

Research Protocol

TITLE: the Mechanism of the Downregulation of Dopamine Receptor in Mechanical Ventilation Induced Lung Injury

PROTOCOL ID: XH-17-011

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Materials and methods

1. Patients recruitment

Research protocol was approved by Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiao Tong University School of Medicine. With written informed consents given by all patients, individual information and lung tissue samples were collected. Forty-six colorectal patients, 18 to 60 years of age, were recruited for this study, meeting all the inclusion criteria shown as followed: undergo elective lobectomy with general anesthesia and mechanical ventilation; classified as physical status I to III according to the American Society of Anesthesiologists Physical Status Classification System; Written informed consent is approved. Participating subjects with any one of criteria shown as followed were excluded: Distant metastases: recent anaesthetics or mechanical ventilation treatment; children; women during pregnancy or lactation; being involved in other clinical subjects.

2. Anesthetic management and mechanical ventilation

General anesthesia protocol: anesthesia induction (midazolam 0.1mg/kg, sufentanil 0.5ug/kg, etomidate 0.3mg/kg, cisatracurium 0.2mg/kg); anesthesia maintenance [sevoflurane 1.5-3%, cisatracurium 0.1mg/kg/h, sufentanil is supplemented during the entire surgical procedure according to patients' anesthetic situation, dexmedetomidine (used conditionally) 0.4ug/kg/h].

Mechanical ventilation protocol: tidal volume 6-8 ml/kg, positive end-expiratory pressure 5 cmH₂O, oxygen concentration 40%; respiratory rate 10-15/min, inspiratory/expiratory ratio 1:1.5.

3. Lung tissues collection and preparation:

The lung tissues were homogenized, and incubated in cold RIPA lysis buffer (Beyotime, China) containing proteinase Inhibitor Cocktail (Sigma-Aldrich) to isolate the total proteins. The tissue extracts (40-50 μ g protein) were separated by 10%SDS-PAGE and subsequently transferred onto a PVDF membrane (Millipore Corp, Bedford, MA). After blocking, immunoblots were incubated with primary antibody against DRD1(1:1000; Abcam), DRD2(1:1000; Proteintech), TH (1:1000; Proteintech), DDC (1:1000; Abcam), Ac-a-tubulin (1:1000; Santa Cruz Biotechnology), β -actin (1:1000; Santa Cruz) at 4°C overnight. The secondary antibodies employed were HRP-conjugated IgG (1:3000; Proteintech) for 1h at room temperature. The antibody-reactive bands were visualized by using the enhanced chemiluminescence Western blotting detection system(Millipore). Total β -actin protein levels were detected for sample loading correction and normalization. In determination of the expression level of above protein, we had a bulk preparation of normal placental protein which was set as a control.

4. Statistical analysis

ImageJ software were used to get the Integrated Optical Density(IOD) of the chemiluminescent signal from the membranes of dopamine receptor 1(DRD1), dopamine receptor 2(DRD2), Tyrosine hydroxylase(TH), Dopa decarboxylase(DDC), acetylated-a-tubulin(Ac-a-

tubulin). The normalization was carried out by DRD1, DRD2, TH, DDC and Ac-a-tubulin IOD/total β -actin IOD for sample loading correction respectively. In determination of the expression level of DRD1, DRD2, TH, DDC and Ac-a-tubulin in different time points, the investigators had a bulk preparation of normal placental protein which was set as a control. Analyze the correlation between duration of mechanical ventilation with DRD1, DRD2, TH, DDC and Ac-a-tubulin expression respectively. If p value is less than 0.05, then its change is statically significant.