

Original Article

Rhein lysinate increases the median survival time of SAMP10 mice: protective role in the kidney

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Aim: To investigate the protective effects of rhein lysinate (RHL), a major bioactive constituent of the rhizome of rhubarb (*Rheum palmatum* Linn or *Rheum tanguticum* Maxim), against kidney impairment in senescence-prone inbred strain 10 (SAMP10) mice.

Methods: SAMP10 mice were orally administered RHL (25 or 50 mg/kg) daily until 50% of the mice died. Senescence-resistant inbred strain 1 (SAMR1) mice administered no drug were taken as control. The kidneys were harvested after animal death, and examined morphologically and with immunochemical assays. The levels of MAD, SOD and GSH-px in the kidneys were measured with a photometric method. The expression of inflammatory factors and related proteins in the kidneys was analyzed using Western blotting.

Results: Treatment of SAMP10 mice with RHL had no effect on the body weight or phenotype. However, RHL significantly prolonged the median survival time of SAMP10 mice by approximately 25%, as compared to untreated SAMP10 mice. Compared SAMR1 mice, SAMP10 mice had a significantly lower level of SOD in the kidneys, but had no significant difference in the MDA or GSH-px levels. Treatment of SAMP10 mice with RHL significantly reduced the MAD level, and increased the SOD and GSH-px levels in the kidneys. Glomerulonephritis was observed in SAMP10 mice but not in SAMR1 mice. RHL decreased the incidence of glomerulonephritis, and significantly decreased the levels of TNF- α , IL-6, NF- κ B, collagen types I and III in the kidneys.

Conclusion: Accelerated senescence is associated with glomerulonephritis in SAMP10 mice, and RHL prolongs their median survival time by reducing the severity of glomerulonephritis.

Keywords: rhein lysinate; SAMP10; aging; kidney; glomerulonephritis; superoxide dismutase; malondialdehyde; glutathione peroxidase; TNF- α ; IL-6; NF- κ B; collagen

Acta Pharmacologica Sinica (2013) 34: 515–521; doi: 10.1038/aps.2012.177; published online 11 Mar 2013

Introduction

It has been demonstrated that approximately 50% of patients over 75 years of age have a nephrotic syndrome involving extramembranous glomerulonephritis or minimal-change glomerulopathy, as demonstrated by a renal biopsy^[1, 2]. Moreover, a number of structural changes occur in the kidney with aging. The aging kidney is characterized by loss of renal mass, arterial sclerosis, arteriolar hyalinosis, an increased number of sclerotic glomeruli, loss of tubules, and interstitial fibrosis^[3–5]. The permanent and irreversible growth arrest of cell senescence is a central paradigm of aging. Various pathophysiologic pressures, such as oxidative stress and mitochondrial injury, can also induce senescence. Senescent

cells secrete altered levels of growth factors, exhibit increased susceptibility to apoptosis, and are associated with delayed repair and regeneration in the aging kidney^[6]. In addition, chronic inflammation (characterized by increased serum levels of tumor necrosis factor- α , interleukin-6, C-reactive protein and plasminogen activator inhibitor-1) and inflammatory diseases are commonly observed in aging. Moreover, senescent cells are an important source of inflammatory factors. Because the kidney is the major site for excretion and perhaps degradation of small inflammatory molecules, reduced renal function during the aging process may promote oxidative stress and inflammation. Chronic inflammation, in turn, may potentiate the initiation and progression of lesions in the aging kidney^[7, 8].

The senescence-accelerated mouse (SAM) model was developed by M Hosokawa's team at Kyoto University in 1970. At present, the senescence-prone inbred (SAMP) strains include SAMP1, SAMP1/Ka, SAMP1/Ta, SAMP2, SAMP3, SAMP6,

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Received 2012-10-06 Accepted 2012-11-25

SAMP6/Ta, SAMP7, SAMP8, SAMP8/Ta, SAMP9, SAMP10, SAMP10/Ta, and SAMP11, and the senescence-resistant inbred (SAMR) strains include SAMR1, SAMR1TA, SAMR4, and SAMR5^[9]. SAMP10 mice are characterized by brain atrophy, deficits in learning and memory, emotional disorders (depressive behavior), contracted kidneys, and senile amyloidosis. Contraction of the kidney is the most frequent cause of death in SAMP10 mice (47.5%)^[10]. Contracted kidneys have a granular surface and are reduced in size based on the severity of the lesions. Destroyed nephrons are replaced by fibrous tissue. Degenerated renal tubules display vacuolar degeneration in their proximal convoluted sections. In addition, angioneurotic lesions are observed in the contracted kidneys. SAMP10 mice are an ideal model of senescence and glomerulonephritis among SAM strains. In this study, SAMR1 mice without contracted kidneys were selected as control mice^[11].

Rhein lysinate, which is the salt of lysine and rhein, is one of the major bioactive constituents of the rhizome of rhubarb (*Rheum palmatum* Linn or *Rheum tanguticum* Maxim)^[12]. Rhein is an anthraquinone molecule that enhances the synthesis of matrix components and inhibits the inflammatory response. Recently, the metabolic precursor of rhein, called diacerein, has been demonstrated to significantly relieve pain and improve function in individuals with osteoarthritis. In the present study, we investigated the protective effects of rhein lysinate against kidney impairment in SAMP10 mice.

Materials and methods

Chemicals and reagents

Rhein lysinate (RHL), which is the salt of rhein and lysine, was prepared with a purity of 95% in our department. The structural formula of this compound is presented in our previous article^[12]. Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) kits were purchased from Nan Jing Jian Cheng Company (Nanjing, Jiangsu Province, China). An insulin radioimmunoassay kit was obtained from Tianjin Nine Tripods Medical & Bioengineering Company (Tianjin, China). Antibodies targeting TNF- α , IL-6, NF- κ B, phospho-NF- κ B, COL1A1, COL3A1, COL4A2, and β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies against rabbit or mouse IgG were obtained from Cell Signaling Technology (Danvers, MA, USA). Prestained protein marker p7708V was purchased from New England Biolabs, Ltd (Beijing, China). Western Blot Luminol Reagent and PVDF membranes were purchased from Millipore (Billerica, MA, USA).

Animal and measurement of serum chemistry

Male SAMP10 and SAMR1 mice were obtained from the Tianjin University of Traditional Chinese Medicine (Tianjin, China). The animal experiments were approved by our institutional review board (animal experiments ethics board, Beijing Hospital). The mice were housed and maintained in a room at 22 \pm 2°C with automatic light cycles (12 h light/dark) and a relative humidity of 40%–60%. Food was provided *ad libitum* throughout the study. The mice were divided into four

groups: the SAMR1 control group ($n=12$), the SAMP10 control group ($n=12$), the 25 mg/kg weight RHL-treated SAMP10 group ($n=12$) and the 50 mg/kg weight RHL-treated SAMP10 group ($n=12$). In these groups, 20-week-old mice were daily administered drinking water (SAMR1 control group and SAMP10 control group), RHL (25 mg/kg) and RHL (50 mg/kg), respectively, until 50% of the mice in each group died. The median survival time of SAMP10 mice is approximately 9.7 months^[9]. At the end of the experiment, serum was collected. The blood glucose, insulin, total cholesterol, triglyceride, creatinine, and urea in the serum were measured using standard protocols in the clinical laboratory.

Antioxidant assay

When mice died, their kidneys were harvested. The remaining mice were anesthetized with chloral hydrate and killed by cervical dislocation; the kidneys were quickly harvested and placed on ice. In total, 50 mg of kidney tissue was dissected and homogenized in a glass Teflon homogenizer containing 9 volumes of precooled physiological saline and centrifuged at 3000 \times g for 15 min at 4°C. The antioxidant enzyme activities (including the activities of SOD and GSH-px) and the MDA content in the obtained supernatant was measured using assay kits, according to the manufacturer's instructions.

Histology and immunohistochemistry

For histological analysis, kidney tissues fixed with 4% buffered paraformaldehyde were embedded in paraffin, and 3- μ m-thick sections were prepared. The sections were then stained with hematoxylin-eosin. Immunohistochemical analyses were performed using antibodies against TNF- α and IL-6. The sections were deparaffinized and quenched in 3% H₂O₂ for 15 min to block endogenous peroxidase and then washed in PBS. The sections were subsequently incubated with anti-TNF- α or anti-IL-6 antibodies for 2 h, followed by incubation with a biotinylated secondary antibody and ABC reagent (Biomed Company, Beijing, China), as recommended by the vendor. The color was developed by incubating the sections with diaminobenzidine as a substrate. The slides were counterstained with Mayer's hematoxylin, and slides that were preincubated with BSA served as negative controls.

Western blotting

Western blots were employed to detect the levels of TNF α , IL-6, NF- κ B, COL1A1, COL3A1, and COL4A2, as well as the phosphorylation of NF- κ B in the kidneys. Briefly, the kidney tissues were treated with a lysis buffer and a cocktail of phosphatase inhibitors (Roche, Indianapolis, IN, USA). Samples (30 μ g) were fractionated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the proteins were transferred to a PVDF membrane, the membrane was incubated in a blocking buffer containing BSA (1%) and Tween 20 (0.1%, *v/v*) in PBS (PBS/Tween 20) at room temperature for 1 h. Next, the membrane was incubated overnight at 4°C with the appropriate primary antibodies and then incubated with the appropriate secondary antibodies at room temperature

for 2 h. Each membrane was developed using an enhanced ChemiImager5500 chemiluminescence system (Alpha Innotech Corporation, Miami, FL, USA).

Statistical analysis

All values are presented as the means±SEM of the indicated number of measurements. A one-way ANOVA test was used to determine significance, with values of $P<0.05$ indicating statistical significance.

Results

RHL increased the median survival time of SAMP10 mice

In the SAMP10 control group, the median survival time was 10.0 ± 1.2 months. However, in the SAMP10 groups treated with 25 mg/kg RHL or 50 mg/kg RHL, the median survival times were 12.5 ± 2.1 and 12.5 ± 1.7 months, respectively. The median survival time of SAMR1 mice was greater than 12.5 months. Furthermore, RHL (25 mg/kg and 50 mg/kg) increased the median survival time of SAMP10 mice by approximately 25% ($P<0.05$), compared with the SAMP10 control group.

RHL had no effect on the weight or phenotype of SAMP10 mice

To assess the effects of RHL on the median survival time of SAMP10 mice, we first observed the effect of RHL on the body weights of the mice. There was no difference between the SAMP10 control group and the RHL-treated group. However, the body weights of SAMR1 mice were higher than those of SAMP10 mice (Figure 1A). The kidney weight/body weight ratio was decreased in the SAMP10 control mice compared with SAMR1 mice. RHL (25 mg/kg and 50 mg/kg body weight) prevented the decrease in the ratio of kidney weight/body weight (Figure 1B). In addition, compared with SAMR1 mice, most of the SAMP10 mice exhibited hair loss and markedly decreased activity, as well as a lack of glossiness, increased lordokyphosis, and early death. Moreover, RHL had no effect on the phenotype of SAMP10 mice.

The effects of RHL on the levels of blood glucose, insulin, total cholesterol, triglycerides, creatinine, and urea

The blood glucose and total cholesterol levels in the senescence-prone inbred mice were lower than in the senescence-resistant inbred mice. RHL (25 mg/kg and 50 mg/kg) had

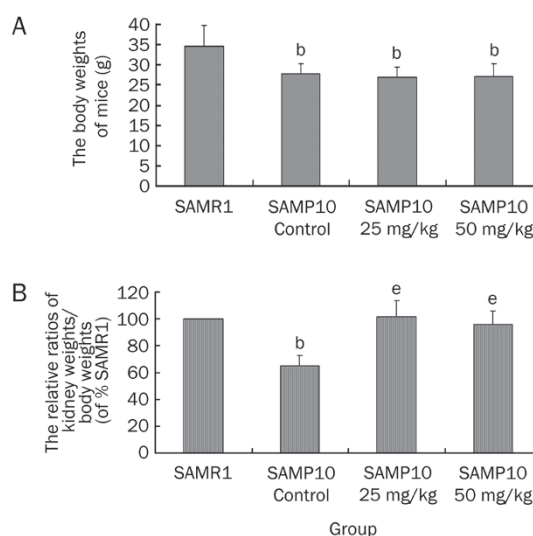


Figure 1. The body weights of the mice and the kidney weight/body weight ratios. When the mice died, their body weights and kidney weights were measured. When 50% of the mice had died, the remaining mice were anesthetized with chloral hydrate and killed by cervical dislocation. The body weights and kidney weights of these mice were measured, and the kidney weight/body weight ratios were calculated. $n=12$. Mean±SEM. ^b $P<0.05$, compared with the SAMR1 group. ^e $P<0.05$, compared with the SAMR10 control group.

no effect on the blood glucose or total cholesterol levels of SAMP10 mice. The triglyceride levels in the senescence-prone inbred mice were higher than those in the senescence-resistant inbred mice. The administration of 50 mg/kg RHL decreased the triglyceride levels in SAMP10 mice relative to the levels in the SAMP10 control mice. However, the insulin levels after RHL administration did not significantly differ among the SAMR1, SAMP10 control, and both RHL treatment groups. Compared with the levels in SAMR1 mice, the levels of creatinine and urea were increased in the SAMP10 control mice. RHL (25 mg/kg and 50 mg/kg) reduced the upregulation of creatinine and urea in SAMP10 mice (Table 1).

RHL decreased the incidence of glomerulonephritis

In the present study, we observed that SAMP10 mice gradually became emaciated. The necropsy revealed glomerulone-

Table 1. The effect of RHL on blood glucose, insulin, total cholesterol, triglyceride, creatinine, and urea. ^b $P<0.05$, compared with SAMR1 group. ^e $P<0.05$, compared with SAMP10 control group. $n=12$. Mean±SEM.

	SAMR1	Control	SAMP10 RHL 25 mg/kg	SAMP10 RHL50 mg/kg
Blood glucose (mmol/L)	9.55±1.01	6.28±0.51 ^b	6.87±0.63 ^b	6.80±0.68 ^b
Insulin (mIU/L)	43.8±10.3	43.6±8.9	40.4±10.8	41.5±11.4
Total cholesterol (mmol/L)	3.80±0.42	2.40±0.35 ^b	2.14±0.27 ^b	2.68±0.28 ^b
Triglyceride (mmol/L)	2.93±0.31	3.65±0.45 ^b	4.03±0.52 ^b	2.95±0.25 ^e
Creatinine (μmol/L)	92.3±10.5	150.0±12.3 ^b	123.2±10.7 ^{be}	120±13.8 ^{be}
Urea (mmol/L)	8.02±1.24	18.5±1.40 ^b	15.3±2.16 ^{be}	14.9±2.41 ^{be}

phritis in most of the SAMP10 control mice but not in SAMR1 mice. The kidney volumes were decreased, and contracted kidneys were observed in the SAMP10 control mice. These results suggest that RHL blocked the progression of contracted kidneys in SAMP10 mice (Figure 2).

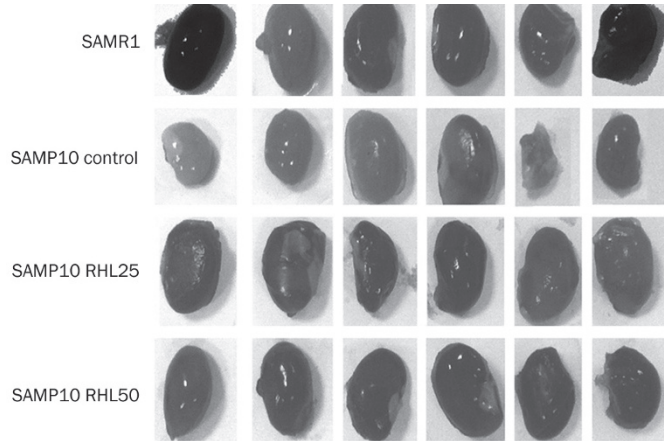


Figure 2. The kidney sizes in the different groups. At the end of the experiment, the mice were anesthetized with chloral hydrate and killed by cervical dislocation. The kidneys were removed and imaged.

Aging induced kidney diseases, and RHL impaired the invasion of mononuclear macrophages

Compared with SAMR1 mice, SAMP10 mice exhibited increased invasion of mononuclear macrophages. Hematoxylin-eosin revealed that RHL (25 mg/kg and 50 mg/kg) administered in the drinking water impaired the invasion of mononuclear macrophages (red arrow, Figure 3A). As shown in Figure 3B, contracted and destroyed renal glomeruli were observed in the SAMP10 control mice but not in SAMR1 mice or RHL-treated mice (25 mg/kg and 50 mg/kg). Lipofuscin deposition was also observed in the nephric tubules of the SAMP10 control mice (blue arrow, Figure 3C) but not in the nephric tubules of SAMR1 mice or RHL-treated mice (25 mg/kg and 50 mg/kg).

RHL improved the MDA, SOD, and GSH-px levels in the kidney tissue

Compared with the SAMR1 group, the SAMP10 control group exhibited SOD and GSH-px levels that were 20% and 5% lower, respectively, in the kidney tissues; however, the MDA level was 6% higher. Compared with the SAMP10 control group, the SAMP10 group treated with 25 mg/kg RHL had 10% and 20% higher SOD and GSH-px levels, respectively, in the kidney tissues; however, the MDA level was 8% lower. Moreover, compared with the SAMP10 control group, the SAMP10 group treated with 50 mg/kg RHL had 15% and 30% higher SOD and GSH-px levels, respectively, in the kidney tissues; however, the MDA level was 20% lower (Figure 4).

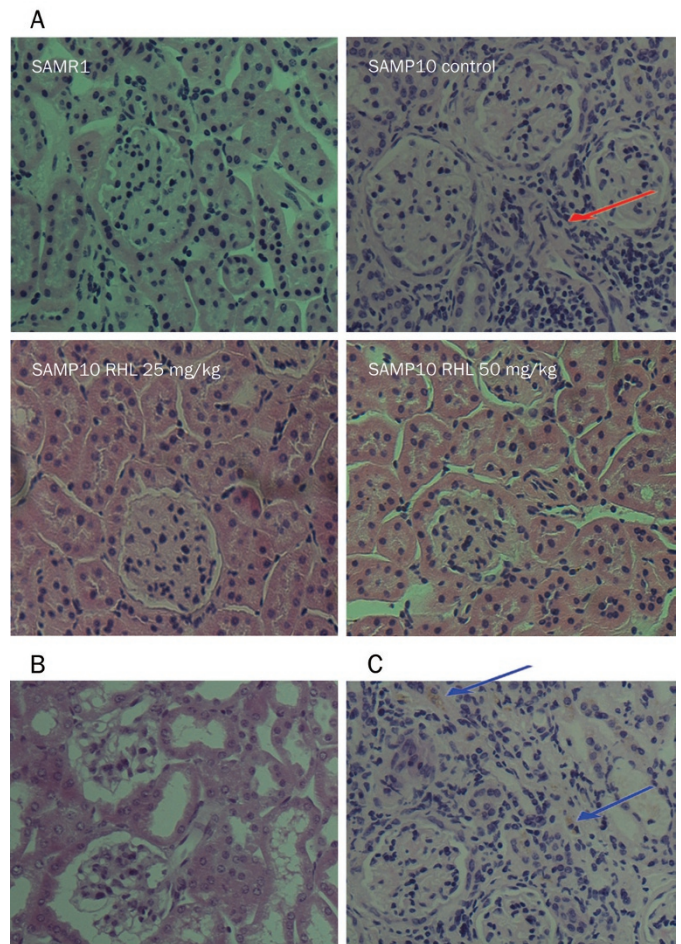


Figure 3. Evaluation of the kidney morphology. Representative examples of hematoxylin-eosin-stained renal sections from the SAMR1, SAMP10 control, 25 mg/kg RHL-treated SAMP10 and 50 mg/kg RHL-treated SAMP10 groups. Magnification $\times 200$ (A). Contracted and destroyed renal glomeruli were present in the SAMP10 control mice (B). Deposition of lipofuscin in the SAMP10 control mice (C).

RHL suppressed the expression of TNF- α , IL-6, and NF- κ B and the phosphorylation of NF- κ B in the kidney tissues

Compared with TNF- α expression in SAMR1 mice, the TNF- α levels were increased in SAMP10 mice. RHL (25 mg/kg and 50 mg/kg) administration reduced expression of TNF- α , as revealed by the immunohistochemical and Western blot analysis (Figure 5A and Figure 5C). The IL-6 levels did not differ between SAMR1 mice and SAMP10 mice. However, compared with the SAMP10 control mice, mice administered RHL (25 mg/kg and 50 mg/kg) in their drinking water had lower levels of IL-6, as indicated by immunohistochemical analyses (Figure 5B and Figure 5C). Moreover, compared with SAMR1 mice, SAMP10 mice exhibited increased phosphorylation of NF- κ B, but not the expression of NF- κ B. However, compared with the SAMP10 control group, the RHL groups (25 mg/kg and 50 mg/kg) exhibited decreased expression of NF- κ B. RHL (50 mg/kg) also decreased the phosphorylation of NF- κ B (Figure 5C).

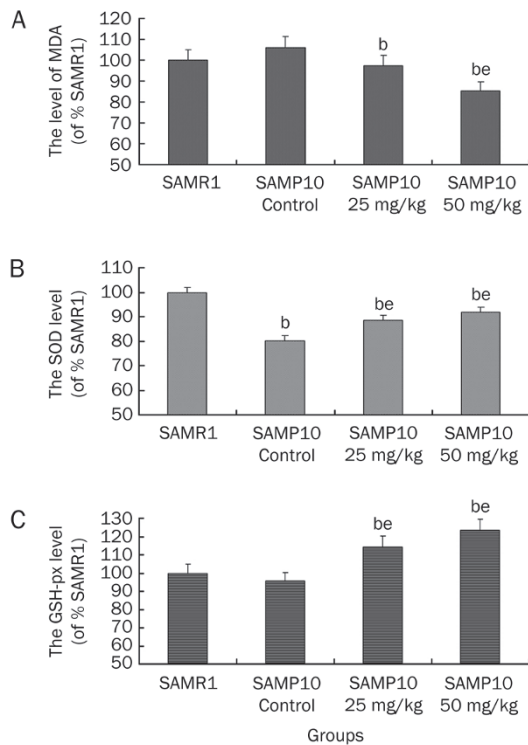


Figure 4. The MDA, SOD, and GSH-px levels in the different groups. The mice were anesthetized with chloral hydrate, and 50 mg of kidney tissue was dissected and homogenized. The supernatant obtained was used to measure the antioxidant enzyme activities, including the MDA (A) content and the activities of SOD (B) and GSH-px (C) with assay kits (according to the manufacturer's instructions). $n=12$. Mean \pm SEM. ^b $P<0.05$ vs the SAMR1 group. ^e $P<0.05$ vs the SAMP10 control group.

RHL inhibited the expression of collagen types I and III

Compared with the expression of collagen types I, III, and IV in SAMR1 mice, the levels of type I and III were increased in SAMP10 mice; however, the level of type IV was not different. RHL (25 mg/kg and 50 mg/kg) administration decreased the expression of types I and III but not type IV, based on Western blotting (Figure 6).

Discussion

The function and morphology of the kidney change markedly with aging. These anatomical and functional changes have been considered to cause increased susceptibility of the elderly to acute or chronic renal failure^[5]. SAMP10 mice exhibit brain atrophy and contracted kidneys. Moreover, contraction of the kidney is the most frequent cause of death in SAMP10 mice (47.5%)^[10]. Therefore, SAMP10 mice are an ideal model of senescence and glomerulonephritis among the SAM strains. In the present study, we observed that RHL (25 mg/kg and 50 mg/kg) administration increased the median survival time of SAMP10 mice. In the biopsy, we found that the SAMP10 control mice had contracted kidneys, which was not the case in the RHL-treated SAMP10 mice or SAMR1 mice. Therefore, we hypothesize that RHL enhances the median survival time

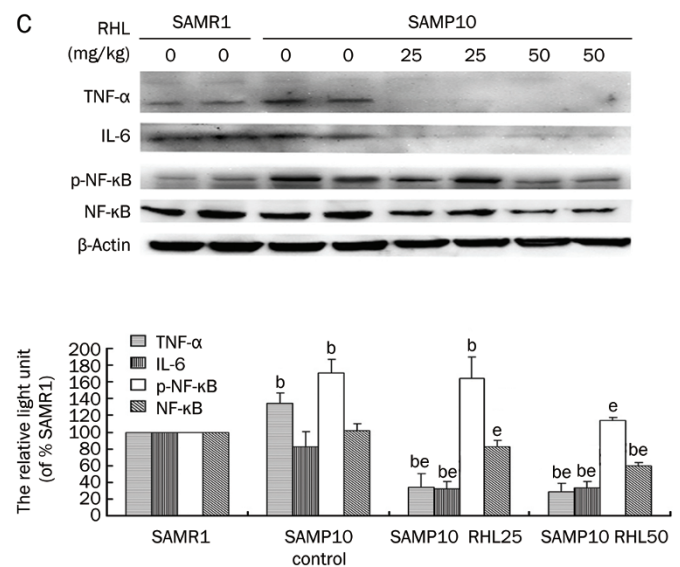
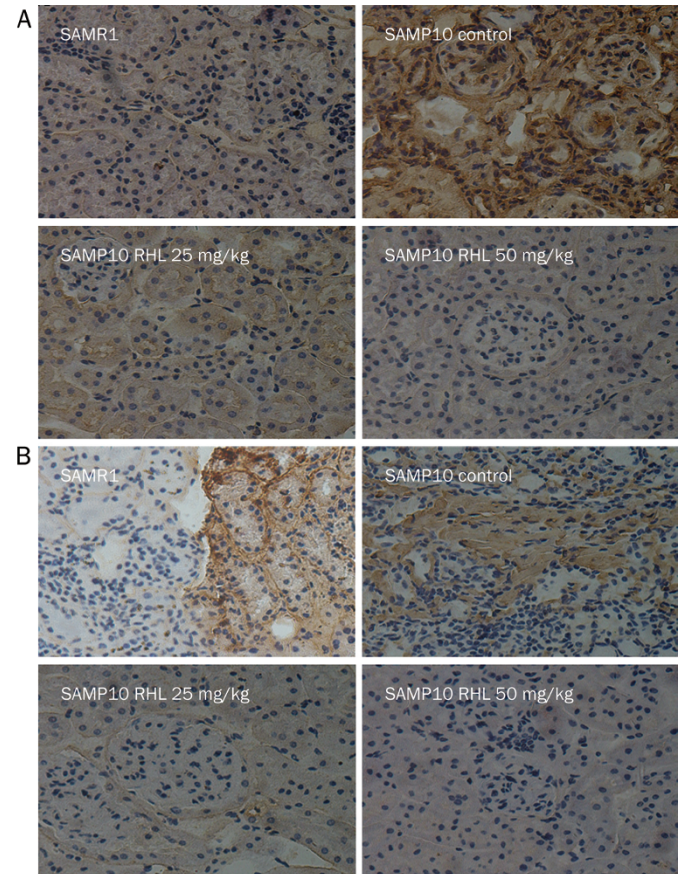


Figure 5. Effects of RHL on the expression of TNF- α , IL-6, and NF- κ B and on the phosphorylation of NF- κ B in the kidney tissues of the SAMP10 mice. Immunohistochemical staining of representative kidney tissue sections from the SAMR1, SAMP10 control, 25 mg/kg RHL-treated SAMP10 and 50 mg/kg RHL-treated SAMP10 mice. Positive (yellow) staining indicates the expression of TNF- α (A) and IL-6 (B). The effects of RHL on the expression of TNF- α , IL-6, and NF- κ B and on the phosphorylation of NF- κ B were analyzed by Western blotting. β -Actin was used as the internal control (C). $n=12$. Mean \pm SEM. ^b $P<0.05$ compared with the SAMR1 group. ^e $P<0.05$ compared with the SAMP10 control group.

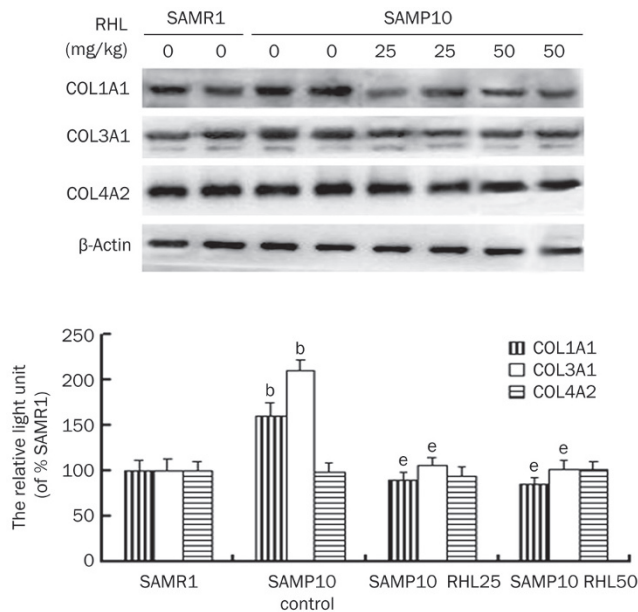


Figure 6. The effects of RHL on the expression of collagen types I, III, and IV were analyzed by Western blotting. β-Actin was used as the internal control. $n=12$. Mean±SEM. ^b $P<0.05$ compared with the SAMR1 group. ^e $P<0.05$ compared with the SAMP10 control group.

of SAMP10 mice by protecting the kidney and preventing the occurrence of uremia, which is characterized by an increase in creatinine and urea, because kidney dysfunction is known to decrease life expectancy in the elderly^[13].

The active component of RHL is rhein (4,5-dihydroxy-anthraquinone-2-carboxylic acid), which is one of the major bioactive constituents of the rhizome of rhubarb (*Rheum palmatum* Linn or *Rheum tanguticum* Maxim). In previous studies, rhein was found to exhibit a variety of bioactivities, such as inhibiting IL-1-induced chondrocyte activation^[14], decreasing the cellular hypertrophy of mesangial cells^[15], suppressing tumor cell proliferation, and inducing tumor cell apoptosis^[16]. In addition, rhein exhibits synergy with mitomycin^[17]. In our previous study, we also determined that RHL inhibited cancer cell proliferation, but the effect of RHL on glomerulonephritis was unclear. In the present study, we found that RHL administered in drinking water increased the median survival time of SAMP10 mice and improved their kidney function, as revealed by decreased creatinine and urea levels (Table 1). Moreover, we concluded that RHL decreased the occurrence of uremia by reducing the severity of glomerulonephritis in SAMP10 mice, and this discovery motivated us to elucidate the mechanism. Oxidative stress plays an important role in kidney injury during aging. The MDA, SOD, and GSH-px levels are indicators of the oxidative stress status. Our results indicate that RHL decreases the level of MDA and increases the levels of SOD and GSH-px in kidney tissues. Thus, it can be deduced that RHL protects the kidney by reducing the level of reactive oxygen species.

The progression of IgA nephropathy (IgAN), the most

frequent type of primary glomerulonephritis, is associated with high levels of mononuclear leukocyte infiltration into the kidneys. These cells primarily consist of T cells and macrophages^[18]. In this study, compared with SAMR1 mice, the SAMP10 control mice displayed kidney disease associated with the invasion of mononuclear macrophages, atrophy and kidney destruction. This disease might be related to inflammation due to autoimmune reactions. In addition, one of the characteristics of aging is deposition of lipofuscin in the tissue. In the present study, we observed that RHL protected the kidney and delayed senescence in the mice by preventing the development of mouse autoimmune glomerulonephritis through potent immune-modulating effects and by decreasing the deposition of lipofuscin in the kidney.

Inflammatory factors reportedly play a role in aging and in the progression of kidney disease. The mRNA expression of TNF-α is up-regulated in chronic-kidney-disease patients with a higher glomerular filtration rate relative to patients with a low glomerular filtration rate^[19]. Moreover, renal insufficiency is associated with increased levels of inflammatory biomarkers, including IL-6 and TNF-α^[20, 21]. However, the effect of RHL on inflammatory factors related to chronic kidney disease is unclear. Our results demonstrated that RHL markedly suppressed the expression of TNF-α and IL-6 in SAMP10 mice (Figure 5). Therefore, we conclude that inflammatory factors (TNF-α and IL-6) play a role in the progression of aging in SAMP10 mice and that RHL administration reduces the production of inflammatory factors, protects the kidneys of SAMP10 mice and delays the senescence of SAMP10 mice.

TNF-α is a proinflammatory cytokine that is involved in a number of biochemical pathways, including the activation of the nuclear factor kappa B (NF-κB) transcription factor. NF-κB acts in two ways. First, it has anti-apoptotic properties and prevents cell death in cells with malignant potential. Second, NF-κB stimulates the immune response, specifically, the production of pro-inflammatory cytokines^[22], and NF-κB activation is associated with inflammation in experimental nephritis^[23]. In the present study, we observed that TNF-α indirectly induced glomerulonephritis via the TNF-α/NF-κB signaling pathway. RHL inhibited not only the expression of TNF-α but also the expression and phosphorylation of NF-κB, a signal downstream of TNF-α. Therefore, RHL was able to inhibit the immune response by blocking the TNF-α/NF-κB signaling pathway.

In most circumstances, when patients are diagnosed with chronic kidney disease, their kidneys also display various degrees of renal fibrosis. Hence, a key to effective therapy for chronic kidney disease is to develop a strategy that blocks the progression of established renal fibrosis^[24]. Collagen types I and III are fibril-forming interstitial collagens, whereas collagen type IV is a basement-membrane collagen. In our study, we determined that the levels of collagen types I and III were increased in SAMP10 mice and that RHL blocked this increase (Figure 6). These results suggest that renal interstitial fibrosis is related to kidney contraction in SAMP10 mice and that RHL blocks the progression of renal interstitial fibrosis.

In conclusion, inflammation and reactive oxygen species played a role in the aging process and the progression of glomerulonephritis in SAMP10 mice. RHL (doses of 25 mg/kg and 50 mg/kg have the same effects) significantly suppresses kidney inflammation by reducing the levels of reactive oxygen species, blocking the TNF- α /NF- κ B signaling pathway and delaying the senescence of SAMP10 mice. We anticipate that the results of the current study will promote future investigations that explore the therapeutic potential of RHL in glomerulonephritis.

Acknowledgements

This work was supported by a grant from the National Natural Science Foundation of China (81001439).

Author contribution

Dr Ya-jun LIN and Dr Yong-zhan ZHEN designed the research. Gang HU, Jiang LIU, Rong XU, and Yu QIAO performed the research. Jie WEI and Ping TU wrote the paper.

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