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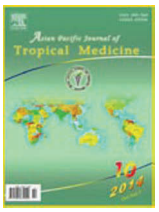

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Effect of simvastatin on expression of IL17, HMGB1 and TLR4 in LN kidney tissues of rats

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ABSTRACT

Objective: To observe the intervention influence and effect of simvastatin on the expression of interleukin 17 (LI17), high mobility group protein 1 (HMGB1) and TLR4 path in Lupus nephritis (LN) rats. **Methods:** A total of 28 BSXSB male mice with LN (16 weeks) were randomly divided into observation group and the comparison group, observation group was given 6 $mg \cdot kg^{-1} \cdot d^{-1}$ simvastatin in 0.1% PBS lavage for 4 weeks, the comparison group was not given any treatment. Blood urea nitrogen (BUN) level and urine trace albumin (Scr) level of two groups were determined. The expression of IL17, HMGB1 and TLR4 protein was detected using immune histochemical method, and the kidney histological damage was observed. **Results:** BNU, LI17, HMGB1, TLR4 protein and HMGB1 mRNA in observation group was significantly lower than that in control group ($P<0.05$); There was no statistical difference of Scr level between two groups ($P>0.05$). Histological observation showed glomerular lesions integral of observation group was obviously lower than that of control group. **Conclusions:** Simvastatin can reduce the expression of IL17, HMGB1 and TLR4 protein in LN mice, thereby can inhibit the autoimmune response as a potential treatment function of LN.

1. Introduction

Systemic lupus erythematosus (SLE) is a common autoimmune disease, can be involving multiple organ[1-3], its complication lupus nephritis (LN) is due to kidney involved in SLE pathogenesis process, not-in-time treatment would cause poor prognosis, even lead to death[4,5]. At present, the pathogenesis of LN is not completely clear. It is generally recognized cytokine secretion imbalance caused immune dysfunction is the core pathogenesis of SLE[6]. Studies have shown that[7], HMGB1 plays a vital role in LN disease, and TLR4 receptor and expression of IL17 are closely correlated with HMGB1. Another study confirmed that[8], statins have not only lipid-lowering effect, but also the effect of anti-inflammatory and immune regulation. To observe the intervention influence and treatment effect of simvastatin on the expression of LI17, HMGB1 and TLR4, and glomerular damage in LN rats, we selected BSXSB mice for observation of simvastatin intervention treatment, results are reported as follows.

2. Materials and methods

2.1. Experimental animal

A total of 28 BSXSB male LN mice of 11 weeks, weight 18-24 g with average of (20±3) g were purchased from Jackson laboratory, and second-level breed by immune department of Peking University, free food, drinking water, temperature (22±2)℃, humidity (55±2)%. Prior to experiment, animals are raised one week until acclimation, then the experiment lasted from 12 weeks to 16 weeks under same breeding environment.

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2.2. Instrument and reagent

Sysmex CHEMIX-180 fully automatic biochemical analyser, optical microscope (BH-2) push around (German); inverted microscope (Push Around Company, Japan); gel scan imaging system (American BioRad Company); morphometric analysis software (Imagepro-plus). Immunohistochemical kit, DAB chromogenic agent and PBS were provided by Beijing Chinese Golden Bridge Biotechnology Co.. Rabbit antimouse TLR4 polyclonal antibody, rabbit antimouse HMGB1 polyclonal antibody, rabbit antimouse IL17 polyclonal antibody and simvastatin were bought from Wuhan Zhongmei BCTC.

2.3. Experiment method

A total of 28 BSXSB male mice with LN (16 weeks) were randomly divided into observation group and the comparison group, observation group was given 6 $mg \cdot kg^{-1} \cdot d^{-1}$ simvastatin in 0.1% PBS lavage for 4 weeks, the comparison group was not given any treatment. Serum was extracted from angular vein under conventional separation, bilateral renal cortical tissue was extracted after sacrificing the mouse, then fixed with 4% paraformaldehyde and 2% glutaric alcohol. Meanwhile, parts of tissue samples were frozen for further test at -72 ℃.

2.4. Indexes observation

Blood of angular vein was centrifuged, supernatant was taken for determination of BUN; Scr extracted from bladder puncture was detected using chemiluminescenceion method; protein expression of kidney tissue was tested by immune histochemical method, bilateral renal cortical tissue was made into paraffin sections. They were dewaxed and HE stained. Antigen was retrieved and added to IL17 PcAb, HMGB1 PcAb and TLR4 PcAb for incubation at 4 ℃. PBS was used instead of primary antibodies in negative control, positive signals were recorded afterwards. HMGB1mRNA expression level was examined using RNA extraction kit. Total of 30 glomerular and corresponding renal tubular, interstitial and blood vessels were observed under light microscope and used -, +, ++, +++ as evaluation criteria of tissue lesion severity.

2.5. Statistical analysis

SPSS19.0 statistical software was used to analyze the measurement data in (mean±sd), data between groups were compared with t test; $P<0.05$ was regarded as statistically significant difference.

3. Results

3.1. BNU and Ser

BNU of observation group was significantly lower than that of control group ($P<0.05$). There was no statistical difference in Ser between two groups ($P<0.05$) (Table 1).

Table 1
Comparison of renal function between two groups.

Groups	n	BNU (mmol/L)	Ser (g/ L)
Observation group	14	6.95±0.96*	47.80±2.66
Control group	14	9.62±0.81	48.33±2.94

Note: * $P<0.05$ when compared with control group.

3.2. Expression level of IL17, HMGB1 and TLR4 protein between two groups

Expression level of IL17, HMGB1 and TLR4 protein in observation group was significantly lower than that of control group ($P<0.05$) (Table 2 and Figure 1). The HMGB1 mRNA relative expression (0.949 9±0.079 2) in observation group was significantly lower than that in control group (0.024 1± 0.079 6) ($P<0.05$).

Table 2
Expression level of IL17, HMGB1 and TLR4 protein between two groups.

Groups	IL17	HMGB1	TLR4
Observation group	1.29±0.09*	1.17±0.05*	1.19±0.09*
Control group	1.51±0.11	1.33±0.07	1.37±0.11

Note: * $P<0.05$ when compared with control group.

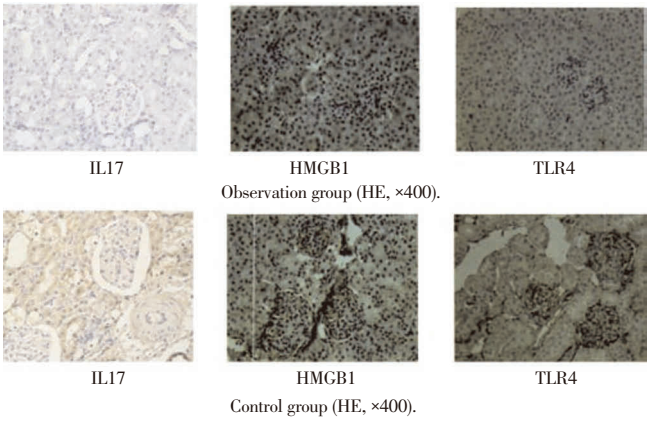
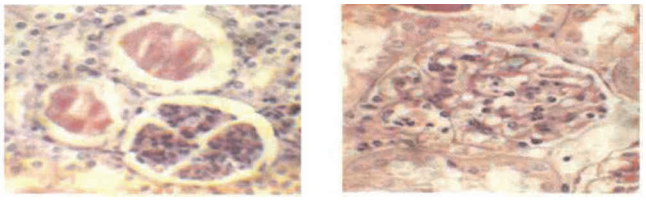


Figure 1. Expression level of IL17, HMGB1 and TLR4 protein between two groups.

3.3. Histological observation

The kidney tissue of observation group was diagnosed as mild diffuse hyperplastic glomerular nephritis: nearly half of the glomerular mesangial cells and matrix were presented with mild hyperplasia, there was no obvious inflammatory cell infiltration in glomerular, renal tubular had no obvious abnormalities, with 182 points of glomerular damage total score and 1 point of tubular damage total score. The kidney tissue of control group was diagnosed as moderate diffuse hyperplastic glomerular and mild interstitial nephritis nephritis, similar to type IV of LN in human: almost half of the glomerular mesangial cells and matrix were presented with moderate hyperplasia, inflammatory cell infiltration was observed in some glomerular, nearly half of renal tubular had abnormalities with expansion and protein tube type, with 281 points of glomerular damage total score and 39 points of tubular damage total score. In observation group, the damage total score in glomerular and in tubular of was significantly lower than that of control group ($P<0.05$) (Figure 2).



observation group control group

Figure 2. Histological observation of damaged kidney tissues (HE, ×400).

4. Discussion

Currently, SLE animal model BXSB is used clinically, in which can mimic the human SLE and induce spontaneous autoimmune diseases within 8 weeks of use on mice, and its late kidney change is similar to Ⅳ type LN on human[9]. In the control group, a significant rise of BUN serum level in observation group during the late onset, shows BUN clearance has reduced; and microscopic finding of obvious LN pathological manifestation is also similar to clinical pathological changes of LN, confirming BXSB mice model is an ideal animal model of SLE. Simvastatin tablets are a reductase inhibitor of methyl hydroxy glutaric acyl coenzyme A (HMG-CoA), inhibiting endogenous synthesis of cholesterol and regulating blood lipid. Studies have confirmed that[8], statins not only have lipid-lowering effect, but also has the effect of anti-inflammatory and immune regulation.

HMGB1 is one of the members of the HMG super family, participating in DNA replication, cell differentiation and various life activities[10]. Studies have shown that[11-14], HMGB1 expressed outside nucleus can be directly involved in innate immune effect as inflammatory cytokines, also can improve the adaptive immune response, and participate in a variety of disease occurrence and development process. Another study reported[15,16], expression of HMGB1 in the peripheral blood of LN patients enhanced, suggesting that HMGB1 is an important cytokines, associated with kidney damage in LN course. IL-17 is mainly generated by memory T cells, plays an important role in white blood cell migration and activation during the inflammatory response[17-19]. Studies have confirmed that[20], serum IL-17 of SLE patients is significantly higher than that of normal people, prompting that IL-17 is closely associated with the occurrence of autoimmune disease development. TLR4 is the main receptor mediated endotoxin/lipopolysaccharide response, TLR4/CD14 signaling pathway is the most important pathway mediated endotoxin induced inflammatory reaction[21].

Studies have considered[22-23], IL17, HMGB1 and TLR4 present a synergy of inflammatory effect on the pathogenesis of LN. In this study, the control group showed a increased trend of IL17, HMGB1 and TLR4 protein level, prompting a close association with the LN onset, which is consistent with literature reports. after treatment with simvastatin, IL17, HMGB1 and TLR4 protein expression in observation group was significantly lower than that of control group ($P<0.05$), suggesting simvastatin can reduce the expression of L17, HMGB1 and TLR4 protein and inhibiting autoimmune reaction in LN mice. And BUN clearance function of observation group increased significantly after simvastatin lavage, also confirmed that simvastatin can improve kidney function for LN mice.

This study shows that simvastatin can reduce the expression of IL17, HMGB1 and TLR4 in kidney tissue of LN mice, inhibit autoimmune damage, therefore can have certain clinical value for LN treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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