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Effect of Low Molecular Weight Heparin Calcium Combined with Argatroban on the Treatment, Vascular Endothelial Function, Inflammatory Factors and Serum Neurological Function in Patients with Acute Cerebral Infarction

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Authors' Contribution
X Yao and GZ collected the samples, conducted the experiments and analysed the results. X Yue and YY analysed the data. All authors discussed the results and wrote the manuscript.

Key words
Low-molecular-weight heparin calcium, Argatroban, Acute cerebral infarction, Inflammatory factors, Vascular endothelialium, Nerve.

ABSTRACT

The objective of this study was to observe the effects of low-molecular-weight heparin calcium (LMWHC) combined with argatroban on the treatment, vascular endothelial function, inflammatory factors and neurological function in patient with acute cerebral infarction. A total of 80 patients with acute cerebral infarction were randomly divided into 2 groups each of 40 cases, regardless of gender, aged from 55-75 years, who underwent treatment in hospital from January 2018 to December 2020. Each group received routine treatment, in which the treatment group received LMWHC alongwith argatroban. The score of neurological deficit (NIHSS) and serum neurological function, endothelial injury and inflammatory marker levels, and the clinical efficacy after treatment of 14 days were compared between groups. After treatment of 14 days, the vascular endothelial function (NO, ET-1, FMD) and inflammatory factors (hs-CRP, TNF-α, IL-6, MMP-9, Lp-PLA2) in the patients of the treatment group were significantly higher than the control group. NIHSS and serum neurological function (copeptin, NT-proBNP, PAO, S-100B) in the patients of the treatment group were significantly higher than that of the control group (P<0.05). Adverse reaction of LMWHC and argatroban was not found in the course of treatment. We conclude that LMWHC combined with argatroban are safe and effective in the treatment of acute cerebral infarction.

INTRODUCTION

Acute cerebral infarction (ACI), also known as cerebral infarction, refers to ischemic stroke in the brain. As a common neurological disease in the clinic, it accounts for more than 60% of cerebral infarction cases, which has an acute onset and rapid progress. Its clinical manifestations include facial paralysis, upper extremity hemiplegia, etc. In severe cases, diplopia, dizziness and arrhythmia will occur, even threatening life (Sun *et al*., 2019; Huang *et al*., 2021). In the past, ACI mostly occurred in the middle-aged and elderly population. Recent years witnessed its occurrence in younger population. It can occur at any age with no specific morbidity group, and there is no forewarning at the time of onset. Therefore, effective treatment drugs and methods are the key to protecting patients' life and health (Wu *et al*., 2016). Clinical studies have shown that anticoagulant therapy has a good effect in preventing prolongation of thrombus, recurrence of stroke, secondary thrombosis in distal small vessels, and improving collateral microcirculation (Peng and Wu, 2018). At present, studies have confirmed that argatroban and low-molecular-weight heparin (LMWH) have a significant therapeutic effect on ACI, but combined use of the two drugs has not been evaluated (Zhang *et al*., 2020; Chen *et al*., 2018; Qiu *et al*., 2021). Therefore, this study investigates the actual efficacy of low-molecular-weight heparin calcium (LMWHC) combined with argatroban in the treatment of ACI, and determines the basic mechanism of the drug by detecting changes in inflammatory factors, vascular endothelial function, and neural function before and after treatment, and ultimately provides data reference for the clinic.

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MATERIALS AND METHODS

General information

Inclusion criteria: ACI diagnosis was performed according to the standards in the 2018 Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke (Peng and Wu, 2018), and only a single infarct was confirmed by MRI or CT, which had consistent located signs, with no cerebral hemorrhage, brain occupation. The first onset or recurrence left no obvious sequelae. The disease attacked within 3 days, and no thrombolytic drug therapy was used. NIHSS score was 2～15. There was no history of allergy to argatroban, and no statins, immune or inflammation inhibitors were taken within 4 weeks before the treatment.

Exclusion criteria: those younger than 18 years old or older than 85 years old, those accompanied with immune system diseases, cancer, hemorrhagic diseases or abnormal liver and kidney functions, as well as pregnant or lactating women.

Case selection: According to the above criteria, 80 patients who received ACI treatment at the First Hospital of XX City from January 2018 to December 2020 were selected, who were divided into two groups by random table method-treatment group (LMWHC + argatroban + conventional treatment) and control group (conventional treatment). The treatment lasted 7 days. The clinical efficacy, changes in inflammatory factors (hs-CRP, TNF-α, IL-6, IL-8, MMP-9, Lp-PLA2), vascular endothelial function indicators (NO, ET-1, FMD), neurological impairment (NIHSS score) were compared between the two groups at 14 days after treatment. The requirements for blood collection were that patients should not eat high-fat, high-tyramine foods for the first 3 days, start fasting at 12h before blood collection. 8mL of anticoagulant blood was collected in the morning on an empty stomach before treatment and on the 14th day after treatment, which was centrifuged at 4°C 3000 r/min for 10 min to separate plasma and then stored at -70°C for future inspection.

Treatment method

For treatment group, the dosage was calculated according to body weight, and 3000～5000AU LMWHC (Shenzhen Sciprogen Bio-Pharmaceutical Co., Ltd., National Medicine Permission Number H20060190) was injected subcutaneously, twice per person per day, with an interval of 12h for 7 days. At the same time, on the first and second days of treatment, continuous intravenous pumping of 60mg argatroban (Tianjin Institute of Pharmaceutical Research Co., Ltd., approval number: National Medicine Permission Number H20050918) was given for 24 h per day per person without interruption; From the 3rd to 7th days of treatment, intravenous infusion of 10mg argatroban was given, 2 times a day and 3 h per time.

For control group, when giving conventional treatment, enteric-coated aspirin (0.1g) was taken orally every night, intravenous drip of edaravone injection (30mg+9g/L sodium chloride injection 250mL, twice a day) and Danshen ligustrazin for injection (10mL+50g/L glucose injection 250mL, once a day) was given, and 10mg atorvastatin calcium was orally taken once a day after supper. In addition, according to the state of illness and complications, increase the amount of other drugs appropriately for symptomatic treatment and maintain water and electrolyte balance.

Evaluation of efficacy

Score was given according to the National Institutes of Health Stroke Scale (NIHSS) before and at 14 days after treatment, with reference to the Scoring Criteria for Clinical Neurological Impairment in Stroke Patients formulated in 1995: (i) Basic recovery: NIHSS score decreases by 91%～100%, grade 0 disability; (ii) Significant progress: NIHSS score decreases by 46%～90%, grade 1～2 disability; (iii) Progress: NIHSS score decreases by 18%～45%; (iv) Invalid: NIHSS score decreases by 0%～17%; (v) Death. Total effective rate = (basic recovery + significant progress + progress) / total number of cases x 100%.

Detection of neurological function factors

ELISA method (with reagent purchased from Shanghai Keshun Biotechnology Co., Ltd.) was used to detect copeptin, N-terminal pro-brain natriuretic peptide (NT-proBNP), polyamine oxidase (PAO), S -100B protein before and after the treatment.

Determination of cerebral blood flow

The average blood flow velocity of the middle cerebral artery (MCA) and the anterior cerebral artery (ACA) was evaluated according to the cranial Doppler (TCD) detection results of the cerebral blood flow status before and after treatment.

Detection of vascular endothelial function

Nitric oxide (NO) was detected by nitrate reductase (purchased from Nanjing Jiancheng Bio-Engineering Institute). Endothelin-1 (ET-1) was detected by radioimmunoassay (purchased from East Asia Institute of Immunology, General Hospital of the People's Liberation Army). When testing the endothelium-dependent vasodilation function (FMD) mediated by brachial artery blood flow, the color ultrasound diagnostic instrument (HP 5500, USA) was used to detect vasodilator drugs

discontinued 12 h ago. The patient needs to lie supine 10 min before measurement of basic diameter of the brachial artery (d_0); when measuring the inner diameter of reactive hyperemia induced brachial artery (d_1), place the sphygmomanometer in the cuff on the distal end of the target artery, inflate and pressurize to 300mmHg, deflate at 4min, and read the result between 60～90s. FMD calculation formula: $(d_{1-} d_0)/ d_0$ x 100%.

Inflammatory factor detection

High-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), IL-8, tumor necrosis factor (TNF-a), matrix metalloproteinase (MMP-9) and lipoprotein-associated phospholipid A2 (Lp-PLA2) in the collected plasma samples were detected, respectively. The ELISA kit was purchased from Shenzhen Xinbosheng Biological Technology Co., Ltd. The BIO-RAD microplate reader was of Model 680.

Statistical analysis

SPS S16.0 statistical analysis software was used to compare the data of each group. When the measurement data conformed to normal distribution, ($\bar{x}$±s) was used for χ^2 test; when the measurement data were not normally distributed, the median and inter-quartile range was compared. The difference was statistically significant when P<0.05.

RESULTS

There was no statistically significant difference in comparison of age, gender, underlying disease (hypertension, diabetes), and cerebral infarction site between the groups (P>0.05) (Table I).

Table I.- Comparison of the 2 groups of patients with acute cerebral infarction.

Project indicators	Control group (n=40)	Treatment group (n=40)
Age ($\bar{x}$±s, years)	63.6±3.6	63.9±3.8
Gender (male/female, n/n)	20/20	21/19
Infarction site		
Anterior circulation	29	27
Posterior circulation	11	13
Hypertension	31	33
Diabetes	20	18

Table II shows the effect of LMWHC and argatroban on score on neurological deficit (NIHSS), cerebral blood flow (MCA, ACA), neurological function indicators (copeptin, NT-proBNP, PAO, S-100B ET-1), vascular endothelial function (NO, ET-1, FMD) and inflammatory factors (hs-CRP, TNF-α, IL-6, MMP-9, LP-PLA2) of the two groups of patients before and after treatment. All parameters in both the control and treatment

Table II.- Effect of low molecular weight heparin-calcium and argatroban on NIHSS, cerebral blood flow (MCA, ACA), neurological function indicators (copeptin, NT-proBNP, PAO, S-100B ET-1), vascular endothelial function (NO, ET-1, FMD) and inflammatory factors (hs-CRP, TNF-α, IL-6, MMP-9, LP-PLA2) of the two groups of patients before and after treatment.

Project indicators		Control group (n=40)	Treatment group (n=40)
Score of neurological deficit			
NIHSS ($\bar{x}$±s)	Before treatment	14.0±2.1	13.5±2.2*
	After treatment	8.7±2.2	6.1±1.4***
Effective rate		80.32	97.01***
Cerebral blood flow			
MCA ($cm•s^{-1}$)	Before treatment	42.0±4.1	41.7±5.2
	After treatment	47.8±5.5	53.9±5.4***
ACA ($cm•s^{-1}$)	Before treatment	34.3±5.0	34.5±3.3
	After treatment	40.6±4.1	51.4±4.7***
Neurological function indicators			
Copeptin ($pmol•L^{-1}$)	Before treatment	5.3±0.6	5.5±0.7
	After treatment	4.3±0.5	2.2±0.3***
NT-proBNP ($pmol•L^{-1}$)	Before treatment	221.4±31.7	226.9±34.5
	After treatment	199.4±24.7	107.6±14.4***
PAO ($U•L^{-1}$)	Before treatment	9.6±1.0	9.9±1.2
	After treatment	8.1±0.9	3.9±0.5***
S-100B ET-1 ($μg•L^{-1}$)	Before treatment	2.2±0.3	2.4±0.3
	After treatment	1.2±0.2	0.9±0.1*
Vascular endothelial function			
NO ($μmol•L^{-1}$)	Before treatment	49.2±14.1	48.7±13.2
	After treatment	50.7±12.5	63.1±11.4***
ET-1 ($ng•L^{-1}$)	Before treatment	79.0±12.8	77.9±12.2
	After treatment	78.3±12.2	69.3±12.7***
FMD (%)	Before treatment	8.3±1.0	8.5±1.3
	After treatment	8.6±1.1	11.4±1.7***
Inflammatory factors			
hs-CRP ($m•L^{-1}$)	Before treatment	7.2±2.1	6.7±1.2
	After treatment	6.0±1.8	4.1±1.4*
TNF-α ($μg•L^{-1}$)	Before treatment	4.8±1.7	4.9±1.5
	After treatment	3.3±1.2	2.3±0.7*
IL-6 ($ng•L^{-1}$)	Before treatment	38.9±8.0	38.5±8.3
	After treatment	18.6±5.1	11.1±4.7***
IL-8 ($ng•L^{-1}$)	Before treatment	39.0±8.1	39.2±7.9
	After treatment	19.1±6.4	12.0±4.1***
MMP-9 ($μg•L^{-1}$)	Before treatment	334.5±35.2	323.6±33.9*
	After treatment	151.4±29.8	105.6±17.7***
Lp-PLA2 ($pg•mL^{-1}$)	Before treatment	17.2±6.3	16.3±5.5
	After treatment	12.0±2.6	5.5±1.0***

groups showed insignificant differences between the two groups. After treatment with LMWHC+argatroban, all these parameters show statistically significant differences.

The NIHSS of the treatment group, on the 14th day after treatment shows statistically significant difference between the groups (P<0.05). The total effective rate was 97.01% in the treatment group after treatment compared to 80.32% in the control group, showing significant difference (P<0.05).

The cerebral blood flow indicators (MCA, ACA) and NO and FMD as vascular endothelial function shows significant increase after treatment with LMWHC and argatroban. On the other hand all neurological indicators (copeptin, NT-proBNP, PAO, S-100B ET-1), ET-1 as endothelial function and all inflammatory factors showed significant decrease after treatment with LMWHC and argatroban.

DISCUSSION

Currently, anticoagulant and antiplatelet aggregation drugs are common clinical drugs for infarction treatment, such as low-molecular-weight heparin, which has the characteristics of long biological half-life, low bleeding risk, and good safety. It does not affect vascular permeability when it exists in the form of calcium salt, and can play a role in improving cerebral hemodynamics and producing antithrombotic effects, but it is not ideal for the treatment of progressive cerebral infarction when used alone, so its combined use with other anticoagulant drugs in clinical application is currently the main treatment strategy (Arahata and Asakura, 2018; Zhu et al., 2018). According to literature reports, low-molecular-weight heparin calcium combined with urokinase can effectively produce antithrombotic, anticoagulant effects and improve whole blood viscosity, thus significantly improving the overall efficacy of cerebral infarction (Huang and Pan, 2012); when vinpocetine is combined with LMWHC for the treatment of senile chronic cerebral circulatory insufficiency, clinical symptoms can be significantly improved, blood rheology and viscosity indicators have improved significantly without any negative effects (Fatima *et al*., 2020). LMWH therapy combined with clopidogrel hydrogen sulfate has significant effect in treating progressive cerebral infarction, which produces better effect than use of LMWH alone and significantly improves the patient's cerebral hemodynamics. Moreover, the risk of adverse reactions has not increased (Arahata and Asakura, 2018).

Argatroban is used as a thrombin inhibitor (with molecular weight 527Da), which can reversibly bind to the active site of thrombin. After binding, it can inactivate thrombin to alleviate the patient's circulating hypercoagulable state and avoid the formation of secondary embolism. It is highly selective and does not require aid of antithrombin to produce anticoagulant effect (Yi-Cui *et al*., 2015; Mohamed and Coombe, 2017). Argatroban has good safety in clinical use. The therapeutic dose does not interfere with platelet function, nor will it cause bleeding. At the same time, it is not immunogenic and does not have cross immunoreaction with heparin-induced antibodies. The metabolic pathway of argatroban is liver, so those with renal insufficiency do not need to reduce the dosage. Moreover, with short half-life and fast onset, it can regulate vascular endothelial cell function and reduce inflammation (Ishibashi *et al*., 2013). According to literature reports, when argatroban is used to treat ACI, the damage to vascular endothelial function can be reduced and the inflammatory response can be significantly reduced, so the patient's nerve function is improved, showing significantly improved therapeutic effect (Xu *et al*., 2017). In addition, edaravone combined with argatroban for the treatment of ACI can also significantly improve the patient's nerve function and body hypercoagulability by reducing the level of inflammatory factors (Huang *et al*., 2021).

The thrombus formed by fibrin contains a lot of thrombin, which will be released when the thrombus is dissolved, thereby causing reformation of the thrombus. LMWHC has a molecular weight 10 times bigger than that of argatroban, and it cannot exert antagonistic effect on bound thrombin. However, because of its small molecular weight, argatroban can inactivate thrombin inside the thrombus, thus producing good thrombolytic effect (Denorme *et al*., 2016). By contrast, LMWHC also has its own advantages, such as longer half-life, anticoagulation and fibrinolytic effects. Related studies have reported that LMWHC, argatroban produce unobvious clinical efficacy in the treatment of acute cerebral infarction. Therefore, this study clinically combines LMWHC and argatroban for the first time, and has achieved ideal treatment effect (Hirsh and Raschke, 2004; Zhang *et al*., 2020).

This study compares the clinical efficacy (NIHSS score) of acute cerebral infarction, TCD cerebral blood flow, serum neurological function indicators (copeptin, NT-proBNP, PAO, S-100B), changes in inflammatory factors (hs-CRP, TNF-α, IL-6, IL-8, MMP-9, Lp-PLA2) and vascular endothelial function indicators (NO, ET-1, FMD) between combined medication and conventional therapy. The results indicate that on the 14th day after treatment, the treatment group has significantly higher total effective rate than the control group, showing significant differences in vascular endothelial function, inflammatory factor levels, serum neurological function indicators and NIHSS between the two groups (P<0.05). Moreover, no

clinical adverse reactions associated with argatroban and LMWHC was found during treatment (Yang *et al*., 2020).

The indicators tested in this study, such as MCA and ACA, can reflect blood supply condition of brain tissue. If the value decreases, it indicates ischemia and hypoxia of brain tissue in the infarct area, suggesting severe brain function damage. Concentration of copeptin, NT-proBNP, PAO and S-100B in serum reflects the functional status of brain tissue, which is also a common clinical evaluation item. Vascular endothelial function damage and inflammation will promote the occurrence of ACI. Where, NO, ET-1 and FMD are typical endothelial function indicators, hs-CRP, TNF-α, IL-6, IL-8, MMP-9 and Lp-PLA2 are the most reported inflammatory markers in clinic. The detection of the above indicators features simple sampling and strong representativeness, which can clarify the basic intervention mechanism of therapeutic drugs on ACI patients. ACI patients mainly manifest neurological damage. The results of this study indicate that the combined application of LMWHC and argatroban not only plays an anticoagulant role in treatment, but also can significantly improve vascular endothelial function and antagonize the inflammatory response, thereby restoring the patient's neurological function (Wu *et al*., 2020; Gao *et al*., 2018).

To conclude, the combination of LMWHC and argatroban for clinical treatment of ACI can improve patients' condition in overall and protect patients' brain tissue function, which means practical significance for improving surgical treatment effect and reducing postoperative complications in clinical application (Gao *et al*., 2019).

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Statement of conflict of interest

The authors have declared no conflict of interests.

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