Research on Cerebral Protection Effect and Mechanism of Dex Combined with Shengmai Injection on CPB Rates

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Abstract: Objective: To explore the cerebral protection effect and mechanism of dextrometadiazine (Dex) combined with Shengmai injection on CPB rates. Method: 10 healthy, adult and male SD rats were classified into CPB group and drug intervention group according to random number table, and each group included 5 rates. CPB group: equivalent normal saline + CPB 120min; drug intervention group: Dex combined with Shengmai injection + CPB 120min. IT-6 (interleukin), IL-10 and S100 β were monitored at two points of time: before CPB (T0) and CPB 2h (T1). Water content and calcium content of rats' brain tissues were determined. Results: Compared with CPB group, IL-6 and S100 β of drug intervention group lowered at T1, and Water content and calcium content of brain tissues decreased. The differences had statistical significance (P＜0.05). Conclusion: Dex combined with Shengmai injection can relieve inflammatory response and alleviate rats' CPB cerebral injury through effectively reducing water content and calcium content of rats' brain tissues after CPB.

Keywords: Dex; Shengmai injection; CPB rat; cerebral protection

In China, nearly 100000 patients receive CPB surgery every year. After CPB surgery, the incidence rate of brain damage is high, and the mechanism is very complicated so that cerebral protection becomes the research hotspot in the field of cardiopulmonary bypass [1]. In this study, CPB model of rats was established, and Dex combined with Shengmai injection was given in CPB. Cerebral injury marker gene and inflammation signal of rats with CPB cerebral injury were detected to explore the protective effect and mechanism of Dex combined with Shengmai injection on CPB cerebral injury, in the hope of providing the new thought for prevention and treatment of CPB cerebral injury.

1. Materials and Methods

1.1. Experimental Animal and Grouping

10 healthy, adult, male and clean (SD) rats (Laboratory Animal Center, Huazhong University of Science and Technology) were chosen, with the weight of (230-320)g and average weight of (287.5±25.8)g. The whole animal experiment was approved by Ethics Committee for Animal Experiments, and executed in strict accordance with the animal experiment guideline. The rats were classified into two groups according to random number table, and each group had 5 rats.

(1) VPB group: equivalent normal saline + CPB 120min;

(2) Drug intervention group: Dex combined with Shengmai injection + CPB 120min.

1.2. Equipment and Materials

(1) The rats were intubated and transfused in vitro before the experiment, and usually the venous drainage tube was used (18G venous indwelling needle);

(2) Small animal membrane lung (provided by Trauson Medical Instrument Co., Ltd., qi and blood exchange area $0.05m^2$);

(3) Constant flow pump, silica gel pump pipe (provided by Baoding Qili Precision Pump Co., Ltd., model: BT100-02);

(4) Connecting pipe (provided by Yangzhou Yangsheng Medical Science & Technology Co., Ltd., model: LJG-J);

(5) Monitor (provided by Shenzhen Creative Medical Co., Ltd., model: PC-5000A);

(6) Qi and blood analyzer (provided by American GEM, model: GEM3000).

1.3. Preparation of CPB Mode of Rats

60ml 20% urethane was intraperitoneally injected for rat anesthesia. The rat was fixed at the supine position, and a longitudinal incision was made along anterior midline, right and left groins. Then, the right external jugular vein, right femoral vein, left and right arteria femoralis were exposed and separated. The venous indwelling needle was retained in the right femoral vein, left and right arteria femoralis, respectively. CPB model was established and circulated for 2h. The following indexes of rats were monitored: mean arterial pressure

(MAP), survival rate, and blood gas parameters [PH (arterial hydrogen ion concentration), PCO_2 (partial arterial pressure of carbon dioxide), PO_2 (Partial pressure of oxygen artery), Hb (hemoglobin), Hc (hematocrit value), Glu (blood glucose), Lac (lactic acid), k (kalium), Na (sodium), BE (base excess), HCO_3 (bicarbonate radical)]. CPB modeling process and rats' survival rate were recorded. Intra-operative parameter maintenance: erythrocyte pressure about 25%, MAP (60-80)mmHg, nasopharyngeal temperature about 36℃, perfusion flow (100-120)ml/ (kg·min).

1.4. Administration Method

10min before CPB, 5ug/(kg·h) Dex (Hengrui Medicine Co., Ltd., GYZZ Z15011032) combined with 5ug/(kg·h) Shengmai injection (Shandong Taihang Pharmaceutical Co., Ltd., GYZZ Z14020812) was pumped into the drug intervention group through vein within 10min. Later, CPB circulation was maintained for 2h at the rate of 5ug/(kg·h) venous pump injection.

1.5. Monitoring Index

2ml venous blood specimen was collected through right external vein at T0 and T1. After each blood sampling, equivalent fresh heparinized rat blood was supplemented. After high speed centrifugation for 15min within 30min, the supernate was taken and kept in the -20 °C refrigerator to determine IL-6, IL-10, S100β, water content of brain tissue and calcium content.

Determination of water content of brain tissue: the method of dislocating the spine was used to kill the rat and take out the complete brain tissue. After the measuring glass was used to weigh the wet weight, the brain tissue was put in the special handling beaker and dried in the 85 °C constant temperature drying oven till the constant weight. Then, the dry weight was weighed. Water content = [(wet weight – dry weight)/ wet weight] x100%.

Determination of calcium content in brain tissue: 1:3 perchloric acid: nitric acid was used to digest the dry brain tissue which was weighed accurately into colorless and transparent liquid. Later, sub-boiling water was used for metering the volume. The calcium content was determined by flame atomic absorption spectroscopy method.

1.6. Statistical Method

SPSS19.0 software was used for statistical analysis. The measurement data were expressed with $\bar{x} \pm s$ and used to test data distribution state. In this study, the data were normally distributed. The homoscedasticity was analyzed. Finally, t test was carried out. $P < 0.05$ means the difference has statistical significance.

2. Results

2.1. CPB Modeling Result

All 10 rats survived. PO2, Glu and Lac rose obviously 0.5h after the surgery ($P < 0.05$), while Hb and Hct lowered obviously compared with pre-operation ($P < 0.05$). Pulmonary alveoli structure appeared to CPB rats, and suffered extensive damages. Erythrocyte exudation occurred in the pulmonary alveoli, and a lot of inflammatory cells were generated. The process is shown in Fig. 1.
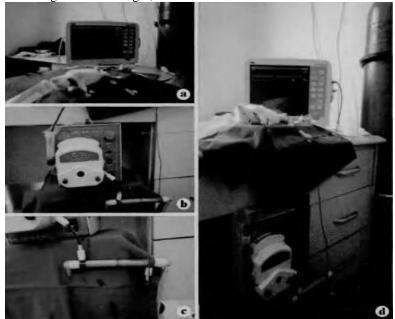

Figure 1. CPB process of rats**.**

2.2 Inflammatory factors

At T1, IL-6 of drug intervention group was lower than that of CPB group, and the comparison difference had statistical significance ($P < 0.05$). The difference of CPB group in IL-10 had no statistical significance ($P > 0.05$), as shown in Table 1.

Table 1. Comparison of inflammatory factor changes.

Group	IL-6(pg/mL)		IL-10(pg/mL)	
	T0	T1	T0	T1
CPB group	9.8±1.4	20.0±2.6	136.8±24.8	1721.0±100.5
Drug intervention group	9.5±1.2	14.3±2.3	140.5±24.0	1899.4±99.8
t	0.364	3.672	0.240	2.817
P	0.862	0.042	0.992	0.103

2.3 S100β, water content and calcium content of brain tissues

At T1, S100β of drug intervention group was lower than that of CPB group. Water content and calcium statistical significance (P＜0.05).

content of brain tissues in the drug intervention group were lower than those of CPB group, and the differences had

Table 2. Comparison of changes in S100β, water content and calcium content of brain tissues.

Group	S100β (pg/mL)		Water content of brain tissue		Calcium content of brain tissue	
	T0	T1	T0	T1	T0	T1
CPB group	133.7±21.4	515.3±109	76.6±0.4	78.8±0.9	103.5±9.0	176.5±15.2
Drug intervention group	135.2±22.8	282.0±66.5	76.0±0.5	76.7±0.6	103.8±9.2	135.8±15.8
t	0.107	4.086	2.095	4.341	0.052	4.151
P	0.921	0.032	0.201	0.028	0.992	0.030

3. Discussion

After CPB surgery, the specific mechanism of cerebral injury is still unclear, thus directly leading to the passivity of corresponding prevention and treatment measures. Thus, the influencing mechanism of CPB on brain tissue needs to be further studied to provide theoretical basis for clinical prevention and treatment of MODS caused by CPB operation, thus improving brain tissue functions.

Rats' cardiovascular anatomical structure is similar to human body, and has very high homology matching with Human genome [2]. Rat resources are rich and the body is small. Clinically, it is easy to establish the CPB model of rats, and the CPB model of rats can well reflect post-operation situations of rats. Dex is α_2-adrenergic receptor stimulant and has the functions of analgesia, calming, and inhibition of sympathetic excitation [3]. Shengmai injection (the main compositions include red ginseng and radix ophiopogonis) is a kind of Chinese herbal preparation. Clinical practice has verified that Shengmai injection has the effect of fast improving hemodynamics of animals with hemorrhagic shock and relieving stock symptoms [4]. However, since there is lack of clinical research, we still do not know whether Dex combined with Shengmai injection can protect brain tissues of rats. The results of this study showed that, at T1, S100β content of rats in CPB group increased, indicating that cerebral injury happens to CPB rats, while S100β content of rats in the drug intervention group was lower than that of CPB group. S100β is one of biochemical markers of central nervous system damage, and mainly exists in neurogliocyte [5]. After cell damage in the central nervous system, it can enter cerebrospinal fluid form cell sap and then enters blood through blood brain barrier. S100β content lowered, indicating that Dex combined with Shengmai injection protects the brain.

The author considers that the action mechanism of cerebral protection may include the following two aspects. Firstly, CPB can cause inflammatory response of the whole body. The Anti-inflammatory factor (IL-10) can improve cell survival rate, while proinflammatory factor (IL-6) can induce cell death [6]. In CPB modeling process, the two indexes can be chosen to observe rats' inflammatory reaction. It can be seen from Table 1 that, IL-6 level of drug intervention group was lower than that of CPB group at T1, demonstrating that Dex combined with Shengmai injection plays a role in inhibiting CPB-related inflammation. It is reported that Dex and Shengmai injection can effectively lower patients' IL-6 and TNF-α inflammatory factors. Shengmai injection has the effect of easing patients' inflammatory response, reducing inflammatory factor expression and protecting cranial nerve system functions. This study conforms to the previous researches [7-8].

Secondly, after CPB, CA^{2+} accumulates in cells in quantity, thus causing overload, which is the central link and the common path of cell death [9]. Hence, it is believed clinically that, reduction of intracellular CA^{2+} overload degree after CPB cerebral injury has certain protection function for cerebral injury. The results of this study showed that, at T1, water content and calcium content of brain tissues in CPB group rose obviously, compared with T0. Water content and calcium content of brain tissues in the drug intervention group were significantly lower than those of CPB group (P＜0.05). CA^{2+} overload channels include VOC (voltage dependence) and ROC (receptor dependence) [10]. After CA^{2+} overload, cytotoxicity can be generated in many ways, including destroying Na^+-K^+-ATP enzyme activity. This method increases osmotic pressure and Cell water content through increasing permeability other ions. Moreover, CA^{2+} inflow increase in cerebrovascular smooth muscle and endothelial cells will give rise to blood brain barrier opening, thus causing

encephaledema and damaging collateral circulation. After drug use, water content and calcium content of brain tissues were obviously lower than those of CPB group, indicating that the drug inhibits extracellular CA^{2+} inflow, and effectively lowers water content and calcium content of brain tissue.

In conclusion, Dex combined with Shengmai injection can relieve inflammatory response and alleviate rats' CPB cerebral injury through effectively reducing water content and calcium content of brain tissues after CPB.

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