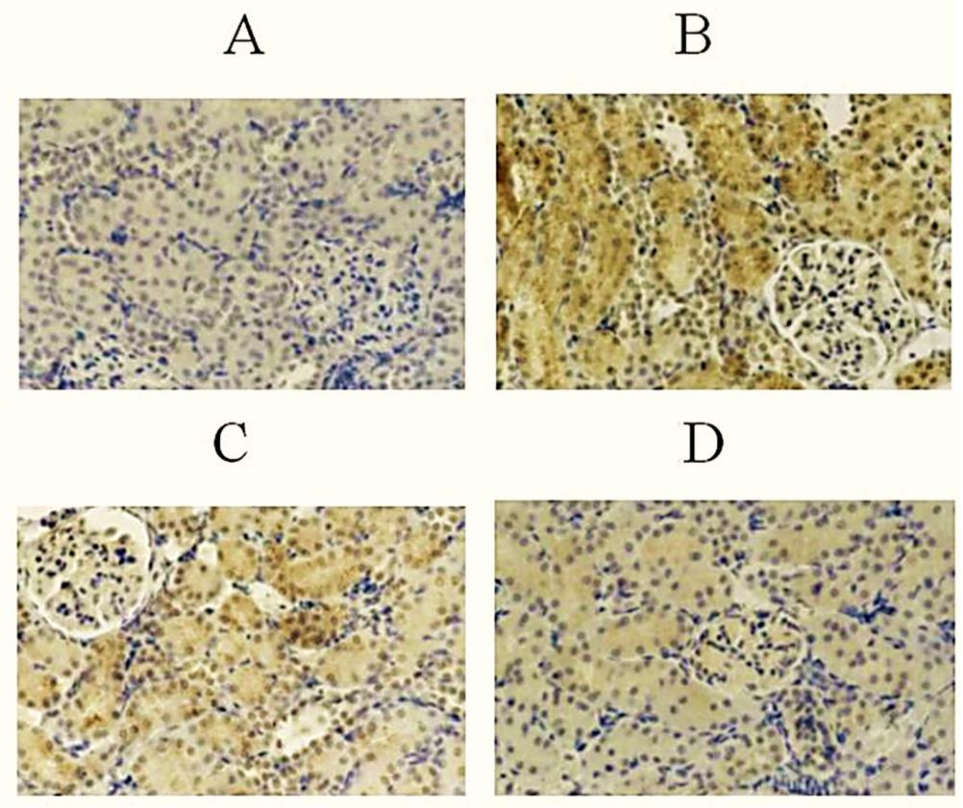

Fig. 2: The effect of cordycepin on MCP-1 expression.

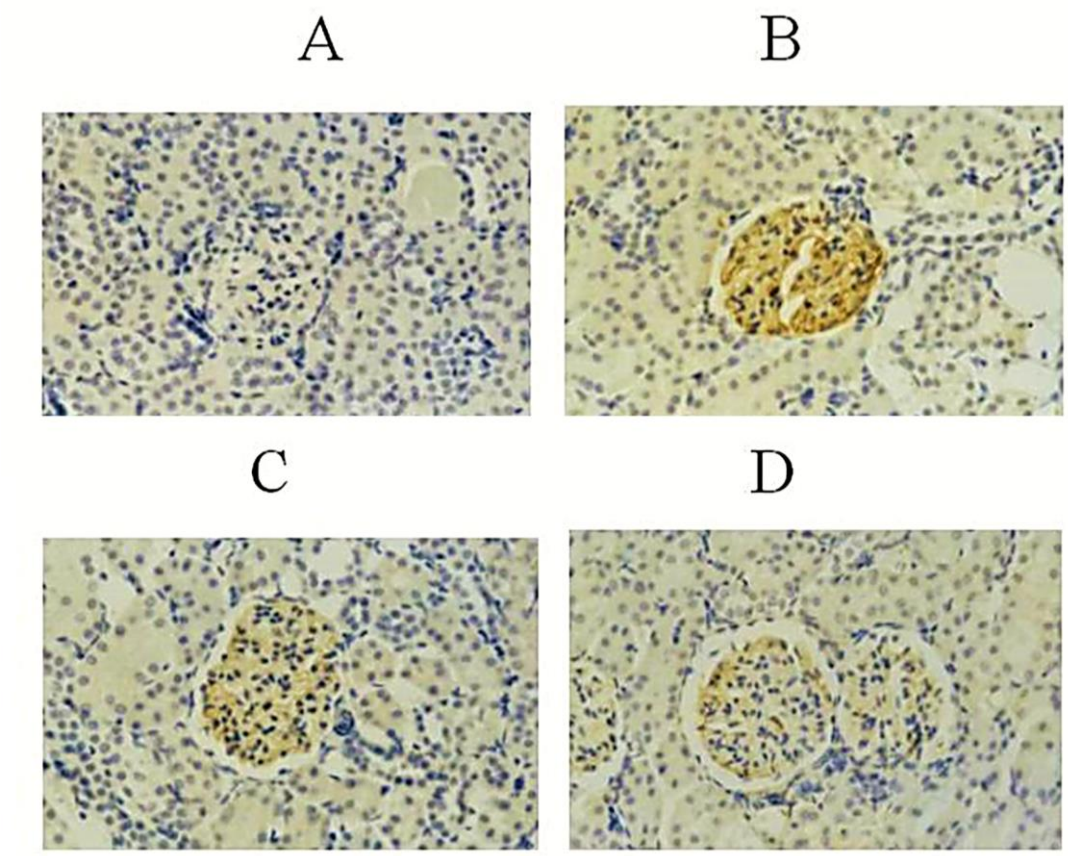

Fig. 3: The effect of cordycepin on desmin expression.

In hypothyroidism, insufficient thyroid hormone may affect renal function and hemodynamics. At the initial stage, the renal blood flow and glomerular filtration rate decreased, the reabsorption and maximum secretion capacity of renal tubules changed, the urine volume decreased and the water excretion load was delayed. Long term hypothyroidism leads to renal blood flow and a more obvious decline in glomerular filtration rate. In addition to the serious reduction in renal tubular resorption, hyperuricemia also causes interstitial nephritis, interstitial fibrosis, and further aggravation of renal damage, which will cause the increase of blood urea nitrogen and creatinine (Jabbar *et al*., 2017; Baum & Quigley, 2004; Textor, 2004).

Monocyte chemoattractant protein-1 is also called monocyte chemoattractant activator. As a major member of the chemokine family, it can be synthesized and secreted by a variety of cells, such as monocytes, macrophages, endothelial cells and mesangial cells in kidney tissue. Podocytes are an important structure that constitutes the glomerular filtration barrier. Podocyte damage has been found in many human and animal models of glomerular diseases. Podocyte plays an important role in the process of glomerulosclerosis and renal dysfunction. At present, studies have confirmed that gene mutation, immune factors, hemodynamic abnormalities, high glucose, high fat and urine protein overload and other multiple factors can lead to abnormal function, apoptosis and decreased number of podocytes. Desmin is a kind of intermediate filament protein of cytoskeleton, which is usually used as a marker of myogenic cells. Normally, a small amount of Desmin is occasionally expressed in glomerular mesangial cells, but not in podocytes. When podocytes are damaged for various reasons, Desmin can be expressed in large quantities and phenotypic transformation occurs. Therefore, Desmin can be used as a sign of podocyte damage under light microscope (Textor, 2004; Chade *et al*., 2002; Attia *et al*., 2004; Cappola & Ladenson, 2003; Iglesias *et al*., 2017).

In our study, propylthiouracil was used to establish the hypothyroid rat model. The thyroid function examination of the rats conformed to the characteristics of hypothyroidism, and the hypothyroid rats exhibited kidney damage and lipid metabolism disorder with the progression of the disease, which was manifested by renal damage and increased MCP-1 expression. Using this model to study the pathogenesis of hypothyroid kidney damage is practically clinical. The dynamic observation of renal histomorphology and the detection of Desmin protein, as well as the renal function and MCP-1 expression during the development of hypothyroidism, showed that, with the extension of the course of the disease, renal damage was parallel to the progression of

hypothyroidism, indicating that renal damage was closely related to thyroid function. The effect of Cordycepin on correcting thyroid function is better than that of slowly correcting thyroid function. It can be seen that rapidly correcting thyroid function during hypothyroidism can reduce renal damage and blood lipid and reduce the expressions of MCP-1 and Desmin protein, thus delaying the progress of kidney damage and protecting the kidney.

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