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The Construction of A Sheep Acute Respiratory Distress Syndrome Model Induced by Smoke Inhalation Injury

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ABSTRACT

Objective To establish a model of acute respiratory distress syndrome induced by sheep smoke inhalation lung injury.

Methods Nine healthy, 12-24 month old, non-pregnant female sheep were randomly selected, the sheep were divided into three groups, through the internal control of smoke generator device components (CO, CO₂, O₂, particle concentration and smoke temperature, etc.) to adjust the exposure time of the sheep model construction of smoke. The chest CT were examined before and after exposure to smoke at 6h, 12h, 24h and 48h, respectively. From the smoke exposure 12h endotracheal intubation and mechanical ventilation, by adjusting the ventilator parameters of hypoxemia and hypercapnia, statistical analysis of PaO₂/FiO₂, PEEP and other indicators, according to the ARDS definition of diagnosis in Berlin definition.

Results The CT showed mild inflammation of the lungs in first group, oxygen index of 95% confidence interval [331.13, 353.53]; In second groups, oxygenation index 95% confidence interval [220.09, 383.90]; CT showed airway edema, pulmonary inflammatory exudation and lung injury in third group. Oxygenation index 95% confidence interval of [195.16, 244.84], PEEP>5cm. There was significant difference between the two groups ($P<0.05$).

Conclusions The severity of lung injury can be changed by adjusting the exposure time of smoke inhalation; when the smoke exposure time up to 45 minutes, a stable model of smoke inhalation lung injury in sheep ARDS can be successfully established.

Key words: Sheep; smoke inhalation injury; acute respiratory distress syndrome; model; mechanical ventilation

INTRODUCTION

The generation of human civilization by fire brings many conveniences to life, but at the same time it also brings people the hidden danger of fire. According to statistics, in the past ten years, there have been 180,000 fires and 1,698 deaths per year^[1]. According to the American Burn Database, more than 200,000 burn patients were admitted from 2005 to 2014^[2]. 30% of burn patients are associated with smoke inhalation lung injury,

Yu Fei, Tan Yuan-fei et al. The construction of a sheep acute respiratory distress syndrome model induced by smoke inhalation injury and the mortality rate of such patients increased by 24 times compared with patients with simple burns^[3,4]. In addition, 20% of these patients will develop acute respiratory distress syndrome^[3,5,6]. An average of 190,000 people are diagnosed with ARDS in the United States each year, with a mortality rate of 26-36%, while domestically > 40%^[7]. These data indicate that SILI and the ARDS caused by it are imminent medical problems, which urgently need to be studied and solved by scholars.

At present, there is no authoritative international standard for the diagnosis, severity classification and prognosis of SILI, and the treatment methods are also varied. This also shows that people do not pay much attention to smoke injuries, and some related research is also basic research. There is an urgent need for a SILI rapid diagnosis method and an authoritative severity classification standard.

In 2012, the European Society of Critical Care Medicine proposed the ARDS "Berlin Definition"^[8]. It provides a basis for the classification of ARDS, but there is no matching animal experimental model. The domestic SILI model has the following characteristics: mostly small animal models, no research reports on the SILI model of large animals; no uniform model establishment methods, such as the establishment of a cotton smoke inhalation acute lung injury rat model, the smoke inhalation made by the smoke generator Rat models of lung injury, etc.; most of them stay on the research of injury mechanism; there is no clear diagnostic criteria or classification criteria to determine the degree of lung injury. The foreign models are mainly large animals, and the most widely used one is still the goat SILI model that Hubbard GB has established in 1988^[9]. Although this method has stabilized after continuous improvement, there are still some problems. Although the temperature of smoke is controlled within a certain range, it is seriously inconsistent with the actual situation of burns; in addition, the concentration of smoke cannot be controlled.

In order to better study the ARDS caused by SILI and provide theoretical and experimental support for its treatment, we designed and made an ARDS model of sheep smoke inhalation lung injury.

MATERIALS AND METHODS

Twelve 12-24-month-old female non-pregnant small-tailed Han sheep were selected, requiring a weight of (30 ± 5) kg, in good health, without fever, cough, diarrhea and other uncomfortable symptoms. The experiment was approved by the hospital ethics committee. All laboratory animals strictly abide by the "Guidebook for the Use and Care of Laboratory Animals" published by the National Institutes of Health in 1985. The feeding of laboratory animals is carried out in accordance with the Swiss Laboratory Animal Health Act.

Sheep selected in good condition 3 days before the experiment were placed in cages and fed individually, and 200 g of feed was given at 10:00 and 16:00 respectively, and water was continuously supplied. Fasting 36 hours before the experiment, drinking fast for the first 12 hours.

Medication before anesthesia, sedation (1 hour before anesthesia, intramuscular injection of midazolam—0.05 mg / kg), anticholinergic drugs (30 minutes before anesthesia: intramuscular injection of atropine—0.05 mg / kg); induced anesthesia, xylazine hydrochloride (Lu Mianxin 0.1~0.15ml / kg intramuscular injection), the anesthesia effect can be maintained for 2~3h. If there is a pain reaction, the drug can be added intravenously, and the additional amount is generally 1/5 to 1/4 of the first injection.

Turn on the air conditioner 2 hours in advance for preheating, to maintain the CT indoor temperature at 26~30 °C, turn on the CT 15 minutes in advance, and prepare for the air meter. After the anesthesia was placed, the sheep was lifted to the CT room for CT examination. The head was placed in the prone position. The CT scan was performed before the smoke exposure to observe the CT performance of the lungs of normal sheep. At the same time, the existence of lung lesions was excluded.

Set the temperature parameter to 37 °C, and turn on the temperature control system (to make the temperature

in the smoke box fluctuate less than 2 ° C around the set parameter) to avoid the compound injury caused by smoke inhalation and thermal burn. The oxygen concentration fluctuates between 19-21% according to the set value, which can avoid animal suffocation due to space constriction and lack of oxygen. Through the observation window of the smoke box, you can observe the reaction and breathing frequency of the tested animal. After the set time, remove the animal and fully expose it to the air.

After the sheep get out of the smoke, they are transferred to the intensive care unit of the animal, and then they are monitored (the ECG monitor continuously monitors the pulse oxygen saturation and heart rate, the tee connects to the animal physiological monitor to continuously monitor the arterial blood pressure), and the intravenous fluid (10% glucose 1000ml + 0.9 % Sodium chloride injection 500ml + sodium potassium magnesium calcium glucose injection 500ml + 10% potassium chloride 10ml, prepare low molecular weight dextran injection and lactated Ringer's solution), 0.5-1ml Lu Mianning intravenously every two hours. Lower urinary tube, stomach tube. Mechanical ventilation: adopt volume control mode, initial setting (tidal volume TV = 10ml / kg, respiratory rate RR = 12 times / min, maintain PCO₂ at 35-45mmHg by adjusting respiratory rate, PEEP = 5cm, FiO₂ = 21 %) The goal is to maintain the airway platform pressure Pplat <35mmH₂O; the goal to correct hypercapnia is: PaCO₂ <60, pH > 7.2, increase the respiratory rate by 2 times / min, up to 30 times / min, if Pplat > 35mmH₂O , Then reduce TV 1ml / kg to maintain Pplat = 35mmH₂O; the goal of correcting hypoxemia is: PaO₂ > 70, SpO₂ > 92%, if the arterial blood gas analysis suggests SpO₂ <92%, then adjust by increasing FiO₂ by 10% Until SpO₂ > 92%, refer to the ARDSNet PEEP titration table; the goal of correcting hypocapnia: PaCO₂ > 30, pH <7.5, reduce the respiratory rate by 2 times / min, the slowest is 10 times / min, at 1ml / The rate of kg reduces the tidal volume, and the final tidal volume cannot be lower than 4ml / kg.

Intraoperative infusion used compound sodium lactate Ringer injection, sodium chloride injection, hydroxyethyl starch (130 / 0.4) electrolyte injection, 10% glucose injection, sodium potassium magnesium calcium glucose injection. The input liquid is mainly crystal liquid, the total amount of hydroxyethyl starch is not more than 10ml / kg, and the total amount of daily rehydration is not less than 75ml / kg. Intraoperative blood pressure was maintained at more than 80% of basal blood pressure, CVP range was 10-15mm Hg and or PAWP range was 15-20mm Hg. During the experiment, actively correct the acid-base balance and electrolyte disorders, adjust the depth of anesthesia and control blood pressure.

STATISTICAL METHOD

Statistical analysis was performed using SPSS17.0 statistical software, and quantitative data was expressed as mean ± standard deviation $\bar{x} \pm s$; comparison between groups was analyzed by variance; P <0.05 indicated that the difference was statistically significant.

RESULT

Nine sheep were purchased from the Experimental Animal Center of the Chinese Academy of Military Medical Sciences and divided into three groups (Group A, Group B, and Group C), each group containing 3 sheep. Table 1 is the basic situation of experimental sheep.

Table 1 Basic situation of experimental animals

index	data
Age (month)	17.20±4.18
Gender (M / F)	0/6
Body height (cm)	80.53±3.49
Body length (cm)	82.01±5.98
Weight(kg)	32.4±4.7
Heart rate (bpm)	90±5

Breathing frequency (times / min)	15±4
Body temperature (°C)	38.7±1.5

Note: All data in Table 1 except $\bar{x}$ is the corresponding quantity is expressed as $\bar{x} \pm s$

The first group of sheep set a smoke exposure time of 15 minutes, and the main monitoring index results are as follows: all three sheep in this group continuously monitored 48h heart rate (HR), blood pressure (systolic pressure, diastolic pressure, average arterial pressure), and respiratory rate (BR), Blood oxygen saturation (SpO₂) is relatively stable, blood oxygen saturation is always higher than 90, blood gas analysis results are shown in Table 2, caused by acute lung injury can not meet the ARDS diagnostic criteria, that is, oxygenation index > 300.

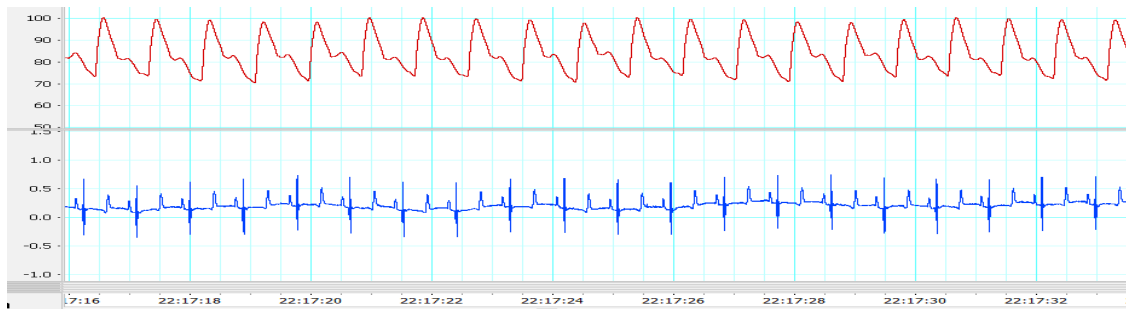


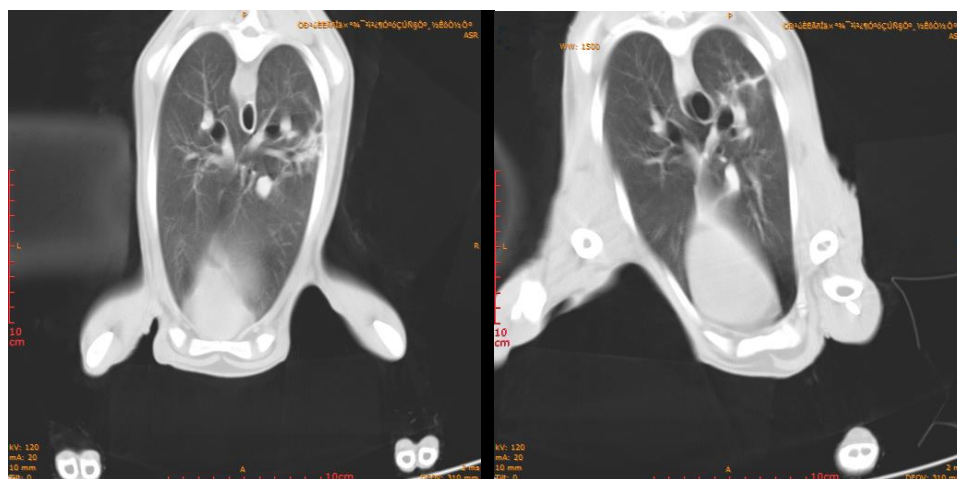
Figure 1 Results of heart rate and blood pressure monitoring (excerpt)

Figure 2 CT examination results



A Before smoke exposure

B 6h after smoke exposure



C 12h after smoke exposure

D 24h after smoke exposure

Table 2 Blood gas analysis and oxygenation index results

	12h	18h	24h	30h	36h
pH	7.476±0.011	7.43±0.015	7.417±0.008	7.391±0.034	7.385±0.056
PCO ₂	35±3	37±2	41±1	40±3	40±2
SO ₂	94±1	95±1	96±1	95±1	94±1
FiO ₂	21	21	21	21	21
PO ₂	70±1	72±1	74±1	76±1	79±1
PO ₂ / FiO ₂	337±6	342±5	352±3	363±4	376±6

The smoke exposure time of the second group of sheep was set at 30 minutes. The main monitoring indicators were as follows: all three sheep in this group continuously monitored 48h heart rate (HR), blood pressure (systolic pressure, diastolic pressure, average arterial pressure), and respiratory rate (BR), blood oxygen saturation (SpO₂). One of the sheep showed symptoms of shortness of breath and wheezing. The blood gas analysis results are shown in Table 3. The sheep met the ARDS diagnostic criteria, that is, the oxygenation index was <300. The remaining two did not experience discomfort and did not meet the ARDS diagnostic criteria.

Figure 3 Heart rate and blood pressure monitoring results (excerpt)

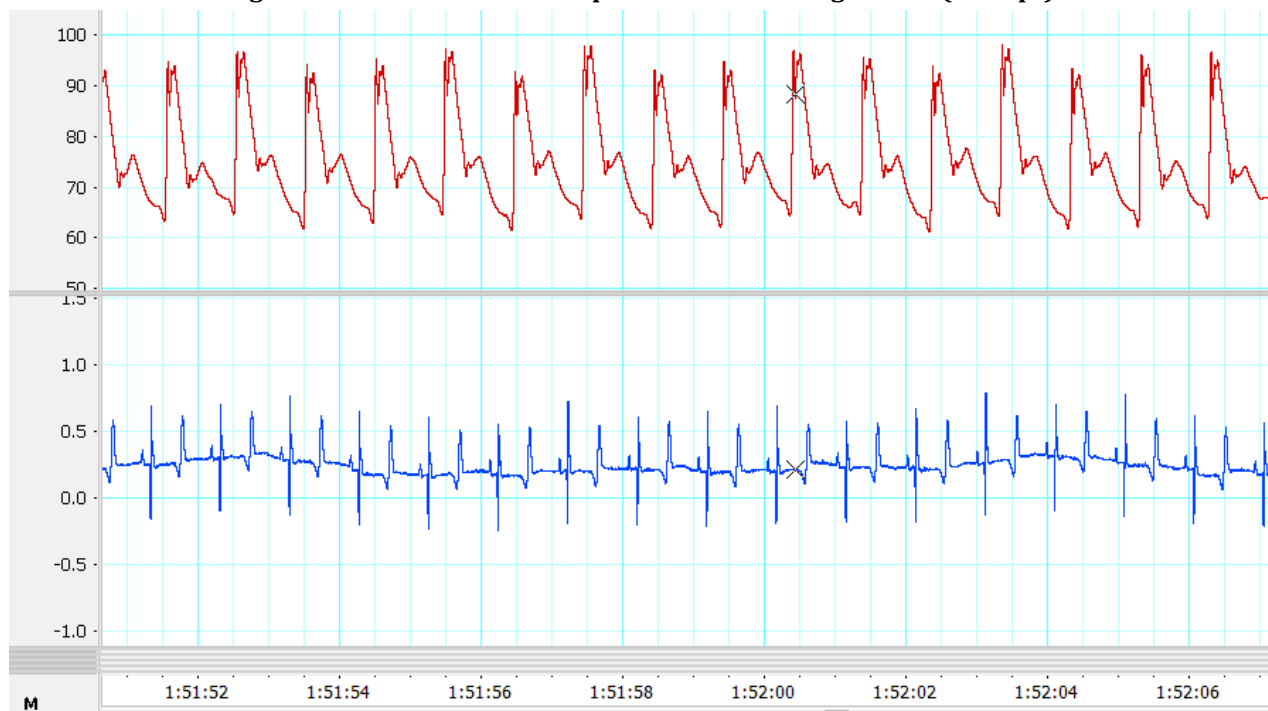


Figure 4 CT examination results



Table 3 Blood gas analysis and oxygenation index results

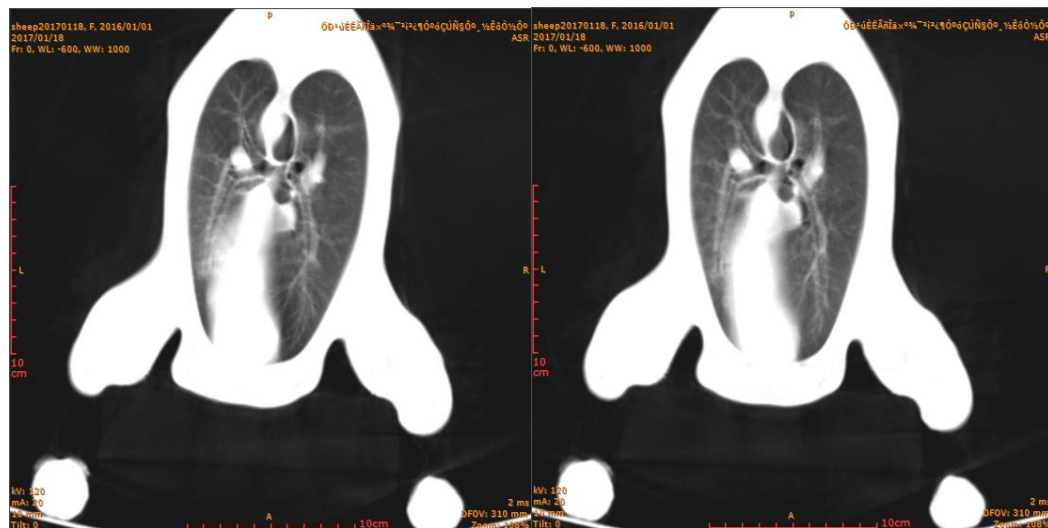
	12h	18h	24h	30h	36h
pH	7.480±0.037	7.416±0.011	7.380±0.026	7.389±0.019	7.415±0.064
PCO ₂	34±3	37±3	43±2	40±2	41±5
SO ₂	92±1	93±1	92±1	91±1	90±1
FiO ₂	21	21	21	21	21
PO ₂	62±2	65±2	64±2	65±2	66±2
PO ₂ / FiO ₂	298±9	307±10	303±12	310±11	315±8

The smoke exposure time set for the third group of sheep is 45 minutes. The main monitoring index results are as follows:

Figure 5 Heart rate and blood pressure monitoring results (excerpt)

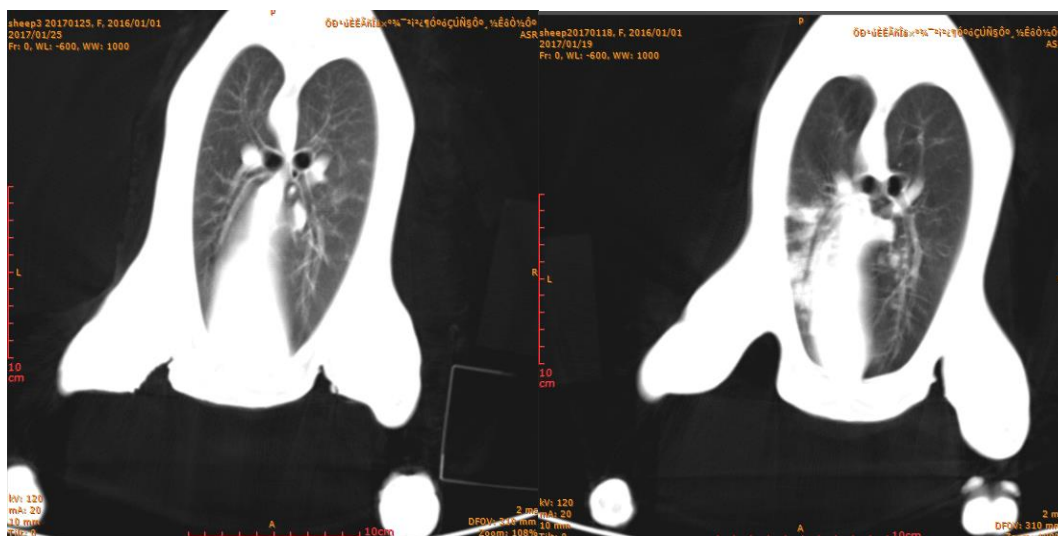


Figure 6 CT examination results



A Before smoke exposure

B 6h after smoke exposure



C 12h after smoke exposure

D 24h after smoke exposure

Figure 7 Visual observation of lung tissue and pathological results

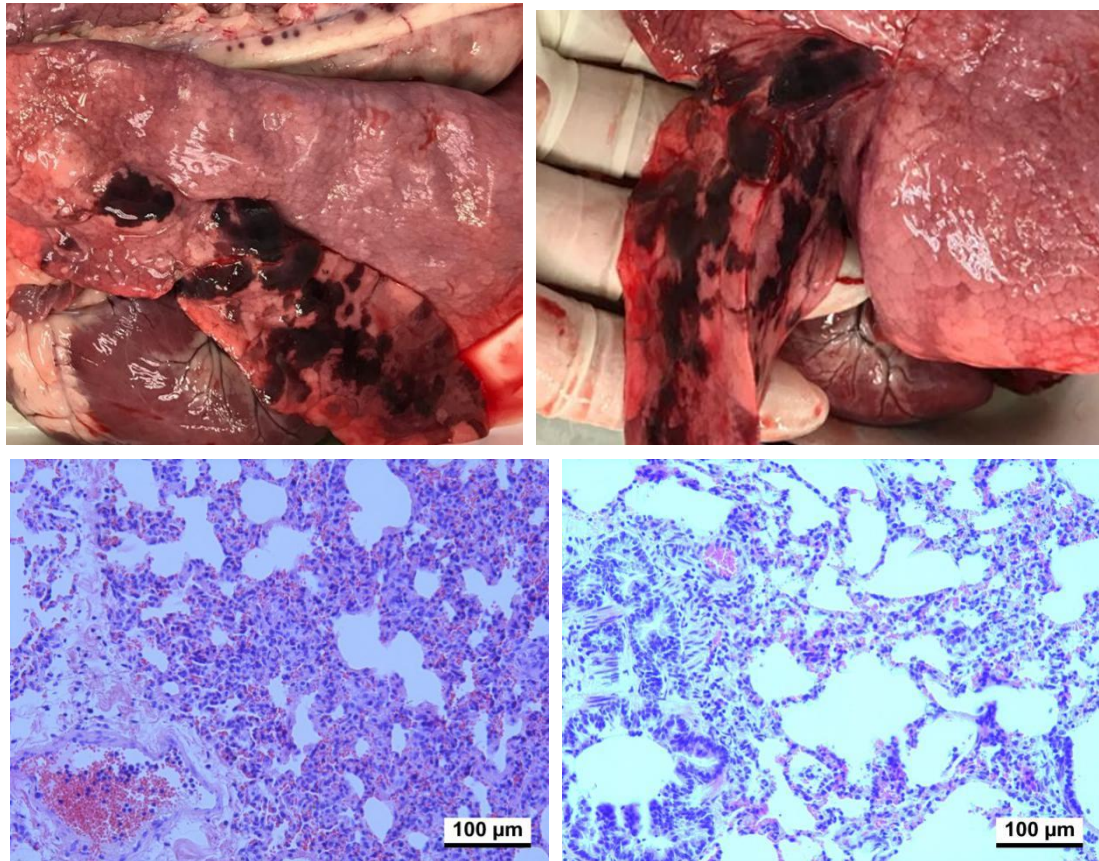
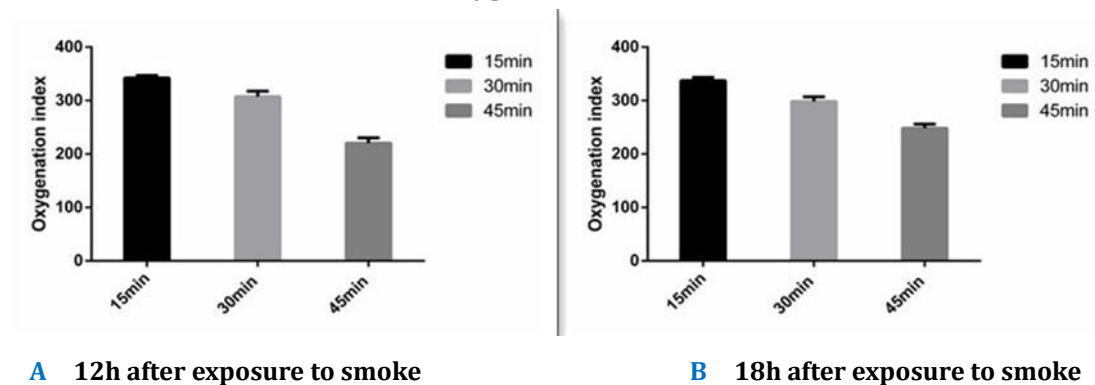
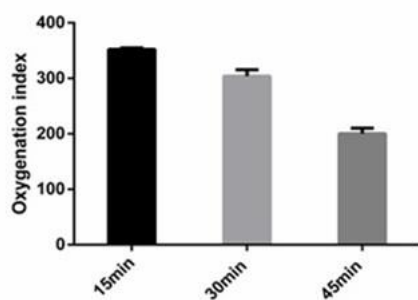


Table 4 Blood gas analysis and oxygenation index results

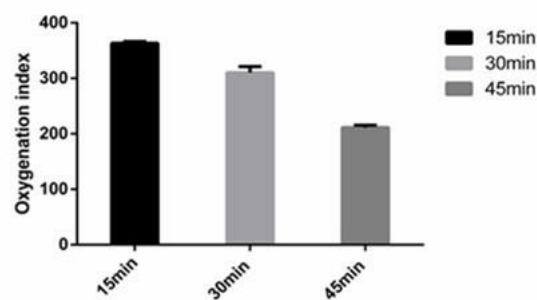
	12h	18h	24h	30h	36h
pH	7.428±0.014	7.294±0.240	7.357±0.011	7.426±0.029	7.433±0.333
PCO ₂	32±2	36±1	44±2	42±3	45±3
SO ₂	92±1	93±1	92±1	91±1	90±1
FiO ₂	21	21	21	21	21
PO ₂	62±2	65±2	64±2	65±2	66±2
PO ₂ / FiO ₂	248±8	220±10	200±10	211±4	223±8

Oxygenation index

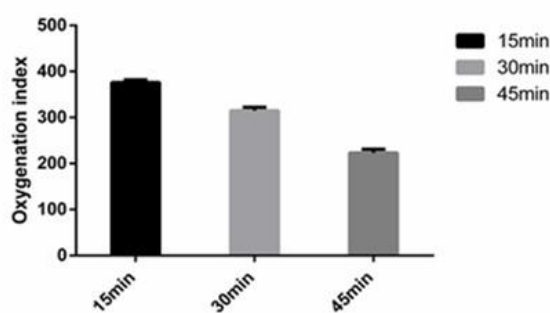




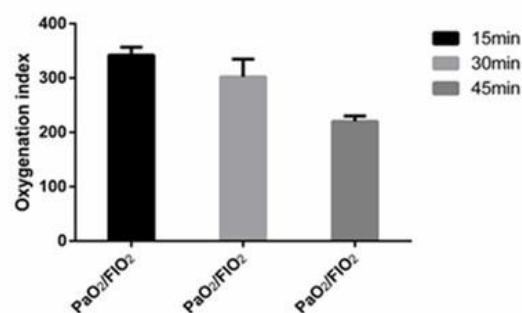
C 24h after exposure to smoke



D 30h after exposure to smoke



E 36h after exposure to smoke



F 48h after exposure to smoke

Note: The comparison of the average number of oxygenation indexes among multiple groups is by analysis of variance. The oxygenation index of each group after mechanical ventilation ($P < 0.05$)

According to the Berlin definition standard, the diagnosis results of the three groups of sheep are as follows: The 95% confidence interval of the oxygenation index of the smoke exposure group for 15 minutes: [331.13, 353.53]; The 95% confidence interval of the oxygenation index of the smoke exposure group for 30 minutes: [220.09, 383.90]; The 95% confidence interval for the oxygen exposure index of the smoke exposure group for 45 minutes: [195.16, 244.84]. Among them, the 95% confidence interval of the oxygenation index in the 45min group is within the ARDS "Berlin definition" diagnostic criteria, and the 95% confidence interval of the oxygenation index in the 30min group is within the ARDS "Berlin definition" diagnostic criteria, and the oxygenation index is 95% in the 15min group The confidence interval is outside the ARDS "Berlin Definition" diagnostic criteria.

DISCUSSION

Burns can not only cause pathophysiological changes of a certain system or a single organ, but also cause the destruction of the function and structure of multiple organ systems^[10]. Because burn is a complex injury that can cause damage to multiple organs, its complex pathophysiological process cannot be captured in in vitro experiments. Smoke inhalation lung injury In accidental burns, the incidence rate is 20% -30%, and some of these patients will develop ARDS with a mortality rate of up to 40%^[3,7,11]. In order to study the impact of burns on various aspects, scholars have established a large number of animal burn models in the past few decades^[12]. The main function and purpose of these models is to study the pathophysiological mechanism and treatment of burns. It is particularly important to Understand the advantages and limitations of these models for the treatment of human-related diseases.

The experimental animals used in the smoke inhalation lung injury model are: mice, rats, rabbits, sheep, dogs, pigs, etc^[12]. Rats dominate in China, abroad is dominated by sheep^[13-16]. Rats have the characteristics of fast

reproduction speed, low economic cost, easy modeling, easy operation, good repeatability and stability, etc.^[12]. In particular, many studies have shown that certain organ structures, physiological characteristics, and immune mechanisms of rats are similar to humans. The tolerance of rats to hypoxia is higher than that of mice. Blood samples can be collected continuously for a certain period of time after injury to make dynamic observations. The pathological specimens are larger and can meet the requirements for the number of specimens in the experiment. It is widely used in research. However, the body shape of rats and humans is quite different. Many new scientific and technological achievements equipment (such as ECMO, Da Vinci robots, etc.) have not been miniaturized and cannot be applied to rats, which limits the application of new technologies and new technologies in experiments. Clinically instructive experimental research. Large animals are closer to humans first in shape and structure, and their tolerance is similar to humans in the process of injury. When large animals are used in the modeling of burn injury or smoke inhalation lung injury, they are closer to human ALI / ARDS after smoke inhalation lung injury in physiology, pathogenesis, pathology, and pathophysiology^[17]. Abdullahi A, in reviewing smoke inhalation lung injury in burn animal models, proposed that sheep are the gold standard for smoke inhalation lung injury^[12]. The goat smoke inhalation lung injury model established in 1988, which has been continuously used in foreign experimental research, although this model can control the temperature of the smoke within a certain range (35 °C -40 °C), it is seriously inconsistent with the actual situation of the burn site. In addition, although the model can determine the composition of each component in smoke (such as CO, CO₂, O₂, particles, etc.), it cannot control the concentration of each component in smoke within a certain range. Finally, the various animal models currently available are mainly used to study acute lung injury (ALI) caused by smoke inhalation, which is a relatively mild stage of lung injury, and may even involve ARDS, but there is no model that specifically studies ARDS caused by smoke inhalation^[18]. The high mortality rate of ARDS caused by smoke inhalation injury and its impact on the prognosis of burns are all problems that people need to solve urgently.

This study uses the second-generation smoke generation device self-developed by the previous research group^[19, 20], real-time control and adjustment of indoor temperature (in the smoke generator cabin), temperature in the box, humidity in the box, operating time, smoke concentration, etc. When the smoke exposure time was 15 minutes, a chest CT scan revealed lung inflammation, bronchial edema, and exudation. However, the vital signs and other monitoring indicators of the sheep did not change significantly, nor did the respiratory dysfunction appear. And after 12 hours from smoke exposure, the oxygenation index is always greater than 300, and its 95% confidence interval is [331.13, 353.53], which does not meet the ARDS diagnostic criteria. It shows that the smoke exposure time of 15 minutes can cause lung damage, but the severity of the damage is less. When the smoke exposure time is 30 minutes, one of the sheep can be diagnosed with ARDS. When the smoke exposure time is 45 minutes, all can be diagnosed as ARDS, and the 95% confidence interval of its oxygenation index is [195.16, 244.84]. It shows that when the smoke exposure time is 45 minutes, a stable ARDS model of smoke inhalation lung injury can be formed. Analysis of variance of the oxygenation index of the three groups of sheep showed that $P < 0.05$, that is, the differences between the groups were statistically significant, indicating that as the smoke exposure time was prolonged, the degree of sheep lung damage gradually increased.

This experiment successfully obtained a stable model of ARDS caused by smoke inhalation lung injury, and further confirmed that lung injury during burns is mainly due to particles and toxic components in the inhaled gas, and the degree of lung injury and the time of smoke exposure Proportional to. The ARDS model directly mimics the clinical pathophysiology of human smoke injury, making it flexible for clinical treatment of burns. The model currently does not have an accurate severity classification for ARDS caused by smoke inhalation lung injury, but with the deepening of research, its classification of smoke inhalation lung injury severity and exploration of ARDS treatment will be of great significance.

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STATEMENT

There is no conflict of interest in this article.

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1,8-Cineol Attenuated A β ₂₅₋₃₅-Induced PC12 Cell Injury through Reducing Caspase 3 Expression and NO Production

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ABSTRACT

Objective To investigate the effect of 1,8-cineol on caspase 3 expression and NO production induced by A β ₂₅₋₃₅ in PC12 cells.

Methods PC12 cells were cultured *in vitro*, and cell injury was induced by A β ₂₅₋₃₅ with a concentration of 20 μ M. 1,8-cineol (1, 3, 10 μ M) was pretreated before A β ₂₅₋₃₅ treatment. PC12 cell viability was evaluated by MTT detection assay. Caspase 3 protein expression was detected by Western blotting. The level of NO production in PC12 cells was measured using ELISA detection assay kit.

Results In cultured PC12 cells *in vitro*, MTT results showed that 20 μ M of A β ₂₅₋₃₅ reduced cell viability significantly compared with control group. The cell viability was increased by pretreatment with 1,8-cineol with concentrations of 3 and 10 μ M compared with A β ₂₅₋₃₅ only group. Western blotting results showed compared with control group, caspase 3 expression was increased significantly in 20 μ M A β ₂₅₋₃₅ group. Compared with A β ₂₅₋₃₅ group, 1,8-cineol of 3 and 10 μ M group reduced caspase 3 protein expression significantly. The level of NO production in PC12 cells was increased significantly, which was decreased by pretreatment with 3 and 10 μ M of 1,8-cineol.

Conclusions: Our results revealed a protective effect of 1,8-cineol on A β ₂₅₋₃₅ induced PC12 cell injury through inhibition of caspase 3 expression and NO production.

Keywords Amyloid beta, cineol, caspase 3

INTRODUCTION

Alzheimer's disease (AD) is an age-related and progressive neurodegenerative disorder characterized by cognitive impairment. Amyloid beta (A β) which aggregate into oligomers are found in the brains of patients with AD [1]. A β deposition leads to diversities of toxic mechanisms including oxidative stress, mitochondrial diffusion, and excitotoxicity [2]. It could cause neuron loss and synaptic lesion. A β -induced neurotoxicity has emerged a possible therapeutic approach to slow the progression of AD [3]. However, there are still lack of effective drugs for treatment. Recent studies of small molecules acting on neuronal cells has been proposed as an alternative for the treatment of AD [4].

1,8-cineol, also known as eucalyptol, is a major monoterpene principally from Eucalyptus essential oils [5]. 1,8-

cineol has been proven clinical efficacy on therapeutic benefits in inflammatory airway diseases such as chronic obstructive pulmonary disease (COPD) [6]. Our previous study indicated anti-inflammatory action of 1,8-cineol through TLR4 expression inhibition [7]. Little is known about the effect of 1,8-cineol in A β -induced neuronal injury. Thus, the present study was to investigate the role of 1,8-cineol against A β ₂₅₋₃₅-induced PC12 cell injury and possible mechanisms.

MATERIALS AND METHODS

REAGENTS

1,8-cineol and A β ₂₅₋₃₅ were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Thermo scientific. (Hyclone, USA). NO detection assay was purchased from Beyotime Biotechnology (Shanghai, China). Trypsin was purchased from Sangon Biotech (Shanghai, China). Rabbit polyclonal antibodies against cleaved caspase 3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). GAPDH antibody was purchased from Kangcheng Bioengineering Co., Ltd (Shanghai, China). Anti-rabbit IRDye700DX®-conjugated antibody or anti-mouse IRDye800DX®-conjugated antibody was purchased from Rockland (LICORE, USA).

DRUG ADMINISTRATION

PC12 cells were randomly divided into five groups, then incubated with A β ₂₅₋₃₅ oligomers at concentration of 20 μ M for 24 h. Soluble oligomeric forms of A β ₂₅₋₃₅ were prepared as reported previously. The peptide was dissolved into sterile PBS and make a final concentration of 100 μ M, incubated under 37 °C for 24 h. To observe the effect of 1,8-cineol against A β ₂₅₋₃₅ induced cell injury, PC12 cells were incubated with 1,8-cineol at concentrations of 1, 3, 10 μ M for 1 h, then co-administrated with A β ₂₅₋₃₅ for 24 h. 1,8-cineol was diluted with sterile PBS.

CELL CULTURE

PC12 cells were cultured in DMEM medium containing 10% fetal bovine serum at 37 °C in humidified air containing 5% CO₂. The cultured medium was changed each 24 hours and the cells in the exponential phase of growth were used in all experiments.

MTT DETECTION ASSAY

PC12 cell viability was detected by MTT reduction assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) was dissolved in dimethyl sulfoxide (DMSO) and added in the medium with a concentration of 0.5 mg/ml. The cells were kept in the incubator for 4 h at 37 °C. The medium was removed and 100 μ l

DMSO was added to dissolve the formazan precipitates in 96-well plates. The absorbance was measured at a wavelength of 570 nm using multiscan spectrum. Cell viability was expressed as percentage of viable cells relative to that of non-treated cells in the control group.

WESTERN BLOT ANALYSIS

After treatment, PC12 cells were collected and total protein was extracted using protein extraction kit. Cell lysates were centrifuged at 12000 g for 30 min at 4°C. The protein concentration was determined by coomassie brilliant blue method. The protein samples were diluted with loading buffer and separated by 10% SDS-PAGE, then transferred onto a nitrocellulose membrane. The membrane was incubated with 7.5% defatted milk for 2 h, and then incubated with rabbit polyclonal anti-cleaved caspase 3 antibody and mouse monoclonal anti-GAPDH antibody overnight. The blots were incubated with with anti-rabbit IRDye700DX®-conjugated antibody or anti-mouse IRDye800DX®-conjugated antibody (1:5000, Rockland, USA). The membranes were washed and scanned by an Odyssey infrared imaging system. The protein bands were quantitatively evaluated

DETERMINATION OF NO PRODUCTION

After treated with A β ₂₅₋₃₅ and 1,8-cineol for 24 h, the cells were collected and crushed. The level of NO in PC12 cells was evaluated using nitric oxide assay kit. The detection procedure was performed according to manufacturers' instructions.

STATISTICAL ANALYSIS

All data are expressed as means $\pm$ SD. The significance of differences among groups was performed using one-way ANOVA followed by Dunnett's post hoc test (SPSS 15.0 for Windows, SPSS inc., USA). $P < 0.05$ was considered statistically significant.

RESULTS

Protective effect of 1,8-cineol on A β ₂₅₋₃₅-induced toxicity in PC12 cells

To investigate the effect of 1,8-cineol on neurotoxicity, PC12 cells were pretreated with different concentrations of (1, 3, 10 μ M) for 1 h, and then exposed to 20 μ M of A β ₂₅₋₃₅ for 24 h. The cell viability was evaluated by MTT reduction assay. As Fig.1 shown, cell viability of the cells in A β ₂₅₋₃₅ group was significantly decreased compared with the control group. As compared with untreated cells in A β ₂₅₋₃₅ group, cell viability of the cells pretreated with 1, 8-cineol (3, 10 μ M) was significantly reduced (Fig.1, $P < 0.01$). 1,8-cineol at the concentration of 1 μ M also increased cell viability but not significant.

Effect of 1,8-cineol on A β ₂₅₋₃₅-induced caspase-3 expression in PC12 cells

The effect of 1,8-cineol on protein expression of caspase 3 was detected by Western blotting. As shown in Fig.2, after exposed to 20 μ M A β ₂₅₋₃₅ for 24 h, cleaved caspase 3 expression increased significantly compared to control group. As compared with untreated A β ₂₅₋₃₅ group, the cleaved caspase 3 expression in 1,8-cineol (3, 10 μ M) group significantly decreased. 1 μ M of 1,8-cineol also reduced cleaved caspase 3 expression. No significance was found between 1 μ M 1,8-cineol group and A β ₂₅₋₃₅ group.

Effect of 1,8-cineol on A β ₂₅₋₃₅-induced NO content in PC12 cells

Varieties of evidences have reported that NO play an important role in neural cell injury in Alzheimer's disease. In the present study, we struggled to investigate whether 1,8-cineol could affect the elevated level of NO production in A β ₂₅₋₃₅-treated PC12 cells. A β ₂₅₋₃₅ 20 μ M caused a higher level of NO production compared with control group. Pretreatment with 3, 10 μ M of 1,8-cineol could significantly decreased the level of NO content as compared with A β ₂₅₋₃₅ group (Fig.3, $P < 0.01$).

DISCUSSION

1,8-cineol is a natural component clinically applied as the active ingredient of soledum. As previously reported, 1,8-cineol is well established for the therapy of airway diseases such as chronic sinusitis and bronchitis [8]. Although clinical trials underline the beneficial effects of 1,8-cineol in treating respiratory diseases, the molecular mechanisms still remains unclear. 1,8-cineol inhibit nuclear NF- κ B translocation, cytokine production and oxidative stress in many inflammatory conditions [9].

In the present study, A β ₂₅₋₃₅ treatment at 20 μ M decreased the cell viability of PC12 cells while pretreatment with 1,8-cineol reversed the effect at 3 and 10 μ M. A previous study showed 1,8-cineol exerted protective effect against ischemic neuron injury at concentration of 10 μ M [10]. The effective concentration is similar to that observed in our study. Here we showed that 1,8-cineol could protect PC12 neuronal cells against A β ₂₅₋₃₅-induced toxicity.

Cell apoptosis may contribute to neuronal degeneration in the progression of AD [11]. It is well known that

apoptosis in neurons is a leading pathway for A β -induced toxicity. Thus prevention of A β -induced apoptosis is regarded as a potential and reasonable therapeutic strategy for AD therapy. Caspase 3 plays a pivotal role in the neuronal cell apoptosis^[12]. In the study we found A β ₂₅₋₃₅ treatment increased cleaved caspase 3 protein expression in agreement with previous studies^[13]. 1,8-cineol pretreatment downregulated the expression of cleaved caspase 3 induced by A β ₂₅₋₃₅. Thus our results indicated that the mitochondrial apoptosis pathway is involved with protective effect of 1,8-cineol against A β ₂₅₋₃₅-induced PC12 cell apoptosis.

Low concentrations of NO protect neural cells from apoptosis, whereas excessive NO causes apoptosis^[14]. And excessive ROS generation initiated the apoptotic cell death by regulating iNOS and increasing apoptosis-related protein expression^[15]. It's reported NO could have vital functions in central nervous system. 1,8-cineol affected the eNOS phosphorylation and thus its activation^[16]. In this study, we found that 1,8-cineol significantly reduced the production of NO in PC12 cells induced by A β ₂₅₋₃₅.

In summary, we evaluated the protective potential of 1,8-cineol against A β ₂₅₋₃₅-induced toxicity and investigated the underlying mechanisms. Our results indicated that 1,8-cineol inhibits A β ₂₅₋₃₅-induced cytotoxicity by suppressing caspase 3 expression and NO production. Further study are needed to focus the mechanisms of 1,8-cineol in the pathogenesis of Alzheimer's disease.

Figures and legends

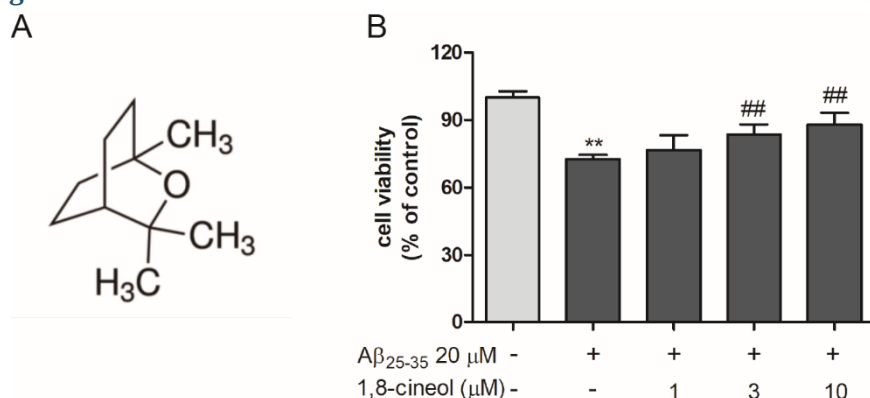


Fig.1 Chemical structure and the effect of 1,8-cineol on cell viability of PC12 cells treated by A β ₂₅₋₃₅. PC12 cells were pretreated with 1,8-cineol, a monoterpene structure shown as A, with concentrations of 1, 3, 10 μ M, and incubated with 20 μ M A β ₂₅₋₃₅ for 24 h. Cell viability was detected by MTT reduction assay and expressed as percentage of control group (B). ** P < 0.01 vs control group, ## P < 0.01 vs A β ₂₅₋₃₅-treated only group.

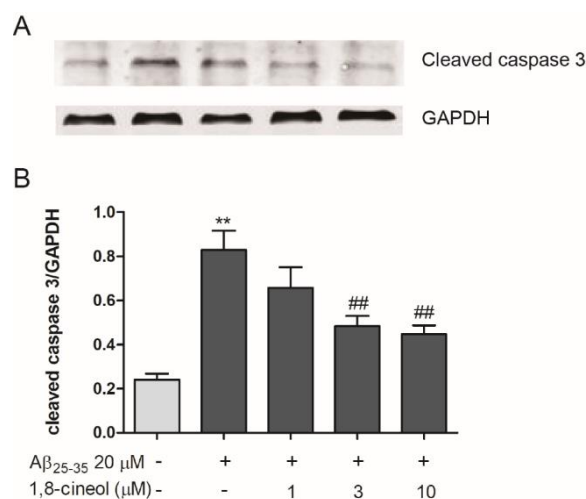


Fig.2 Effect of 1,8-cineol on caspase 3 protein expression in A β ₂₅₋₃₅-induced PC12 cells Cells were pretreated with 1,8-cineol for 1 h with concentrations of 1, 3, 10 μ M , and then incubated with 20 μ M A β ₂₅₋₃₅ for 24 h. Cleaved caspase 3 protein expression was detected by Western blotting (A) and the ratio of cleaved caspase 3 to GAPDH was calculated (B). ***P* < 0.01 vs control group, ##*P* < 0.01 vs A β ₂₅₋₃₅-treated only group.

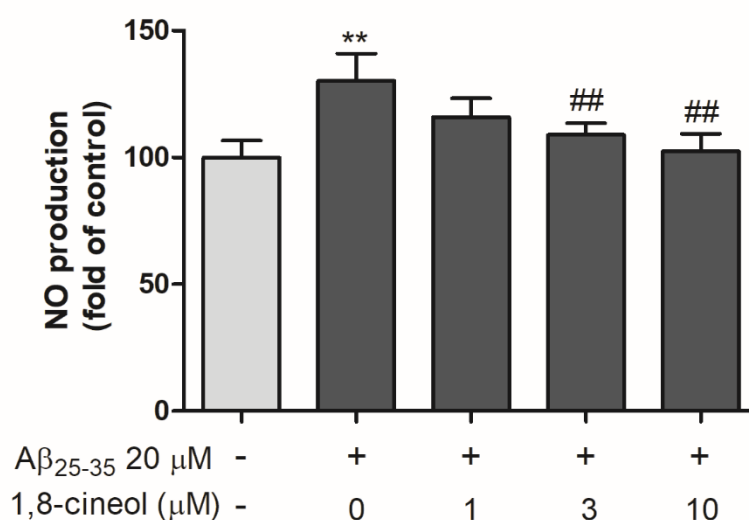


Fig.3 Effect of 1,8-cineol on NO production in A β ₂₅₋₃₅-induced PC12 cells Cells were collected and the level of NO content was evaluated using nitric oxide assay kit. ***P* < 0.01 vs control group, ##*P* < 0.01 vs A β ₂₅₋₃₅-treated only group.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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Analysis on The Curative Effect of Urinary Fistula Repair with Penile Flip Flap

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ABSTRACT

Objective To investigate the effect of urinary fistula repair with penile flip flap after hypospadias correction in children.

Methods Anastomotic fistula and coronal groove fistula were repaired with penile flip flap, and the results were analyzed.

Results The correction rate of anastomotic fistula repair was significantly higher than that of coronal groove fistula group.

Discussion The penile flip flap is safe and reliable for the repair of anastomotic fistula and coronal groove fistula after hypospadias correction. The correction rate is high, especially in the repair of anastomotic fistula, related to local circulation and scar hyperplasia.

Keywords urinary fistula; repair; children

INTRODUCTION

Urinary fistula is the most common complication post-operatively of hypospadias repair. The incidence rate varies from literature to literature, ranging from 5% to 30%, and the failure rate of urinary fistula repair is 20% to 40%^[1-5]. Many children with urinary fistula can not achieve the goal of healing after repeated repair, and families are physically and mentally exhausted. Therefore, increasing the cure rate of urinary fistula correction is the direction of surgeon. The common postoperative urinary fistula in hypospadias includes anastomotic fistula and coronal groove fistula. We used penile flip flap to repair the two urinary fistulas and analyzed the results.

COMMON DATA

From Jan. 2014 to Sept. 2019, 54 cases of urinary fistula repair postoperatively of hypospadias urethroplasty were performed, aged 3-13 years old (mean 5.5 y).

Table 1 Case number of anastomotic fistula /coronal groove fistula

category	anastomotic fistulas	coronal groove fistulas
Case number	18	36

METHODS

Hospitalization flow and perioperation step chart.(see Fig. 1)

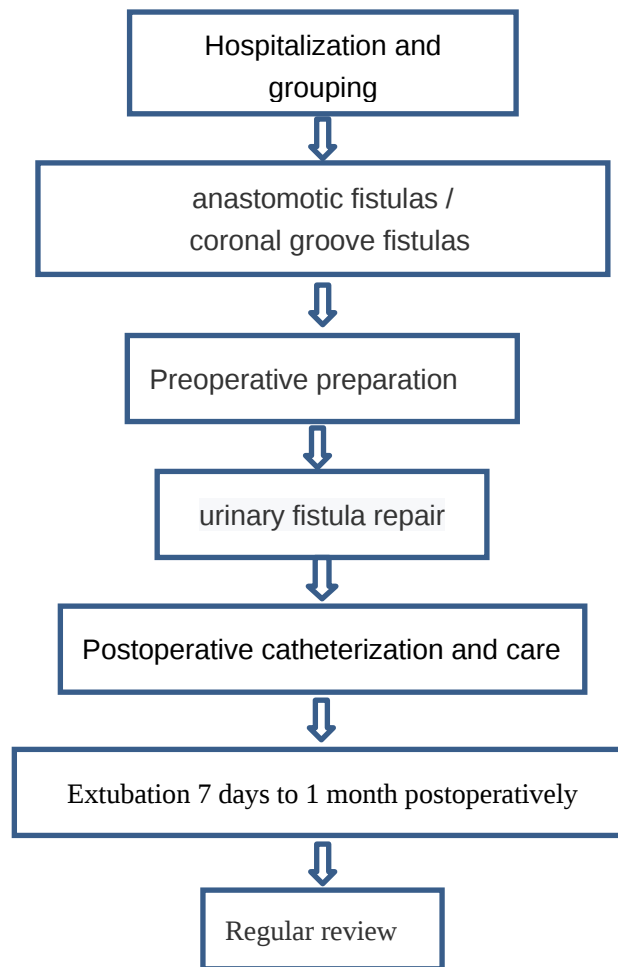


Fig 1 Hospitalization flow and perioperation step chart

SURGERY STEPS

Preoperative examination was completed for all children, and the operation was performed under tracheal intubation general anesthesia. Double-chamber urethral catheter was inserted before surgery.

Anastomotic urethral fistula: the fistula is mostly located in the proximal or middle segment of the penile ventral. Firstly, the subcutaneous tissue around the fistula should be trimmed, the scar tissue cut off, the fistula exposed and the urethra protected. The distal penile/scrotal midline middle-thick flap was revised via an inverted U-shaped incision and turned over to cover the fistula with 6/0 absorbable continuously suture. And then the flip flap was capped with distal subcutaneous fascia with intermittent suture. In the end Prune the skin and suture the incision continuously.

Coronary groove fistula: The skin scar around the fistula were trimmed first, the urethra protected. The fistula was closed, under the oblique 45° rectangular incision, with the proximal lateral phallic flip flap, continuous suture and intermittent reinforced. Another incision was cut on the other proximal side of the fistula, the subcutaneous fascia turned-over and covered the flap. The skin incision was closed with continuous and intermittent suture.

RESULT

The 54 children were grouped according to the fistula, and undergone the repair surgery In accordance with the operation steps above. Preoperative and postoperative treatment process were performed in accordance

with the procedure. The postoperative catheter indwelling duration is basically 14 days(7 days-1 month) . Anastomotic fistula is located at the proximal part of the penis, and the local scar is light and few. The subcutaneous tissue around the fistula is healthy and distinct. The blood supply of pedicled flap is well. The coronal groove fistula is located at the distal end of the penis with poor local tissue hierarchy, heavy scar and poor flap condition. After repair, some cases of skin flap necrosis and then scar healing, continued indwelling catheter is conducive to local scar healing. So, the indwelling catheter should be maintained for up to 1 month, and the distal urathra should be dilatated regularly after extubation. Urethral dilatation can be instructed to parents after health education. Regular follow-up adjustment.

In this study, the maximal frequency of repair was: anastomotic fistula 3 times, while coronal groove fistula 8 times.

Table 2 . Frequency of urinary fistula repair

Frequency	1	2	3	4	5	6	7	8
cases	36	13	2	1	1	0	0	

Table 3 . The success ratio of fistula repair in two groups

	anastomotic fistula group	coronal groove fistula group
achievement ratio of operation	17/18 (94.4%)	23/36 (63.8%)

The comparison of fistula repair success ratio in two groups was statistically significant (SPSS 19.0) ($P<0.05$). There was a significant difference in the healing rate between anastomotic fistula and coronal groove fistula repair . The result was related to the severity of local anatomical scar and distribution of blood supply.

CONCLUSION

Urinary fistula is the most common complication post-operative of hypospadias urethroplasty, and the cause include: distal urethral stricture of new urethra, urethral outer-opening stricture, urethral diverticulum , lack of support tissue in the new urethra, poor blood supply in the operative flap , necrosis or infection of the flap and poor urine drainage, etc^[7].

Most scholars believe that the surgical timing of urethral fistula repair should be performed 6 months after the last operation and preferably 1 year later, when the local scar tissue ,fixed, will not develop, and the tissue around will return to health without inflammation. At the same time, there should be no obvious signs of urine infection preoperatively .At least 3 months after the last surgery, the scar around the urine fistula turned softened and limited, so that a new blood supply could be established.

At the same time, the distal urethral stricture should also be estimated , and urethral stenosis should be treated. Otherwise, morbidity of fistula repair will increase obviously^[8].

To improve the cure rate of urinary fistula repair is the key to improve hypospadias family medical-satisfaction and physical and mental health .

Urinary fistula after hypospadias surgery included anastomotic fistula and coronal groove fistula. The surgery of urinary fistula included direct repair, flap repair and urinary reconstruction. Flap repair is most adopted , with less trauma, higher success rate or lower reoperative rate ,without causing urethral stricture

For anastomotic fistulas and coronal groove fistulas, we used penile proximal over- flip flap repair technique. The proximal penile and scrotal midline flap for the anastomotic fistula repair had no surgical scar tissue, with good blood supply. The scar around anastomotic fistula was lighter than the coronal groove fistula, and

subcutaneous tissue still existed around the fistula. The penis- scrotal junction and the scrotal midline flap are axial island flaps with the anterior and posterior scrotal arteries and their anastomotic branches as the vascular pedicles^[6]. After the fistula freed, the inverted u-shaped proximal rectangular flap of peri-fistula was reversed and the fistula was continuous varus suture, with small tension and good blood supply. Then covered with a healthy peripherally pedicled fascia, the skin incision was reshaped. It is beneficial to repair and heal the fistula.

In the ventral side of the coronal groove of the penis, the coronal groove fistula scar contracture is obvious, because the coronal groove of the penis itself is a natural scar with few adjacent healthy flaps and subcutaneous fascia. There is no subcutaneous tissue between the skin scar and urethral fistula, and the incision and release around the fistula can easily damage the urethra and cause the enlarged fistula, making repair more difficult.

Therefore, it is impossible to adopt the proximal flap of the fistula. The completely vertical lateral flap does not meet the optimal distribution of blood supply, but the reversed- "Y" rectangular flap that avoids the central axis of the urethra, not only preserves the excellent blood supply and collateral circulation of the proximal end, but also avoids the damage to the urethra. At the same time, the free coronary groove fistula was as far away from the fistula as possible, free to healthy tissue outside the urethral tube around the fistula, fully cut out the remaining skin scar on the fistula, and then closed the fistula with the inverted flap varus, and the healthy fascia on the other side was flipped and covered with a layer. Skin incisions were cut and then sutured.

We used double - chamber super - slip urinary catheters for hypospadias or urethroplasty in our center. Normal saline lubrication is convenient for implantation, and 5-7 days after the operation, urinary tube and wound adhesion is separated, which is easy to be removed, causing little damage to the urethra, and there is no damage to the early tube removal, or difficult extubation damage to the urethra. The operation quality is guaranteed, and the nursing is simple. Reduce expenses and average hospital stay. According to the literature, there was no significant difference in the effectiveness of the single-chamber silicone catheter sewn into the penis head with a traction line, or the double-chamber catheter of the corresponding size.

Through the comparative study of this group, the penile flap is safe and reliable for the repair of anastomotic fistula and coronal groove fistula after hypospadias, with a high success rate, especially in the repair of anastomotic fistula has a very obvious advantage. Due to the good local anatomical conditions, the healing rate of stage 1 repair operation is high, and the total number of repair operations is also low. However, due to poor local conditions (scar, blood supply, etc.) and poor overall healing rate in stage 1, coronal groove fistula often requires multiple surgeries.

"The more sewing repair, the more leakage", looking forward to more improved surgical skills and even tissue engineering materials to improve the success rate of the repair of urinary fistula, improve the experience of medical treatment for children, and improve the physical and mental health of children and their families.

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Observation of Postoperative Analgesia in Elderly Patients with PFNA By Routine Pulsed Lumbar Plexus Administration

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Abstract

Objective to observe the postoperative analgesia of elderly patients with PFNA by pulsed lumbar plexus administration. **Methods:** 90 patients with PFNA postoperative analgesia were selected and divided into three groups on average. The first group, 30 routine PCIA. The second group received PCEA analgesia after spinal anesthesia. In the third group, the intraoperative lumbar plexus nerve block was completed and the anesthetic catheter was indwelling. The drug administration mode was programmed pulse administration without background dose. The pain index, movement tissue index and the incidence of various adverse reactions were observed after PFNA.

Results the pain score of peripheral nerve block program pulse group was significantly lower than that of general anesthesia group, the incidence of motor tissue was lower than that of epidural group, and the incidence of side effects was lower than that of general anesthesia group and epidural group.

Conclusion patients with PFNA is mostly elderly patients with varying degrees of system disease and organ function degradation, extra week nerve block program pulse to VAS score is lower and less motor block, fewer side effects, is PFNA more effective postoperative analgesia mode, you can reply as soon as possible to the patient's activity ability, improve the prognosis of patients.

Key Words Observation of Postoperative Analgesia ; Elderly Patients;PFNA

China has fully entered the aging society. With the increase of age, the elderly are prone to femoral neck fracture, femoral trochanteric fracture, etc. These hip fractures are the most serious fracture types caused by fracture osteoporosis. After the fracture, the patient's mobility and quality of life are reduced. Moreover, elderly patients are prone to recurrent osteoporotic fractures after surgery, such as contralateral hip fractures. As the elderly patients have many complications before injury, it has a serious impact on the body. Elderly patients with progressive organ function decline and decompensation, are prone to complications of cardiopulmonary and renal diseases, and are prone to unpredicted adverse events related to drugs, anesthesia and surgery during perioperative period. For such patients, the short-term goal is to effectively relieve pain, restore the patients' living ability as soon as possible, and avoid bed rest and complications caused by it. Self-control analgesia method has the characteristics of reliable effect, quick effect, therefore in the process of the analgesic treatment of orthopaedic surgery, the way of the wider application range methods mainly include epidural drug dosing and intravenous drug delivery as well as the peripheral nerve block, dosing way

mostly load plus continuous as well as the additional mode of self-control analgesia, program pulse to drugs used in the study of postoperative analgesia. In this study, the analgesic effects and adverse reactions of PCEA and peripheral nerves in patients with postoperative analgesia of orthopedic PFNA under the mode of PCIA and programmed pulse administration were observed, providing a more suitable method for clinical management of postoperative pain of PFNA.

GENERAL INFORMATION AND METHODS

General data selection of 90 cases with PFNA in orthopaedic surgery, aged 60 ~ 90 years old, ASA grade I ~ III were randomly divided into PCIA group (group I), the PCEA group (group E) and peripheral nerve postoperative analgesia group (N group), 30 people in each group. Exclusion criteria: if the original anesthesia method fails to change the anesthesia method, the postoperative analgesia score cannot be matched.

Anesthesia and analgesia methods epidural analgesia was performed in the PCEA group after the operation. The analgesia pump with a load of 1mg morphine was formulated with 0.125% bupivacaine +0.5ug/ml sufentanil. In the PCIA group, sufentanil 3-5ug analgesic formula was 2ug/ml+ tropisetron 10mg after general anesthesia. Each additional dose of the above two groups was 0.5ml, and the locking time was 15 minutes. The analgesic and analgesic formula for peripheral nerve block was 0.15% bupivacaine with no load. The dosage was added once per hour for 4ml each time, and the dosage was not more than 30 seconds. The dosage was added for 3ml each time, and the locking time was 15min.

Visual analogue score (VAS) was observed and recorded at 2, 4, 8, 24 and 48 hours after the operation, indicating sharp pain when the result was 10, satisfied with analgesia when the result was less than or equal to 3, and painless during the operation and analgesia when the result was zero. Postoperative motor block was evaluated by modified Bromage score. No leg lift at level 1; Level 2 cannot extend the knees; Level 3 does not extend or bend the ankle. Meanwhile, the incidence of adverse reactions such as nausea and vomiting during analgesia was recorded. Postoperative recovery was followed up 1 week, 1 month and 6 months after surgery.

STATISTICAL METHOD

SPSS12.00 statistical software was used for data processing. The standard deviation of measurement indexes in this study was represented by ($\bar{x} \pm s$), and variances were analyzed for various data in this study. For inter-group data, t was used to test, and x was used ²To test the counting data, and the comparison between them is statistically significant.($P < 0.05$).

THE RESULTS

In general, there were no statistically significant differences in age, weight and operation time between each group.

VAS scores of postoperative 4, 8 and 24 in group I were higher than those in group E and group N, and there was no significant difference in scores at 2 and 48h (table 1).

In the three groups, muscle strength of lower limbs could be completely recovered within 6h after the operation. 3 patients in group E had motor block, 4 patients in group I had severe nausea, vomiting and itching, and no motor block and adverse reactions occurred in group N (table 1).

Table 1 postoperative pain scores and adverse reactions of patients in the three groups (case)

The number of patients in the group was 2h, 4h, 8h, 24h and 48h				
Group I	30	1±0.5	2 4±0.5	1 4±0.5
Group E	30	1±0.5	2±0.5	2 4±0.5
Group N	30	1±0.5	2±0.5	2±0.5

Note: compared with N group, $P^1 \geq 0.05$, $P^2 < 0.05$, $P^3 \geq 0.05$, and $P^0 \geq 0.05$ were found

DISCUSS

PFNA is a common type of surgery in clinical practice, and most of the patients are elderly. Although this type of surgery has little trauma, it is characterized by severe pain in orthopedic surgery and requires early postoperative activities. However due to the old people in all kinds of physiological function gradually faded, and often accompanied by a variety of complications, it is to a certain extent of anesthesia and analgesia effect put forward higher request^[1], for such patients, short-term goal for effective analgesia, as soon as possible to restore the ability of the patient's life, avoid bed and the resulting complications; The long-term goal is to help patients return to their pre-injury mobility and function as much as possible and to avoid further falls and fractures^[2,3]. According to relevant statistics, the mortality rate of elderly patients after injury is as high as 30%. Therefore, people from all walks of life pay more and more attention to brittle fracture of the elderly.

Analgesia methods mainly include PCIA and PCEA. When patients use PCEA technology in the process of analgesia, they must learn how to properly use the analgesia pump. However, some patients may not want to control the analgesia by themselves due to fatigue and other reasons, and would rather leave these things to the doctor. In recent years, domestic and foreign studies on the use of PIEB + PCEA for analgesia have gradually increased. With the PIEB technique, the analgesia pump is programmed to be administered in a regular pulse mode rather than in a continuous infusion mode. The analgesia results are excellent, with few bursts of pain, few side effects, no serious complications and major technical errors, and high satisfaction.

PFNA patients are mostly elderly patients with different degrees of systemic diseases and organ function degradation, and peripheral nerve block procedure pulse drug VAS score is lower, less motor block, less side effects, which is a more effective analgesia mode after PFNA surgery, can restore the patient's activity ability as soon as possible, improve the prognosis of patients.

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Precise Application of the Xiao-Chai-Hu-Tang in 98 Cases of Patients with the Major Syndrome of Feeling Pain and Tenderness under the Right Costal Arch

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Abstract

Xiao-Chai-Hu-Tang (XCHT), a representative of previous edition of Formulas of Traditional Chinese Medicine and harmonizing formulas, has multiple pharmacological functions. However, a variety of side effects could be caused by misuse or abuse, ignoring the theory of diagnosis and treatment based on the combination of syndrome and disease differentiation. Therefore, we conducted a retrospective study to evaluate the efficacy of XCHT, depending upon the individual patient's condition. Among 98 patients treated with XCHT, most of the patients of syndromes could be cured after about two course. The therapeutic effect rate of prescribed XCHT in our 98 cases of treatment is 91.84% (90/98). In addition, the high frequently syndromes are consistent with Shanhanlun. With multiple years of experience, we believe that: patient feel pain and tenderness under the right costal arch is one of the symptoms of patients who fit for the treatment with XCHT.

Key words: diagnosis; Xiao-Chai-Hu-Tang (XCHT); syndrome; therapeutic effect

Xiao-Chai-Hu-Tang (XCHT), the famous classical prescription, was earliest reported in the ancient Chinese medical book Shanghanlun over 2000 years ago^[1]. It consists of Bupleurum falcatum, Panax ginseng, Scutellaria baicalensis, Pinellia ternate, Glycyrrhiza glabra, Zingiber officinale, and Zizyphus jujube^[2, 3]. With these 7 simple types of medicinal herbs, XCHT combines the therapy of cooling with warming and is homological in ascending and descending and works on clearing and tonifying^[4]. As the representative of previous edition of Formulas of Traditional Chinese Medicine and harmonizing formulas, XCHT takes “mediation” as a functional feature, which can be used in exogenous febrile diseases and miscellaneous diseases of internal injury. Generations of physicians extend and derive its connotation based on “mediation”. The common saying, “Xiao-Chai-Hu-Tang cure for all disease”, represents XCHT's wide range of applications and its impressive curative effects. However, a variety of side effects could be caused by misuse or abuse, ignoring the theory of diagnosis and treatment based on the combination of syndrome

CLINICAL DATA

In this retrospective study, we reviewed the data of 100 patients (56 male and 44 female, range 3-78 years) who had prescribed Xiao-Chai-Hu-tang from June 2009 to May 2017 at Shouguang Hospital of Traditional Chinese Medicine and Shandong University of Traditional Chinese Medicine Affiliated Hospital.

DIAGNOSTIC CRITERIA

The diagnostic criteria are met with the major syndrome and the one of secondary syndromes. The major syndrome is that the patient feel pain and tenderness under the right costal arch. The secondary syndrome were as follows: (1) intermittent chills and fevers, (2) a feeling of distention and oppression in the chest and costal region, (3) anorexia and disinclination to talk, (4) restless and nauseous, (5) bitterness in the mouth, (6) a parched throat, (7) vertigo, (8) pulse string.

TREATMENTS

The treatment was in accordance with the modification of XCHT in Shanghanlun. To be restless but not nauseous: Take away *Pinellia ternate* and *Panax ginseng*, and add *Fructus Trichosanthis*; With thirst for water: Take away *Pinellia ternate*, increase *Panax ginseng* to four and a half liang, add *Radix Trichosanthis*; With abdominal pain: Take away *Scutellaria baicalensis*, add *Radix Paeoniae*; With mass below the costal margin: Take away *Zizyphus jujube*, and add *Concha Ostreae*; With palpitation and dysuria: Take away *Scutellaria baicalensis*, and add *Poria*; No thirst of water but with a slight exterior fever: Take away *Panax ginseng*, and add *Ramulus Cinnamomi*. Cover the patient with a quilt to obtain a light perspiration; Accompanied by coughing: Take away *Panax ginseng*, *Zingiber officinale* and *Zizyphus jujube*; add *Fructus Schisandrae* and *Rhizoma Zingiberis*. Five days count as a course, and the total treatment time is two courses or until the patient is cured.

THERAPEUTIC CRITERIA

Cured: major syndrome and secondary syndromes disappeared; Effective: major syndrome significantly decreased and secondary syndromes were significantly alleviated; Ineffective: the symptoms were not significantly improved.

RESULTS

With the exception of one patient who was unable to fulfill timely follow-up and another patient who was transferred to another hospital due to aggravation in the primary diseases, the therapeutic effect for the other 98 patients is shown in Table 1. Among these patients, most of the patients of syndromes could be cured after about two course. The high frequently syndromes are consistent with Shanhanlun (Table 2).

Table 1. Therapeutic Effect of Prescribed XCHT

Patients	Cured	Effective	Ineffective	Total Effective Rate(%)
98	84	6	8	91.84

Table 2. The High Frequency Syndromes of XCHT

Syndrome	Frequency	Ratio (%)
Bitterness in the mouth	45	45.92
Reluctant to speak and eat	45	45.92
Nauseous	41	41.84
Intermittent chills and fevers	27	27.55
Parched throat	26	26.53

Vertigo	25	25.51
Feels a distention and a sensation of oppression in the chest and costal region	21	21.43
Restless	20	20.41
Headache	17	17.35

DISCUSSION

Xiao-Chai-Hu-Tang also called Minor Bupleurum Decoction is traditionally used in Japan, Korea and China^[5]. The preparation of formulas in which herbs are combined to achieve greater efficacy than individual herb is a form of oriental herbology. These herbal formulas are commonly used for treatment of a number of disorders^[6]. Previous studies demonstrated that XCHT has multiple pharmacological functions, such as inhibition of hepatitis virus^[7, 8], anti-inflammatory^[9-13], anti-hepatic fibrotic^[14], immune-modulating^[9, 15, 16], antioxidant^[17], anti-cancer^[18-20], liver protective effect^[21-23], and renal protective effect^[24-26]. However, Chinese herbal medicine should be specifically used depending upon a syndrome, not a disease, and a number of side effects could be caused by the misuse or abuse without any consideration of syndrome differentiation^[27]. Reports have raised concerns regarding acute respiratory failure and interstitial pneumonia^[28]. Indeed, the patients who took XCHT for treating chronic liver diseases have cases of interstitial pneumonia^[5]. According to Lee^[29] et al, the incidence of side effects could be increased by coadministration of interferon, the duration of XCHT treatment and increasing age of patients. Therefore, it is very difficult to tell whether or not the apparent side effects are causally linked to XCHT administration.

With multiple years of experience, we believe that: patient feel pain and tenderness under the right costal arch is one of the symptoms of “When one of the symptoms of syndrome is observed, a diagnosis of Xiao-Chai-Hu tang syndrome can be established.” Other examples include Triple-jiao and Gallbladder, which are associated with Couli and vellus hair (Cou is a juncture where the Triple-jiao and Body Resistance converge and a Channel where in vital energy and the blood flow. Li is the texture on skin, viscera and bowels.)^[30]. When the blood is deficient and Vital Resistance weaken, the Couli opens, so the pathogenic factor intrudes into Couli through the vellus hair and enters the triple-jiao channel of hand shaoyang; then the triple-jiao channel of hand shaoyang intersect with the gallbladder channel of foot shaoyang on the neck and shoulder. Consequently, pathogenic factors enter the gallbladder Channel of Foot-Shaoyang; Then through quepen (supraclavicular fossa), gallbladder channel connect to the liver and the gallbladder attaches to the liver, the pathogenic factors enter the gallbladder. On the other hand, liver is located at the left side and lungs are located at the right side, spirit and essence circulation refer to left as ascending and right as descending^[31]. The lung governs qi, externally it connects with skin and body fair. The pathogenic factors pass through upper-jiao with lung qi fall from the right side, then stagnate in the subcostal; consequently, the patient feels pain and tenderness under the right costal arch. The evidence exhibited is caused by the pathogenic factors’ struggles against the body resistance at the costal region. The syndromes include feeling of distention and oppression in the chest and costal region, anorexia and disinclination to talk, restless and nauseous.

In summary, among 98 patients treated with XCHT, most of the patients of syndromes could be cured after about two course. The therapeutic effect rate of prescribed XCHT in our 98 cases of treatment is 91.84% (90/98). In addition, the high frequently syndromes are consistent with Shanhanlun. With multiple years of experience, we believe that: patient feel pain and tenderness under the right costal arch is one of the symptoms of patients who fit for the treatment with XCHT.

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