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Effect of urokinase on cerebral perfusion after cardiopulmonary resuscitation in rabbits

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Abstract. – BACKGROUND: Cerebral resuscitation after cardiac arrest (CA) is always an unresolved problem in medical field. The decreased cerebral perfusion or nonperfusion caused by coagulation and fibrinolytic system function disorder and cerebral microthrombosis after CA and cardiopulmonary resuscitation (CPR) is one of the important reasons.

MATERIALS AND METHODS: TTo investigate the effect of urokinase on cerebral microcirculatory perfusion after CA and CPR in rabbits. 20 rabbits were randomly divided into experimental group and control group, 10 rabbits in each group. Potassium chloride injection combined with asphyxia method was conducted to establish the CA models. CPR and basic life-support were performed on experimental group. Based on above treatments, intervention with urokinase was conducted on experimental group. Dual-slice spiral CT cerebral perfusion imaging was performed to observe the cerebral blood flow (CBF), cerebral blood volume (CBV) and top teep time (TTP).

RESULTS: CBF and CBV in experimental group were significantly higher than those in control group, respectively ($p < 0.01$), and TTP in experimental group was significantly shorter than control group ($p < 0.01$). The cerebral perfusion in experimental group was better than control group.

CONCLUSIONS: Thrombolytic therapy with urokinase in CPR after CA can improve the cerebral microcirculatory perfusion in rabbits.

Key words:
Cardiopulmonary resuscitation, Cerebral perfusion, Microcirculation, Urokinase.

Introduction

Cerebral resuscitation after cardiac arrest (CA) is always an unresolved problem in medical field. The survival rate of patients with cardiopulmonary resuscitation (CPR) due to out-of-hospital cardiac and respiratory arrest is about 2%-9%[1]. The brain function restoration after resuscitation represents the ultimate success of CPR. However, only a few patients can achieve a certain degree of nerve function restoration. It is found that, in Los Angeles, there are only 1.4% of patients with out-of-hospital CA have obtained a good nerve function restoration after CPR[2]. There are many factors affecting brain resuscitation, in which the decreased cerebral perfusion or nonperfusion caused by coagulation and fibrinolytic system function disorders and cerebral microthrombosis after CA and CPR is one of the important factors[3-5].

As found in CPR related researches, treatment with thrombolytic drug can increase the success rate of cerebral resuscitation, and improve the function of the nervous system[6,7]. It is also found that use of thrombolytic drugs can improve the perfusion on cerebral microcirculation and reduce the nonperfusion area, contributing to improvement of neurological prognosis[8]. The effect of thrombolytic drug on cerebral microcirculation in CPR is worthy of further investigation. With development of multislice spiral CT cerebral perfusion imaging technology, the quantitative study of cerebral perfusion has been widely performed. Using this technology, the continuously multidimensional perfusion imaging can be conducted, with an expanded observation scope[9]. Therefore, it is feasible to use this technology for observation of cerebral perfusion. In recent years, rabbit is selected as a more suitable animal for imaging study, and is gradually used in the basic research on CT imaging of cerebral perfusion.

In this study, the rabbit models of CA were established by potassium chloride injection combined with asphyxia method. During the basic life-support (BLS) stage, urokinase was given to the rabbits and the dual-slice spiral CT cerebral perfusion imaging was conducted. The effect of urokinase on rabbit cerebral microcirculatory perfusion was observed.

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Materials and Methods

Experimental animal and grouping

20 healthy New Zealand thoroughbred rabbits (age, 5-6 months; weight, 2-3 kg) were randomly divided into routine CPR group (control group) and thrombolysis group (experimental group), 10 rabbits for each group.

Establishment of rabbit models of CA

Rabbits were treated with fasting overnight before operation, with free access to water. At preoperative 30 min, atropine was intramuscular injected (0.01 mg/kg). After weighing, ketamine combined with Sumianxin (1 mL: 1 mL) was intramuscular injected (0.2-0.3 mL/kg) for anesthesia. Four limbs of rabbit were connected to the ECG electrodes. The femoral artery was separated, and the pediatric cardiac catheter was placed. After catheter heparinization, the femoral artery was connected to the sensor of MINDRAY 9000 multi-channel physiological recorder to monitor the arterial blood pressure and mean arterial pressure (MAP). The femoral vein was separated, and BD22G trocar was placed for intravenous administration. The trachea of rabbit was incised, and the catheter was placed for mechanical ventilation. According to the reported method[10,11], 10% potassium chloride (4°C) was injected in the femoral vein (bolus injection, 7 mg/100 g). After CA, the trachea catheter terminal was clipped at the end-tidal. The criteria of CA were as follows: The arterial pressure wave disappeared and MAP was less than 20 mmHg; the sinus rhythm disappeared in ECG; the ventricular fibrillation wave or straight line appeared; the auscultation sound disappeared. The resuscitation was conducted after 3 min of above manifestations.

Cardiopulmonary resuscitation method

The clipped trachea catheter was loosened and connected to the small animal ventilator for mechanical ventilation (frequency 40 beats/min; tidal volume, 20 mL/kg). The chest compressions with 150-180 beats/min were conducted. When the resuscitation began, epinephrine was intravenously injected (0.02 mg/kg) in each group, once per 5 min for 3 repetitions. If the spontaneous circulation was not restored after 30 min of resuscitation, the observation was ceased, indicating the failure of resuscitation. In experimental group, from the beginning of resuscitation, 30 min of intravenous drip using Urokinase (20 000 U/kg) combined with normal saline (30 ml) was performed[12]. In control group, 30 ml of normal saline was intravenously dripped within 30 min. After restoration of spontaneous circulation, mechanical ventilation was continued until restoration of spontaneous breathing.

Observation indexes

Spontaneous circulation restoration time[13] The judgment standard was as follows: Without chest compression, the systolic blood pressure was maintained above 60 mmHg for 10 min.

Spontaneous breathing restoration time The judgment standard was as follows: The abdominal breathing appeared, and it continued for 5 min after removing the ventilator.

Observation of cerebral microcirculatory perfusion After 30 min since spontaneous breathing restoration, dual-slice spiral CT (Siemens, Munich, Germany) was used for cerebral perfusion imaging and image processing[14,15]. The rabbit was fixed on the plate (supination). The head coronal scanning was performed (layer thickness, 2.5 mm; tube voltage, 120 Kv; tube current, 150 mA). Then the cerebral perfusion scanning was conducted (layer thickness 1.5 mm; tube voltage, 80 Kv; tube current, 200 mA; contrast agent, ioversol, 350 mg/ml, 1.5 ml/kg dosage; delay time, 5 s; dynamic scanning for continued 40 s; rotation time, 1 s, image reconstruction interval, 0.5 s). All images were transmitted to SYNGO ONE workstation (Siemens), and were processed using VTCP software package. The automatic threshold definition was performed to remove the impact of bone and air. The superior sagittal sinus was selected as the outflow vein, and the time-density curve was obtained. After calculation, the cerebral blood flow (CBF), cerebral blood volume (CBV), and top TEEP time (TTP) were obtained. The basal ganglia region was selected as the area of interest (10-15 pixels), and CBF, CBV and TTP of bilateral basal ganglia region were obtained. The mean value of bilateral data was calculated, and was compared with that in left cerebellum using formula was as follows: $[CBF_{left} + CBF_{right}) / 2] / (CBF)_{left\ cerebellum}$ (CBV and TTP were similar with CBF).

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered as statistically significant.

Table I. Comparison of cerebral perfusion by CT imaging between two groups.

Group	Case	CBF	CBV	TTP
Experimental group	10	1.048±0.139[a]	1.206±0.117[a]	0.59 ±0.037[a]
Control group	10	0.90±0.07	0.969±0.067	1.015±0.026

Note: [a]$p < 0.01$ compared to control group.

Results

As show in Table I and Figures 1 and 2, CBF and CBV in experimental group were significantly higher than those in control group, respectively ($p < 0.01$), and TTP in experimental group was significantly shorter than control group ($p < 0.01$). Figure 1 also showed that, the bilateral CBF, CBV and TTP in basal ganglia region in control group were not significant different, respectively. This was the same with experimental group (Figure 2). As shown in Table II, there was no significant difference of spontaneous circulation restoration time, spontaneous breathing restoration time and MAP between two groups, respectively ($p < 0.05$).

Discussion

In this study, potassium chloride injection combined with asphyxia method is used to establish the rabbit models of CA for CPR. These models can simulate the systemic pathophysiological changes induced by CPR after CA, with advantage of stability, reliability and simple operation.

Results find that, there is no significant difference of spontaneous circulation restoration time, spontaneous breathing restoration time and MAP between two groups, respectively. This indicates that, there are the same microcirculation disorders after CPR in two groups. After CA and CPR, the blood stasis, hypoxia, acidosis and vascular endothelial injury activate the coagulation system. So the coagulation and fibrinolytic system function disorders appear, and cause the cerebral microthrombosis, thus, leading to poor cerebral microcirculation perfusion[16-20]. Although the systemic hemodynamics is stable, the regional cerebral metabolic abnormalities and microcirculatory perfusion defects still exist under normal circulatory perfusion pressure. This may aggravate the cerebral injury[21].

As shown in this study, CBF and CBV in experimental group are significantly higher than those in control group, respectively. The reasons may be that, the thrombolytic drugs not only have effect on brain microvascular emboli after CA, but also can improve the cerebral microcirculatiory perfusion and reduce the nonperfusion area. This contributes to improving the neurological prognosis[22]. Aliyev et al[23] find that, during the basic life support (BLS) stage of CPR after CA, the microcirculatory blood flow of rabbit decreases, with blood cell aggregation. Microthrombosis may occur in an instant during ischemia reperfusion[3,17]. Intervention with thrombolytic and anticoagulant drugs can improve the tissue microcirculatory perfusion in BLS stage, and prevent the formation of miocrothrombus in microcirculation. One research had shown[24] that existence of Urokinase receptor on the surface of endothelial cells and mononuclear cells, these cells

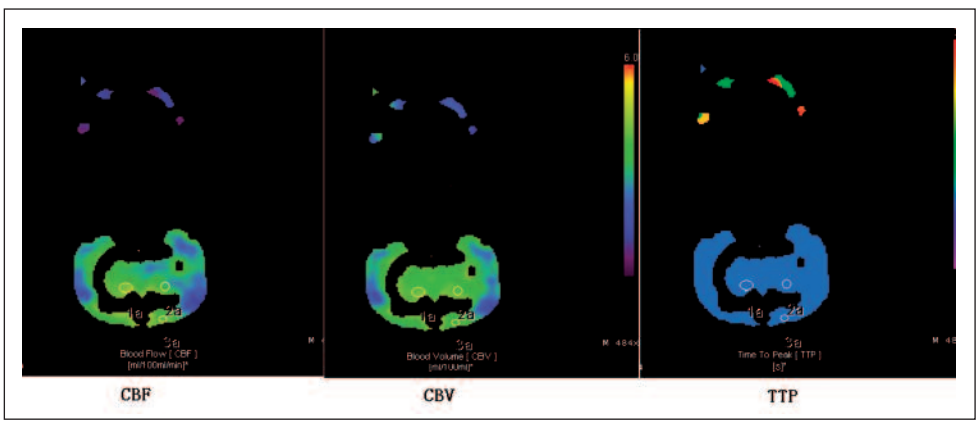

Figure 1. CT images of CBF, CBV and TTP in control group (color strip belt in image of CBV indicated the perfusion level).

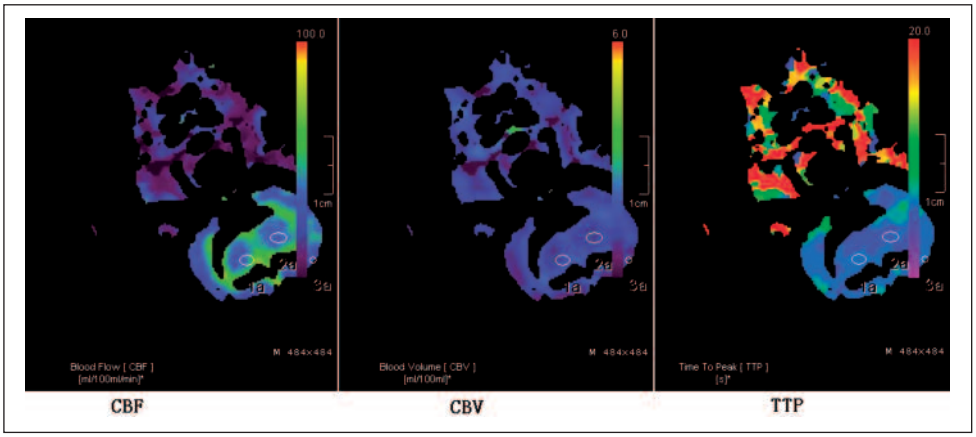

Figure 2. CT images of CBF, CBV and TTP in experimental group (left color strip belt indicated the perfusion level).

which act microthrombosis formation in the process of ischemia reperfusion can increase the catalytic activity of Urokinase. Urokinase can directly transform the plasminogen into plasmin, leading to thrombolysis, especially for new thrombus.

Urokinase has fibrinolytic effect. It can prevent the formation of new thrombosis and dissolve the formed thrombus, thus make the microcirculation relatively unobstructed Urokinase still has a significant effect on the longer formed blood clots, the degradation products that result from Urokinase can be maintained its action for 12-24 hours. So we started the application of Urokinase at recovery in the beginning of CPR. Guo et al[24] have conducted a CPR experiment in rats and find that, just 5-8 min of heartbeat and respiratory arrest can cause the formation a large amount of cerebral intravascular miocrothrombus and degeneration and necrosis of cerebral nerve cells. After intervention with urokinase, the injury of brain nerve cells is reduced, with an improved prognosis.

In this study, the basal ganglia CT scanning is performed 30 min after spontaneous breathing restoration. The main reason may be that, after the spontaneous breathing is restored, the trachea catheter can be extubated for CT examination. In addition, the plasma half-life of intravenously injected urokinase is 15 minutes. Furthermore, the basal ganglia region is located in the central brain. This position is convenient for imaging and analyzing the related results, and reflecting the cerebral perfusion.

Cerebral perfusion imaging by dual-slice spiral CT shows that, treatment with urokinase can increase the cerebral perfusion. CBF and CBV in experimental group are significantly higher than those in control group. This indicates that, the thrombolytic therapy with urokinase can significantly reduce the cerebral ischemia after CA and CPR, improve the cerebral microcirculation and restore the effective perfusion of brain tissue. This can reduce the brain damage caused by cerebral ischemia and hypoxia, thus improving the success rate of cerebral resuscitation. In this study, due to the limited experimental conditions, the sample size is relatively small, and the CT scanning is conducted 30 min after spontaneous breathing restoration. In next study, the observation should be conducted based on multiple stages and multiple parts of brain, for more comprehensively reflecting the cerebral perfusion.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

Table II. Comparison of observation index between two groups.

Group	Case	Spontaneous circulation restoration time (s)	Spontaneous breathing restoration time (min)	MAP (mmHg)
Experimental group	10	242.0±71.0	19.3±10.1	69.0±6.7
Control group	10	307.9±96.4	20.6±12.5	65.5±6.2

Note: $p > 0.05$ by comparison between two groups

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