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Effects of inflammatory response on renal function and TGF-β1 pathway of rats with aging-related kidney damage by upregulating the expression of CD36

J. PENG, X.-F. REN, C. YANG, T.-B. LAN, Z.-Y. SHAO, Y. CHEN

Department of Nephrology, Central Theater Command General Hospital of the Chinese People's Liberation Army, Wuhan, China

Abstract. – OBJECTIVE: The aim of this study was to explore the effects of inflammatory response on renal function and TGF-β1 pathway of rats with aging-related kidney damage by upregulating the CD36 expression.

MATERIALS AND METHODS: A total of 70 pathogen free (PF) Sprague-Dawley (SD) male rats were enrolled. The rats injected with normal saline and D-galactose were assigned to a control group and a model group, respectively. Those injected with both D-galactose and different concentrations of casein were assigned to casein A, B, and C groups accordingly, and 16 rats injected with D-galactose and with CD36 gene knocked out were assigned to a treatment group. The following methods were employed to determine the following factors of the rats: ELISA for serum inflammatory factors, Western blot for CD36 in kidney tissues, Real Time-PCR for TGF-β1, and Smad (2, 3, and 7) mRNA, radioimmunoassay for hyaluronic acid (HA) and laminin (LN), and colorimetry for the expression quantity of plasma superoxide dismutase (SOD) and malondialdehyde (MDA). An automatic biochemical analyzer was used to determine blood, urine, and renal function indexes.

RESULTS: After successful modeling, the model group showed significantly higher inflammatory indexes than the control group. The relative expression of CD36 in the model group was significantly higher than that in the control group and treatment group, and significantly lower than that in the casein groups. Both inflammatory indexes and relative expression of CD36 increased with the increase of casein concentration in the casein groups. Groups with severer inflammatory response showed higher renal function indexes, and higher expression of TGF-β1, Smad2, Smad3, HA, LN, and MDA, and those with decreased CD36 level showed lower renal function index levels. The Smad7 expression and SOD were contrary.

CONCLUSIONS: Inflammatory stress can promote the CD36 expression in renal tissues of aging rats and oxidative stress and affect TGF-β1/Smad pathway, thus aggravating renal fibrosis and renal damage in rats.

Key Words:
Inflammatory reaction, CD36, Rat, Renal function, TGF-β1 pathway.

Introduction

Aging is an inevitable process for natural organisms, and its occurrence time varies with tissue types and is greatly affected by environmental conditions[1,2]. The development of science, technology, and medical care has brought about substantial changes to human life expectancy and population structure, including a sharp increase of the elderly population[3,4]. As we grow old, our body shows a series of irreversible degenerative changes in physiology and anatomy, which manifests as the decline of the ability of adapting to the external environment and increase of the susceptibility to malignant tumors, cardiovascular diseases, and nervous system diseases[5]. Aging is characterized by increased oxidative stress, declined antioxidant capacity, oxidative phosphorylation disorder, severe mitochondrial dysfunction, cell apoptosis, and death caused by protein, DNA, and lipid peroxidation. If the whole aging process is in balance, the aging will be normal[6,7]. Kidney is an important organ of the body, and is also the organ with the most evident aging state. Its aging is accompanied by renal volume reduction, cortex atrophy, glomerulosclerosis, and glomerular filtration function decline[8,9]. A severer renal aging indicates a higher possibility of renal hypoperfusion. A long term of hypoperfusion is likely to cause adverse symptoms and signs, such as myocardial infarction, renal arteriosclerosis, and dehydration[10,11].

Previous reports[12,13] on genes responsible for kidney aging have pointed out that the genes with increasing expression in rats with aging-related

Corresponding Author: Xingfeng Ren, MD; e-mail: e7610t@163.com

damage mainly include genes related to inflammation, stress response, and extracellular matrix metabolism. However, different levels of inflammatory response cause different damages to cells. Mild inflammatory response can repair cells to a certain extent, but the inflammatory response exceeding the functional limitation of autophagic cells will trigger apoptosis reaction[14]. In this study, rat models similar to rats with kidney aging were constructed by injecting rats with D-galactose, and casein was also injected into some rats to aggravate their inflammatory response. Then, the changes of CD36 and TGF-β1 pathway in the rats were evaluated, so as to explore the way of suppressing the effects of inflammatory response on aging-related kidney damage.

Materials and Methods

Experimental Materials and Instruments

D-galactose granules (the Solarbio Company, Beijing, China, product code: D8310), casein (Lanpai Biotechnology Co., Ltd., Shanghai, China), blood separator (Feilong Medical Equipment Co., Ltd., Zhengzhou, China), microplate reader (Biobase Medical Devices Co., Ltd., Jinan, China), all enzyme-linked immuno-sorbent assay (ELISA) kits (the Abcam Company, Shanghai, China), bicinchoninic acid (BCA) kits (the Sigma-Aldrich Company, Shanghai, China), sodium-dodecyl-sulfate-polyacrylamide gel-electrophoresis (SDS-PAGE; Guchen Biotechnology Co., Ltd., Shanghai, China), CD36 antibodies (Yuanmu Biotechnology Co., Ltd., Shanghai, China), TRIzol reagent kits (Mrcing Biotechnology Co., Ltd., Jiangsu, Shanghai), reverse transcription kits (Biopeony Beijing Co., Ltd., Beijing, China), radioimmunoassay kit (Furui Runze Biotechnology Co., Ltd., Beijing, China), colorimetry reagent kits (Ruiqi Biotechnology Co., Ltd., Shaanxi, China), and automatic biochemical analyzer (Ali Road Medical Equipment Co., Ltd., Wuhan, China). All primers and sequencing were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

Experimental Animals

The experimental animals included 24 pathogen free (PF) Sprague-Dawley (SD) male rats and 8 rats (8-9 weeks old) with CD36 knocked out and weight of (185±15) g from the Guangdong Medical Laboratory Animal Center. Those rats were raised at the constant temperature of 25°C and humidity of 40%-65% in separate cages by groups and allowed to drink sterile water freely.

Animal Modeling and Grouping

D-galactose granules (10 g) were dissolved in 1500 ml normal saline, and injected subcutaneously into the back neck of each rat at a standard dose of 1500 mg/kg/d. The rats in all groups except for rats in the normal group injected with the normal saline were injected with it at the same dose for 2 continuous weeks to establish rat models of aging-related kidney damage. Before modeling, the 32 rats were divided into 4 groups (each n=8). The rats injected with 1500 mg/kg normal saline subcutaneously at the back neck were assigned to a control group, and those only injected with 1500 mg/kg D-galactose were assigned to a model group. In addition, the rats injected with 1500 mg/kg D-galactose combined with separate 1 mg/mL casein, 2 mg/mL casein, or 3 mg/m casein were assigned into a casein A group, casein B group, and casein C group, respectively, and those only injected with 200 mg/kg D-galactose and with CD36 gene knocked out were assigned to a treatment group. The treatments of rats were all in line with regulations concerning experimental animals, and according to the regulations on the protection and euthanasia of experimental animals, all rats were euthanized after all examinations were completed. All animal experiments have been approved by the Animal Ethics Committee of the Central Theater Command General Hospital of the Chinese People's Liberation Army.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum (100 μl) sampled from each group was added into plates coated with antibodies, and the standard wells and blank wells were set in the plates at the same time. The serum in each well was subject to reaction at 37°C for 90 min, and then, the liquid in each well was discarded. Subsequently, each well was added with 150 μl chromogenic reagent after three times of drying and washing, and developed through reaction at 37°C for 30 min. Then, 50 μl serum was taken out from each well, and the average optical density of each well was measured at 500 nm wavelength using a microplate reader. The C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and procalcitonin (PCT) in the serum were detected in strict accordance with ELISA kit instructions.

Determination of CD36 in Renal Tissues by Western Blot

Renal tissues sampled from the rats were sealed with liquid nitrogen, and pyrolyzed to collect the supernatant. The supernatant was frozen for later

analysis. The bicinchoninic acid assay (BCA) kit was employed to determine the protein concentration. The protein extract was heated at 100°C for 5 min after being diluted with buffer solution, and then, centrifuged at 12000 r/min for 10 min, and stored at -20°C. The protein was separated through SDS-PAGE, and transferred to a polyvinylidene difluoride (PVDF) membrane. Subsequently, the protein was blocked with 5% skim milk, and incubated with primary antibodies overnight, and then, added with secondary antibodies after the membrane was washed with buffer solution. The protein was incubated with the antibodies at room temperature, followed by membrane washing. The fluorescence protein bands were scanned using an Odyssey double-color infrared laser scanning developer. The target protein level of CD36 was recorded as the gray value of target protein band/the gray value of β-Actin protein band.

Real Time-PCR

The total mRNA was extracted using the TRIzol method, and synthesized into cDNA through reverse transcription at 37°C for 15 min and at 85°C for 5 s. It was subjected to amplification under pre-denaturation at 95°C for 30 s, and polymerase chain reaction (PCR) under 40 cycles of denaturation at 95°C for 5 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) (ΔΔCt comparison method) was used for analysis. The average relatively content was $2^{-\Delta\Delta Ct}\times100\%$. Primer information of qRT-PCR are shown in Table I.

Determination Methods

The pathological changes of kidney was analyzed using the periodic acid-Schiff (PAS) staining under an optical microscope (10×20). The radioimmunoassay was used to detect the hyaluronic acid (HA) and laminin (LN), and the colorimetry to determine oxidative stress injury indexes, superoxide dismutase (SOD) and malondialdehyde (MDA). An automatic biochemical analyzer was employed to determine the indexes of blood, urine, and renal function, including the contents of serum creatinine (Cr), urea nitrogen (BUN), serum cystatin C (Cys C), blood β2-microglobulin (β2-MG), and urinary albumin.

Statistical Analysis

In the experiment, SPSS 19.0 software (Beijing NDTimes Technology Co., Ltd.) was adopted for statistical analysis of experiment data. Enumeration data were analyzed using the Chi-square test, and measurement data were expressed as the mean ± standard deviation (SD). Comparison between two groups was carried out using the independent *t*-test, and multiple-group comparison was carried out using the ANOVA back testing. In addition, comparison between different measured values was carried out using the repeated measures analysis of variance. The data were visualized to figures using GraphPad Prism 8 (La Jolla, CA, USA). $p<0.05$ indicates a significant difference.

Results

Comparison of Renal Function Between the Control Group and the Model Group

After the aging model was established, the renal function indexes (serum Cr, BUN, Cys C, β2-MG, and urinary albumin content) of the model group were higher than those of the control group (all $p<0.05$) (Figure 1). PAS staining revealed that the control group did not show evident glomerulosclerosis, but showed normal basement membrane

Table I. Gene primer sequence.

Gene sequence		
TGF-β1	Upstream sequence	AGGCGGTGCTCGCTTTGTA
	Downstream sequence	GATTGCGTTGTTGCGGTCC
Smad2	Upstream sequence	GCAGGAAGAAACCGTTGGAGT
	Downstream sequence	CAAGTCTGAGGCGGGAGGTAG
Smad3	Upstream sequence	GCACAGCCACCATGAGTTACG
	Downstream sequence	GCACCAACACTGGAGGTAGCA
Smad7	Upstream sequence	TACCTTCCTCCGATGAAACCG
	Downstream sequence	GAGTCTTCTCCTCCCAGTATGCC
β-Actin	Upstream sequence	ACCGTGAAAAGATGACCCAGAT
	Downstream sequence	GTAACCCTXATAGATGGGACA

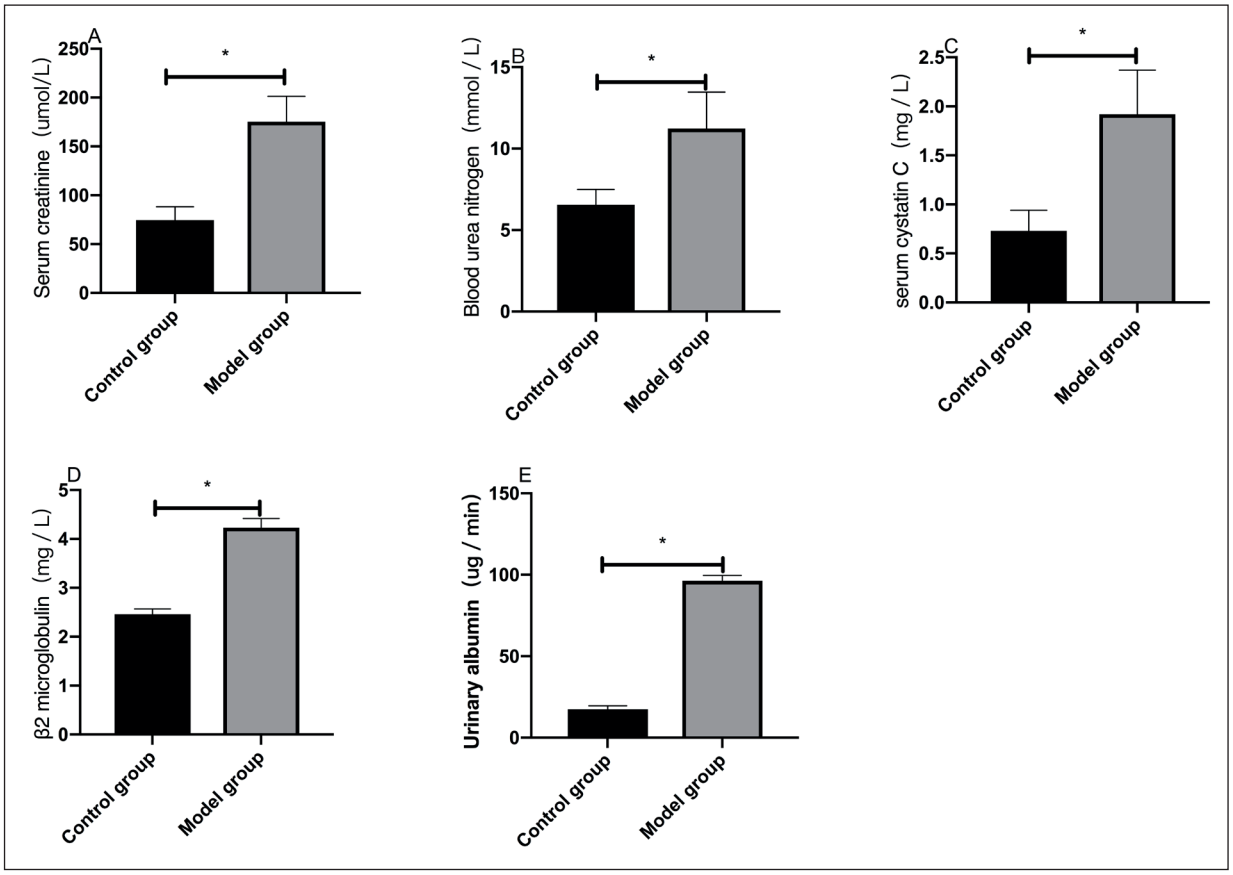

Figure 1. Comparison of renal function between the control group and the model group. **A**, After the aging model was established, the serum Cr of the model group was higher than that of the control group. **B**, After the aging model was established, the serum BUN of the model group was higher than that of the control group. **C**, After the aging model was established, the serum Cys C of the model group was higher than that of the control group. **D**, After the aging model was established, the serum β2-MG of the model group was higher than that of the control group. **E**, After the aging model was established, the serum urinary albumin content of the model group was higher than that of the control group. Note: *indicates $p<0.05$.

morphology and renal tubular epithelial cells, and normal thickness of vascular intimal wall, and the model group showed flat renal tubular epithelial cells in the renal tissues with brush-like margins detached, significantly expanded tubular lumen, increased the number of protein casts, and infiltrated interstitial inflammatory cells. It indicated that the modeling was successful.

Comparison of Inflammatory Response Indexes Among Groups

Comparison of inflammatory response indexes, CRP, TNF-α, IL-6 and PCT, revealed that compared with the control group, the aging model group showed significantly increased inflammatory response indexes, and the inflammatory response in the casein groups with different casein contents were intensified along with the increase of the casein content (Figure 2).

Comparison of the Relative Expression CD36

The relative expression of CD36 protein in the model group was significantly higher than that in the control group. The relative expression of it in the three casein groups was higher than that in the model group, and the relative expression in the three groups increased with the increase of casein concentration. In addition, the relative expression of it in the treatment group was significantly lower than that in the model group ($p<0.05$) (Figure 3).

Comparison of Renal Function Among the Casein Groups with Different Casein Concentrations, the Treatment group, and the Model Group

The contents of serum Cr, BUN, Cys C, β2-MG, and urinary albumin in the three casein groups were significantly higher than those in the

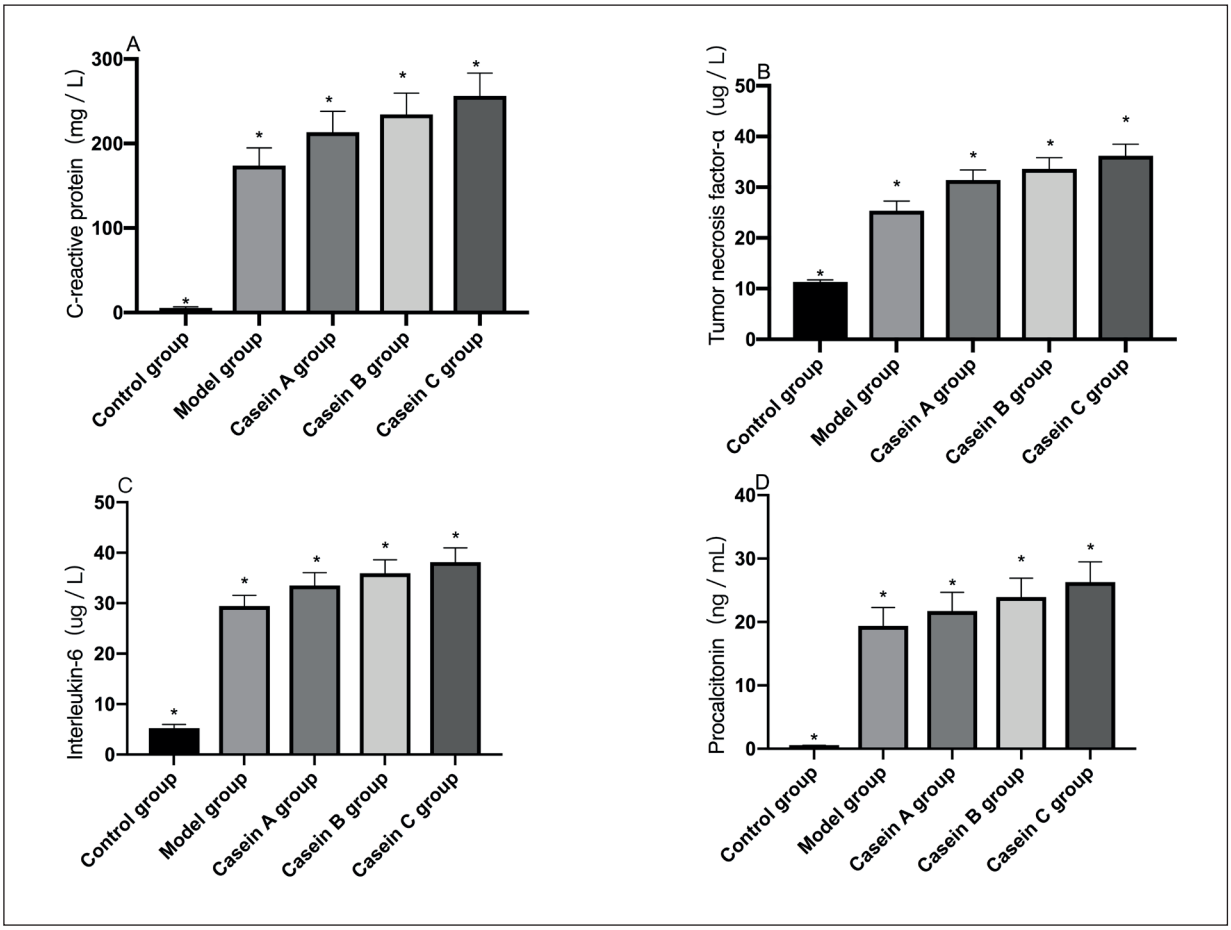

Figure 2. Comparison of inflammatory response indexes among groups. **A**, The CRP of the model group was significantly higher than that of the control group, and that of the three casein groups was higher than that of the model group. The CRP of the casein B group was higher than that of the casein A group, and that of the casein C group was higher than that of other two casein groups. **B**, The TNF-α of the model group was significantly higher than that of the control group, and that of the three casein groups was higher than that of the model group. The TNF-α of the casein B group was higher than that of the casein A group, and that of the casein C group was higher than that of other two casein groups. **C**, The IL-6 of the model group was significantly higher than that of the control group, and that of the three casein groups was higher than that of the model group. The IL-6 of the casein B group was higher than that of the casein A group, and that of the casein C group was higher than that of other two casein groups. **D**, The PCT of the model group was significantly higher than that of the control group, and that of the three casein groups was higher than that of the model group. The PCT of the casein B group was higher than that of the casein A group, and that of the casein C group was higher than that of other two casein groups. Note: *indicates that in terms of the comparison among multiple groups, $p<0.05$.

model group. Those indexes in the casein B group were significantly higher than those in the casein A group, and those in the casein C group were significantly higher than those in the casein A and B groups. In addition, those indexes in the treatment group were significantly lower than those in the model group (all $p<0.05$) (Figure 4).

Changes of TGF-β1 Pathway and Fibrosis in Each Group

The TGF-β1, Smad2, Smad3, HA, and LN in the casein groups were significantly higher than those in the model group, and the relative expression of Smad7 was contrary. The TGF-β1, Smad2, Smad3, HA, and LN in the casein B group were significantly higher than those in the casein A group, and those in the casein C group were significantly higher than those in the casein A and B groups. The relative expression of Smad7 in the three casein groups was contrary to those indexes in the three groups, and those indexes in the treatment group were significantly lower than those in the model group (all $p<0.05$) (Figure 5).

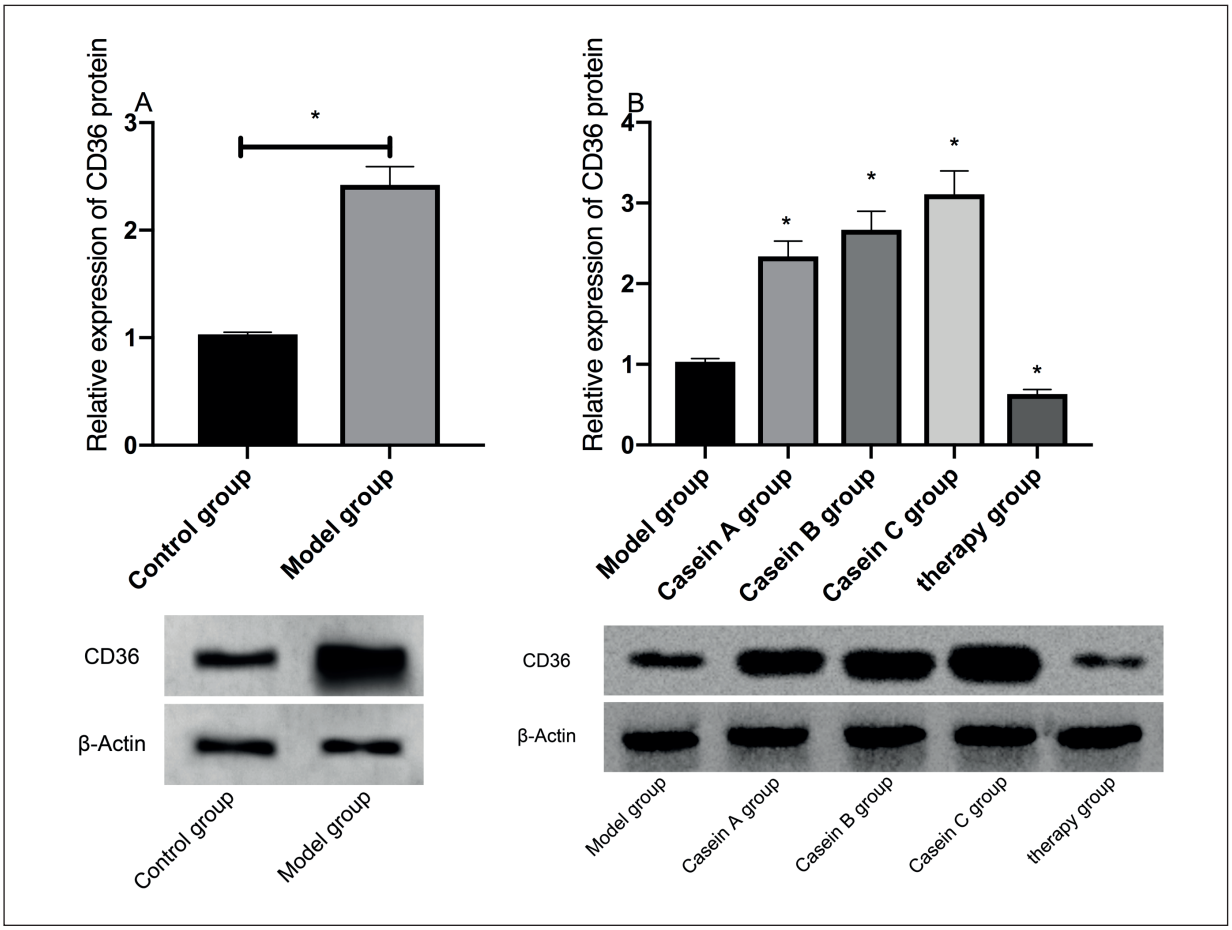

Figure 3. Comparison of the relative expression of CD36. **A**, The relative expression of CD36 in the model group was significantly higher than that in the control group. **B**, The relative expression of CD36 in the three casein groups was higher than that in the model group, and the relative expression in the three groups increased with the increase of casein concentration. The relative expression of it in the treatment group was dramatically lower than that in the model group. Note: *indicates $p<0.05$.

Comparison of Oxidative Stress Parameters Among Groups

The SOD in the model group was significantly lower than that in the control group and the treatment group, but significantly higher than that in the casein groups. The situation of MDA was contrary to that of SOD in those groups ($p<0.05$) (Table II).

Discussion

Body aging is the overall manifestation of the aging of various organs, which is an inevitable result in the natural process. Most people gradually get older when they are fully developed and mature at the age of 25. The elderly face a relatively high incidence of chronic kidney disease, and their chronic kidney disease may develop into end-stage renal diseases. Kidney aging has become a major disease affecting the development of human society and healthy life[15]. During aging, the body shows adaptive function changes of immune response and is in a steady aging state for chronic inflammatory[16]. From a microscopic point of view, the most basic cell cycle changes in cell biology can regulate and dominate the correlation of embryonic development and growth with tissue regeneration and aging[17]. One study found that under the treatment against inflammation, the content of bone marrow mesenchymal stem cells at G1 phase and their overall apoptosis were increased, while their biological function was inhibited, which affected the cell cycle[18]. Analysis on kidney aging revealed that inflammatory response may promote aging by affecting the periodic changes of relevant bone marrow mesenchymal stem cells. However,

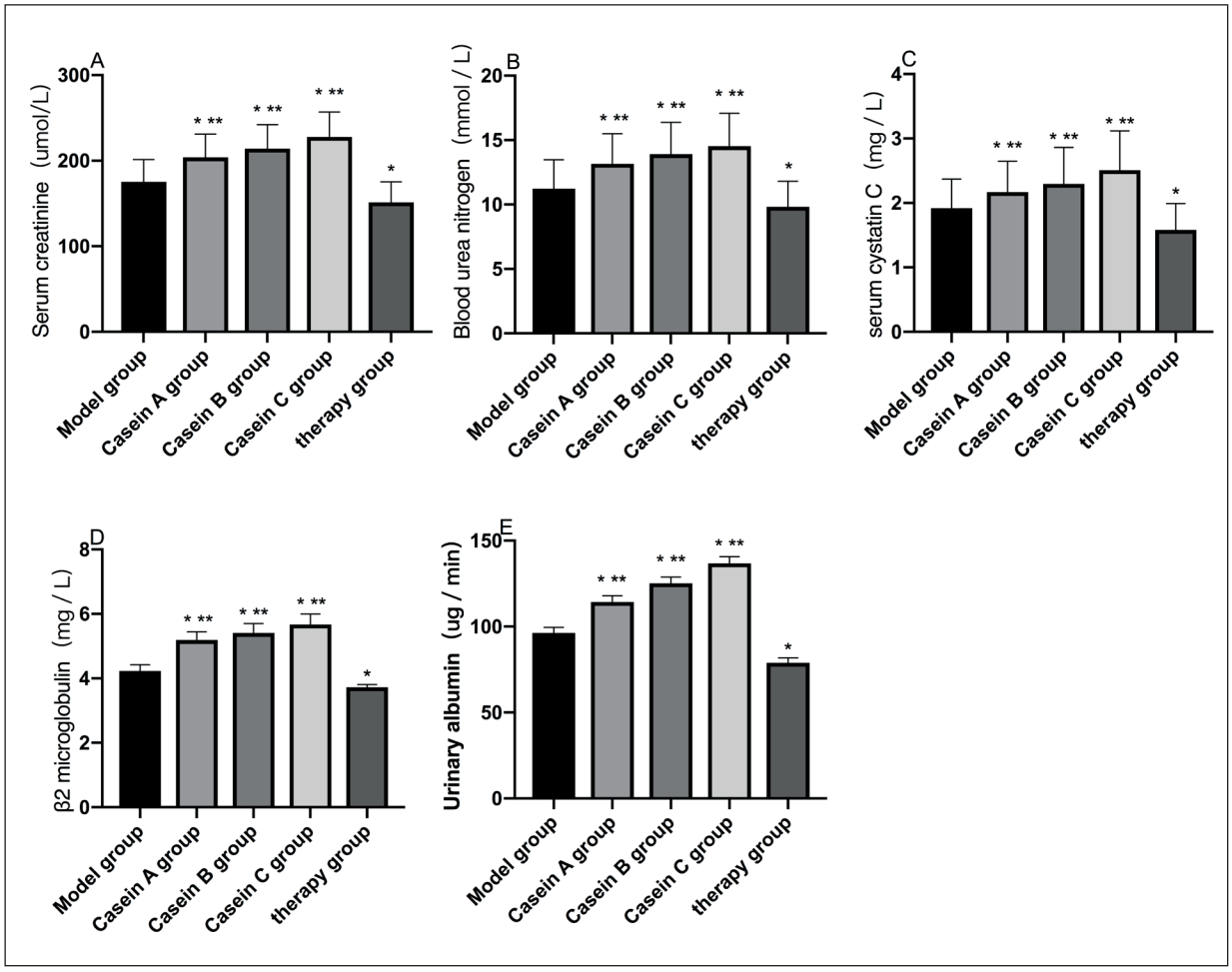

Figure 4. Comparison of renal function among the casein groups with different casein concentrations, the treatment group, and the model group. **A**, The serum Cr in the casein groups was significantly higher than that in the model group. The serum Cr in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. The serum Cr in the treatment group was lower than that in the model group. **B**, The serum BUN in the casein groups was significantly higher than that in the model group. The serum BUN in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. The serum BUN in the treatment group was lower than that in the model group. **C**, The serum Cys C in the casein groups was significantly higher than that in the model group. The serum Cys C in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. The serum Cys C in the treatment group was lower than that in the model group. **D**, The serum β2-MG in the casein groups was significantly higher than that in the model group. The serum β2-MG in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. The serum β2-MG in the treatment group was lower than that in the model group. **E**, The urinary albumin content in the casein groups was significantly higher than that in the model group. The urinary albumin content in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. The urinary albumin content in the treatment group was lower than that in the model group. Notes: *indicates that in comparison with the model group, $p<0.05$, and ** indicates that in terms of the comparison among casein groups, $p<0.05$.

there is currently a lack of relevant study on the mechanism between inflammatory response and kidney aging. Therefore, this study mainly explored the effects of inflammatory response on CD36 expression in rats with aging-related kidney damage and the regulatory changes of renal function and TGF-β1 pathway.

Aging models were constructed to monitor the changes in renal histopathology and renal function indexes. After successful modeling, the rats were additionally injected with casein based on the measures to the model group to intensify their inflammatory response. Based on the comparative monitoring and analysis on

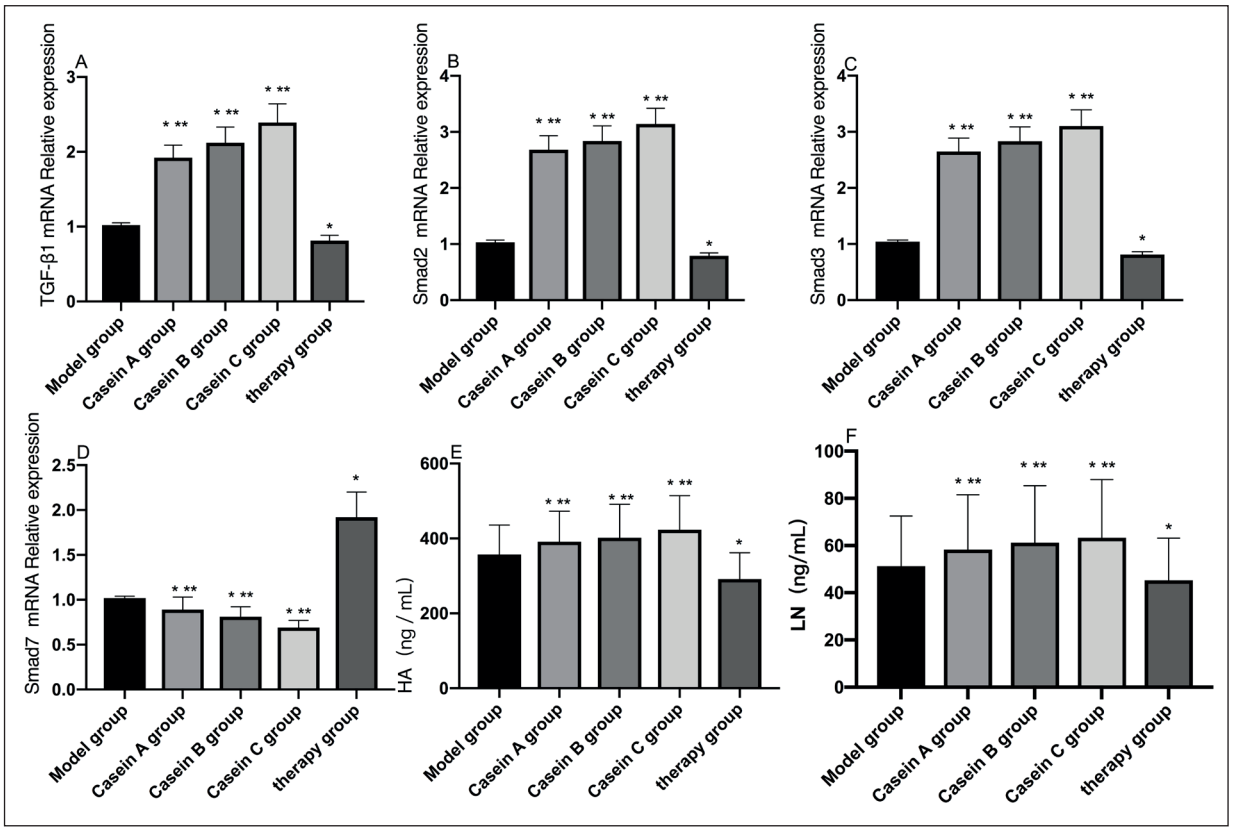

Figure 5. Changes of TGF-β1 pathway and fibrosis in each group. **A**, The TGF-β1 in the casein groups was significantly higher than that in the model group. TGF-β1 in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. TGF-β1 in the treatment group was lower than that in the model group. **B**, Smad2 in the casein groups was significantly higher than that in the model group. Smad2 in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. Smad2 in the treatment group was lower than that in the model group. **C**, Smad3 in the casein groups was significantly higher than that in the model group. Smad3 in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. Smad3 in the treatment group was lower than that in the model group. **D**, Smad7 in the casein groups was significantly lower than that in the model group. Smad7 in the casein B group was lower than that in the casein A group, and that in the casein C group was lower than that in the casein A and B groups. Smad7 in the treatment group was higher than that in the model group. **E**, HA in the casein groups was significantly higher than that in the model group. HA in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and B groups. HA in the treatment group was lower than that in the model group. **F**, LN in the casein groups was significantly higher than that in the model group. LN in the casein B group was higher than that in the casein A group, and that in the casein C group was higher than that in the casein A and casein B groups. LN in the treatment group was lower than that in the model group. Notes: *indicates that in comparison with the model group, $p<0.05$, and **indicates that in terms of the comparison among casein groups, $p<0.05$.

the rats according to different casein concentrations, CRP, TNF-α, IL-6, and PCT in rats were compared, which revealed that the aging model group showed significantly higher inflammatory response indexes than the control group, and the inflammatory response indexes in the casein groups with different casein concentrations increased with the increase of casein concentra-

Table II. Comparison of oxidative stress parameters among groups.

Group	The control group	The model group	Casein groups	The treatment group
SOD (U/ml)	103.42±21.38*	91.38±19.28	82.93±17.28*	98.19±19.86*
MDA (μmol/L)	4.24±1.02*	5.17±1.23	5.92±1.28*	4.71±0.93*

Note: *represents that in comparison with the model group, $p<0.05$.

tion accordingly. IL-6 and TNF-α have different immune functions and participate in various inflammatory diseases. PCT generated under the stimulation by the inflammatory factor (IL-6) and various bacterial endotoxins release after acting on neuroendocrine cells, and its level significantly increased in the early stage of inflammation[19,20]. CRP is an anti-inflammatory factor related to specific reactions originating from the liver, and its concentration increases after bacterial infection[21]. Analysis on the above studies and the results in this study revealed that aging-related kidney damage was accompanied by certain inflammatory kidney damage. The changes of CD36 expression on the surface of bone marrow mesenchymal stem cells were further analyzed by intensifying the inflammatory damage. It was found that the relative expression of CD36 in the model group was significantly higher than that in the control group, and its relative expression in all casein groups was higher than that in the model group. Additionally, it was also observed that the expression of CD36 in the three casein groups increased with the increase of casein concentration, and the expression in the treatment group was significantly lower than that in the model group. It suggested that inflammatory response severity was positively correlated with the expression of CD36. Previous scholars[22] pointed out that the secretion of pro-inflammatory factors and inflammatory response promoted the expression of CD36, contributed to the forming of heteromultimers with Toll-like receptors, and activated transcription factors by signaling cascade. It indicated that inflammatory reaction had an upregulation effect on CD36, and CD36 level in rats injected with D-galactose would decrease accordingly when their inflammatory reaction was controlled. We monitored the renal function of each group after inducing inflammation and regulating CD36, and found the following results: the casein groups showed significantly higher indexes of renal function than the model group, and the difference of the indexes among the three casein groups were significant. In addition, the indexes in the treatment group were lower than those in the model group. A long-term inflammation without evident inducement and without interruption can develop into inflammatory response without high titer of auto-antibodies or antigen-specific T lymphocytes, causing renal insufficiency with protein A amyloidosis, gradual decrease of renal function, and even renal failure[23,24]. Moreover, some studies have shown that the progress of many kidney diseases is closely related to CD36[25]. In order to explore the way by which CD36 level increases in plasma and urine reflects the CKD degree, we studied the TGF-β1 pathway and found that TGF-β1, Smad2, and Smad3 were expressed at higher levels in the groups with severer inflammatory response, while the expression trend of Smad7 was contrary. It has been proved that in patients with chronic kidney disease, many damage factors induce the upregulation of CD36 expression, thus activating Scr/Lyn/Fyn and TGF-β signal pathways related to NLRP3 inflammatory bodies and participating in renal inflammatory reactions[26,27]. Smad protein is a signal transduction protein downstream of the TGF-β1 family. TGF-β1/Smad pathway formed jointly can induce renal fibrosis by mediating vascular endothelial cells to differentiate into myofibroblasts. Renal fibrosis progress can be inhibited by knocking down Smad2 and Smad3 and overexpressing Smad7[28-30]. According to relevant references, inflammatory cells and fibroblasts need accumulation of HA matrix for changes during the formation of fibrous tissues, but LN is distributed in the basement membrane, and when its expression is upregulated, inflammatory cells can accumulate around the basement membrane to construct tissue fibrosis[31,32]. The results of this study further supported the conclusion that inflammatory response may lead to renal fibrosis damage by affecting CD36 and various signal pathways. In addition, we also studied and compared the oxidative stress parameters of each group, finding that SOD in a group with severer inflammatory response was significantly lower than that in a group with milder inflammatory response, while the situation of MDA value was opposite. SOD can protect cell membrane from free radical oxidative damage. MDA is usually generated by peroxidation of polyunsaturated fatty acids. The downregulation of its expression can indirectly relieve the free radical metabolic changes of the body and alleviate the damage to cells[33,34]. There is a close correlation between oxidative stress and inflammation. The increase of oxidative stress level can promote the production of collagen fibers, pro-fibrogenic cytokines, and growth factors[35,36]. It indicated that the repeated oxidative stress reactions induced the aggravation of inflammatory stimulation, and the severity of renal injury caused by renal fibrosis injury and aging itself was higher, which was consistent with this study.

Conclusions

To sum up, inflammatory stress can up regulate the CD36 expression in renal tissues of aging rats, promote oxidative stress, and affect the TGF-β1/Smad pathway, thus aggravating renal fibrosis and renal injury in rats. This study provides a novel therapeutic idea that may be applied to renal protection for renal injury patients based on clinical experiments of observing CD36 expression in renal tissues of renal injury rats. However, there are still some unsolved problems in the process of studying rats. For rats in the treatment group, CD36 knockout is selected first for comparison, which is different from the traditional treatment sequence and may result in inaccurate data. On the other hand, this study has subdivided the inflammatory response groups with different concentrations, but has not made analysis on the association of data about pathways and oxidative stress in different groups. We will take it as the direction of further exploration, so as to provide a reasonable scheme to improve the anti-inflammatory mechanism for patients with severe aging-related renal injury caused by inflammatory response.

Conflict of Interests

The authors declare that they have no conflict of interest.

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