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Original Research Article

Ameliorative Effects of Neurolytic Celiac Plexus Block on Stress and Inflammation in Rats with Partial Hepatectomy

Jun Li[1], Fu-Xiang Li[2], Jian-Xiang Che[3], Xiao-Hong Wei[4], Qing-Ming Qiu[3], Peng Zha[1], Shu-Rong Bai[1], Xu-Dong Tang[5] and Tao Chen[6]*

[1]Department of Anesthesia, [2]Department of ICU, Chengdu Military General Hospital, No. 270 Rongdu Avenue, Jinniu District, Chengdu 610083, Sichuan Province, [3]Department of Anesthesia, The 44th Hospital of PLA, Guiyang 550009, Guizhou Province, [4]Department of Cardiovascular Surgery, Chengdu Military General Hospital, No. 270 Rongdu Avenue, Jinniu District, Chengdu 610083, Sichuan Province, [5]Department of Cardiothoracic Surgery, The 44th Hospital of PLA, Guiyang 550009, Guizhou Province, [6]Department of General Surgery Center, Chengdu Military General Hospital, No. 270, Rongdu Avenue, Jinniu District, Chengdu 610083, Sichuan Province, PR China

For correspondence:* ***Email:* *taochencz@126.com, xutang44@163.com;* ***Tel:*** *+86 28 86571251;* ***Fax:*** *+86 28 86570421*

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Abstract

Purpose: *To investigate effects of neurolytic celiac plexus block (NCPB) on stress and inflammation in rats with partial hepatectomy (PH).*
Methods: *A model of PH rat was established, and serum C-reactive protein (CRP); corticosterone (GC); adrenocorticotropin (ACTH); noradrenaline (NA); adrenalin (AD); aspartate transaminase (AST); alanine transaminase (ALT); as well as tumor necrosis factor-α (TNF-α); interleukin (IL)-1β and IL-6; high mobility group box1 (HMGB1); and nitric oxide (NO) concentrations in serum assessed after PH. Additionally, Western blotting was performed to determine the effect of NCPB on expressions of glucocorticoid receptors (GR), inhibitor of nuclear factor kappa B (IκB), p65, c-Jun and inducible nitric oxide synthase (iNOS) of PH rats, as well as assay effects of NCPB on nuclear translocation of GR, c-Jun and p65. DNA binding activities of nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) were also determined.*
Results: *NCPB reduced AST and ALT ($P < 0.05$), decreased secretion of inflammatory cytokines and NO ($P < 0.05$), as well as decreased CRP, GC, ACTH, NA and AD after PH ($p < 0.05$). NCPB increased expressions of GR and IκB, but expressions of p65, c-Jun, and iNOS ($p < 0.05$). Additionally, NCPB increased nuclear translocation of GR ($p < 0.01$), but decreased nuclear translocation of p65 and c-Jun after PH ($p < 0.05$). Additionally, DNA binding activity of NF-κB and AP-1 was decreased by NCPB ($p < 0.05$).*
Conclusion: *The results indicate that NCPB treatment can significantly inhibit stress and inflammation in PH rats.*

Keywords: *Neurolytic celiac plexus block, Cytokine, Nuclear translocation, Partial hepatectomy, Stress, Inflammation*

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INTRODUCTION

Autonomic nervous system (ANS) plays important roles in stress and inflammatory reaction after severe trauma [1,2].The parasympathetic nervous system (PNS) can regulate the inflammatory reactions effectively through acetylcholine [3]. Solar plexus, the largest human autonomic plexuses, consisted of sympathetic nerve, parasympathetic nerve and

sensory nerve. Moreover, the solar plexus play crucial effects on regulation the functions of organ in epigastric region including liver, guts, pancreas, etc. Neurolytic celiac plexus block (NCPB) has been used for treating pain of pancreatic diseases for very long time, and is widely performed to treat visceral pain related to retroperitoneal and metastatic tumors [4-7].

Severe trauma can induce strong stress and inflammatory reactions, leading to over-activation of inflammatory cascade reaction, even the systemic inflammatory response syndrome (SIRS) [8,9]. The SIRS is one of the major causes of multiple organ dysfunction syndrome (MODS) as well as death. Thus, it's important to alleviate the damages of severe liver trauma and liver cancer surgery in order to suppress or control the levels of stress and inflammatory reaction. In our previous investigation, we found that NCPB can improve the liver regeneration after PH, as well as decrease the pro-inflammatory cytokines in serum [10]. As a part of our continuing investigation of NCPB, this study was designed to investigate the ameliorative effects of NCPB on stress and inflammation in PH rats.

EXPERIMENTAL

Chemicals and Regents

Corticosterone (GC) ELISA Kit, Adrenocorticotropin (ACTH) ELISA Kit, Noradrenaline (NA) ELISA Kit, Adrenalin (AD) ELISA Kit, Trans AM^{TM} NF-κB p65 kit, Trans AM^{TM} AP-1 c-Jun kit were purchased from the Beijing north institute of Biological Technology (Beijing, China); Rat TNF-α ELISA kit, Rat IL-1β ELISA kit, Rat IL-6 ELISA kit, Rat C-Reactive Protein (CRP) ELISA kit were purchased from Cusabio Life Science Ltd. (Wuhan, China); Rat HMGB1 ELISA Kit was purchased from Biovendor R&D (Shanghai, China); Nitric Oxide assay kit was purchased from the Nanjing Jiancheng Bioengeering Institute (Nanjing, China); Cell protein extracts and Nuclear protein extracts were purchased from Pierce Protein Biology (Rockford, USA); Anti-GR rabbit polyclonal IgG, Anti-NF-κB p65 rabbit polyclonal IgG, Anti-NF-κB p65 rabbit polyclonal IgG, Anti-c-Jun rabbit polyclonal IgG, Anti-β-Actin rabbit polyclonal IgG, Anti- Lamin B rabbit polyclonal IgG were purchased from the Santa Cruz Biotech, Inc. (California, USA). The Rabbit anti-goat IgG/HRP and Goat anti-rabbit IgG/HRP were obtained from Zhongshan Biotech Co. Ltd. (Guangdong, China). SDS-PAGE Molecular high weight markers for proteins were purchased from the Shanghai Lizhudongfeng Biotech Ltd. (Shanghai, China). The prestained protein molecular weight marker was purchased from Xian Runde Biotech Ltd. (Xian, China). All other chemicals used in this study were of analytical reagent grade.

Ethics statement

All animal treatments were strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals, and the experiments were carried out with the approval of the Animal Experimentation Ethics Committee of the General Hospital of Chengdu Military Command Area (No. CDZYY2012KX25).

Animals

The animals were provided by the medical laboratory animal center of the Third Military Medical University and followed the Principles of Laboratory Animal Care [11]. Male Sprague-Dawley rats, weighing 200 ± 20 g, were used in our study. Rats were kept on a 12-h light/dark cycle with free access to standard laboratory chow and water. Humidity was maintained at 50 % and the temperature at 25 °C. Each animal was used only once in the experiment.

Partial hepatectomy

Our rat PH model was prepared using the method described previously by Higgins and Anderson [12,13] with minor modifications. In brief, approximately 70 % of the liver (left and middle hepatic lobe) was surgically removed. Rats were anesthetized with sodium pentobarbital (40 mg/kg, ip). Thereafter, the left and medial lobes of liver were resected after a midline laparotomy. Subsequently, the abdominal wound was closed.

Neurolytic celiac plexus block

The method to achieve percutaneous NCPB in rats was performed as described previously [14], with minor modifications. In brief, 0.5 % xylocaine was injected once the needle tip reached the anatomic site of the celiac plexus once per day, over a total of 7 days. For the control group, rats underwent the same surgical procedure, but physiological saline was injected instead of 0.5 % xylocaine.

Experimental design

A total of 30 rats underwent PH as described above, and the rats were equally divided into the

following two groups (n = 15): (1) Control group, and (2) NCPB-treated group. For the NCPB-treated group, percutaneous NCPB was performed with 0.5 % xylocaine after PH. Five rats were used to collect blood and liver tissues at each time point (1, 3, and 7 days), [under pentobarbital sodium (60 mg/kg)] after percutaneous NCPB. The serum samples were separated by natural deposition, and were stored at -70 °C until further analysis. Additionally, the peritoneal macrophage was collected following the method described previously [15], and the cells were cultured in 6 pore plate containing DMEM supplemented with 10 % heat-inactivated FBS for 24 h (at 37 °C with 5 % CO_2) for further investigations.

Determination of the biochemical indicators of serum

The serum corticosterone (GC), adrenocorticotropin (ACTH), noradrenaline (NA), adrenalin (AD), aspartate transaminase (AST), alanine transaminase (ALT), CRP, TNF-α, IL-1β, IL-6 HMGB1 concentrations in serum were determined by ELISA and quantified using a microplate reader (Bio-Tek ELX800) at 450 nm with commercial kits.

Determination of the NO production

The method of nitrate reductase (Griess method) was performed to determine the total contents of NO_3^- and NO_2^- (which can be used to evaluate the NO levels in serum), based on the instructions of commercial nitric oxide assay kits [13]. Briefly, following the introduction of the commercial kit, serum samples (100 µL) was added to 100 µL Griess reagents, and incubated at room temperature for 10 min; then absorbance of the mixture measured at 540 nm, and calculated the contents of nitrites based on the standard curve of standard solution.

Western blotting

Cells were harvested, and the total protein/nucleoprotein was extracted, and equal amounts of protein (50 µg) were separated by SDS-PAGE, blotted on polyvinylidene difluoride membranes, and probed with Anti-GR rabbit polyclonal IgG, Anti-NF-κB p65 rabbit polyclonal IgG, Anti-NF-κB p65 rabbit polyclonal IgG, Anti-c-Jun rabbit polyclonal IgG, Anti-β-Actin rabbit polyclonal IgG, Anti- Lamin B rabbit polyclonal IgG, following by incubation with a goat anti-rabbit/HRP secondary antibody, and detected by chemiluminescence. To measure protein loading, antibodies directed against β-Actin and Lamin B were used.

Determination of the DNA binding activities of NF-κB and AP-1

The nucleoprotein of the peritoneal macrophage was isolated. Then, the DNA binding activities of NF-κB and AP-1 were determined as the introductions of the commercial kits and quantified using a microplate reader (Bio-Tek ELX800) at 450 nm.

Statistical analysis

All the experiments were conducted at least in triplicate, and the data presented as mean ± SD. The statistical significance of differences was analyzed by using SPSS software (SPSS for Windows 18.0, SPSS Inc., USA), and performed using the two-tailed Student's t test with a significance level of $p < 0.05$.

RESULTS

Effect of NCPB on functions of liver and kidney of rats after PH

As can be seen from the Table 1, the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the control and NCPB groups were all decreased gradually; the AST and ALT levels of the NCPB mice group at 1, 3 and 7 days after PH were significantly lower than that in the control group mice ($p < 0.05$). In addition, the levels of creatinine (Cr) and blood urea nitrogen (BUN) of the NCPB mice group at 1 and 3 days after PH were also significantly lower than that in the control group mice ($p < 0.05$). The results of our present study indicated that the NCPB can improve the functions of liver and kidney of rats after PH surgery.

Effect of NCPB on the CRP, GC, ACTH, NA, and AD in serum of rats after PH

The concentration of CRP was measured in the serum of rats after PH (Table 2). The results indicated that CRP level increased until 3rd days after PH, and then decreased until the 7th day after PH. However, the serum concentration of CRP in the NCPB group was significantly lower significantly than that of the control group at each time point ($p < 0.05$). Furthermore, the concentrations of the GC, ACTH, NA, and AD in serum of rats after PH were also determined, and our results suggested that the levels of these biochemical indicators were all progressively decreased until the end of the observation period in both the control and NCPB groups;

Table 1: Effect of NCPB on functions of liver and kidney of rats after PH (n = 5, mean ± SD)

Parameter			Day 1	Day 3	Day 7
Liver functions (U/L)	AST	Control	927.4 ± 82.6	558.6 ± 51.2	214.8 ± 19.3
		NCPB	509.1 ± 46.1 [b]	256.6 ± 24.6 [a]	154.3 ± 14.9 [a]
	ALT	Control	474.1 ± 38.3	112.5 ± 11.9	43.9 ± 3.5
		NCPB	361.0 ± 27.0 [a]	78.5 ± 6.3 [b]	41.6 ± 3.1
Kidney functions (μmol/L)	Cr	Control	104.6 ± 8.3	134.5 ± 11.7	87.3 ± 7.2
		NCPB	78.2 ± 6.7 [b]	95.1 ± 9. 4 [b]	79.1 ± 6.7
	BUN	Control	5.91 ± 0.44	7.34 ± 0.64	5.35 ± 0.43
		NCPB	5.12 ± 0.38 [a]	5.71 ± 0.51 [b]	5.24 ± 0.37

[a]$p < 0.05$; [b]$p < 0.01$, compared with the control

despite this similar trend, the serum concentrations of GC, ACTH, NA, and AD in the NCPB group was significantly lower significantly than that of the control group at each time point ($p < 0.05$).

Effect of NCPB on the inflammatory cytokines and NO in serum of rats after PH

As the results showed in Table 3, the concentrations of inflammatory cytokines and NO in serum of rats after PH surgery were measured. The concentrations of all the TNF-α, IL-6, IL-1β were at their maximum at 1 day after PH, and diminished progressively until the end of the observation period. The serum concentrations of IL-1β and TNF-α in the NCPB group were significantly lower than that of the control group at each time point ($p < 0.05$); additionally, the IL-6 concentration in the NCPB group was significantly lower than that the control group at 1 day after PH ($p < 0.05$). The serum concentration of HMGB1 was also determined after PH, and the results indicated that HMGB1 concentration of the NCPB group was significantly lower than that of the control group at each time point ($p < 0.05$). Furthermore, the NO levels were determined, and our study demonstrated that the serum NO levels of both the two groups were decreased gradually, however the NO level of the NCPB mice were obviously lower than that of the control mice at 1 and 3 days after PH ($p < 0.05$).

Table 2: Effect of NCPB on stress index of rats after PH (pg/mL, n=5, mean ± SD)

Parameter		Day 1	Day 3	Day 7
CRP	Control	552.9 ± 50.8	723.3 ± 63.0	383.2 ± 37.1
	NCPB	182.9 ± 16.8 [b]	342.5 ± 31.9 [b]	321.9 ± 31.5 [a]
GC	Control	43.12 ± 3.45	21.23 ± 2.02	13.72 ± 1.13
	NCPB	29.31 ± 2.87 [b]	16.22 ± 1.56 [b]	9.82 ± 0.91 [b]
ACTH	Control	32.72 ± 3.11	11.62 ± 1.06	7.22 ± 0.68
	NCPB	13.23 ± 1.24 [b]	6.73 ± 0.49 [b]	3.35 ± 0.31 [b]
NA	Control	753.6 ± 62.4	368.6 ± 51.0	313.2 ± 28.8
	NCPB	325.4 ± 29.7 [b]	241.7 ± 22.1 [b]	267.9 ± 23.0 [a]
AD	Control	821.3 ± 74.2	650.7 ± 58.1	258.2 ± 24.5
	NCPB	425.6 ± 39.6 [b]	341.1 ± 33.2 [b]	198.9 ± 18.7 [b]

[a]$p < 0.05$; [b]$p < 0.01$, compared with the control

Table 3: Effect of NCPB on inflammatory cytokines and NO of rats after PH (n=5, mean ± SD)

Parameter		Day 1	Day 3	Day 7
NO	Control	49.2 ± 4.5	24.1 ± 3.1	15.2 ± 1.2
(μmol/L)	NCPB	29.5 ± 2.3 [a]	15.0±1.3 [a]	13.6 ± 1.1
TNF-α	Control	1823.6 ± 166.2	1654.0 ± 129.0	1142.8 ± 104.1
(pg/mL)	NCPB	1471.8 ± 133.6 [a]	1105.4 ± 98.4 [a]	861.4 ± 76.0 [a]
IL-1β	Control	53.2 ± 5.2	44.5 ± 3.9	34.0 ± 3.4
(pg/mL)	NCPB	34.6 ± 3.2 [a]	29.1 ± 2.7 [a]	23.7 ± 2.1 [a]
IL-6	Control	21.4 ± 1.9	13.2 ± 1.8	10.9 ± 0.9
(pg/mL)	NCPB	14.3 ± 1.3 [a]	11.7 ± 1.1	9.3 ± 0.8
HMGB1	Control	4.13 ± 0.32	13.25 ± 1.32	5.02 ± 0.47
(pg/mL)	NCPB	2.13 ± 0.14 [a]	3.11 ± 0.24 [a]	3.34 ± 0.28 [a]

[a]$p < 0.05$; [b]$p < 0.01$, compared with the control

Effect of NCPB on expressions of GR, IκB, p65, c-Jun, and iNOS in peritoneal macrophage

The protein expressions of GR, IκB, p65, c-Jun, and iNOS were showed in Figure 1 and Table 4. The protein expression level of GR in the NCPB group was obviously higher than that of control group ($p < 0.01$). Moreover, the c-Jun level was significantly lower than that of control group at 1 and 3 days after PH ($p < 0.01$). Similar with the c-Jun, the protein expression levels of p65 and iNOS were significantly lower in the NCPB group compared to the control group at each time point after PH ($p < 0.01$); whereas the protein expressions of IκB were higher in the NCPB compared with control group at 1 and 3 days after PH ($p < 0.01$).

Effect of NCPB on nuclear translocation of GR, c-Jun, p65 in peritoneal macrophage

In our present study, the nuclear translocation of GR was evaluated by determination the protein expressions of GR in ribonucleoprotein. As can be seen from the Figure 2 and Table 5, the expressions of GR in ribonucleoprotein of the NCPB group was obviously higher than that of control group at 1, and 3 days after PH ($p < 0.01$). Additionally, we determined the p65 and c-Jun to evaluate the nuclear translocation levels of NF-κB and AP-1. Our present results showed that the levels of c-Jun were significantly lower than that of the control group ($p < .0.01$), and the levels of p65 were obviously lower in the NCPB group compared to the control group at each time point after PH ($p < 0.05$).

Table 4: Effect of NCPB on expressions of GR, IκB, p65, c-Jun, and iNOS (Int×mm^2, n=5, mean ± SD)

Parameter		Day 1	Day 3	Day 7
GR	Control	1.54 ± 0.06	1.76 ± 0.05	1.96 ± 0.05
	NCPB	1.79 ± 0.06 [b]	2.22 ± 0.06 [b]	2.24 ± 0.08 [b]
c-Jun	Control	2.41 ± 0.11	1.54 ± 0.10	1.48 ± 0.11
	NCPB	0.78 ± 0.05 [b]	1.04 ± 0.09 [b]	1.43 ± 0.12
IκB	Control	1.93 ± 0.12	1.13 ± 0.09	1.28 ± 0.11
	NCPB	2.29 ± 0.14 [b]	2.01 ± 0.12 [b]	1.96 ± 0.14 [b]
p65	Control	2.36 ± 0.13	2.99 ± 0.18	2.68 ± 0.16
	NCPB	0.76 ± 0.08 [b]	0.89 ± 0.06 [b]	0.95 ± 0.08 [b]
iNOS	Control	1.78 ± 0.11	2.28 ± 0.16	2.36 ± 0.19
	NCPB	1.49 ± 0.09 [b]	1.44 ± 0.13 [b]	0.87 ± 0.04 [b]

[b] $P < 0.01$ compared with the control

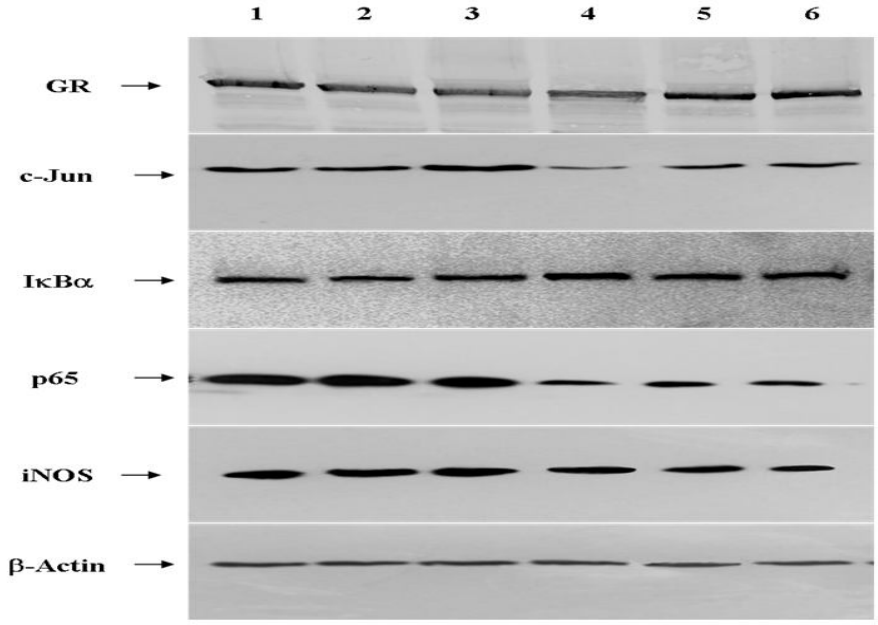

Figure 1: Effects of NCPB on expressions of GR, IκB, p65, c-Jun, and iNOS. Lanes 1-3 represented the protein expressions in the control group at 7, 3 and 1 days after PH, respectively. Lanes 4-6 represented the protein expressions level in NCPB group at 1, 3 and 7 days after PH, respectively

Table 5: Effect of NCPB on nuclear translocation of GR, c-Jun, p65 (Int×mm^2, n=5, mean ± SD)

Parameter		Day 1	Day 3	Day 7
GR	Control	0.65 ± 0.05	0.77 ± 0.04	1.04 ± 0.07
	NCPB	0.92 ± 0.08 [b]	0.95 ± 0.06 [b]	1.02 ± 0.09
c-Jun	Control	0.75 ± 0.06	1.27 ± 0.10	1.15 ± 0.09
	NCPB	0.83 ± 0.08	0.71 ± 0.07 [b]	0.89 ± 0.08 [b]
p65	Control	1.35 ± 0.12	1.47 ± 0.11	2.23 ± 0.18
	NCPB	1.15 ± 0.11 [a]	0.83 ± 0.09 [b]	0.91 ± 0.05 [b]

[a]$p < 0.05$; [b]$p < 0.01$, compared with the control

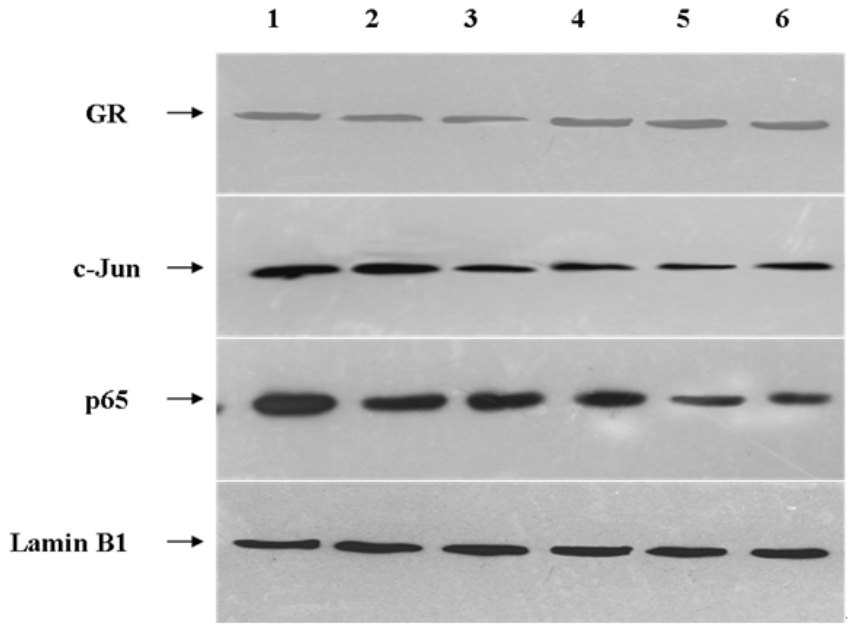

Figure 2: Effect of NCPB on nuclear translocation of GR, c-Jun, p65. Lanes 1-3 represented the protein expressions in the control group at 7, 3 and 1 days after PH, respectively. Lanes 4-6 represented the protein expressions level in NCPB group at 1, 3 and 7 days after PH, respectively

Table 6: Effect of NCPB on the activities of NF-κB and AP-1 of rats after PH (n=5, mean ± SD)

Parameter		Day 1	Day 3	Day 7
	Control	1.21 ± 0.11	1.75± 0.15	1.52 ± 0.12
c-Jun	NCPB	1.03 ± 0.09 [a]	0.97 ± 0.08 [b]	1.07 ± 0.08 [b]
	Control	1.89 ± 0.14	1.93 ± 0.16	2.32 ± 0.22
p65	NCPB	1.24 ± 0.11 [b]	1.03 ± 0.07 [b]	0.98 ± 0.09 [b]

[a]$p < 0.05$; [b]$p < 0.01$, compared with the control

Effect of NCPB on the activities of NF-κB and AP-1 of peritoneal macrophage

From the result of our present investigation (Table 6), we can come to the conclusion that the DNA binding activities of NF-κB and AP-1 of peritoneal macrophage were significantly lower than that in the control group ($p < 0.05$).

DISCUSSION

Results of our present investigation revealed that NCPB is an effective way to relieve pain in clinic, and we found that the NCPB induced by 0.5 % lidocaine can not only significantly decrease the serum AST and ALT concentrations after 70 % PH surgery to reduce the hepatocellular damage, but also obviously alleviate the damage of kidney by decreasing the levels of Cr and BUN. Furthermore, the mechanisms of this protective function of NCPB above may possibly be related to the inhibitory effects of NCPB on stress and inflammatory reaction after severe trauma.

Neuroendocrine responses refers to the stress process started immediately after trauma, and a measurable neuroendocrine response is key for maintaining body homeostasis [16,17], however, SIRS and immune function disorders can be

induced by over-response. The over-stress reactions after severe trauma mainly manifest as the over-activations of hypothalamic-pituitary-adrenal (HPA) sympathetic-adrenal medulla (SAM). The activation of HPA can be evaluated by levels of serum ACTH and GC. In our present study, we demonstrated that NCPB can significantly decrease the serum concentrations of GC and ACTH at all the tested time points compared with the control. What's more, the concentrations of serum NA and AD will be continually increased by over-activation of SAM after trauma, and our results indicated that NCPB treatment can obviously reduce the serum NA and AD levels of PH rats, compared with the control. All these results above showed that NCPB operation can alleviate significantly the stress response level, and help to maintain homeostasis after trauma. CRP is an acute phase reactive protein CRP, and can partly reflect the level of stress reaction after trauma. Our work demonstrated that NCPB can effectively decrease the serum CRP level, which is another evidence for demonstrating that NCPB can reduce the stress reaction level.

GC reaction is one of the most important stress reactions of body, and GR is the key point GC. Currently studies reported that severe trauma can induce the down-regulation of GR expression, and GR decrease can possible induce the multiple organ failure (MOF) after trauma [18]. The results of our present study showed that NCPB treatment can significantly increase the level of serum GR, compared to the control. After severe trauma, the secretion of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, commonly increased obviously [19]. Our present results demonstrated that NCPB treatment can reduce the concentrations of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, which indicated that NCPB can alleviate the function damages of liver and kidney after PH. HMGB1 is an important late inflammatory medium, and plays a crucial role in delayed lethal effect of sepsis [20,21]. In our investigation, NCPB can down-regulate the expression of HMGB1 obviously. Combined with the results of early inflammatory cytokines, we can come to the conclusion that the NCPB can suppress the secretions of cytokines in the whole process of inflammatory reaction.

NF-κB and AP-1 signal transduction paths play the important roles in inflammatory reactions [19]. The p65/p50 heterodimers are the main active pattern of NF-κB, and the degradation of IκB is the key link of the activation of NF-κB. Under quiescent condition, the heterodimers of NF-κB showed no activity and combined with IκB in the cytoplasm. The degradation of IκB can be induced by some signal stimulus, and then the phosphorylation of the heterodimers of NF-κB induced immediately, resulting in various inflammatory genes' transcription. The results of western blotting showed that NCPB treatment can significantly inhibit the degradation of IκB and decrease the expression of p65, as well as decreasing the nuclear translocation of p65. The expressions of c-Jun are correlated to the activity of AP-1 [22], our work demonstrated that NCPB can decrease the expressions of c-Jun of the macrophages, and suppress its nuclear translocation. In addition, our results also indicated that NCPB can also decrease the DNA binding activities of NF-κB and AP-1. These results indicated that NCPB might block the NF-κB and AP-1signal transduction paths, which is also one of the mechanisms that NCPB can inhibit the development of SIRS after PH surgery. NO is one of the effector molecules for activation of the macrophages to kill pathogenic microorganism and tumors, and is also an important inflammatory medium which plays the pro motive effect in the development of inflammation [23]. Our present study revealed that NCPB can not only significantly suppress the production of NO, but also obviously down-regulate the iNOS expressions in the peritoneal macrophage. The activation of NF-κB is the important cause of productions of iNOS by macrophage [24,25], therefore, the result mentioned above also indicated that NCPB might block the NF-κB signal transduction paths.

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