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Effects of ulinastatin on inflammatory response and cognitive function after hip arthroplasty for the elderly patients with femoral neck fracture

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Abstract. – OBJECTIVE: To investigate the effects of ulinastatin on inflammatory response and cognitive function after hip arthroplasty for the elderly patients with femoral neck fracture.

PATIENTS AND METHODS: A total of 80 patients with femoral neck fracture receiving hip arthroplasty in our hospital from August 2016 to February 2017 were selected and divided into observation group (n=40) and control group (n=40) using a random number table. The control group was treated with hip arthroplasty and symptomatic and supportive treatment after operation, while the observation group was treated with ulinastatin based on the treatment means of control group. The changes in antioxidant capacities, plasma noradrenaline (NA) and adrenaline (A) levels between the two groups before and after intervention were compared. The changes in neuron-specific enolase (NSE) and plasma S-100B protein levels before intervention and at 48 h after intervention were also compared. Moreover, the changes in mini-mental state examination (MMSE) scores during intervention and the Harris hip scores before intervention and at discharge between the two groups were compared. Finally, the off-bed walking time and postoperative discharge time of the two groups were recorded.

RESULTS: After intervention, the levels of malondialdehyde (MDA) and superoxide dismutase (SOD) and the total antioxidant capacity in observation group were significantly superior to those in observation group before intervention and control group after intervention ($p<0.05$). After intervention, the levels of NA and A in observation group were lower than those in control group ($p<0.05$), and the levels of interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and high-sensitivity C-reactive protein (hs-CRP) in observation group were also lower than those in control group ($p<0.05$). At 48 h after intervention, the levels of NSE and plasma S-100B protein in observation group were significantly lower than those in observation group before intervention and control group at 48 h after intervention ($p<0.05$). At 12 h, 24 h and 48 h after intervention, the MMSE scores of observation group were superior to those of control group in the same period ($p<0.05$). After intervention, the Harris hip score of observation group was superior to that of control group before and after intervention ($p<0.05$). The postoperative discharge time of observation group was earlier than that of control group ($p<0.05$), and the off-bed walking time was also earlier than that of control group ($p<0.05$).

CONCLUSIONS: The combined application of ulinastatin could effectively reduce the oxidative stress and inflammatory response, improve the neurological functions, and promote the postoperative recovery in the elderly patients with femoral neck fracture after hip arthroplasty.

Key Words:
Ulinastatin, Elderly, Femoral neck fracture, Hip arthroplasty, Inflammatory response, Cognitive function.

Introduction

Osteoporosis is the most common orthopedic complication in the middle-aged and aged population with the chronic pain, pathological fractures, shortening of height and spinal deformity as main manifestations[1]. Its pathogenesis is the decreased cell level in bone tissues per unit volume with the

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bone metabolic disorder[2]. Besides, osteoporosis often occurs in the elderly postmenopausal women[3]. With the social aging in China, the incidence rate of osteoporosis is increasing significantly. Meanwhile, the fracture is the most common and most serious complication of osteoporosis patients. Femoral neck fracture belongs to the violent fracture type, and the surgical treatment is the first choice for the elderly patients complicated with osteoporosis. However, multiple stimuli, such as trauma, surgery and anesthesia, increase postoperative inflammatory response and stress response, affecting the recovery of the patients. Meanwhile, the elderly people belong to a high-risk group of postoperative cognitive dysfunction[4]. Ulinastatin has effects of removing the oxygen or hydroxyl free radicals, reducing the level of inflammatory factors and the stress response, and improving the tissue perfusion[5]. It is mainly used in the treatment of severe infection, acute multi-stress stimulation. However, the effects of ulinastatin on inflammatory response and cognitive function after hip arthroplasty are not clear. The primary purpose of this study was to investigate the effects of ulinastatin on inflammatory response and cognitive function after hip arthroplasty for the elderly patients with femoral neck fracture.

Patients and Methods

Patients

A total of 80 patients with femoral neck fracture receiving hip arthroplasty in our hospital from August 2016 to February 2017 were selected. All the patients were diagnosed via clinical manifestation and imaging examination. Before enrollment, the patients or their guardians signed the informed consent of operation, anesthesia and enrollment. This study was approved by the Ethics Committee of Changzhou No. 2 People's Hospital, the Affiliated Hospital of Nanjing Medical University. Inclusion criteria: patients aged below 70 years old with normal mind and consciousness. Exclusion criteria: patients complicated with systemic infection, cardiopulmonary insufficiency or dysfunction, hepatic-renal insufficiency or dysfunction, coagulation disorders or immune system disease; patients who used glucocorticoids and/or immunosuppressive agents in the past 3 months or who refused to be enrolled. Patients enrolled were divided into observation group (n=40) and control group (n=40) using a random number table. In observation group, there were 23 males and 17 females aged 34-70 years with an average of (63.5±1.0) years old; in terms of injured site, there were 19 cases on the left and 21 cases on the right. In control group, there were 21 males and 19 females aged 60-85 years old with an average of (73.6±1.3) years old; in terms of injured site, there were 15 cases on the left and 25 cases on the right. There were no statistically significant differences in the gender, age and injured site between the two groups (p>0.05).

Research Methods

All the patients in control group underwent the hip arthroplasty, followed by anti-inflammation, analgesia, alleviation of tissue edema, wound dressing, and prevention of venous thrombosis after operation. The observation group was treated with the combined application of ulinastatin (Guangdong Techpool Biochemical; NMPN H20040476, Guangdong, China) via intravenous drip of 0.9% 100 mL normal saline with 200000 U ulinastatin added each time (once every 12 h) for 7 consecutive days as one course of treatment.

Observational Indexes

The changes in antioxidant capacities, plasma noradrenaline (NA) and adrenaline (A) levels between the two groups before and after intervention were compared, and the changes in neuron-specific enolase (NSE) and plasma S-100B protein levels before intervention and at 48 h after intervention were also compared. Moreover, the changes in mini-mental state examination (MMSE) scores during intervention and the Harris hip scores before intervention and at discharge between the two groups were compared. The correlation between high-sensitivity C-reactive protein (hs-CRP) level and MMSE score was analyzed. Finally, the off-bed walking time and postoperative discharge time of the two groups were recorded.

Evaluation Criteria

The oxidative stress factors were examined using the 450 full-automatic biochemical analyzer (Bio-Rad, Hercules, CA, USA). The reference value of malondialdehyde (MDA) in adults (TBA method) was 3.52 nmol/mL-4.78 nmol/mL; that of superoxide dismutase (SOD) in adults (WST method) was 0.242 μU/mL-0.620 μU/mL, while for the total antioxidant capacity in adults (FRAP method) was 2.34 μU/mL-26.96 μU/mL. Inflammatory factors were detected by enzyme-linked immunosorbent assay (ELISA). The reference value of tumor necrosis factor-α (TNF-α) in adults was 1 ng/mL-10 ng/mL; that of interleukin-1 (IL-1) in adults was

130 ng/mL-250 ng/mL, while that of hs-CRP in adults was <10 mg/L. The plasma NA and A levels were measured via radioimmunoassay. The reference value of NA in adults was 380 pmol/L-2365 pmol/L while that of A in adults was 0 pmol/L-380 pmol/L. About plasma S-100B protein in adults, the reference value of NA was <0.5 μg/L while for the neuron-specific enolase (NSE) in adults was <12.5 μg/L. The mini-mental state examination (MMSE) included 6 major items, namely the time, place orientation ability, expression and calculation ability, attention and memory. The total score was 30 points; the comprehensive evaluation was performed combined with the educational condition; the lower the score was, the better cognitive function will be.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 21.0 (IBM, Armonk, NY, USA) was used for statistical treatment. Measurement data were presented as mean ± standard deviation ($\bar{x}\pm s$), while the enumeration data were presented as percentage (%). *t*-test was performed for the intergroup comparison of means, analysis of variance was performed for the intragroup comparison of means, and x^2-test was used for the intergroup comparison of rates. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of Antioxidant Capacity and Stress Response Between the Two Groups Before and After Intervention

There were no significant differences in the comparisons of MDA, SOD, and total antioxidant capacity between the two groups before intervention, respectively ($p>0.05$). After intervention, the levels of MDA, SOD and the total antioxidant capacity in observation group were significantly higher than those in observation group before intervention and control group after intervention (t=47.725, 202.000 and 205.061; t=30.358, 144.250 and 75.895, $p<0.05$). There were no significant differences in the comparisons of NA and A levels between the two groups before intervention ($p>0.05$). After intervention, the levels of NA and A in observation group were lower than those in control group (t=63.607 and 205.061; t=72.831 and 51.430, $p<0.05$).

Comparisons of Inflammatory Factors Between the Two Groups After Intervention

After intervention, the levels of hs-CRP, IL-1 and TNF-α in observation group were significant lower than those in control group ($p<0.05$).

Comparisons of NSE and Plasma S-100B Protein Levels Between the Two Groups Before Intervention and at 48 H After Intervention

Before intervention, the levels of NSE and plasma S-100B protein were (8.1±0.9) ng/mL and (2.5±0.3) ng/mL in observation group, and (8.0±0.9) ng/mL and (2.5±0.4) ng/mL in control group; the differences were not statistically significant ($p>0.05$). At 48 h after intervention, the levels of NSE and plasma S-100B protein were (2.3±0.2) ng/mL and (0.5±0.1) ng/mL in observation group, and (5.3±0.6) ng/mL and (1.5±0.6) ng/mL in control group; at 48 h after intervention, the levels of NSE and plasma S-100B protein in observation group were significantly lower than those in observation group before intervention and control group at 48 h after intervention (t=39.788 and 30.000, 40.000 and 9.428, $p<0.05$) (Figure 1).

Comparisons of MMSE Scores Between Two Groups During Intervention

Before the intervention, the MMSE scores of the two groups were (29.5±1.7) points and (29.6±1.7) points, respectively; there was no significant difference (t=0.263, p=0.793>0.05). At 12 h, 24 h and 48 h after intervention, the MMSE scores of observation group were (23.5±1.5) points, (20.3±1.3) points and (16.3±1.0) points, respectively, which were superior to those of control group in the same period [(26.8±1.6) points, (24.2±1.4) points and (20.1±1.2) points] (t=9.516, 12.911 and 15.386, $p<0.05$) (Figure 2).

Correlation Analysis of hs-CRP Level and MMSE Score

The hs-CRP level was positively correlated with the MMSE score (r=0.9070, p=0.000<0.05) (Figure 3).

Comparisons of Harris Hip Scores Between the Two Groups Before Intervention and at Discharge

There was no statistically significant difference in the Harris hip score between the two groups

Table I. Comparisons of antioxidant capacity and stress response between the two groups before and after intervention ($\bar{x}$±s).

		MDA (nmol/mL)	SOD (μU/mL)	Total antioxidant capacity (U/mg)	NA	A
Observation group	Before intervention	1.65±0.05	1.36±0.03	2.11±0.02	1506.2±113.5	4812.5±306.9
	After intervention	1.21±0.03	0.35±0.01	3.56±0.04	335.6±25.8	1253.3±36.6
Control group	Before intervention	1.66±0.06	1.35±0.03	2.12±0.02	1500.3±113.9	4810.6±307.0
	After intervention	1.45±0.04	0.86±0.02	2.96±0.03	425.1±53.6	2586.4±159.8

Table II. Comparisons of inflammatory factors between the two groups after intervention ($\bar{x}$±s).

	hs-CRP (mg/L)	IL-1 (ng/mL)	TNF-α (ng/mL)
Observation group	7.2±0.6	158.6±13.2	8.1±0.3
Control group	25.5±0.9	238.6±15.6	15.0±0.6
t	107.001	24.759	65.054
p	0.000	0.000	0.000

Table III. Comparisons of Harris hip scores between the two groups before intervention and at discharge ($\bar{x}$±s).

	Before intervention	After intervention	*t*	*p*
Observation group	68.6±6.2	85.1±7.0	11.160	0.000
Control group	56.5±4.0	70.5±5.1	13.661	0.000
t	12.215	10.661	-	-
p	0.000	0.000	-	-

Table IV. Comparisons of off-bed walking time and postoperative discharge time between the two groups (d, $\bar{x}$±s).

	Off-bed walking time	Postoperative discharge time
Observation group	2.5±0.2	6.5±0.5
Control group	3.6±0.3	7.9±0.7
t	19.295	10.293
p	0.000	0.000

before treatment ($p>0.05$). After intervention, the Harris hip score of observation group was significant superior to that in control group before and after intervention ($p<0.05$).

Comparisons of Off-bed Walking Time and Postoperative Discharge Time Between the two Groups

The postoperative discharge time of observation group was earlier than that of control group ($p<0.05$), and the off-bed walking time was also earlier than that of control group ($p<0.05$).

Discussion

With the aging of population in China, the proportion of the elderly people is getting higher, and the medical diseases are getting more complicated, especially for the middle-aged women with osteoporosis who suffer from femoral neck fracture easily[6,7]. Surgery is the first choice in the treatment of the femoral neck fracture in the elderly, which can effectively improve the life quality and motor function of patients, and avoid the deep vein thrombosis, malnutrition and bed sores due to immobilization. The hip arthroplasty is the ideal method in the treatment of femoral neck fracture for elderly people[8]. Such patients often suffer from severe inflammatory response. Meanwhile, the antioxidant capacity is significantly reduced. The postoperative neurological function recovery is also affected by trauma, surgery, anesthesia and other stimuli combined with a variety of medical diseases[9]. Ulinastatin is obtained from the fresh urine of healthy adults[10], and its effect is to improve the stability of intracellular lysosomal membrane[11], remove oxygen and/or hydroxyl free radicals[12] and inhibit the body's inflammatory response[13].

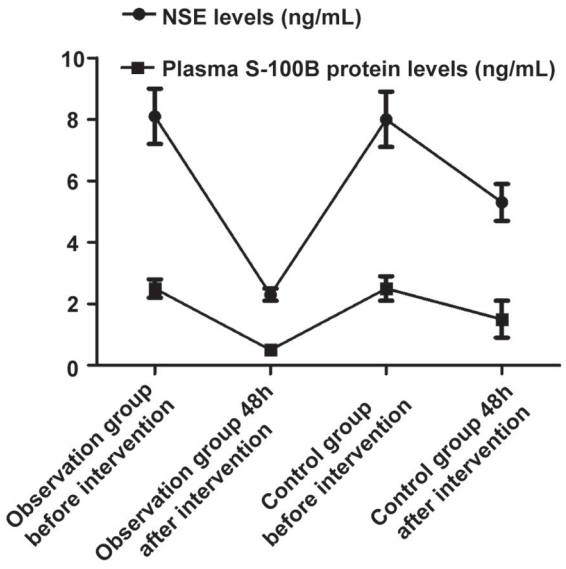

Figure 1. Comparisons of NSE and plasma S-100B protein levels before intervention and at 48 h after intervention. The differences in levels of NSE and plasma S-100B protein before intervention and at 48 h after intervention between the two groups are not statistically significant ($p>0.05$); at 48 h after intervention, the levels of NSE and plasma S-100B protein in observation group are significantly lower than those in observation group before intervention and control group at 48 h after intervention ($p<0.05$).

In this study, the comparisons of antioxidant capacity and stress response between the two groups before and after intervention were done. Our results showed that after intervention, the levels of MDA and SOD and the total antioxidant capacity in observation group were significantly superior to those in observation group before intervention and control group after intervention. The levels of NA and A in observation group were lower than those in control group, suggesting that the application of ulinastatin can effectively improve the body's antioxidant capacity and reduce stress response. In addition, the levels of IL-1, TNF-α and hs-CRP in observation group were lower than those in control group after intervention, indicating that the application of ulinastatin could reduce the body's inflammatory response significantly. At the same time, the levels of NSE and plasma S-100B protein in observation group at 48 h after intervention were significantly lower than those in observation group before intervention and control group at 48 h after intervention; at 12 h, 24 h and 48 h after intervention, the MMSE scores of observation group were superior to those of control group in the same period, suggesting that the application of ulinastatin is important for reducing the inhibitory neurotransmitter or protein and improving the neurological function of elderly patients. It was found that the hs-CRP level was positively correlated with the MMSE score, indicating that the higher the level of hs-CRP in the elderly patients with femoral neck fracture receiving hip arthroplasty is, the higher the MMSE score is, and the worse the cognitive function will be. Finally, it was also found that the Harris hip score of observation group after intervention was superior to that of control group before and after intervention. The postoperative discharge time of observation group was earlier than that of control group, and the off-bed walking time was also earlier than that of control group, which further indicated that the application of ulinastatin has a positive significance in improving the patient's hip function, promoting the postoperative off-bed walking time as soon as possible and early discharge. Ulinastatin is a kind of broad-spectrum protease inhibitor extracted from the urine of normal adult males, which can effectively inhibit the body's inflammatory response[14] and reduce the stress response[15], thus achieving the purpose of anti-oxidation[16]. The intravenous application of ulinastatin effectively inhibits a variety of proteolytic enzymes in the body, reduces the dam-

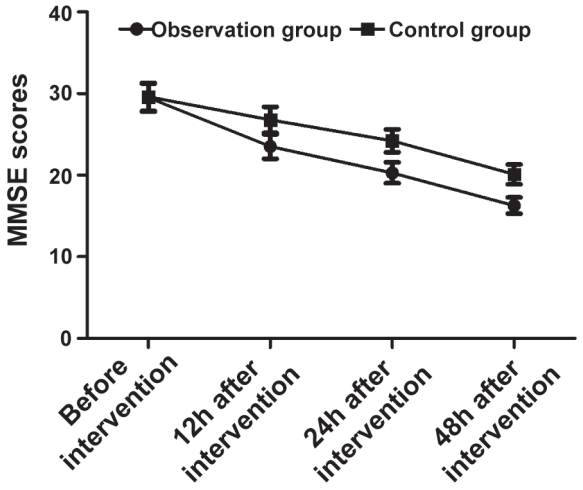

Figure 2. Comparisons of MMSE scores between two groups during intervention. There is no statistically significant difference in the comparison of MMSE score between the two groups before intervention ($p>0.05$); at 12 h, 24 h and 48 h after intervention, the MMSE scores of observation group are superior to those of control group in the same period ($p<0.05$).

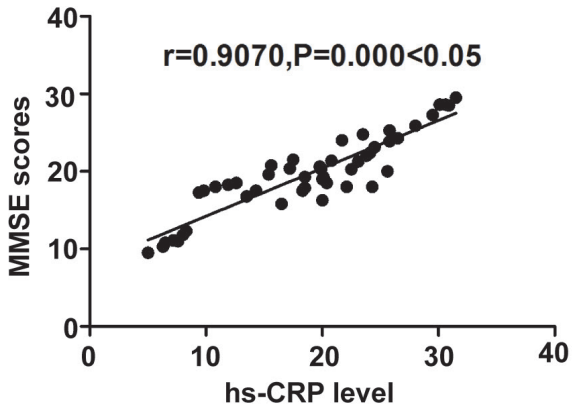

Figure 3. Correlation analysis of hs-CRP level and MMSE score.

age of proteolytic enzymes to the normal tissues and organs[16], and stabilizes the lysosomal membrane in the organelles. Besides, it can also reduce the proteolytic enzyme activity, thereby improving the microcirculation and tissue perfusion function. Therefore, after intervention, the body's antioxidant capacity is enhanced[17], but the stress and inflammatory responses are inhibited. At the same time, after the application of ulinastatin for elderly patients, the lysosomal membrane in neural cells is effectively stabilized, the mitochondrial activity in organelles is increased[18], the respiratory function of nerve cells is improved, the elastase activity is enhanced[19], and the nerve cell regeneration ability is effectively improved[20], so the neurological function of patients is significantly improved after intervention, thereby improving the overall therapeutic effect.

Conclusions

The combined application of ulinastatin could effectively reduce the oxidative stress and inflammatory response, improve the neurological functions, and promote the postoperative recovery in the elderly patients with femoral neck fracture after hip arthroplasty.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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