Int J Clin Exp Med 2017;10(1):1080-1086
www.ijcem.com /ISSN:1940-5901/IJCEM0038044

Original Article

Application of ropivacaine combineddexmedetomidine is beneficial for rat spinal cord injury

Xinping Yang[1,2*], Ye Zhang[1], Yongqiang Zhan[3*], Zhiheng Liu[2], Mingfa Xiang[2]

[1]Department of Anesthesiology, The Second Hospital of Anhui Medical University, Hefei, P. R. China; Departments of [2]Anesthesiology, [3]Surgery, Shenzhen Second People's Hospital, Shenzhen, P. R. China. [*]Equal contributors.

Received August 17, 2016; Accepted October 24, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: As one of the most serious complications of spinal injury, spinal cord injury shows increasing trend in recent years, leading to severe limb dysfunction under the damaged section. Since the surgical trauma is large (?) for spinal cord injury, the reasonable choice of anesthetic is particularly important. This article intends to analyze the effect of anesthesia and postoperative analgesia of ropivacaine combined dexmedetomidine in rat spinal cord injury model. Modified ALLEN striking method was applied to establish rat spinal cord injury model. The rats were randomly divided into three groups, including ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group. Intraoperative heart rate, breathing, blood oxygen saturation, temperature, onset time of anesthesia, duration, postoperative analgesia time, and adverse reactions were compared. BBB score was used to evaluate motor function. Reuter score was adopted to assess spinal cord injury feeling function. Caspase 3 activity was detected. ELISA was performed to test TNF-α and IL-2 expressions. Intraoperative heart rate, breathing, blood oxygen saturation, and temperature showed no statistical difference among each groups. Ropivacaine combined dexmedetomidine resulted in significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups. It obviously inhibited Caspase 3 activity, declined TNF-α and IL-2 secretions, and reduced adverse reactions compared with single application group ($P < 0.05$). In summary, Ropivacaine combined dexmedetomidine can promote rat spinal cord injury recover through regulating apoptosis and inhibiting inflammation to improve intra- and postoperative anesthetic and analgesic effect.

Keywords: Ropivacaine, dexmedetomidine, spinal cord injury, analgesia, anesthesia

Introduction

The incidence of spinal cord injury (SCI) caused by car accident or falling injury gradually increased following society development [1, 2]. There are about hundreds of thousands of new cases of spinal injury every year worldwide, mainly in patients younger than 40 [3, 4]. SCI is one of the most serious complications of spinal injury, leading to high disability rate [5]. Because of high incidence, morbidity, and cost, SCI may cause severe limb dysfunction under the damaged section and bring huge economic burden to the society [6, 7]. Since the surgical trauma is large for spinal cord injury, the reasonable choice of anesthetic is particularly important along with the increase surgery number and requirements [8]. Appropriate choice of anaesthetics, anesthesia method, and anesthesia time is of great significance to reduce surgical damage and improve anesthetic and postoperative analgesia effect [9, 10].

Anesthetic selection and combination are critical to guarantee the anesthesia effect in spinal surgery. Traditional local anesthetics widely applied in clinic include tetracaine, procaine, ropivacaine and dexmedetomidine [11]. Dexmedetomidine is a relatively selective α2 adrenergic receptor agonist belonging to excited isopyrazole subtype [11, 12]. It has the pharmacological effects of anti-anxiety, sedation, analgesia, hypnosis, sympathetic block. FDA has approved it to be applied in adult mechanical ventilation in ICU, pediatrics, neurosurgery, and obese surgeries, and fiberoptic bronchos-

copy [13, 14]. Ropivacaine, belonging to long-term local anesthetics of amide derivatives, is a traditional intraspinal anesthesia drug for decades. Ropivacaine inhibits nerve impulses and conduction, improves electrical nerve stimulation threshold, and reduce the rate of action potential mainly by stabilizing the sodium ion channels on the nerve cell membrane. However, both ropivacaine and dexmedetomidine are limited by long onset time, short analgesia time, and more adverse reactions [15, 16]. Therefore, this article intends to analyze the effect of anesthesia and postoperative analgesia of ropivacaine combined dexmedetomidine in rat spinal cord injury model.

Materials and methods

Experimental animals

SPF grade male Wistar rats with 2-month old and weighted 250±20 g were bought and fed in the experimental animal center of Anhui Medical University. The feeding condition maintained constant temperature at 21±1°C and relative humidity at 50-70%. Day/night cycle was 12 h.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of The Affiliated Clinical College Shenzhen Second People Hospital, Anhui Medical University.

Main materials and instruments

Pentobarbital sodium and lidocaine were got from Shanghai Zhaohui Pharmaceutical co., Ltd. TNF-α and IL-2 ELISA kits were purchased from R&D. Ropivacaine and dexmedetomidine were bought from Sigma. Caspase 3 activity detection kit was purchased from Cell Signaling. Surgery microscopic instruments were from Medical Instrument Factory in Suzhou. Multi-Parameter Monitor small animal physiological monitor was from Yuyanbio. Microplate reader was from BD. Other common reagents were purchased from Sangon.

Methods

Animal grouping and treatment: Modified ALLEN striking method was applied to establish rat SCI model. The rats were equally randomly divided into three groups, including ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group with 30 in each group.

Rat SCI model establishment: Rat SCI model was established by modified ALLEN striking method [17]. After anesthetized by 30 mg/kg pentobarbital intraperitoneal injection, the rat was fixed on the operating floor to resect the vertebral plate and spinous process of T9-11. T10 spinal cord segment was set as central to expose the damage zone with diameter at 4 mm. A plastic buckling gasket at 3×2×1 cm according to the physiological curvature of dorsal rat spinal cord was put to the epidural of T10 spinal cord segment. Next, a sleeve was vertically put on the center of the gasket and the stick directly hit on the gasket at 5 cm high through the sleeve. The marker of modeling success included retraction flapping of body and lower limbs, and myoclonic swing of tail. The incision was closed and the rat received conventional antibiotics to diminish inflammation.

Anesthesia treatment: Rats in ropivacaine group received ropivacaine femoralis injection at 100 mg/kg. Rats in dexmedetomidine group received dexmedetomidine femoralis injection at 50 mg/kg. Rats in combined group received ropivacaine (100 mg/kg) and dexmedetomidine (50 mg/kg) femoralis injection. Duration of anesthesia was 1 h in each group.

Intraoperative index and anesthetic effect observation

Intraoperative heart rate (300-600 bpm), breathing (70-110 bpm), blood oxygen saturation, and temperature (38.5-39.5°C) were recorded by small animal physiological monitor. Adverse reaction including bradycardia (heart rate < 300 bpm), itching, hypotension (systolic blood pressure < 80 mmHg), chills, and respiratory depression was observed and recorded. Onset time of anesthesia, duration, and postoperative analgesic time were evaluated. BBB score was used to evaluate joints and lower limb motion recovery on the 20th day after surgery. The higher BBB score, the better recovery is. Reuter score was adopted to evaluate pain withdrawal reflex, myotatic reflex, muscle strength, muscle tone, and back sense. The

Table 1. The impact of anesthesia on intraoperative vital signs

Group	Heart rate (bpm)	Breathing (bpm)	Blood oxygen saturation	Temperature (°C)
Ropivacaine	437±68	82±12	89.7±2.5	38.2±1.6
Dexmedetomidine	461±22	86±17	85.6±1.6	38.8±1.2
Ropivacaine combined dexmedetomidine	412±32	76±15	81.4±2.1	37.9±1.1

Table 2. The influence of different anesthesia methods on anesthesia effects of SCI rat

Group	Onset time of anesthesia (min)	Duration (min)	Analgesia time (min)	BBB	Reuter
Ropivacaine	21±5	65±13	83±17	12.1±1.5	5.2±0.4
Dexmedetomidine	19±3	62±11	81±15	13.2±1.6	5.1±0.5
Ropivacaine combined dexmedetomidine	11±6*	78±16*	98±15*	17.9±1.8*	3.1±0.6*

*P < 0.05, vs ropivacaine group or dexmedetomidine group.

higher Reuter score, the worse of sensory function recover [17, 18].

Specimen collection

On the 20th day after surgery, a total of 5 ml blood was extracted from caudal vein and centrifuged at 3000 rpm for 15 min. The serum was collected in Eppendorf tube and stored at -80°C. Spinal cord tissue was extracted and stored at -80°C.

ELISA

TNF-α and IL-2 expressions were detected by ELISA according to the manual. A total of 50 μl diluted standard substance or sample was added to the 96-well plate with three repeats. After washed for five times, 50 μl enzyme-labelled reagents were added to the well and incubated at 37°C for 30 min. Next, the plate was treated by 50 μl color agent A and 50 μl color agent B at 37°C for 10 min. At last, the plate was added with 50 μl stop buffer and tested on microplate reader at 450 nm to obtain the absorbance. The linear regression equation was made based on the absorbance value of standard substance. The sample concentration was calculated according to the equation.

Caspase 3 activity

Caspase 3 activity in spinal cord tissue was tested by the kit. The cells was digested by enzyme and centrifuged at 600 g and 4°C for 5 min. Then the cells were treated by lysis on ice for 15 min. After centrifuged at 20,000 g and 4°C for 5 min, the sample was added with 2 mM Ac-DEVD-pNA and detected at 405 nm to calculate Caspase 3 activity.

Data analysis

All data analysis was performed on SPSS 19.0 software. The measurement data was presented as mean ± standard deviation and compared by one-way ANOVA. P < 0.05 was depicted as statistical significance.

Results

The impact of anesthesia on intraoperative heart rate, breathing, blood oxygen saturation, and temperature

Intraoperative heart rate (300-600 bpm), breathing (70-110 bpm), blood oxygen saturation, and temperature (38.5-39.5°C) in ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group were analyzed. Though ropivacaine combined dexmedetomidine reduced intraoperative heart rate, breathing, blood oxygen saturation, and temperature compared with single drug group, the difference was lack of statistical significance (**Table 1**). It suggested that the application of three anesthetic drug groups showed no obvious impact on vital signs.

The influence of different anesthesia methods on anesthesia effects of SCI rat

The influence of ropivacaine, dexmedetomidine, and ropivacaine combined dexmedetomidine on anesthesia effects of SCI rat was ob-

Table 3. The effect of different anesthesia methods on intraoperative adverse reaction of SCI rat (n%)

Group	Bradycardia (heart rate < 300 bpm)	Itching	Hypotension	Chills	Respirator depression	Incidence (%)
Ropivacaine (30)	2 (6.6%)	5 (16.6%)	4 (13.3%)	6 (20.0%)	0	17 (56.6%)
Dexmedetomidine (30)	2 (6.6%)	4 (13.3%)	6 (20.0%)	3 (10.0%)	0	15 (50.0%)
Ropivacaine combined dexmedetomidine (30)	1 (3.3%)	1 (3.3%)*	2 (6.6%)*	1 (3.3%)*	0	5 (16.6%)*

*P < 0.05, vs ropivacaine group or dexmedetomidine group.

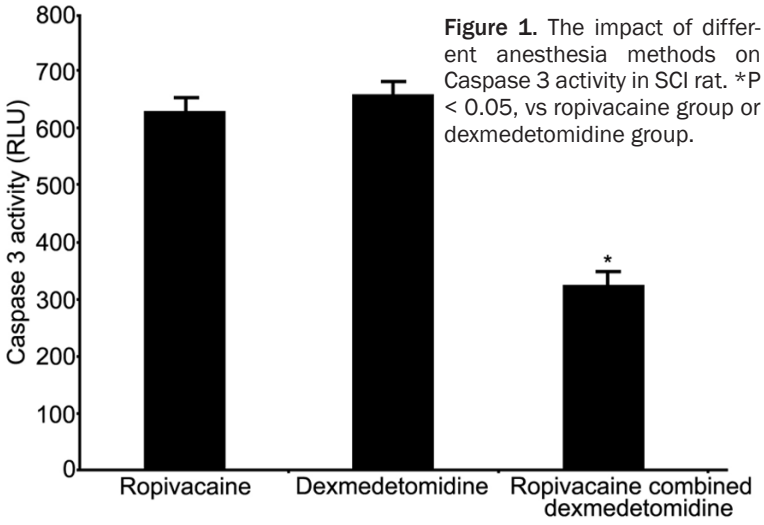

Figure 1. The impact of different anesthesia methods on Caspase 3 activity in SCI rat. *P < 0.05, vs ropivacaine group or dexmedetomidine group.

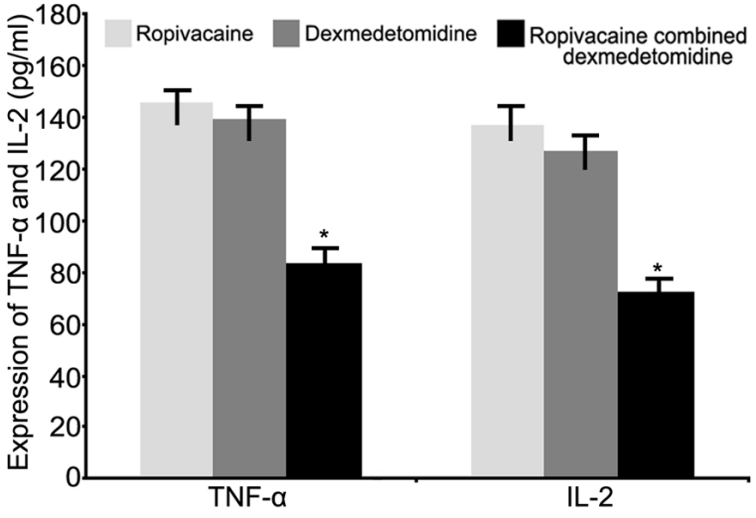

Figure 2. The impact of different anesthesia methods on TNF-α and IL-2 expressions in SCI rat. *P < 0.05, vs ropivacaine group or dexmedetomidine group.

served. It was revealed that ropivacaine combined dexmedetomidine was presented as significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups (P < 0.05) (**Table 2**).

The effect of different anesthesia methods on intraoperative adverse reaction of SCI rat

The impact of ropivacaine, dexmedetomidine, and ropivacaine combined dexmedetomidine on intraoperative adverse reaction of SCI rat was analyzed. No bradycardia or itching was found after basal anesthesia by phenobarbital sodium. Ropivacaine combined dexmedetomidine obviously reduced intraoperative adverse reaction in SCI rat compared with single drug groups (P < 0.05) (**Table 3**).

The impact of different anesthesia methods on Caspase 3 activity in SCI rat

Caspase 3 activity in spinal cord tissue was tested by kit. It was demonstrated that ropivacaine combined dexmedetomidine markedly suppressed Caspase 3 activity compared with single drug groups (P < 0.05) (**Figure 1**). It indicated that ropivacaine combined dexmedetomidine can reduce apoptosis to alleviate spinal tissue damage.

The impact of different anesthesia methods on TNF-α and IL-2 expressions in SCI rat

Serum TNF-α and IL-2 expressions in ropivacaine group, dexmedetomidine group, and ropivacaine combined dexmedetomidine group were tested by ELISA. The results showed that ropivacaine combined dexmedetomidine significantly declined TNF-α and IL-2 secretions

compared with single drug groups ($P < 0.05$) (**Figure 2**). It suggested that ropivacaine combined dexmedetomidine can decrease inflammatory injury in SCI rat through suppressing inflammatory cytokines secretion.

Discussion

As SCI seriously damages human health, and brings heavy spirit and economic pressure, it is a vital step to timely and effective treat SCI. Surgery can prevent the further injury of the spinal cord by stabilizing the spine and relieving the compression [19]. However, the huge trauma of surgery may cause severe inflammation and trigger further immunosuppression. The patient's own factors, as well as low temperature, the application of anaesthetics, and mechanical ventilation can aggravate inflammation. Rational choice of anesthetic drugs can alleviate stress reaction to different level and reduce the immunosuppression caused by surgery [20].

Ropivacaine is the first discovered local anaesthetic in the pure enantiomers form that featured as fewer side effects, lower central nervous system toxicity and heart toxicity, and long-effectiveness [21]. Dexmedetomidine plays its role in antinociceptive effects, hypnosis, antisympathetic activity, and calm by binding with α2A receptor distributed in the brain. It plays a role in promoting vasoconstriction and increasing blood pressure by binding with α2B receptor distributed in vascular smooth muscle. At last, it can induce hypothermia by acting on α2C receptor to regulate dopaminergic nerve. In clinic, dexmedetomidine plays its function by synergistically activating receptors [22]. Dexmedetomidine can be used for clinical analgesia and sedation, which may reduce the drug dosage. Reasonable application of anesthetics may reduce the occurrence of postoperative complications and accelerate the efficacy of anesthesia, so as to promote SCI improvement and recovery [23]. This study established rat SCI model and applied ropivacaine and dexmedetomidine for intervention. Intraoperative heart rate, breathing, blood oxygen saturation, and temperature exhibited no statistical difference among each groups. Ropivacaine combined dexmedetomidine was presented as significantly shorter onset time of anesthesia, longer duration and postoperative analgesia time, higher BBB score, and lower Reuter score compared with other groups. It suggested that ropivacaine combined dexmedetomidine can enhance anesthetic effect, shorten postoperative pain course, and promote rat SCI recovery.

Complication after anaesthesia is one of the problems in operation and postoperative recovery, mainly including itching, postoperative pain, respiratory depression, chills, and hypotension. This study found that ropivacaine combined dexmedetomidine can reduce the adverse reaction of anesthesia. Inflammatory cytokines IL-2 and TNF-α secretion can trigger inflammation, facilitating leukocytes adhesion to provide the conditions for the further development of inflammation. Caspase is one of the most important proteases during apoptosis process. It can induce apoptosis through decomposing and activating DNA cleavage related proteins. Caspase-3 is the most critical protease in the process of apoptosis, and also the common downstream effect part of different apoptosis pathways [24, 25]. Further analysis revealed that ropivacaine combined dexmedetomidine can decrease TNF-α and IL-2 secretion and suppress Caspase-3 activity. It suggested that their combination can regulate apoptosis and suppress inflammation cytokines secretion to alleviate inflammatory injury to SCI rat.

Conclusion

Ropivacaine combined dexmedetomidine can facillitate rat SCI recover by regulating apoptosis and inhibiting inflammation to improve intra- and postoperative anesthetic and analgesic effects.

Acknowledgements

This work was supported by the Medical Research Project of Shenzhen Science and Technology Innovation Committee [No. GJHZ2014-041470821201].

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ye Zhang, Department of Anesthesiology, The Second Hospital of Anhui Medical University, 678 Furong Road, Economy

& Technology Development Zone, Hefei 230032, P. R. China. Tel: +86-0551-63869420; Fax: +86-0551-63869420; E-mail: YeZhangzxc@163.com

References

[1] Fang H, Zhang JC, Yang M, Li HF, Zhang JP, Zhang FX, Wang QY, Wang RR, Liu J. Perfusion of gastrodin in abdominal aorta for alleviating spinal cord ischemia reperfusion injury. Asian Pac J Trop Med 2016; 9: 688-693.

[2] Cheah M, Andrews MR, Chew DJ, Moloney EB, Verhaagen J, Fassler R, Fawcett JW. Expression of an activated integrin promotes long-distance sensory axon regeneration in the spinal cord. J Neurosci 2016; 36: 7283-7297.

[3] Harsha KJ, Parameswaran K. Permanent spinal cord injury during lumbar spinal anesthesia: A report of two cases. Neurol India 2016; 64: 808-811.

[4] Schussler-Fiorenza Rose SM, Eslinger JG, Zimmerman L, Scaccia J, Lai BS, Lewis C, Alisic E. Adverse childhood experiences, support, and the perception of ability to work in adults with disability. PLoS One 2016; 11: e0157726.

[5] Qin W, Li X, Peng Y, Harlow LM, Ren Y, Wu Y, Li J, Qin Y, Sun J, Zheng S, Brown T, Feng JQ, Ke HZ, Bauman WA, Cardozo CP. Sclerostin antibody preserves the morphology and structure of osteocytes and blocks the severe skeletal deterioration after motor-complete spinal cord injury in rats. J Bone Miner Res 2016; 31: 1482.

[6] Nunnerley J, Gupta S, Snell D, King M. Training wheelchair navigation in immersive virtual environments for patients with spinal cord injury - end-user input to design an effective system. Disabil Rehabil Assist Technol 2016; [Epub ahead of print].

[7] Sachdeva R, Farrell K, McMullen MK, Twiss JL, Houle JD. Dynamic changes in local protein synthetic machinery in regenerating central nervous system axons after spinal cord injury. Neural Plast 2016; 2016: 4087254.

[8] Rao SN, Pearse DD. Regulating axonal responses to injury: the intersection between signaling pathways involved in axon myelination and the inhibition of axon regeneration. Front Mol Neurosci 2016; 9: 33.

[9] Burkovskiy I, Zhou J, Lehmann C. Experimental cannabinoid 2 receptor inhibition in CNS injury-induced immunodeficiency syndrome. Microcirculation 2016; 23: 283-292.

[10] Samantaray S, Das A, Matzelle DC, Yu SP, Wei L, Varma A, Ray SK, Banik NL. Administration of low dose estrogen attenuates persistent inflammation, promotes angiogenesis, and improves locomotor function following chronic spinal cord injury in rats. J Neurochem 2016; 137: 604-617.

[11] Fan L, Zhang J, Lv Z, Guo H, Zhao Y. Clinical research on the dexmedetomidine applied for patient-controlled sedation during the lower limbs operation under combined spinal-epidural anesthesia. Pak J Pharm Sci 2016; 29: 1095-1100.

[12] Sayed E, Yassen KA. Intraoperative effect of dexmedetomidine infusion during living donor liver transplantation: A randomized control trial. Saudi J Anaesth 2016; 10: 288-294.

[13] Das A, Chhaule S, Bhattacharya S, Basunia SR, Mitra T, Halder PS, Chattopadhyay S, Mandal SK. Controlled hypotension in day care functional endoscopic sinus surgery: A comparison between esmolol and dexmedetomidine: A prospective, double-blind, and randomized study. Saudi J Anaesth 2016; 10: 276-282.

[14] Conti G, Ranieri VM, Costa R, Garratt C, Wighton A, Spinazzola G, Urbino R, Mascia L, Ferrone G, Pohjanjousi P, Ferreyra G, Antonelli M. Effects of dexmedetomidine and propofol on patient-ventilator interaction in difficult-to-wean, mechanically ventilated patients: a prospective, open-label, randomised, multicentre study. Crit Care 2016; 20: 206.

[15] Lim HJ, Hasan MS, Chinna K. Faster onset time of supraclavicular brachial plexus block using local anesthetic diluted with dextrose. Braz J Anesthesiol 2016; 66: 341-345.

[16] Faria-Silva R, de Rezende DC, Ribeiro JM, Gomes TH, Oliveira BA, Pereira FM, de Almeida Filho IA, de Carvalho Junior AE. Association of clonidine and ropivacaine in brachial plexus block for shoulder arthroscopy. Braz J Anesthesiol 2016; 66: 335-340.

[17] McDonald T, Liang HA, Sanoja R, Gotter AL, Kuduk SD, Coleman PJ, Smith KM, Winrow CJ, Renger JJ. Pharmacological evaluation of orexin receptor antagonists in preclinical animal models of pain. J Neurogenet 2016; 30: 32-41.

[18] Wei ZJ, Zhou XH, Fan BY, Lin W, Ren YM, Feng SQ. Proteomic and bioinformatic analyses of spinal cord injuryinduced skeletal muscle atrophy in rats. Mol Med Rep 2016; 14: 165-174.

[19] Khankan RR, Griffis KG, Haggerty-Skeans JR, Zhong H, Roy RR, Edgerton VR, Phelps PE. Olfactory ensheathing cell transplantation after a complete spinal cord transection mediates neuroprotective and immunomodulatory mechanisms to facilitate regeneration. J Neurosci 2016; 36: 6269-6286.

[20] Mishori R, Groah SL, Otubu O, Raffoul M, Stolarz K. Improving your care of patients with spinal cord injury/disease. J Fam Pract 2016; 65: 302-309.

[21] Bawdane KD, Magar JS, Tendolkar BA. Double blind comparison of combination of 0.1% ropivacaine and fentanyl to combination of 0.1%

bupivacaine and fentanyl for extradural analgesia in labour. J Anaesthesiol Clin Pharmacol 2016; 32: 38-43.

[22] Kundra TS, Nagaraja PS, Singh NG, Dhananjaya M, Sathish N, Manjunatha N. Effect of dexmedetomidine on diseased coronary vessel diameter and myocardial protection in percutaneous coronary interventional patients. Ann Card Anaesth 2016; 19: 394-398.

[23] Yuan F, Fu H, Yang P, Sun K, Wu S, Lv M, Dong Z, Dong T. Dexmedetomidine-fentanyl versus propofol-fentanyl in flexible bronchoscopy: A randomized study. Exp Ther Med 2016; 12: 506-512.

[24] Simpson S Jr, Stewart N, van der Mei I, Blizzard L, Taylor BV. Synergetic and antagonistic effects of combined calcitriol and interferon-beta treatment on cytokine production by stimulated PBMCs. J Neuroimmunol 2016; 297: 148-155.

[25] Liu YH, Liu GH, Mei JJ, Wang J. The preventive effects of hyperoside on lung cancer in vitro by inducing apoptosis and inhibiting proliferation through Caspase-3 and P53 signaling pathway. Biomed Pharmacother 2016; 83: 381-391.