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Original Article

The effects of dexmedetomidine on the cognitive function and TGF β/Smad pathway in propofol-anesthetized rats

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Abstract: Objective: To investigate the effects of dexmedetomidine on the cognitive function and the TGF-β/Smad pathway in propofol-anesthetized rats. Methods: A total of 36 20-month-old Sprague-Dawley (SD) rats were randomly assigned to the following three groups: the dexmedetomidine + propofol anesthetized group (D group), the propofol-anesthetized group (P group), or the blank control group (C group). The rats in D group were infused with dexmedetomidine and propofol via the caudal vein using an infusion pump for 4 hours, those in P group received an infusion of propofol alone via the caudal vein for 4 hours, but those in C group did not receive any treatment. All the rats in D and P groups underwent exploratory laparotomies. Morris water maze experiments were conducted on the rats on the 1st, 3rd, and 5th days after surgery. The rats were sacrificed on the 6th day, and then some serum and hippocampal tissues were collected. The protein expressions (TGF-β1, Smad3, and Smad7) associated with the TGF-β/Smad pathway were measured using an enzyme linked immunosorbent assay (ELISA). Results: During the water maze experiment, compared with C group, the escape latencies of the rats in D and P groups were significantly prolonged, the time spent in finding the target quadrant was significantly shortened, and the number of times crossing the platform were considerably reduced (all $P<0.05$). D group showed significantly shorter escape latency, a remarkably longer time spent in finding the target quadrant, and significantly more times crossing the platform than the P group ($P<0.05$). Compared with C group, D and P groups had significantly elevated levels of TGF-β1, Smad3, and Smad7 in their serum and hippocampal tissues (all $P<0.05$); however, the levels of the above-mentioned proteins were markedly lower in D group than in P group ($P<0.05$). Conclusion: Anesthesia with propofol alone led to postoperative cognitive dysfunction (POCD) in elderly rats; however, the addition of dexmedetomidine relieved POCD and elevated the levels of proteins associated with the TGF-β/Smad pathway in the serum and hippocampal tissues of the propofol-anesthetized rats, suggesting that the TGF-β/Smad pathway was activated, and the addition of dexmedetomidine to propofol anesthesia reduced the protein levels. Hence, we speculate that dexmedetomidine relieves POCD in elderly rats by inhibiting the TGF-β/Smad pathway.

Keywords: Dexmedetomidine, cognitive dysfunction, propofol, TGF-β/Smad pathway

Introduction

Postoperative cognitive dysfunction (POCD) refers to the symptoms in the central nervous system associated with attention, mental activity, learning and problem-solving abilities in patients after surgery or anesthesia [1, 2]. The symptoms last for days to weeks, or even exist permanently. They may have serious impacts on the patients' prognosis and quality of life and increase their mortality after surgery. Age is a confirmed risk factor for long-term POCD. According to a previous study, nearly 20% of elderly patients have POCD, which greatly affects their prognosis [3]. Therefore, it is urgent to solve the problem of POCD in elderly patients. We can reduce the incidence of POCD through preventive intervention.

Exploratory laparotomy is a common clinical surgery technique [4]. It is necessary to perform exploratory laparotomies in abdominal surgeries for intestinal obstructions, gastrointestinal tumors, and gynecological diseases. Old age is a risk factor for intestinal tumors and other abdominal diseases, so exploratory laparotomy is also very common in elderly patients [5]. Additionally, surgery is also a high-risk factor for the occurrence of POCD because there will be stress reactions during surgery [6]. Stimulation affects patients' central nervous systems, especially the hippocampus, which controls memory and learning and is closely related to the presence of POCD. Therefore, determining how to reduce the incidence of POCD after surgery and anesthesia is very important for postoperative rehabilitation in elderly patients.

Propofol is one of the most widely used intravenous anesthetics in clinical practice. The drug is valuable because of its rapid effect, short action time, fast clearance, and few adverse reactions [7, 8]. However, it has been confirmed that propofol suppresses the long-term potentiation of the hippocampus, which is the basis of memory and learning. Moreover, propofol can also affect many normal neurotransmitters in the human brain, which may lead to POCD [9]. Dexmedetomidine, a newly-developed alpha 2-adrenoreceptor agonist, has analgesic, sedative, and anti-sympathetic effects, and it can be used in patients who need clinical sedation or intraoperative sedation [10-12]. Dexmedetomidine has been shown to reduce the incidence of POCD, but its specific mechanisms of action remain unclear [13, 14].

Transforming growth factor-β (TGF-β) is a multifunctional cytokine [15]. Smads are its main signal transduction molecules, and the TGF-β/Smad pathway is involved in embryonic development, tumor proliferation and growth, as well as tissue fibrosis [16, 17]. They have a wide range of biological actions and can be expressed in all cells. Multiple studies show that the expressions of TGF-β1 and Smad7 are associated with cognitive dysfunction, including the cognitive dysfunctions resulting from diabetes, cerebral infarction or the use of sevoflurane (anesthetics) [18, 19]. However, few studies have explored on the association between dexmedetomidine and POCD. Therefore, the present study was designed to investigate the effects of dexmedetomidine on the cognitive function and TGF-β/Smad pathway in propofol-anesthetized rats.

Materials and methods

Study subject

This study was approved by the animal ethics committee. Thirty-six Sprague-Dawley (SD) rats (age, 20 months old; weight, 800±100 g) were selected for this study. The rats were housed in a sterile animal room at a room temperature of 22.0±0.5°C. The animals were maintained in a 12:12-hr light/dark cycle and had free access to water and food in a quiet environment. All the rats adapted to the environment for one week before modeling. After that, they were randomly assigned to the following three groups: the Dexmedetomidine + propofol anesthetized group (D group), the propofol-anesthetized group (P group), or the blank control group (C group). Each group had 12 rats.

Rat model establishment

All the rats were catheterized for infusion via the caudal vein before surgery. Rats in the P group were induced with an intravenous infusion of propofol at 19 mg/kg (Xi'an Libang Pharmaceutical Co., Ltd., China), followed by a propofol infusion with an infusion pump at 54 mg/kg/hour for 4 hours. The rats in D group were induced with an intravenous infusion of propofol at 19 mg/kg, and administered with concomitant dextromethoridine using an infusion pump at a loading dose of 10 μg/kg within one minute (Jiangsu Enhua Pharmaceutical Co., Ltd., China), followed by a propofol infusion via an infusion pump at 54 mg/kg per hour and a concomitant dextromethoridine infusion at 10 μg/kg per hour for 4 hours. The rats in C group received no treatment. Subsequently, the rats in D and P groups underwent exploratory laparotomy under anesthesia. The surgery was completed 2 hours after exploratory laparotomy, and then propofol or dextromethomidine were administered for 2 hours.

Outcome measures

Observation of the intraoperative vital signs in rats: The heart rates (HR), mean arterial pressures (MAP), and respiration rates (RR) of the

rats were measured with a monitor (Shanghai Yuyan Instruments Co., Ltd., China) during the surgery, and the measurements of the rats were recorded before the surgery, at 0 h, 1 h and 3 h after the induction, at the completion of anesthesia, and upon waking, respectively. The dead rats were calculated as invalid cases.

Morris water maze experiment: The Morris water maze experiment was used to assess the rats' memory and spatial learning abilities. A Morris water maze experiment consists of two parts: place navigation and spatial probe. In the place navigation test, the rats started the water maze training 3 days before surgery, but no training was conducted on the day of the surgery; after surgery, the training was resumed for 5 consecutive days. The rats were trained at fixed times 3 times a day. The average values of the tests were taken. The results of the test were recorded on the 1st day before the surgery, and the 1st, 3rd and 5th days after surgery, respectively. The rats entered the water maze from any of the four entry points with different patterns, and the time taken by each rat to find and climb onto the hidden platform (the escape latency) was observed and recorded. If a rat did not succeed in finding the platform within 60 s, they were allowed to be guided to the platform and rest on it for 10 s; then its escape latency was recorded as 60 s. A shorter escape latency indicates a better memory ability. In the spatial probe test, on the 6th day after surgery, after the removal of the platform in the pool, the rats were trained as described previously. The time spent in the target quadrant within 1 min and the times of crossing the exact position of the former platform, i.e., the number of times crossing the platform, were observed and recorded. A longer time spent in the target quadrant and a higher number of times crossing the platform suggest better abilities of learning and memory [20, 21].

Measurement by the enzyme linked immunosorbent assay (ELISA): After the completion of the spatial probe test, the rats were sacrificed on the 6th day after surgery, and serum and hippocampal tissues were collected. The ELISA kits (Shanghai Senxiong Biotech, China) were used to determine the expressions of TGF-β1, Smad3 and Smad7 in the rats' serum and hippocampi.

Statistical analysis

The data from all the experiments were analyzed and plotted using SPSS, version 23.0 (IBM, USA) and GraphPad Prism 5 (GraphPad, USA). The measurement data were expressed as the mean ± standard deviation. The comparisons among the three groups were conducted using one-way analyses of variance (ANOVA), while the comparisons between two groups were made using Bonferroni posttests. A value of $P<0.05$ was considered statistically significant.

Results

Comparison of the intraoperative parameters in the rats

There were no significant differences in HR, RR, and MAP among the D, P and C groups before surgery (all P>0.05). After the induction of anesthesia, lower HR, RR and MAP were observed in both the D and P groups, but no significant differences in the RR and MAP were found (P>0.05); HR was lowered more significantly in the D group than it was in P group (P<0.05); however, the HR, RR and MAP did not differ significantly between the two groups one hour after the induction of anesthesia (P>0.05). One rat in P group died, and it was calculated as an invalid case. No deaths occurred among the rest of the rats (**Table 1**).

Escape latency of the rats in the water maze experiment

No significant differences were observed in the escape latency among the three groups before surgery (P>0.05). The escape latencies in D and P groups were significantly longer on the 1st day after surgery than they were before the surgery, and also longer than that of C group on the 1st day after surgery (all P<0.05); the escape latency of D group on the 1st day after surgery was significantly shorter than that of P group (P<0.05; **Figure 1**).

Time the rats spent in the target quadrant in the water maze experiment

The time spent in the target quadrant was significantly shorter in D and P groups than in C group (P<0.05), but the time spent in the target quadrant was considerably prolonged in D group compared to P group (P<0.05; **Figure 2**).

Table 1. Comparison of the intraoperative vital signs of the three groups in rats

Vital signs	Groups	Pre-operation	After induction	One hour after induction	There hours after induction	End of anesthesia	After waking up
HR (times/scores)	D group	361.6±36.34	295.5±32.7$^{\#\#,*,\&\&}$	328.2±32.56	327.6±33.8	333.2±26.9	354.4±33.7
	P group	358.7±37.25	326.1±29.8$^{\#,\&}$	336.8±28.7	331.9±30.7	329.5±29.8	361.1±32.3
	C group	349.2±40.6	352.5±39.5	354.8±36.1	349.8±38.2	351.1±38.7	350.6±39.7
RR (times/scores)	D group	85.3±5.8	76.8±6.1$^{\#,\&}$	77.4±5.8	76.4±6.6	78.6±6.9	83.2±6.5
	P group	84.6±6.3	77.3±6.5$^{\#,\&}$	79.2±4.9	77.3±5.2	77.5±5.8	85.5±4.8
	C group	87.5±5.2	86.9±5.9	85.6±6.1	87.1±5.3	86.4±5.6	84.1±6.2
MAP (times/scores)	D group	118.2±8.7	96.7±7.2$^{\#,\&}$	99.3±6.1	98.9±6.4	102.3±6.1	120.5±7.7
	P group	117.9±7.6	98.5±6.3$^{\#,\&}$	97.5±7.8	98.6±7.2	99.5±7.3	116.7±7.9
	C group	120.3±7.2	117.2±7.1	121.4±6.8	116.8±5.6	117.4±6.5	118.9±8.2

Note: $^{\#}$P<0.05, $^{\#\#}$P<0.01, compared with C group; *P<0.05, compared with P group; $^{\&}$P<0.05, $^{\&\&}$P<0.01, compared with pre-operation. HR: heart rate; MAP: mean arterial pressure; RR: respiration rate.

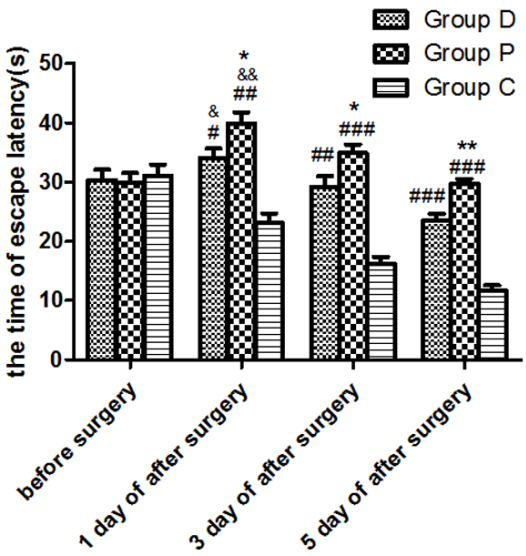

Figure 1. Comparison of the rats' escape latency in each group. $^{\&}$P<0.05, $^{\&\&}$P<0.01, compared with pre-operation; *P<0.05, **P<0.01, compared with D group; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, compared with C group.

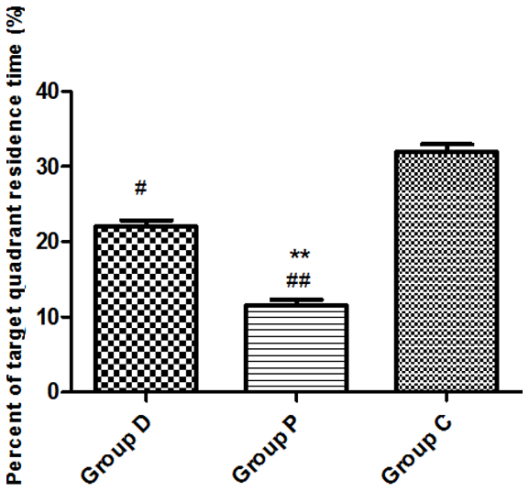

Figure 2. Comparison of the time the rats spent in the target quadrant in each group. $^{\#}$P<0.05, $^{\#\#}$P<0.01, compared with C group; **P<0.01, compared with D group.

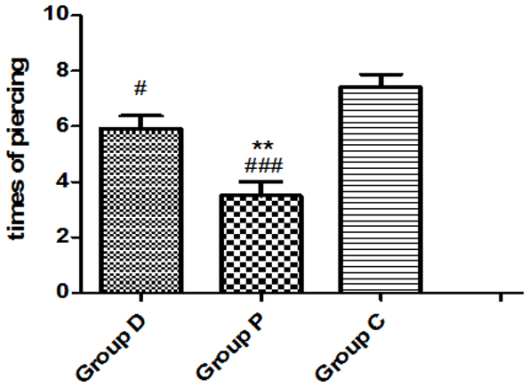

Figure 3. The perforation times of the rats in each group were compared. $^{\#}$P<0.05, $^{\#\#\#}$P<0.001, compared with C group; **P<0.01, compared with D group.

The number of time the rats crossed the platform in the water maze experiment

Compared with C group, the number of times crossing the platform by the D and P group rats were significantly reduced (P<0.05); however, compared to P group, the number of times crossing the platform in D group was significantly increased (P<0.05; **Figure 3**).

The expressions of TGF-β1, Smad3, and Smad7 in the rats' serum and hippocampal tissues

The levels of TGF-β1, Smad3, and Smad7 in the rats' serum and hippocampal tissues were significantly elevated in both D and P groups com-

Table 2. The expressions of TGF-β1, Smad3, and Smad7 in the rats' serum

Grouping	D group	P group	C group
TGF-β1 (ng/mL)	67.7±5.8$^{\#}$	89.5±7.2$^{\#\#\#,**}$	54.3±6.4
Smad3 (ng/mL)	44.3±6.9$^{\#}$	52.8±7.1$^{\#\#,*}$	35.4±6.2
Smad7 (ng/mL)	30.2±4.9$^{\#}$	39.7±5.1$^{\#\#,*}$	25.5±4.3

Note: $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, compared with C group; *P<0.05, **P<0.01, compared with D group. TGF-β1: Transforming growth factor β-1.

Table 3. The expressions of TGF-β1, Smad3, and Smad7 in the rats' hippocampal tissues

Grouping	D group	P group	C group
TGF-β1 (ng/mL)	82.4±6.8$^{\#}$	95.8±8.3$^{\#\#,*}$	74.5±7.7
Smad3 (ng/mL)	62.3±7.1$^{\#}$	75.1±7.9$^{\#\#\#,*}$	55.6±6.7
Smad7 (ng/mL)	46.2±5.8$^{\#}$	55.1±6.3$^{\#\#,*}$	38.2±4.9

Note: $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, compared with C group; *P<0.05, compared with D group. TGF-β1: transforming growth factor β-1.

pared to C group (all P<0.05), but the levels in D group were considerably lower than the levels in P group (P<0.05; **Tables 2** and **3**).

Discussion

POCD is a mental symptom prevalent in elderly patients after surgery or anesthesia, and there are no clear and effective ways to avoid the presence of POCD [22]. Aibeining (Dexmedetomidine Hydrochloride Injection) is a newly-developed drug used for sedation and analgesia in recent years. The drug has been widely used in clinical practice, and it is especially indicated for patients who need long-term sedation and analgesia. It is also indicated for elderly patients and has minor side effects. Previous studies have confirmed that Aibeining reduces the incidence of POCD, but its mechanisms of action are not clear. In the present study, dexmedetomidine was further confirmed to reduce the incidence of POCD, and the possible mechanisms of action were also discussed.

The results of the present study show that learning and memory abilities were reduced in rats undergoing exploratory laparotomy under anesthesia with propofol alone. In the water maze experiment, on the 1st day after surgery, the rats' escape latencies were significantly prolonged compared to before the surgery, with substantially longer escape latencies in the D and P groups than in the C group; the time spent in the target quadrant was significantly shorter, and the number of times crossing the platform was also significantly fewer. After addition of dexmedetomidine to propofol anesthesia, although the rats had prolonged escape latency, shortened time spent in the target quadrant and a reduced number of times of crossing the platform than before surgery and C group, the dexmedetomidine-anesthetized rats had shorter escape latency, longer time spent in the target quadrant and a higher number of times crossing the platform than those without dexmedetomidine anesthesia. Moreover, such trend of improvement (shortened escape latency, prolonged time spent in the target quadrant and an increased number of times crossing the platform) was still observed in the dexmedetomidine-anesthetized rats on the 3rd and 5th days after surgery, suggesting that dexmedetomidine definitely alleviates the rats' decrease in postoperative memory and learning abilities caused by propofol anesthesia and surgery. It is speculated that dexmedetomidine can reduce the incidence of POCD. The TGF-β/Smad signal pathway is involved in multiple biological processes in the body, and the proteins (TGF-β, Smad3 and Smad7) are the essential proteins for this pathway to be involved in biological processes [23]. The TGF-β/Smad signal pathway has been shown to play a decisive role in the cognitive dysfunction associated with diabetes mellitus. TGF-β, Smad3 and Smad7 are primarily expressed in astrocytes in the hippocampal tissues, and the results of testing the hippocampal tissues reveal that the protein levels are significantly higher than those of the control group [9]. Therefore, we speculate that the TGF-β/Smad pathway may also be involved in the presence and development of POCD. The results of the current study indicate that rats undergoing exploratory laparotomy under anesthesia with propofol alone had significantly elevated levels of TGF-β, Smad3 and Smad7 compared with those in the control group. This demonstrates that it might activate the TGF-β/Smad signal pathway. However, the levels of TGF-β, Smad3 and Smad7 in the rats were significantly reduced after addition of dextromethomidine to propofol anesthesia, indicating that dexmedetomidine inhibits the TGF-β/Smad pathway. This has proven that dexmedetomidine relieves the decline in the memory and learning abilities and reduces the incidence of

POCD in rats by inhibiting the TGF-β/Smad pathway.

There are still many limitations in the present study. We only investigated the effect of dexmedetomidine on rats at 5 days after surgery, and we only found that dexmedetomidine reduces the incidence of POCD in a short period of time. However, POCD lasts for several months after surgery. We failed to explore the long-term effect of dexmedetomidine after its application. Therefore, more clinical trials and follow-ups are needed in future research.

In conclusion, dexmedetomidine improves POCD in rats undergoing exploratory laparotomy under propofol anesthesia, an improvement that might be achieved by the activation of the TGF-β/Smad pathway.

Disclosure of conflict of interest

None.

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