

RESEARCH ARTICLE

The effect of continuous blood purification on P38MAPK signaling pathway in patients with multiple organ dysfunction syndrome

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Objective: The aim of the study was to investigate the role of p38MAPK signaling pathway in patients with multiple organ dysfunction syndrome treated with continuous blood purification.

Methods: Blood samples were obtained to analyze the protein level of inflammatory factors (IL-1, IL-8, IL-10, and TNF- α) and phosphorylated p38MAPK by utilizing ELISA assay and Western blotting, respectively. The relative mRNA level of iNOS was detected by using RT-PCR. In vitro study was conducted in Caco-2 cells, which were treated with serum from patients subjected to continuous blood purification. Serum-induced inflammatory factors and phosphorylated p38MAPK were also analyzed in Caco-2 cells.

Results: The protein levels of IL-1, IL-8, IL-10, and TNF- α were significantly decreased in Caco-2 cells treated with serum obtained from patients who were subjected to continuous blood purification therapy at the time course of 12 and 24 hours. A drastic decrease ($P < 0.05$) was observed in the level of IL-8 and TNF- α after continuous blood purification therapy in the patients treated with continuous blood purification therapy compared with control group.

Conclusion: Our study conducted in vivo and in vitro demonstrated that the continuous blood purification therapy could ameliorate the inflammatory response via activating the p38MAPK signaling pathway.

KEYWORDS

MODS, P38MAPK signaling pathway, syndrome

1 | INTRODUCTION

Multiple organ dysfunction syndrome (MODS) is a term which defines the progressive dysfunction of 2 or more organ systems after acute life-threatening disruption of systemic homeostasis.¹ Multiple organ dysfunction syndrome usually attributes to infection, injury, and hypotension, which triggers an inflammatory response. Sepsis is the most common inducer of multiple organ dysfunction and may

give rise to sepsis shock. A sepsis-related disorder is defined as systemic inflammatory response syndrome (SIRS) without the presence of infection. Both sepsis and SIRS could potentially evolve toward MODS.² Recently, several mechanisms have been proposed for the progression of MODS, including cell or tissue hypoxia, immune system dysregulation, mitochondrial dysfunction, and induction of cellular apoptosis. Despite the fact that MODS perhaps results from a complicated synergetic effect of these factors, more and more

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evidence inclines to the theory of immune system dysfunction.³ Immune system dysfunction is the imbalance between pro-inflammatory and anti-inflammatory mechanisms.⁴ To sustain homeostasis, the innate immune system is prepared to respond rapidly to danger signals including pathogen-associated molecular patterns via toll-like receptors, which then activate signaling pathways to stimulate inflammation.⁵ Once activated, pro-inflammatory cytokines like tumor necrosis factor (TNF- α) and interleukin (IL-1 β) will be produced, which in turn, spurs the synthesis of other inflammatory factors, leading to the activation.³

In clinical practice, several scoring systems have been applied to classify the stage of MODS, including Acute Physiology and Chronic Health Evaluation II Scoring System (APACHE II), which was one of the first classification systems and other systems like Multiple Organ Dysfunction Score and the Sequential Organ Failure Assessment.⁶⁻⁸ Continuous blood purification (CBP) has now been utilized for the treatment of systemic inflammatory response syndrome, and MODS,⁹ which is believed to be able to scavenge the inflammatory mediators, restore dynamics of immune homeostasis, and improve the function of endothelial cells.⁹ It was demonstrated that CBP could ameliorate the membrane permeability of the intestinal endothelial cell, promote the recovery of gut barrier function, and improve the prognosis of patients with MODS,¹⁰ but its underlying mechanism remains undetermined.

P38 mitogen-activated protein kinases (p38 MAPK) are a member of mitogen-activated protein kinases (MAPKs) that are sensitive to stress stimuli, and participated in cell differentiation, apoptosis, and autophagy.¹¹ The p38 MAPK has been reported to regulate inflammatory response in rats and in various cells,^{12,13} and other studies indicated that the activation of p38 MAPK could diminish the production of TNF- α .^{12,14} In addition, p38 MAPK activation could also result in the induction of heme oxygenase (HO)-1.^{12,13} HO-1 is protective against oxidative stress through antioxidative, antiapoptotic, and anti-inflammatory actions.¹⁴

Despite the above studies, to our best of knowledge, little is known about the role of p38 MAPK signaling pathway in MODS patients underwent continuous blood purification neither in clinical nor in vitro. The present study aimed to investigate the role of continuous blood purification on P38MAPK signaling pathway in MODS patients. This study might give more clinical evidences as well as better understanding for continuous blood purification in treatment of MODS patients.

2 | MATERIALS AND METHODS

2.1 | Patients

The present study included a total of 25 patients with MODS who were admitted to intensive care unit (ICU) at our hospital, and 20 healthy individuals were also enrolled as control group. The inclusion criteria were as follows: Acute Physiology and Chronic Health Evaluation (APACHE) II Scoring ≥ 20 at admission,¹⁵ simultaneous failure of at least two organs. Patients were excluded from the study

if they had chronic gastrointestinal disease, gastrointestinal malignance, HIV, and immune diseases. The study was approved by the Ethic committee of the Shanghai Ninth People's Hospital. All participants have signed a consent form before the beginning of study.

2.2 | Continuous blood purification

Veno-venous vascular access was established by using a double lumen catheter via femoral vein or jugular vein intubation. Continuous veno-venous hemodiafiltration (CVVHDF) was executed by using PRISMA hemodialysis machine (Sweden), Fresenius AV600S polysulfone membrane filters with a blood flow of 250-300 mL/min, and a 4000 mL/h replacement flow. All patients were injected low molecular heparin, with an initial dose of 2000-4000 IU, and maintained at 200-400 IU/h while in the process of CVVHDF. Patients who suffered from coagulopathy and active bleeding were subjected a injection of anticoagulant instead of heparin. The duration time of the CVVHDF is 2 hours for each session.

2.3 | Serum sample collection

Venous blood of the patients and the control group was collected at the time point of 0, 6, and 12 hours in the process of continuous veno-venous hemodiafiltration and at the time point of 12 and 24 hours after the therapy. The serum was obtained from the venous blood after centrifugation with 2000 g for 10 minutes and stored at the temperature of -80°C .

2.4 | Clinical parameters monitor

Patients were monitored before and after the therapy for the level of heart rate (HR), leukocyte (LEU) count and levels of hemoglobin, aspartate aminotransferase (AST), serum creatinine (SCR), creatine kinase (CK), IgE, alanine aminotransferase (ALT), interleukin-1 (IL-1), IL-8, IL-10, and tumor necrosis factor (TNF)- α in the serum. Enzyme-linked immunosorbent assay (ELISA) assay was performed to analyze the concentrations of IL-1, IL-8, IL-10, and TNF- α utilizing the commercial kits (all purchased from Abcam, Cambridge, MA, USA).

2.5 | Single layer of human epithelial colorectal cell model

Caco-2 cells were inoculated into the 24-well plate and divided into seven subgroups. (a) Control group: Culture medium was replaced by the DMEM with a 10% concentration of serum from healthy participants; (b) 0 hour: Culture medium was replaced by the DMEM with a 10% concentration of serum from patients with MODS before they were subjected to CBP; (c) 12 hours: Culture medium was replaced by the DMEM with a 10% concentration of serum from patients with MODS after 12 hours of CBP; and (d). 24 hours: Culture medium was replaced by the DMEM with a 10% concentration of serum from patients with MODS after 24 hours of CBP. Six hours after intervention, the cells were collected.

2.6 | iNOS mRNA expression level detection

Half of the cells from a, b, c, and d group, respectively, were collected. Briefly, 1 mL of Trizol (Tiangen Biotech, Beijing, China) was added into cell for extraction of total RNA. Reverse transcription reaction was implemented later, and cDNA was obtained using a Prime-Script™ one-step qRT-PCR kit (TAKARA, Dalian, China). The PCRs were conducted in an ABI 7500 Fast RealTime PCR System (Life Technologies, Gaithersburg, MD, USA). The iNOS forward primers and reverse primers are as follows: F: 5'-ACCTCAGCAAGCAGCAGAAT-3', R: 5'-ATCTGG AGGGGTAGGCTTGT-3'. GAPDH was used as an internal reference. Each group had three repeat trials both for iNOS and GAPDH. The relative mRNA level of iNOS in a, b, c, and d group was analyzed by calculating $2^{-\Delta\Delta CT}$.

2.7 | Western blotting analysis

The protein levels of IL-1, IL-8, IL-10, TNF- α , and p-p38MAPK were determined using Western blotting assay. Briefly, total protein was extracted from Caco-2 cells. Proteins were then loaded on 10% SDS-PAGE, transferred to PVDF membranes, and subsequently blocked using 5% non-fat milk at room temperature for 1 hour. Then, membranes were incubated with the specific primary antibodies at 4°C overnight and subsequently incubated with corresponding secondary antibody at 37°C for 45 minutes. Protein bands were scanned with the Pierce ECL Western Blotting Substrate (Pierce, Shanghai, China). GAPDH was served as an internal control.

2.8 | Statistical analysis

The measurement data were expressed by mean \pm SD. Paired *t* test was performed to determine the difference between groups. Comparison among three or more groups was conducted using one-way analysis of variance (ANOVA). *P* < 0.05 was considered significant difference. All calculations were made using SPSS (Version 18; IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Characteristics for all patients

The present study included a total of 25 MODS patients, and 20 healthy individuals were enrolled as control. As shown in Table 1, the mean age of all patients was 47.1 ± 12.9 , with male: female 14:11.

TABLE 1 Characteristic and clinical scores of the patients with at admission

Variables	Value
Age, y	47.1 ± 12.9
Gender, male: female	14:11
APACHE II score	24.3 ± 4.0
SOFA score	13.9 ± 3.7
Treatment duration, h	25.6 ± 7.5

The mean APACHE II score was 24.3 ± 4.0 , mean SOFA score was 13.9 ± 3.7 , and mean treatment duration was 25.6 ± 7.5 hours. The mean age of healthy people was 23.8 ± 3.6 , with male: female 12:8. All the healthy people had no MODS.

3.2 | Clinical index for all participants before and after the treatment

As shown in Table 2, CVVHDF could pose an effect on the number of LEU count, AST, hemoglobin, IgE, CK, SCR, and ALT. There was significant decrease for the levels of all AST, LEU count, IgE, CK, SCR, and ALT after CVVHDF therapy compared with before the treatment (*P* < 0.05). Hemoglobin showed no significant difference before and after treatment.

3.3 | Change in the expression level of p-p38MAPK and the cytokines in blood sample

The blood sample obtained from all participants at the time course of 12 and 24 hours after continuous blood purification was subjected to protein extraction to analyze the expression pattern of phosphorylated p38MAPK and cytokines (IL-1, IL-8, IL-10, and TNF- α). As shown in Table 3, the level of p-p38MAPK showed a gradual decrease in patients with MODS after 12 and 24 hours treatment. The levels of IL-1, IL-8, IL-10, and TNF- α in two groups also showed a drastic decrease (*P* < 0.05) after CVVHDF therapy in the experimental groups.

3.4 | Change in the mRNA level of iNOS in Caco-2 cell

The RNA were obtained from the a, b, c, and d group, whose culture medium was replaced by DMEM with a 10% concentration of serum from healthy participants, a 10% concentration of serum from patients with MODS before they were subjected to CBP, a 10% concentration of serum from patients with MODS after 12 hours of CBP, and a 10% concentration of serum from patients with MODS after 24 hours of CBP, respectively. RT-PCR results (Figure 1) revealed that the relative mRNA level of iNOS was rather low in the control group (a group) compared to b, c, and d group. When Caco-2 cells were treated with serum from patients with MODS after CBP treatment, the relative mRNA level of iNOS presented a continuous decrease.

3.5 | Change in the expression level of p-p38MAPK and the cytokines in Caco-2 cell

The protein was obtained from the a, b, c, and d group, whose culture medium was replaced by DMEM with a 10% concentration of serum from healthy participants, a 10% concentration of serum from patients with MODS before they were subjected to CBP, a 10% concentration of serum from patients with MODS after 12 hours of CBP, and a 10% concentration of serum from patients with MODS after 24 hours of CBP, respectively. In comparison with

Variables	Before treatment	After treatment	P value	Control group
LEU count, $\times 10^9/L$	19.4 \pm 7.5	13.7 \pm 6.2	0.024	8.7 \pm 4.5
AST, IU/L	441.6 \pm 187.5	329.4 \pm 126.7	0.005	32.5 \pm 18.2
Hemoglobin, g/L	63.4 \pm 19.8	65.2 \pm 21.8	0.776	141 \pm 41.2
IgE, IU/mL	366.9 \pm 138.5	247.6 \pm 112.7	<0.001	130.2 \pm 83.4
CK, IU/mL	1109.4 \pm 547.8	725.4 \pm 364.4	<0.001	882.4 \pm 259.4
SCR, $\mu\text{mol/L}$	374.2 \pm 131.5	258.6 \pm 109.7	0.003	108.0 \pm 87.1
ALT, IU/L	332.4 \pm 124.6	235.3 \pm 103.9	<0.001	120.5 \pm 98.8

TABLE 2 Clinical index for all participants before and after the treatment

Factors	Before treatment	12 h after treatment	24 h after treatment	Control group
IL-1, pg/mL	57.2 \pm 10.9	35.8 \pm 9.8 [*]	24.9 \pm 10.2 [*]	20.3 \pm 5.1
IL-8, pg/mL	71.4 \pm 19.7	38.7 \pm 11.2 [*]	23.5 \pm 9.7 [*]	19.8 \pm 11.2
IL-10, pg/mL	69.7 \pm 14.6	46.5 \pm 13.4 [*]	34.3 \pm 14.5 [*]	25.0 \pm 10.9
TNF- α , pg/mL	7.0 \pm 2.1	4.9 \pm 1.8 [*]	3.5 \pm 1.2 [*]	2.2 \pm 0.9
p-p38MAPK, pg/mL	545.6 \pm 187.8	207.9 \pm 89.4 [*]	93.4 \pm 37.5 [*]	87.3 \pm 20.6

TABLE 3 Change in the expression level of p-p38MAPK and the cytokines in blood sample

* $P < 0.05$, compared with value = s before treatment.

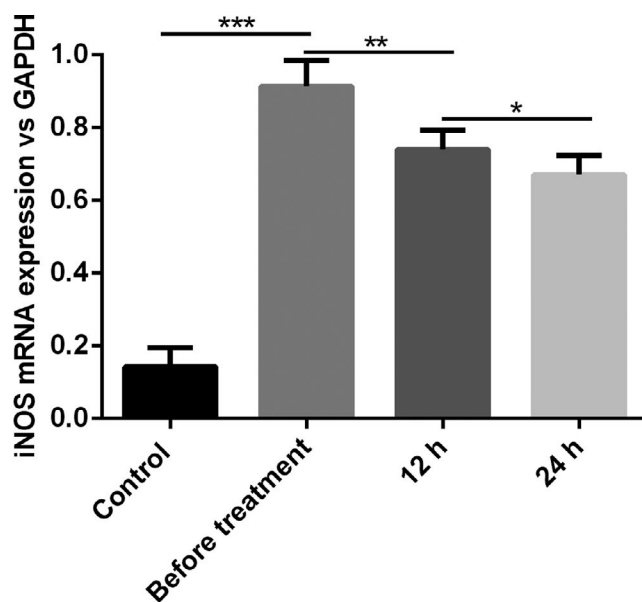


FIGURE 1 Expression of mRNA of iNOS in different group of cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with corresponding groups

group a, the levels of cytokines and p-p38MAPK were significant higher in caco-2 cells treated with serum from patients with MODS before they were subjected to CBP (Figure 2). Western blotting result (Figure 2) indicated that compared to the caco-2 cells treated with serum from healthy participants, the protein levels of IL-1, IL-8, IL-10, and TNF- α were significantly decreased in Caco-2 cells treated with serum obtained from patients who were subjected to CVVHDF therapy at the time course of 12 and 24 hours. These results were consistent with the findings observed in blood samples obtained from the patients with MODS.

4 | DISCUSSION

Gastrointestinal tract is the hub of the bacterium and toxins in the human body. Under the stressful state, such as severe trauma, shock, burn, or ICU, intestinal mucosa is in danger of aseptic injury, which increases the membrane permeability of the intestinal mucosa, leading to the dysfunction of the intestinal mucosa. The change of the membrane permeability is also the trigger causing distal organ injury.¹⁶ It was demonstrated that when the intestinal mucosa was impaired, the pro-inflammatory cytokines from intestinal would flow into intestinal lymphatic system, rendering the systemic inflammatory response,¹⁷ and exacerbating further injury of tissues and organs, which was closely associated with MODS. Many pathogenic mechanisms have been proposed to be involved in the gut barrier dysfunction. Inflammatory response played a key role in the increase of membrane permeability. Research has found that iNOS mediated in the process of the increase in membrane permeability resulted from the cytokines.¹⁸ Our results indicated that the relative mRNA level of iNOS induced by the MODS patients before CBP therapy in caco-2 cells was significantly higher than that in the control group, which gave us a hint that the increase in mRNA could mediate in the process of the uncontrolled inflammatory response and oxidative stress, which damage the barrier function of the intestinal epithelial cells.

The p38 MAPK has been reported to regulate inflammatory response in rats and in various cells,^{12,13} and other studies indicated that the activation of p38 MAPK could diminish the production of TNF- α .^{12,14} Various stimuli, such as pro-inflammatory factors, cytokines, and cellular stress, can phosphorylate p38MAPK via the protein kinase cascade. Phosphorylated p38MAPK will get access to the nucleus, regulating the inflammatory response and cellular immune response.¹⁹ In our study, we found out that the protein level of p-p38MAPK was significantly higher in the blood sample of

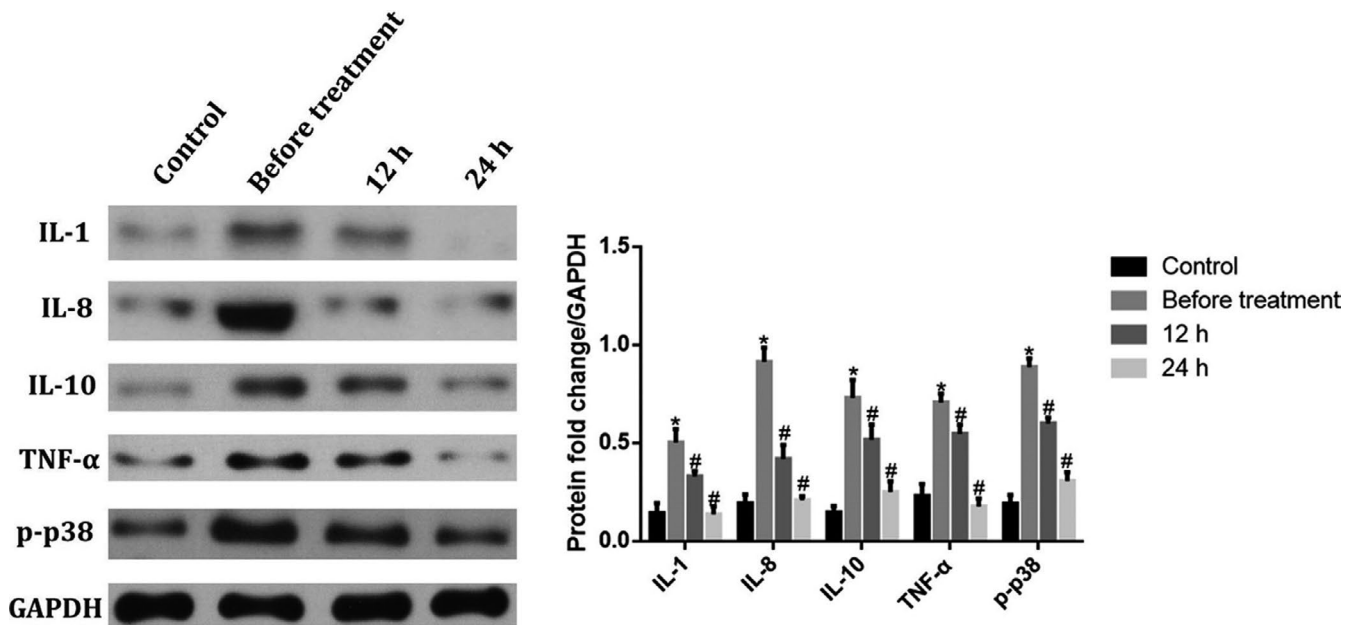


FIGURE 2 Protein levels of IL-1, IL-8, IL-10, TNF- α , and p-p38MAPK in different group of cells. * $P < 0.05$ compared with the control; # $P < 0.05$ compared with the group before treatment

patients with MODS and the p-p38MAPK induced by serum from patients with MODS was also significantly increased compared to control group ($P < 0.05$). Study demonstrated that cellular toxins and pro-inflammatory factors, such as IL-1 and TNF- α , could activate the signaling pathway of p38MAPK, while blocking the p38MAPK signaling pathway could alleviate the inflammatory response, restraining the production of IL-1 and TNF- α .²⁰ These results together indicated that activation of the p38MAPK signaling pathway was involved in the process of intestinal barrier dysfunction in patients with MODS, and p38MAPK activation may be associated with the release of the inflammatory factors in patients with MODS.

5 | CONCLUSION

Our study conducted in vivo and in vitro demonstrated that the CVVHDF therapy could ameliorate the inflammatory response via activating the p38MAPK signaling pathway. The pro-inflammatory factors were decreased after the CVVHDF therapy, and in vivo study in caco-2 cells also indicated that p-p38MAPK induced by the serum from patients treated with MODS was significantly decreased.

ETHICAL APPROVAL

The ethic approval was obtained from the Ethic Committee of Shanghai Ninth People's Hospital.

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