Effects of propofol and dexmedetomidine anesthesia on Th1/Th2 of rat spinal cord injury

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Abstract. – OBJECTIVE: Spinal cord injury (SCI) patients had major trauma during surgery, which thus necessitates optimal choice of anesthesia drugs. The specific selection of anesthesia agents may affect body immune system. Therefore, this study aims to investigate the anesthesia effect of propofol and dexmedetomidine on the rat SCI and their effects of Th1/Th2.

MATERIALS AND METHODS: Improved AL-LEN's pouching method was used to generate rat SCI model. The SCI rat models were further divided into propofol and dexmedetomidine group for analyzing anesthesia time, duration, post-op analgesia time, SCI sensory function Reuter score. Real-time PCR quantified Th1 secreted cytokines interleukin (IL)-2, tumor necrosis factor (TNF)-α, Th2 secreted cytokines IL-4 and IL-10 mRNA expression levels. Enzyme labeled immunosorbent assay (ELISA) quantified serum cytokine levels. Th1 and Th2 cytokines were analyzed for correlation.

RESULTS: Dexmedetomidine had shorter anesthesia onset time, longer duration time, and elongated post-op analgesia time with lower Reuter score ($p<0.05$ compared to propofol group). No significant difference existed between heart rate (HR), respiration rate, SpO_2, and body temperature (T) during surgery. Compared to dexmedetomidine, propofol had elevated mRNA or serum levels of IL-2 and TNF-α, plus significantly lower IL-4 or IL-10 expression ($p<0.05$). IL-2 and TNF-α levels were negatively correlated with IL-4 and IL-10 ($p<0.05$).

CONCLUSIONS: Dexmedetomidine had better effects for improving in-op and post-op anesthesia/analgesia effects than propofol. Both drugs can induce imbalance of Th1/Th2.

Key Words:
Spinal cord injury, Dexmedetomidine, Propofol, Anesthesia, Th1, Th2.

Introduction

Spinal cord injury (SCI) is one severe complication of spine trauma, as it frequently causes severe functional deficit of limbs below injury segment[1,2]. In recent years, development of industry and transportation lead to various trauma, in which SCI is a severe case especially in younger population[3,4]. It is estimated that nearly one million people suffer from spine trauma, for which SCI is the most severe complication and can cause high morbidity[5]. Major approach treating SCI is still surgery. However, it has an expensive cost and unfavorable efficacy, severely affecting patient's life quality and brining heavy burdens for the public health[6,7]. SCI surgery has longer duration and larger trauma, thus requiring stringent anesthesia technique[8]. With increasing cases of SCI surgery and complicated patient status, clinicians need to consider anesthesia drug or route based on patient body status and disease condition, and trying to shorten surgery duration and to reduce surgical complications. The choice of optimal anesthesia drugs thus benefit efficacy of anesthesia and analgesia of patients, and for better post-op recovery[9,10].

Propofol is one rapid and potent agent for inducing/maintaining general anesthesia. Frequently being combined with spinal cord anesthesia or epidural anesthesia, it can stabilize sodium channel on neuronal membrane, thus inhibiting generation or transmission of action spike, elevating stimuli threshold, and lowering potential action velocity[11,12]. Dexmedetomidine belongs to α2-adrenaline receptor agonist, or imidazole subtype of α2 receptor agonist. It has selectivity to exert pluripotent pharmaceutical roles including anti-anxiety, sedation, analgesia, hypnosis and retarding sympathetic nerves[13,14]. The application of various anesthesia drugs may exert unique effects on body immune system. T helper cells (Th) can be divided into Th1 and Th2 sub-populations based on different secreted cytokines[15]. A recent study revealed that Th1 and Th2 cells can maintain a balance via mediating cytokine secretion,

thus maintaining normal immune functions[16]. This study aims to analyze the anesthesia efficiency of propofol and dexmedetomidine on rat SCI surgery, and their effects on Th1/Th2.

Materials and Methods

Experimental Animals

Healthy male Wistar rats (2-month-old, specific pathogen free (SPF) grade, body weight 250 ± 20 g) were purchased from Laboratory Animal Unit of Shanghai Jiao Tong University School of Medicine and were kept in an SPF grade animal facility with fixed temperature ($21 \pm 1°C$) and relative humidity (50~70%), using 12 h/12 h light/dark cycle. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Nanxiang Hospital of Shanghai Jiading District, Shanghai, China.

Major Materials and Equipment

Pentobarbital sodium and lidocaine were purchased from Zhaohui (Shanghai, China). ELISA kits for IL-2, TNF-α, IL-4 and IL-10 were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Dexmedetomidine and propofol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Microscopic surgical instruments were purchased from Suzhou Medical Instrument (Suzhou, China). RNA extraction kit and reverse transcription kit were purchased from Axygen (Tewksbury, MA, USA). The small animal physiological monitor was purchased from Yuyan Instrument Co. Ltd. (Shanghai, China). Labsystem version 1.3.1 microplate reader was purchased from Bio-Rad Laboratories (Hercules, CA, USA). ABI 7700 Fast fluorescent quantitative PCR cycler was purchased from ABI (Foster City, CA, USA). Ultrapure workstation was purchased from Sutai High-tech Materials Co. Ltd. (Shanghai, China). Other common reagents were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China).

Animal Grouping and Treatment

Healthy male Wistar rats were prepared for SCI model using improved ALLEN's method, and were randomly assigned into two groups (n=20 each), namely, propofol group and dexmedetomidine group.

Rat SCI Model Preparation

Improved ALLEN's weight-drop method was used to generate rat SCI model as previously reported[17]. After general anesthesia by 30 mg/kg pentobarbital sodium via intraperitoneal injection, rats were fixed on the table to remove vertebral disc and spines at T9-T11 segments. Using T10 spinal cord segment as the center, a round area (4 mm diameter) was exposed at the injury site. A plastic spacer with 3 mm length, 2 mm width 1 mm thickness with pre-curved processing based on physiological angle of rat spinal cord was placed outside the dura of T10 segment. A sleeve was placed vertically on the center of spacer. Using improved ALLEN's weight-drop apparatus, a pouching rod was made to drop on the spacer in a free-falling manner. Successful generation of SCI model was identified when retraction movement occurred in body and bilateral forelimbs, spastic swings of the tail. Surgical wound was closed by layers, with antibiotics for anti-inflammation.

Anesthesia Treatment of Rat SCI

All SCI rats after surgery were treated. In propofol group, rats were anesthetized by 30 mg/kg pentobarbital sodium via intraperitoneal injection, and were fixed in a supine position. A total of 100 mg/kg propofol was introduced via femoral veins for general anesthesia for SCI injury. In dexmedetomidine group, rats were fixed and injected by 50 mg/kg propofol via femoral veins for general anesthesia before surgery. In ropivacaine plus dexmedetomidine group, 100 mg/kg ropivacaine plus 50 mg/kg dexmedetomidine were applied via femoral veins for general anesthesia. All groups had a similar surgical duration. Rats were maintained at the anesthesia status for 1 h. Incisions were sutured after surgery, with 20,000 U/kg penicillin injection for anti-inflammation.

Monitor of Vital Signs and Anesthesia Effects During Surgery

Small animal physiology monitor was used to record heart rhythm (HR), partial oxygen saturation (SpO_2), body temperature (T) and respiratory rhythm were recorded. Any side effects including bradycardia, itches, hypotension, limb stiffness, or respiratory distress were recorded. Time onset and duration of anesthesia, post-op time duration and analgesia were evaluated, along with Reuter score for SCI sensory. Pain retraction reflex, stretch reflex, muscle strength and back sensation were all evaluated, with a higher score representing a worse recovery[17,18].

Sample Collection

20 days after surgery, rats were harvested for 5 ml tail vein blood. Samples were centrifuged at 3000 r/min for 15 min to collect the serum, which was stored at -80°C for further use. Rats were then sacrificed and collected for spinal cord tissues, which were kept in -80°C fridge for further assays.

ELISA for Serum IL-2, TNF-α, IL-4 and IL-10 Expression Level

Serum samples were tested for the level of IL-2, TNF-α, IL-4 and IL-10 following the manual instruction of ELISA kits. In brief, 96-well plate was added with 50 µl serially diluted samples, which were used to plot standard curves. 50 µl test samples were then added to test wells in triplicates. After washing for 5 times, liquids were discarded to fill with washing buffer for 30 s vortex. The rinsing procedure was repeated for 5 times. 50 µl enzyme labeling reagent was then added to each well except blank control. After gentle mixture, the well was incubated for 30 min at 37°C. Chromogenic substrates A and B were sequentially added (50 µl each), followed by 37°C dark incubation for 10 min. The test plate was then mixed with 50 µl quenching buffer as the blue color turned into yellow. Using blank control well as the reference, absorbance (A) values at 450 nm wavelength were measured by a microplate reader within 15 min after adding quenching buffer. Linear regression model was then plotted based on the concentration of standard samples and respective OD values. Sample concentration was further deduced based on OD values and regression function.

Real-Time PCR for Th1/Th2 Cytokines Expression

Total RNA was extracted from total peripheral blood samples of rats using Trizol reagents (Sigma-Aldrich, St. Louis, MO, USA). Total RNA purity was tested by UV spectrometry along with quantification. cDNA was synthesized reverse transcription. PrimerPremier 6.0 was used to design PCR specific primer (see Table I for sequences), which was synthesized by Invitrogen/Life Technologies (Carlsbad, CA, USA). Real-time PCR was used to test target gene expression under the following conditions: 92°C 30 s, followed by 35 cycles each containing 92°C 30 s, 58°C 45 s and 72°C 35 s. Fluorescent quantitative PCR was used to collect data. CT values of standard samples were calculated based on internal reference gene GAPDH for plotting standard curve. Semi-quantitative analysis was performed by $2^{-\Delta Ct}$ method.

Statistical Analysis

SPSS 19.0 statistical software (SPSS Inc. Chicago, IL, USA) was used for analysis. Measurement data were presented as mean ± standard deviation (SD). Comparison of means among multiple groups was performed by one-way analysis of variance (ANOVA). Linear regression was performed to analyze the correlation among indexes. Statistical significance was defined when $p < 0.05$.

Results

Effects of Propofol/Dexmedetomidine Anesthesia on general body signs of SCI rats During Surgery

We analyzed the effect of propofol/dexmedetomidine anesthesia on general body signs of SCI rats during surgery including HR, respiration, SpO$_2$ and T. Results showed that although dexmedetomidine decreased HR, respiration, SpO$_2$ and T of SCI rats during surgery to certain extents, no significant difference was discovered between dexmedetomidine and propofol groups (p>0.05, Table II). These results showed that propofol and dexmedetomidine had no effects on general body signs of SCI rats during surgery.

Table I. Primer synthesis sequences.

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
GADPH	AGTGCCAGCCTCGTCTCATAG	CGTTGAACTTGCCGTGGGTAG
IL-2	GATCCAGCGATACGAGAACTT	CTCTGCATCTGGACCGTCA
TNF-α	CTAAGCGGAATCTCAATAGCG	GGGACTCTCAATCCTCGTC
IL-4	AGCGGATCTCGGAAACTCAAT	CTGGCAGTCTGACATC
IL-10	GAAGATCTCAATAGCGTCA	AATCTCTCAATCCTCGTC

Table II. Effects of propofol/dexmedetomidine anesthesia on general body signs of SCI rats during surgery.

Group	Heart rhythm (per min)	Respiration (per min)	SpO$_2$	T (°C)
Propofol	321±42	80±15	89.2±4.7	38.1±1.7
Dexmedetomidine	298±32	71±11	82.4±2.5	37.6±1.9

Table III. Anesthesia efficacy of propofol/dexmedetomidine on SCI rats.

Group	Anesthesia onset time (min)	Anesthesia duration (min)	Analgesia time (min)	Reuter score
Propofol	21±5	61±11	81±15	5.5±0.6
Dexmedetomidine	12±3*	82±11*	98±13*	3.2±0.7*

Note: *, $p<0.05$ compared to propofol group.

Table IV. Propofol/dexmedetomidine anesthesia and adverse effects during SCI surgery.

Group	Bradycardia	Itchy	Hypotension	Chill	Respiration distress	Overall incidence (%)
Propofol	2 (6.6%)	5 (16.6%)	4 (13.3%)	6 (20.0%)	0	17 (56.6%)
Dexmedetomidine	2 (6.6%)	2 (6.6%)*	2 (6.6%)*	4 (13.3%)	0	10 (33.3%)*

Note: *, $p<0.05$ compared to propofol group.

Effects of Propofol/Dexmedetomidine on Anesthesia Efficacy of SCI rats

We further observed the anesthesia efficacy of propofol and dexmedetomidine on SCI rats. Results showed shorter onset time and longer duration of general anesthesia in dexmedetomidine group, which also had longer post-op analgesia time and decreased Reuter score (p<0.05 compared to propofol group, Table III).

Propofol/Dexmedetomidine Anesthesia and Adverse Effects During SCI Surgery

The effect of propofol/dexmedetomidine anesthesia on adverse effects during SCI rat surgery was analyzed. Results showed that dexmedetomidine significantly decreased adverse effects of SCI rats during surgery compared to propofol group (33.3% vs. 56.6%, $p<0.05$, Table IV).

Effects of Propofol/Dexmedetomidine Anesthesia on Th1 Cytokines IL-2 and TNF-α mRNA Expression in SCI rats

Real-time PCR was used to test the effect of propofol/dexmedetomidine anesthesia on Th1 cytokines IL-2 and TNF-α mRNA expression in SCI rats. Results indicated significantly higher IL-2 and TNF-α mRNA expression levels in both propofol and dexmedetomidine groups ($p<0.05$ compared to control group). Propofol group had a more potent change ($p<0.05$ compared to dexmedetomidine group, Figure 1).

Effects of Propofol/Dexmedetomidine Anesthesia on Th2 Cytokines IL-4 and IL-10 mRNA Expression in SCI rats

Real-time PCR was used to test the effect of propofol/dexmedetomidine anesthesia on Th2 cytokines IL-4 and IL-10 mRNA expression in SCI rats. Results indicated significantly lower IL-4 and IL-10 mRNA expression levels in both propofol and dexmedetomidine groups ($p<0.05$ compared to control group). Propofol group had a more potent change ($p<0.05$ compared to dexmedetomidine group, Figure 2).

Table V. Correlation analysis between serum Th1 and Th2 cytokines in propofol anesthesia SCI rats.

R value	IL-4	IL-10
IL-2	-0.726	-0.641
TNF-α	-0.572	-0.718

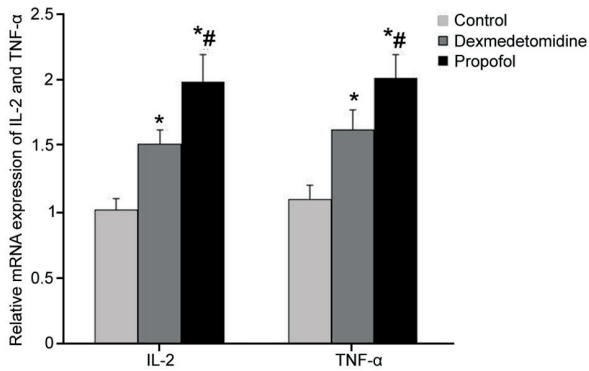Note: *, $p<0.05$ compared to propofol group.

Figure 1. Effects of propofol/dexmedetomidine anesthesia on Th1 cytokines IL-2 and TNF-α mRNA expression in SCI rats. *, $p<0.05$ compared to control group; #, $p<0.05$ compared to dexmedetomidine group.
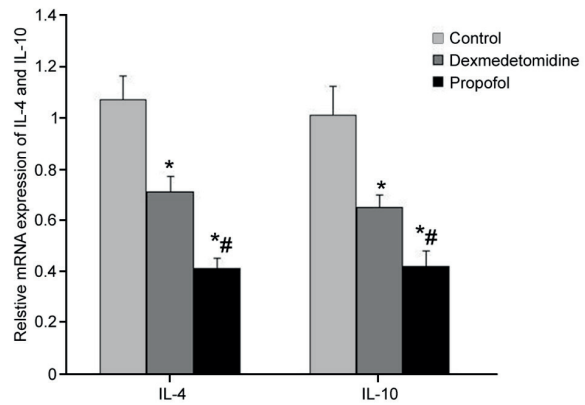

Figure 2. Effects of propofol/dexmedetomidine anesthesia on Th2 cytokines IL-4 and IL-10 mRNA expression in SCI rats. *, $p<0.05$ compared to control group; #, $p<0.05$ compared to dexmedetomidine group.

Effects of Propofol/dexmedetomidine Anesthesia on Serum Th1 and Th2 Cytokines levels in SCI Rats

ELISA was used to test the effect of propofol/dexmedetomidine anesthesia on serum Th1 and Th2 cytokines in SCI rats. Results indicated significantly elevated serum IL-2 and TNF-α, plus lower IL-4 and IL-10 levels in both propofol and dexmedetomidine groups ($p<0.05$ compared to control group). Propofol group had a more potent change ($p<0.05$ compared to dexmedetomidine group, Figure 3).

Correlation Analysis Between Serum Th1 and Th2 Cytokines in Propofol Anesthesia SCI rats

We further analyzed the correlation between serum Th1 and Th2 cytokines in propofol anesthetized SCI rats. Results showed that IL-2 and TNF-α levels were negatively correlated with IL-4 and IL-10 levels, respectively ($p<0.05$, Table V).

Correlation Analysis Between Serum Th1 and Th2 cytokines in Dexmedetomidine Anesthesia SCI rats

We further analyzed the correlation between serum Th1 and Th2 cytokines in dexmedetomidine anesthetized SCI rats. Results showed that

IL-2 and TNF-α levels were negatively correlated with IL-4 and IL-10 levels, respectively ($p<0.05$, Table VI).

Discussion

SCI severely threatens patient's health and affects life quality, so that effective treatments of SCI are necessary. Surgery can help to alleviate SCI, stabilize spine, and decompress spinal cord[19]. However, due to the factor of patient's condition, plus hypothermia and anesthesia drugs, makes it critical for the optimal choice of anesthesia drugs[20]. Propofol has a rapid onset, with fewer adverse/toxic effects, and less toxicity for heart and central nervous system[21]. Dexmedetomidine

Table VI. Correlation analysis between serum Th1 and Th2 cytokines in dexmedetomidine anesthesia SCI rats.

R value	IL-4	IL-10
IL-2	-0.819	-0.572
TNF-α	-0.631	-0.428

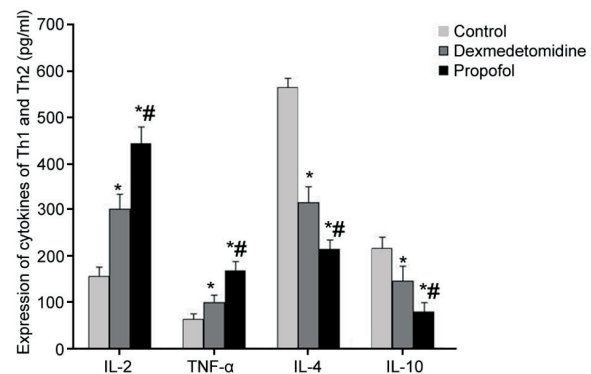Note: *, $p<0.05$ compared to propofol group.

Figure 3. Effects of propofol/dexmedetomidine anesthesia on serum Th1 and Th2 cytokines levels in SCI rats. *, $p<0.05$ compared to control group; #, $p<0.05$ compared to dexmedetomidine group.

can bind with cerebral vascular α2A receptor, thus exerting anti-pain, hypnosis, antagonizing sympathetic activity, sedation and hypothermia, and can be used for analgesia or sedation. Its usage can decrease anesthesia drug dosage, minimize the occurrence of post-op complication, facilitating recovery of SCI patients. Dexmedetomidine can bind with α2B receptor in smooth vascular cells to facilitate vessel constriction and to elevate blood pressure. It can also bind with α2C receptor to mediate dopaminergic neuron for hypothermia induction[22]. In clinics, dexmedetomidine exerts its function via binding with these three receptors to activate receptors[23]. This study generated a SCI rat model, on which anesthesia efficacy of propofol and dexmedetomidine was compared. Between these two methods, general vital signs including HR, SpO_2, T and respiration rhythm were not significantly changed. However, dexmedetomidine anesthesia has a shorter onset time, longer duration, and can elongate post-op analgesia times; moreover, it decreases Reuter score, and suppresses post-op adverse effects including itches, post-op pains, respiratory distress, chilly and hypotension, with better effects than propofol group. These results suggested that dexmedetomidine could enhance anesthesia efficacy, decrease post-op pain duration and facilitate recovery from SCI. Due to major trauma in SCI surgery, a potent stress response can be induced, frequently leading to body inflammation. The optimal choice of anesthesia drugs can alleviate the surgery stress response, further suppressing surgery-induced inflammation[24]. T cell subpopulation Th1-induced immune response is mainly involved in pathogenic immune process, manifesting as increased secretion of IL-2 and TNF-α to activate an inflammatory response. Th2 cell sub-population, however, mainly exerts protective effects via secreting cytokines to induce immune response[25]. Th1/Th2 imbalance can be analyzed by the levels of secreted cytokines. Currently no study has been reported regarding the comparison of dexmedetomidine and propofol anesthesia on SCI surgery or post-op immune function. In this work, we found that both dexmedetomidine and propofol anesthesia can increase Th1 cytokine secretion and decrease Th2 cytokine, leading to imbalance of Th1/Th2 to affect body immune function. Propofol has more potent effects on Th1/Th2, indicating that dexmedetomidine has fever effects than inflammatory factors, and can reduce SCI related inflammatory injury. Further studies can be performed to elu-

cidate the mechanism of dexmedetomidine and propofol anesthesia on immune functions.

Conclusions

Dexmedetomidine has better effects of anesthesia and sedation than propofol during SCI surgery. Both dexmedetomidine and propofol anesthesia can elevate Th1 cytokine secretion and decrease Th2 cytokines, leading to Th1/Th2 imbalance.

Conflict of interest
The authors declare no conflicts of interest.

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