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Effect of anesthesia on cognitive status and MMP-2 expression in rats

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ABSTRACT

Objective: To investigate the effect of anesthesia on the cognitive status damage and MMP-2 expression in rats. **Methods:** A total of 120 healthy rats were selected and randomly divided into the control group, CF3-CH(OCH2F)-CF3 (Sevoflurane) group and CF3-CH2-O-CHF-CF3 group (Sevoflurane) ($n=40$). After training for 3 d by the Morris water maze, the control group were injected with fentanyl for analgesia, the CF3-CH(OCH2F)-CF3 group and the CF3-CH2-O-CHF-CF3 group were anesthesia with CF3-CH(OCH2F)-CF3 and CF3-CH2-O-CHF-CF3 on the basis of fentanyl, then rats in three groups underwent open surgery and suture conventional incision. Morris water maze was used to measure the rats' cognitive ability in three groups on the 1st d, 3rd d, 5th d and 7th d, and the brain tissue MMP-2 expression was detected. **Results:** After 1 d/7 d of the surgery, Morris water maze performance and MMP-2 expression were not significantly different among three groups ($P>0.05$); After 3 d/5 d of the surgery, compared with the control group, the Morris water maze test result was significantly worsened, MMP-2 expression levels were significantly increased ($P<0.05$); After 3 d/5 d of the surgery, compared with the CF3-CH2-O-CHF-CF3 group, Morris water maze test result of CF3-CH(OCH2F)-CF3 group was significantly worsened, MMP-2 expression levels were significantly increased ($P<0.05$). **Conclusions:** Anesthesia can cause some injury on cognitive status, different anesthetic drugs may cause different injury, and the cognitive status injury is related to the MMP-2 expression.

1. Introduction

Postoperative cognitive dysfunction (POCD) refers to the neurocognitive dysfunction caused by anesthesia, which is a common postoperative neurological complication. The clinical symptoms were that patients have personality changes in a few days to several weeks or months postoperative, with decreased memory, attention, social adaptability, cognitive ability and comprehension ability, even long-term cognition disorders, which severely affects patients' life quality[1]. Recently the pathogenesis of POCD is not very clear, but numerous studies showed that anesthesia

can suppress brain function temporality, and this effect is reversible[2]. This study selected adult rats as the research object to explore the impact of anesthesia on the cognitive status and matrix metalloproteinase-2 (MMP-2) expression.

2. Materials and methods

2.1. General information

A total of 120 healthy 14-month-old male rats weighing 346–382 g were selected. They were randomly divided into the control group, the CF3-CH(OCH2F)-CF3 (Sevoflurane) group and the CF3-CH2-O-CHF-CF3 group (Sevoflurane) ($n=40$).

2.2. Anesthesia methods

The control group were injected with fentanyl for

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analgesia, then underwent open operation 20 min later. On the basis of fentanyl, self-made closed circulating organic glass box was used in the CF3-CH(OCH2F)-CF3 (Sevoflurane) group and the CF3-CH2-O-CHF-CF3 group (Sevoflurane), each with one hole on both sides in the box. One hole was connected to the Draeger fabius anesthesia machine (Draeger produced), and the other hole was connected to F-MCI type gas monitors (USA DateOhmeda produced). Concentration of 3% CF3-CH(OCH2F)-CF3 (Sevoflurane) and the CF3-CH2-O-CHF-CF3 (Sevoflurane) was continuously imported respectively, at the speed of 1.5 L/min^[3]. After 10 min of sustained anesthesia, the depth of anesthesia with pain stimulation was assessed. The rats then underwent open surgery with 1 cm incision, suture conventional incision after 3 min. During surgery, the monitor was open to ensure the concentration of anesthetic gas^[4].

2.3. Morris water maze test

Before 1d of the experiment, all rats swam in the water maze in batches for three times, 2 min times to adapt to the water maze. Morris water maze was produced by Beijing Pusheng Science and Technology Ltd. The water maze was constituted of circular pool with 130 cm diameter and 50 cm height and cylindrical platform with 10 cm diameter and 29 cm height. The wall was marked with four cardinal points and the pool was divided into four quadrants. In exercise and experimental tests, the water depth was 30 cm and the temperature was (26±1.3)°C, the outer wall was pasted with reference object. Morris water maze test was performed after 1 d, 3 d, 5 d and 7 d of the operation. Tests included three parts: place navigation test, spatial probe test and visible platform task training, 10 rats were randomly selected in each group every day to participate in the experiment.

Place navigation test was as follows^[1]: A total of 30 rats in three groups were put into the water from four quadrants in sequence, the swim tracks of the rats were recorded as the latency (escape latency). If the rats did not climb the platform till 120 s, it was still counted as 120s^[5]. This test was carried out every morning and afternoon, average value was calculated on that day.

Spatial probe test was as follows^[2]: after the place navigation test, the platform was removed, the rats were put into the pool at the point and swam for 120 s. The times of

crossing the former platform was measured. The average value of the total number from the 4 points was counted as rats' individual score, the mean value of 10 individual scores was counted as the spatial probe test result on that day^[6,7].

Exercise test experiments^[8] was as follows: platform was removed, foreleg of the rats was used as the observed target, the times of two front leg drive in 1 min with a video camera was recorded.

2.4. MMP-2 expression of rat brain tissue

After the Morris water maze test, anesthesia method was performed the same as above. Then the neck was brokento obtain the brain tissue. Tissue was made into 10% homogenate with saline in batches, MMP-2 expression levels were detected by ELISA.

2.5. Data analysis

Excel 2007was used, data were expressed as mean±SD values and analyzed with Spss18.0 software then t test. Each rate was expressed by percentage and was compared by χ^2 test. Measurement data were compared with nonparametric test (*Z* test). *P*<0.05 was considered as statistical significant difference.

3. Results

3.1. Morris water maze test results

3.1.1 Rat escape latency

After 1 d/7 d of the surgery, three groups escape latency had no statistically significant difference(*P*> 0.05); After 3 d/5 d of the surgery, compared with the control group, the escape latency of the CF3-CH2-O-CHF-CF3 group and the CF3-CH(OCH2F)-CF3 group were significantly increased (*P*<0.05); After 3 d/5 d of the surgery, compared with the CF3-CH2-O-CHF-CF3 group, the escape latency of the CF3-CH(OCH2F)-CF3 group was significantly decreased (*P*<0.05) (Table 1).

3.1.2. Times of rats crossed the location of platform

After 1 d/7 d of the surgery, the times of rats crossed the

Table 1

Comparison of the escape latency of rats in three groups (mean±SD, s).

| Groups | 1 d | 3 d | 5 d | 7 d |
|-------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| Control group | 96.57±13.86 | 52.44±10.17 | 29.69±6.25 | 18.36±4.91 |
| CF3-CH(OCH2F)-CF3 group | 99.24±15.72 ^{ab} | 74.73±12.54 ^{ab} | 36.96±7.75 ^{ab} | 19.87±5.14 ^{ab} |
| CF3-CH2-O-CHF-CF3 group | 100.95±15.48 ^a | 81.49±13.22 ^a | 42.03±8.96 ^a | 20.05±4.88 ^a |

Note: Compared with the control group, ^a*P*<0.05; compared with the CF3-CH2-O-CHF-CF3(sevoflurane) group, ^b*P*<0.05.

location of platform in three groups had no statistically significant difference ($P>0.05$); After 3 d/5 d of the surgery, compared with the control group, the times of rats crossed the location of platform of the CF3–CH2–O–CHF–CF3 group and the CF3–CH(OCH2F)–CF3 group were significantly decreased ($P<0.05$); After 3 d/5 d of the surgery, compared with the CF3–CH2–O–CHF–CF3 group, the escape latency of the CF3–CH(OCH2F)–CF3 group was significantly increased ($P<0.05$) (Table 2).

3.1.3. Exercise test experiments of the rats

The times of two front leg drive of rats in three groups were (158.69±25.41) times, (161.36±25.82) times and (159.80±25.35) times respectively, the difference was not statistically significant ($P>0.05$).

3.2. MMP-2 expression of rat brain tissues:

After 1 d/7 d of the surgery, the difference in MMP-2 expressions of three groups were not significant ($P>0.05$); After 3 d/5 d of the surgery, compared with the control group, the MMP-2 expression levels of the CF3–CH2–O–CHF–CF3 group and the CF3–CH(OCH2F)–CF3 group were significantly increased ($P<0.05$); After 3 d/5 d of the surgery, compared with the CF3–CH2–O–CHF–CF3 group, the MMP-2 expression levels of the CF3–CH(OCH2F)–CF3 group was significantly increased ($P<0.05$) (Table 3).

4. Discussion

POCD is the central nervous system complication postanesthetic, which occurs in adult and elderly patients. This disorder may persist for a long time and have negative influence on the rehabilitation and quality of life in patients. The currently foreign researches showed that in elderly patients, the probability of POCD after 7 d of general

anesthesia non-cardiac surgery is higher than 25%^[9], the probability of occurrence of POCD after three months is higher than 9%; in adult patients, the probability of POCD after 7 d of general anesthesia abdominal operation or plastic surgery is higher than 19%, the probability of occurrence of POCD after 3 months more than 6%^[10]. The pathogenesis of POCD is not yet clear, but a lot of studies have shown that it related to perioperative anesthesia. Results of correlation study in China such as Wu *et al*^[11] that surgery anesthetic (particularly inhaled anesthetics) will inhibit the cholinergic system and damage the cognitive status. The results of Egi *et al*^[12], Jing *et al*^[13] and Ni *et al*^[14] showed that the damage of cognitive status is related to the amount of anesthetic and the duration, and the damage of cognitive status is positively correlated to the amount of anesthetic, while Ni *et al* believe that different anesthetics can cause different degrees of cognitive status damage^[14].

The brain stem reticular activating system now considered to be maintain the excitement of the cerebral cortex, so that the body can in a waking state and with clear conscience. Its dysfunction and tissue damage is the important mechanism of cognition disorders. Rats' brain tissue structure is similar with human brain tissue, and the hippocampus is an important part for general anesthesia^[15]. Hippocampus can receive visual, auditory, tactile, pain and other information by brain stem reticular formation and subcutaneous fibrous. MMPs are a class of proteolytic enzyme with similar composition to amino acid and depend on zinc ion. Recently it is found that MMPs involved in tissue damages of aortic aneurysm, rheumatoid arthritis, gastric ulcer, myocardial disease and fibrotic lung disease and the nervous system infections and other diseases. MMPs entry into cellular and finished endocytosis, then participate in the proteolytic cleavage at cytoplasm and organelle. MMP-2 is particularly active in the brain tissue of the rat with transient focal cerebral ischemia, and directly lead to neuronal apoptosis and brain damage. MMP-2 has high expression in cerebral

Table 2

Comparison of times of rats crossed the location of platform in three groups (mean±SD).

| Groups | 1 d(times) | 3 d(times) | 5 d(times) | 7 d(times) |
|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| Control group | 8.65±2.38 | 11.04±3.56 | 14.32±4.64 | 16.30±5.65 |
| CF3–CH(OCH2F)–CF3 group | 8.47±2.35 ^{ab} | 10.23±2.63 ^{ab} | 12.56±3.13 ^{ab} | 16.27±5.30 ^{ab} |
| CF3–CH2–O–CHF–CF3 group | 8.52±2.40 ^a | 9.17±2.34 ^a | 10.85±2.93 ^a | 15.94±4.97 ^a |

Note: Compared with the control group, ^a $P<0.05$; compared with the CF3–CH2–O–CHF–CF3(sevoflurane) group, ^b $P<0.05$.

Table 3

Comparison of MMP-2 expression of rat brain tissues in three groups(mean±SD, mg/L)

| Groups | 1 d | 3 d | 5 d | 7 d |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Control group | 2.76±0.48 | 2.82±0.51 | 2.83±0.50 | 2.85±0.53 |
| CF3–CH(OCH2F)–CF3 group | 2.82±0.55 ^{ab} | 3.95±0.78 ^{ab} | 4.68±0.84 ^{ab} | 2.83±0.52 ^{ab} |
| CF3–CH2–O–CHF–CF3 group | 2.80±0.53 ^a | 4.27±0.86 ^a | 4.95±0.92 ^a | 2.79±0.56 ^a |

Note: Compared with the control group, ^a $P<0.05$; compared with the CF3–CH2–O–CHF–CF3(sevoflurane) group, ^b $P<0.05$.

ischemia, brain injury, hemodynamic changes, inflammation and so on, while MMP-2 has a low expression in normal rat brain. Kim *et al*^[16] and others considered that MMP-2 may be related to the recovery after ischemic injury, which is closed to the results of Istaphanous *et al*^[17].

In this study, after 1 d/7 d of the surgery, three groups Morris water maze performance and MMP-2 expression were not significantly different, the difference had no statistically significant ($P>0.05$), which is consistent with the results of Li *et al*^[8]. After 3 d/5 d of the surgery, compared with the control group, the Morris water maze test of the sevoflurane groups were significantly decreased, these differences have statistically significant ($P<0.05$). This is consistent with the results of Tang *et al*^[18], Lu *et al*^[9]. MMP-2 expression levels of the sevoflurane groups were significantly increased, which is consistent with the results of Kim *et al*^[16] and Istaphanous *et al*^[17]. After 3 d/5 d of the surgery, compared with the CF3-CH2-O-CHF-CF3 group, the CF3-CH(OCH2F)-CF3 Morris water maze test was significantly decreased, MMP-2 expression levels were significantly increased, these differences were statistically significant ($P<0.05$). This is consistent with results of Li *et al*^[4]. The exercise test experiments of the rats in three groups had no significant difference ($P>0.05$), which indicated that the escape latency is not related to the exercise ability.

In summary, anesthesia can cause some damage on the cognitive status, different anesthetic drugs may cause different damages, and the cognitive status injury is related to the MMP-2 expression. But there are lots of different anesthetic drugs and usually combined application in clinical, we will do further research on the cognitive status injury of the combined application of different anesthetics.

Conflict of interest statement

We declare that we have no conflict of interest.

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