

Original Article

Interleukin-18 binding protein attenuates renal injury of adriamycin-induced mouse nephropathy

Menghua Dong^{1*}, Mingfeng Zhao^{1*}, Min Cui^{2*}, Jiuzheng Sun³, Xianghui Meng⁴, Wangnan Sun¹, Lin Wang⁵, Pengchao Du¹

¹School of Basic Medical Sciences, Binzhou Medical University, Yantai, Shandong, China; ²Department of Pediatrics, Binzhou People's Hospital, Binzhou, Shandong, China; ³Jinan Central Hospital Affiliated to Shandong University, Jinan, Shandong, China; ⁴Central Hospital of Zibo (Gaoqing Branch Courts), Zibo, Shandong, China; ⁵The Second Hospital of Shandong University, Jinan, Shandong, China. *Equal contributors.

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Abstract: Nephrotic syndrome is one of the most common kidney diseases in children, most of which were caused by minimal change disease, which could be typically reversible with the use of corticosteroid therapy in steroid-sensitive nephrotic syndrome. At the same time, there still exist some side effects caused by drugs and steroid-resistant nephrotic syndrome. It's urgent to investigate more accurate treatment to improve the situation. In this study, we chose mice model by adriamycin to observe the effect of IL-18BP intervention. It was shown that (1) weak general conditions appeared after adriamycin administration; (2) Proteinuria showed up after adriamycin-administration and then decreased with IL-18 binding protein intervention; (3) the level of triglyceride, cholesterol, IL-18, IFN- γ , and TNF- α in the IL-18 binding protein intervening group were significantly lower than those in the adriamycin-minimal change disease MCD group (all $P < 0.01$), and the levels of serum total protein, albumin, and IL-4 were significantly higher than those in the adriamycin-minimal change disease MCD group ($P < 0.05$, $P < 0.01$, $P < 0.05$); (4) ultramicrostructural examination demonstrated wide fusion of foot processes of glomerular epithelial cells in adriamycin-minimal change disease MCD mice, while only focal fusion occurred in IL-18 binding protein intervening mice. In conclusion, IL-18BP repaired the proteinurine, histopathological injury of kidney, and the induction of serum cytokines in mice models of minimal change disease induced by adriamycin.

Keywords: IL-18, IL-18 binding protein, minimal change nephrosis, IL-4, ultramicrostructural examination

Introduction

Primary nephrotic syndrome is a group of multiple diseases that seriously affect people's health, especially children's. Minimal change disease (MCD) is the most common cause of nephrotic syndrome in children and around 15-20% of cases in adults [1], with the clinical characteristics of heavy proteinuria and hypoalbuminemia, resulting in edema and hypercholesterolemia. However, the pathogenesis of this disease was still not completely elucidated so far. The current treatment was limited to the application of hormones and other immunosuppressive agents, whose efficacy was unstable with possible side effects and drug resistance [2, 3]. So, it's urgent to discover new treatments with fewer side effects and less resistance.

The inflammatory factors produced by various immunocompetent cells might be the direct cause of glomerular pathological damage and renal interstitial inflammation, clinically manifested as characteristic large and recurrent proteinuria [4]. IL-18 is a member of the IL-1 family which is expressed by a wide range of cells, mainly macrophages, Kupffer cells, keratinocytes, osteoblasts, astrocytes, and DCs [5]. IL-18 is synthesized as a biologically inactive precursor that requires cleavage by the enzyme caspase-1 to become a biologically active molecule, sharing structural features with IL-1 [6]. Caspase 1 activation by inflammasomes, such as NLRP3, NLRC4, and AIM2 by CARD-CARD interaction, results in a cell-death program termed pyroptosis, which induces mature IL-1 β and IL-18 release [7-9]. IL-18 could induce IFN- γ

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production in presence of IL-12 [10]. IL-18 also played an important role in the differentiation of naïve T cells into Th2 cells, producing IL-13 and IL-4 [11, 12]. The biological activity of IL-18 can be neutralized by the IL-18-binding protein (IL-18BP), which binds mature IL-18 with a high affinity. The binding equimolar ratios of IL-18BP were reported at 50-70% inhibition of IL-18 [13]. The biological antagonism of IL-18BP against IL-18 has been proved in both *in vitro* and *in vivo* [14-16]. It has been revealed that IL-18 expression correlates with autoimmune diseases or inflammatory disorders, activities of rheumatoid arthritis and Crohn disease notably by NLRP3 [17, 18]. IL-18 was also involved in and aggravated the progress of many primary and secondary kidney diseases [19, 20]. Urinary IL-18 was also thought as a predictive biomarker for the early detection of acute kidney injury [21, 22]. Based on this review, in this study, we chose IL-18 as a target to observe the effect of IL-18BP intervention in mice model of MCD by adriamycin administration, in order to find a new approach for MCD.

Materials and methods

Animal studies

A total of 38 male mice, 4-6 weeks old and weighing (20 ± 2) g, were obtained from our own breeding colony (Binzhou Medical University) and maintained under standard conditions. Adriamycin was obtained from Wanle pharmaceutical Corp (Shenzhen, China). The 36 mice were assigned randomly to three groups: (1) the control group (n=10); (2) the adriamycin-minimal change disease (ADR-MCD group, n=10); (3) the low-dose IL-18BP intervening group (n=9); and (4) the high-dose IL-18BP intervening group (n=9). Adriamycin (7.5 mg/kg body weight) was injected once by a single intravenous injection through the tail vein. IL-18BP was given on day 5, 7, 12 and 21 after adriamycin administration at 0.25 mg/kg (low dose) and 0.5 mg/kg (high dose) by intraperitoneal injection [8]. PBS (0.25 mg/kg and 0.5 mg/kg, intraperitoneal injection) acted as a vehicle. The behavior of mice was observed and recorded, and 24-hour urine was collected on day 14, 28, 42. On day 42, blood samples were obtained from mouse hearts. After reperfusion, the kidney samples were collected in

4% formaldehyde or 5% glutaraldehyde solution for later histological examination on day 42.

Assessment of renal function and biochemical markers

The blood was collected from the heart into self-made anticoagulant tubes with 3.8% sodium citrate, and serum was isolated by centrifuge at $3000 \times g$ for 10 min at 4°C. Total protein (TP), albumin (ALB), cholesterol (CH), triglyceride (TG), blood urea nitrogen (BUN) and serum creatinine (Scr) were analyzed by ck-7 automatic biochemical analyzer (Backman company, America).

Histological assessment

Formalin-fixed kidneys were paraffin-embedded and 4 μ m sections were stained with hematoxylin and eosin. Microstructural examinations were carried out by transmission electron microscope (H-600, Hitachi, Japan).

Cytokines assessment

Mouse IL-18BP and mouse IL-18BP ELISA kit were purchased from R&D Systems (Minneapolis, Minn., USA). Mouse TNF- α , INF- γ , and IL-4 ELISA assay kits were obtained from Jingmei pharmaceutical Corp (Shenzhen, China). The ELISA examinations were carried out by manufacture's instructions.

Statistical analysis

Data are expressed as means \pm s.e.m. The statistical software SPSS12.0 and GraphPad Prism 5 software were used for the statistical analysis. Statistical differences between groups were assessed by one-way ANOVA followed by Duncan's multiple range tests. $P < 0.05$ was considered statistically significant.

Results

Weak general condition appeared after adriamycin administration

After administration of adriamycin, mice were in low spirits with different degree of decreased urine volume, edema, loss of body weight, baldness, and poor activity. Concurrently, these symptoms relieved after IL-18BP intervening.

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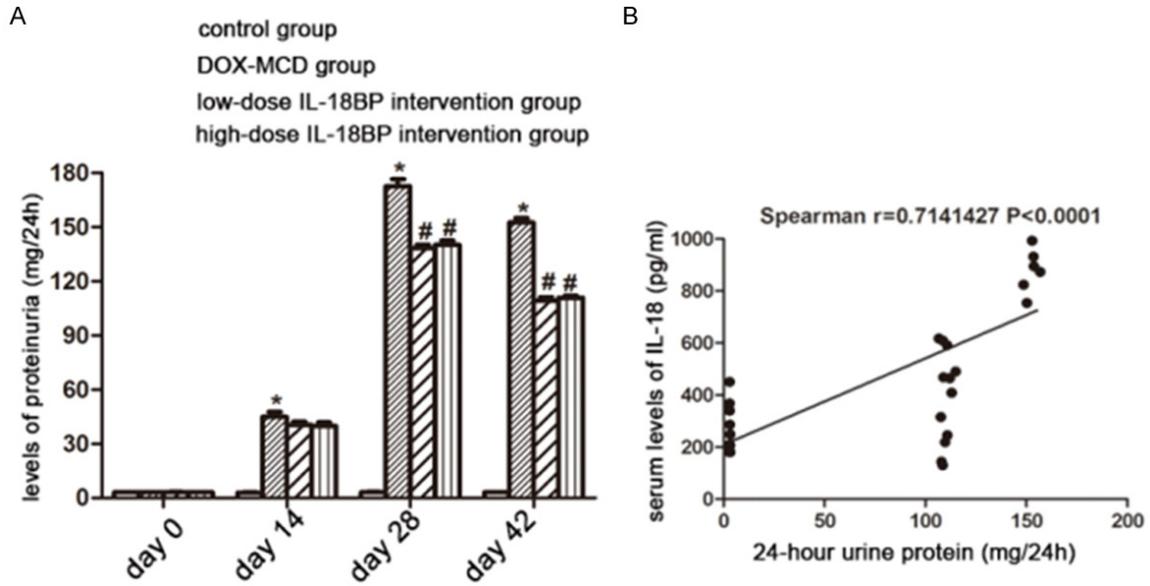


Figure 1. Proteinuria was observed after adriamycin administration and decreased by IL-18BP intervention. A. Levels of overt proteinuria were shown. Overt proteinuria appeared on day 14 after administration of ADR, and peak proteinuria appeared on day 28 after administration of ADR. ADR-MCD group means mice with minimal change nephrosis (MCD) induced by adriamycin (ADR). B. There was a positive relationship between the 24-hour urine protein and levels of serum IL-18 on day 42. *, $P < 0.05$ versus control group; #, $P < 0.05$ versus ADR-MCD group. The control group (n=10); the ADR-MCD group (n=10); the low-dose IL-18BP intervening group (n=9); the high-dose IL-18BP intervening group (n=9).

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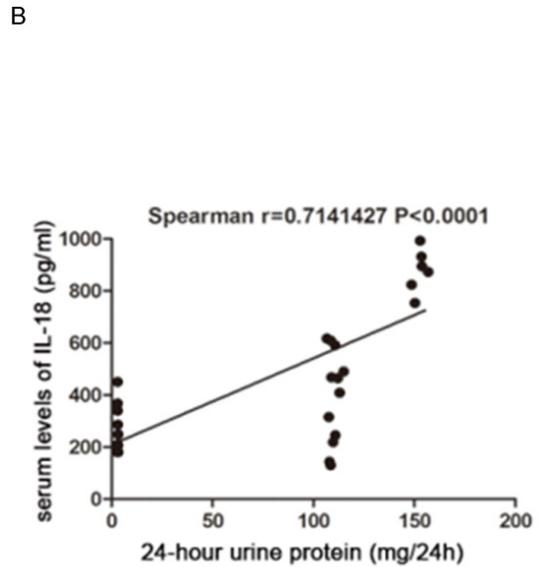
Overt proteinuria appeared on day 14 after administration of adriamycin. The 24-hour urine protein after adriamycin administration was significantly higher than control. Concurrently, there was no significant difference between the ADR-MCD group and the IL-18BP intervention groups.

Peak proteinuria appeared on day 28 after administration of adriamycin, and decreased on day 42. No matter on day 28 or day 42, in different IL-18BP intervention groups, the 24-hour urine protein was significantly lower than that in ADR-MCD group respectively (**Figure 1A**).

There was a positive relationship between the 24-hour urine protein and levels of serum IL-18 on day 42 (**Figure 1B**).

Blood biochemical parameters changed after IL-18BP intervention

Serum levels of TP and ALB in the ADR-MCD group were significantly lower than those in the control group respectively, while there was



no significant difference in the IL-18BP intervening group (**Figure 2A**).

Serum levels of TG and CH in the IL-18BP intervening group were significantly lower than those in the ADR-MCD group, while significantly higher than those in the control group (**Figure 2B**). There was no significant difference in the serum levels of BUN and Scr among the three groups (**Figure 2C, 2D**).

IL-18BP intervention alleviated morphological alterations after adriamycin administration

HE staining showed glomerular mild hyperemia with or without glomerular mesangial cells mild hyperplasia and capillary stenosis or occlusion in ADR-MCD group. There was no obvious glomerular hyperemia and congestion, and the mesangial cell proliferation was obviously reduced after IL-18BP intervention. High doses of IL-18BP showed fewer alterations (**Figure 3A**).

Ultrastructural examination showed that the glomeruli from the ADR-MCD group exhibited intense degenerative changes. Glomerular epithelial foot processes widely spread fused along the capillary basement membrane, which slightly thickened, accompanied by mitochon-

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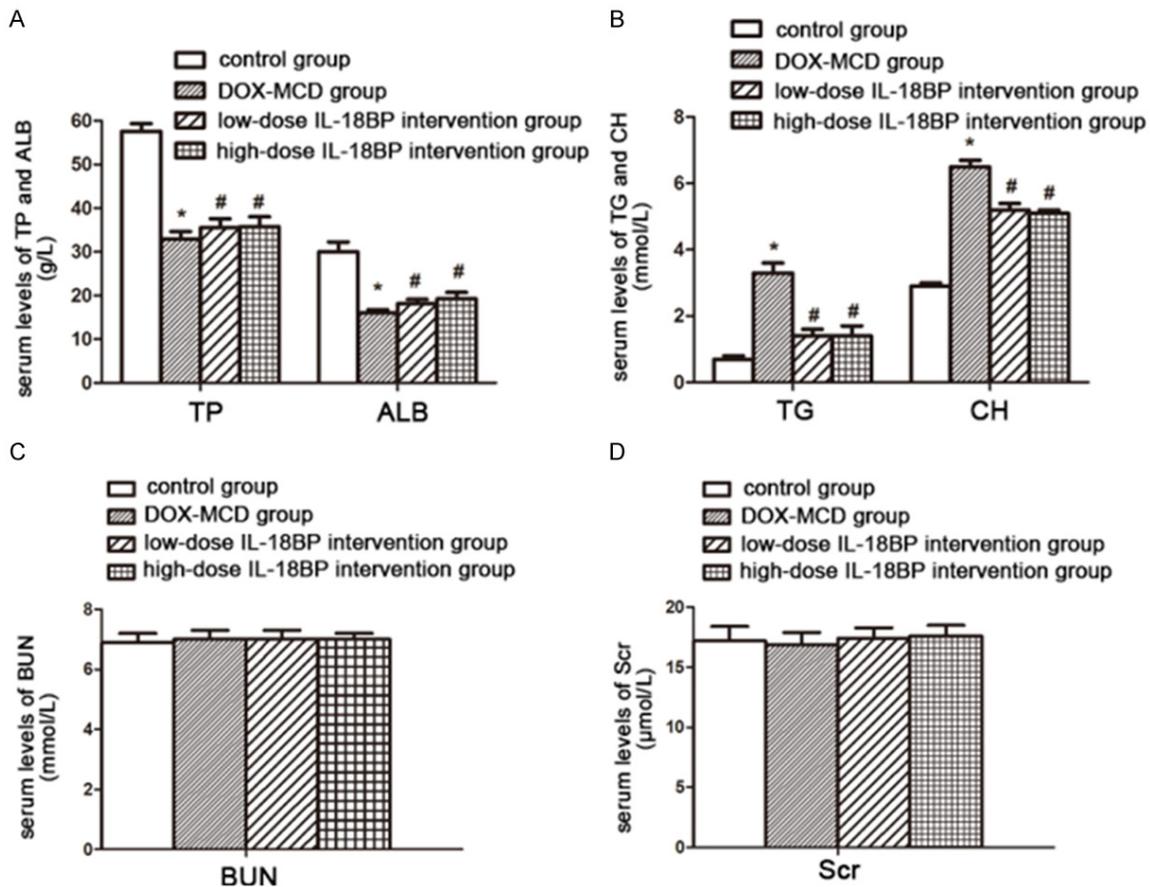


Figure 2. Blood biochemical parameters changed after IL-18BP intervention. A. Serum levels of TP and ALB were shown in different groups. B. Serum levels of TG and CH were shown in different groups. C. Serum levels of BUN and Scr were shown in different groups. D. Serum levels of Scr were shown in different groups. *, $P < 0.05$ versus control group; #, $P < 0.05$ versus ADR-MCD group. The control group ($n = 10$); the ADR-MCD group ($n = 10$); the low-dose IL-18BP intervening group ($n = 9$); the high-dose IL-18BP intervening group ($n = 9$).

dria swelling. While the glomeruli from the IL-18BP intervening group only demonstrated local fusion of the glomerular epithelial foot processes, or approximated the normal renal tissue (Figure 3B).

Serum cytokines after adriamycin administration were intervened by IL-18BP

The serum levels of IL-18 decreased significantly after IL-18BP intervention by two different doses, which represented the effect of IL-18BP intervention (Figure 4A).

Elisa showed that levels of IFN- γ in the IL-18BP intervening group by two different doses were significantly lower than those in the ADR-MCD group (Figure 4B), while the level of TNF- α in the IL-18BP intervening group only by high dose was significantly lower than those in the ADR-MCD group (Figure 4C).

The serum level of IL-4 in the IL-18BP intervening group by high dose was significantly higher than that in the ADR-MCD group (Figure 4D).

Discussion

In the current study, the protective effect of IL-18BP was shown in MCD mice models with clinical features of MCD induced by adriamycin. After IL-18BP administration, the fusion of foot processes of glomerular epithelial cells by ultramicrostructural examination in ADR-MCD mice was relieved. Meanwhile, the level of TG, CH, IL-18, IFN- γ and TNF- α in the IL-18BP intervening group were significantly lower, while the higher level of TP, ALB and IL-4.

The primary target of MCD injury is considered as podocyte, including the extensive effacement of foot processes of the podocytes noted by electron microscopy with normal glomeruli

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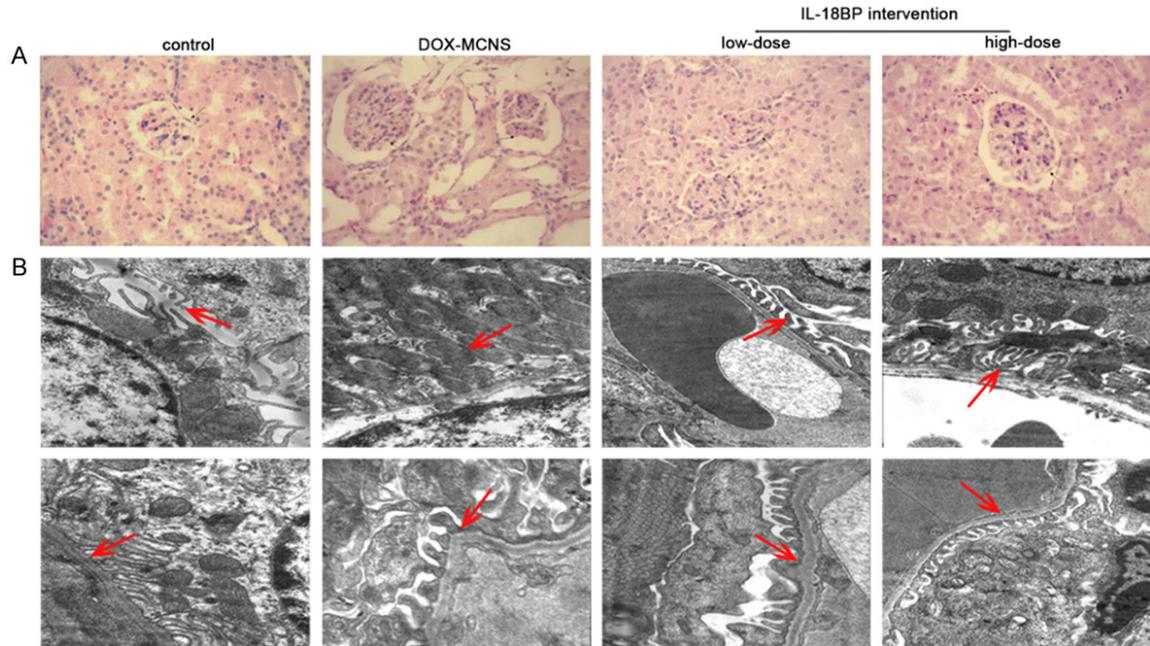


Figure 3. IL-18BP intervention alleviated morphological alterations after adriamycin administration. A. HE staining showed the levels of glomerular mild hyperemia and hyperplasia in glomerular mesangial cells and capillary stenosis or occlusion in different groups. B. Ultrastructural examination showed degenerative changes in glomeruli from different groups. Red arrows showed the changes of podocytes.

by light microscopy [23]. The foot process effacement and disruption of the glomerular slit diaphragm leads to proteinuria [24] and persistent proteinuria is a prognostic marker for the progression to end-stage renal disease [25]. In this study, the levels of 24-hour urine protein in different stages was observed, and it showed the positive relationship between the 24-hour urine protein and levels of serum IL-18 was shown on day 42 (**Figure 1A**), which means that the levels of serum IL-18 is closely related to the degree of the kidney injury of MCD. However, there is lack of clinical evidence.

The point of view that the IL-18BP could exert nephro-protective effects could be confirmed by the results of quantization of urine protein as well as the ultrastructural examination in this study. The serum levels of IL-18 in IL-18BP intervening group were significantly lower than that in the ADR-MCD group. The IL-18BP intervening mice showed overt proteinuria on day 14, there was no significant differentiation compared with the ADR-MCD mice. While on day 28 and later, the IL-18BP intervening mice showed significantly lower level of urine protein than the ADR-MCD mice. Podocyte foot process effacement and disruption of the glomer-

ular barrier are common phenotypes observed in almost all glomerular diseases associated with nephrotic-range proteinuria [26]. The podocyte foot process effacement involves dedifferentiation, direct injury of the slit diaphragm or the actin cytoskeleton, and changes in the glomerular basement membrane and podocyte interactions [27]. The loss of glomerular slit diaphragm function leads to proteinuria. The ultrastructural examinations, which were the criteria for MCD, demonstrated that the podocyte injury in the IL-18BP intervening group was relieved, consistent with morphological examination in Kate R. Wyburn' study [28]. All the data above proved the nephro-protective effects of IL-18BP.

Minimal change disease (MCD) is most commonly associated with nephrotic syndrome, which is a clinical and pathological entity defined by selective proteinuria, hypoalbuminemia, and profound alterations in lipid and lipoprotein metabolism [29, 30]. In the current blood biochemical parameters examinations, TP and ALB, TG and CH, BUN and Scr were detected to explore the renal function and the level of serum albumin, urinary protein excretion and the alterations of lipoprotein metabolism, which were also detected in clinical trials

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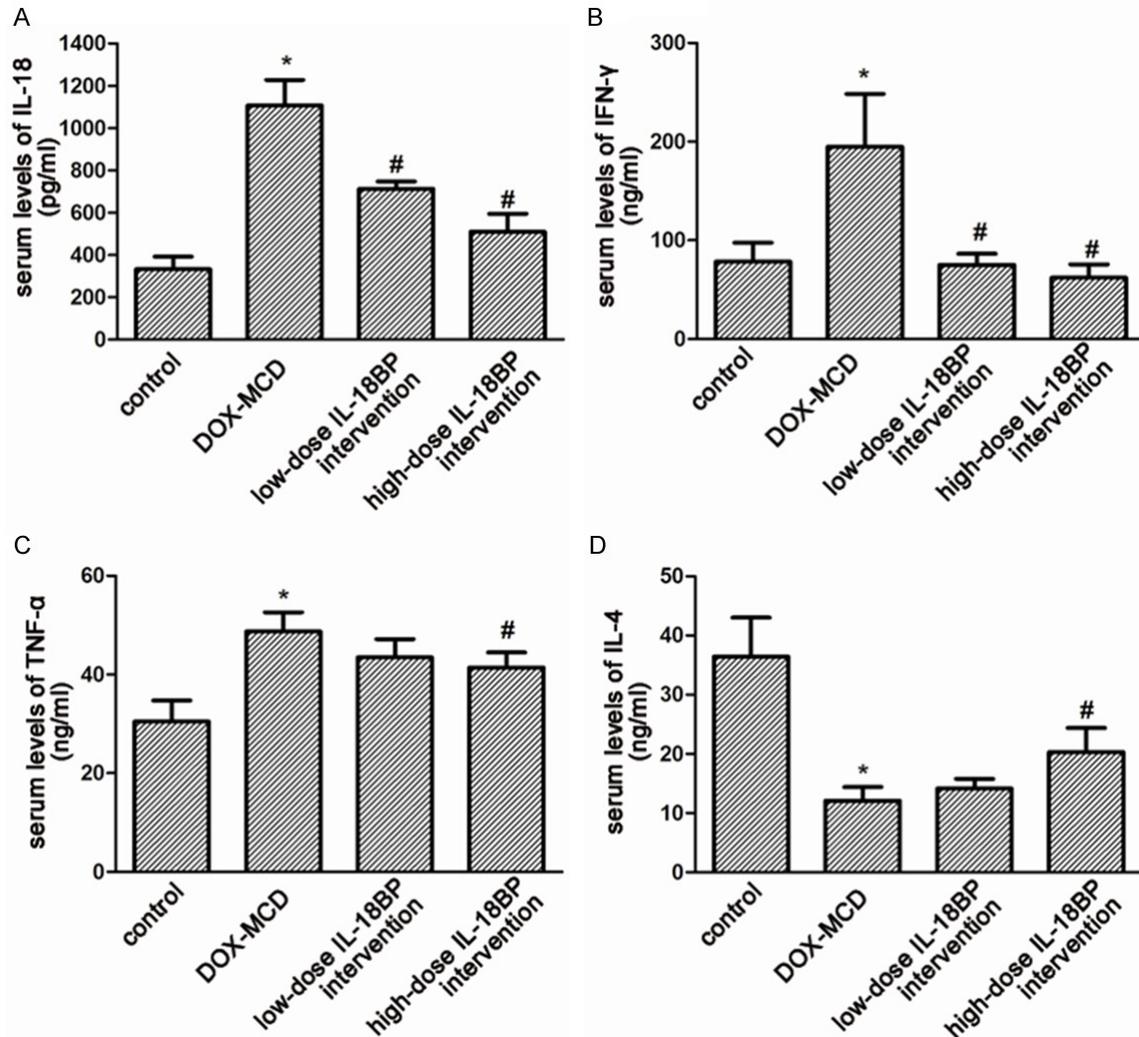


Figure 4. Serum cytokines after adriamycin administration were intervened by IL-18BP. A. The serum levels of IL-18 were shown in different groups. B. Elisa showed that levels of IFN- γ in different groups. C. The levels of TNF- α were shown in different groups. D. The levels of IL-4 were shown in different groups. *, $P < 0.05$ versus control group; #, $P < 0.05$ versus ADR-MCD group. The control group (n = 10); the ADR-MCD group (n = 10); the low-dose IL-18BP intervening group (n = 9); the high-dose IL-18BP intervening group (n = 9).

[31]. The serum levels of TP and ALB increased significantly in IL-18BP intervening group, while TG and CH were decreased significantly. There was no obvious change in the level of BUN and Scr, which was different from the others' report [28]. The serum levels of interferon (IFN)- γ and TNF- α in IL-18BP intervening group were significantly lower than those in the ADR-MCD group, which meant that the induction of IFN- γ and TNF- α by IL-18 was impaired by IL-18BP. On the other hand, in our study, we found the serum level of IL-4 was significantly higher in the IL-18BP intervening group than that in the ADR-MCD group. IL-4 plays a central role in the differentiation of antigen stimulated naive T cells into T_H2 cells and prolong the lives of T and B

lymphocytes in culture [32-34]. IL-4 also has an important role in tissue adhesion and inflammation. It acts with TNF- α to induce expression of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelial cells; and down regulates the expression of E-selection [35]. Additionally, IL-4 plays a major role in the development of protective immune responses to allergy [36]. The role of IL4 in MCD remained unclear. The deep mechanisms of down regulation of IL-4 by IL-18BP was of our interest currently.

Though the doxorubicin-induced MCD models actually caused cardiotoxicity, which would influence the general condition of animals, the kid-

ney injury was still the key point of this study. The involvement of cytokines played an important role was shown in the pathogenesis and pathogenesis of human nephritic syndrome. Cytokine therapy, with strong pertinence and no obvious side effects, provides a new idea and method for clinical treatment of nephrotic syndrome. However, at present, the human IL-18BP is only used for scientific research, which brings certain difficulties to the clinical application of IL-18BP. This problem will be solved gradually in the further research.

In conclusion, IL-18BP impaired the proteinuria, histopathological injury of kidney and the induction of serum cytokines in MCD mice models induced by ADR. It was the first time to show the intervention effect of IL-18BP in MCD mice models. The most important thing was that no data demonstrated obvious side effects by IL-18BP intervention with remissive MCD clinical manifestation. There might be a new way to the MCD treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Pengchao Du, School of Basic Medical Sciences, Binzhou Medical University, Yantai 264003, Shandong, China. Tel: +86-535-6913476; Fax: +86-535-6913163; E-mail: pengchaodu@163.com

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