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Effect of different anesthesia methods on erythrocyte immune function in mice

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ABSTRACT

Objective: To explore effect of different anesthesia methods and different anesthetics on erythrocyte immune function in mice. **Methods:** The mice were anesthetized by isoflurane and ether inhalation, and also under intraperitoneal anesthesia with sodium pentobarbital and chloral hydrate. Blood was collected from the ventro-cardinal vein. Automatic blood cell analyzer was used for routine blood examination, and the canthine oxidase method was used to measure the superoxide dismutase (SOD) activity. Lipid peroxidation product malondialdehyde (MDA) was measured with TBA, and glutathione peroxidase (GSH-Px) was measured with DTNB, and then the effect of different anesthesia methods and different anesthetics on erythrocyte immune function in mice was observed. **Results:** Hct level of chloral hydrate intraperitoneal injection group was significantly higher than the other three groups ($P<0.05$). And the MDA levels in the pentobarbital sodium group were significantly higher than the other three groups ($P<0.05$). SOD and GSH-Px of the chloral hydrate and sodium pentobarbital intraperitoneal injection group were significantly lower than the other two groups; RBC-C 3bRR and RBC-ICR of the chloral hydrate and sodium pentobarbital intraperitoneal injection group were significantly lower than the other two groups. **Conclusions:** Different drugs can induce changes in immune function of mice at different levels. Isoflurane and ether have less damage to animal body, while chloral hydrate and sodium pentobarbital intraperitoneal injection have a certain inhibitory effect on the animal body respiratory system and can cause greater damage to the body. Therefore, the reasonable selection and control of anesthetics are very important in order to avoid the experimental errors caused by anesthesia.

1. Introduction

In recent years, more and more evidence in the research area of laboratory animal suggests different experimental methods can cause animal stress response. If there is no timely control or intervention, that will cause negative effects on the body and then affect the scientific results[1]. Therefore, this experiment aims to study the effect of different anesthesia methods and different anesthetics on immune function in mice, provide an experimental basis and reference for the animal experiments and anesthesia method.

2. Materials and methods

2.1. Animals and reagents

A total of 80 SPF experimental mice, male, 4 week old,

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weighting 19-25 g [average (22±2) g], were purchased from the Experimental Animal Center of Medicine. Isoflurane, 100 mL/bottle, ether, 500 mL/bottle, chloral hydrate, 250 g/bottle, sodium pentobarbital, 25 g/bottle were all purchased from Shanghai WuLian Chemical Reagent procurement and supply chemical works. Blood cell automatic analysis machinery was purchased from NOVA Corporation in the United States. MDA test kit, GSH-Px and MDA test kits were all purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd.

2.2. Grouping and anesthetic methods

The animals were divided into 4 groups according to different methods of anesthesia: isoflurane anesthesia group (group A), ether inhalation anesthesia group (group B), chloral hydrate intraperitoneal injection group (group C) and sodium pentobarbital intraperitoneal injection group (group D) (n=20). Mice in Group A were treated with 2% to 3% volume fraction isoflurane inhalation anesthesia for 3-4 min. Mice in Group B were treated with 2% to 3% volume fraction ether inhalation anesthesia for 3-4 min, Group C were treated with intraperitoneal injection of 10% chloral hydrate anesthesia according to the mice weight at 5 mL/kg, and Group D were treated with intraperitoneal injection of 3% sodium pentobarbital anesthesia according to the mice weight at 1.5 mL/kg. They were disinfected abdominally, had 2 cm longitudinal incision by transrectal incision. Then the abdomen was cut open to expose abdominal veins. 0.6 mL blood were collected into tubes containing EDTA.

2.3. Detection index

Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), mean corpuscular volume (MCV), red blood cell volume distribution width (RDW), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were all detected by the blood cell automatic analysis machinery. Xanthine oxidase method was used to measure the superoxide dismutase (SOD) activity. Lipid peroxidation product malondialdehyde (MDA) was measured with TBA, and glutathione peroxidase (GSH-Px) with DTNB. According to the erythrocyte C3b receptor and the immune complex rosette test by Guo *et al*[2], two or more RBC was adhered to zymosan as a garland. Evenly 200 RBC was counted and the rate of erythrocyte C3b receptor rosette and immune complexes was calculated.

2.4. Statistical analysis

The data were expressed as mean±SD values, all data were analyzed by SPSS 13.0 statistics software, One-Way ANOVA was applied in the comparison between two groups, with a significance level of P<0.05.

3. Results

3.1. Effect of different anesthesia methods on mouse erythrocytes morphology and parameter changes

Hct level of group C were significantly higher than group A, B, D. The differences in RBC, Hb, RDW, MCV, MCH and MCHC among 4 groups were not significant (P>0.05) (Table 1).

Table 1

Mouse erythrocytes parameter test results in the experimental groups.

Indexes	Group A	Group B	Group C	Group D
RBC(×10^{12}/L)	7.5±1.4	7.6±1.7	7.5±1.5	7.3±1.7
Hb (g/L)	107.8±17.2	108.9±18.6	110.3±17.3	106.4±16.9
Hct (%)	38.5±2.3	39.0±1.8	41.2±2.0$^{*△}$	37.8±2.5
RDW (%)	9.3±1.3	9.6±1.4	9.5±1.6	9.6±1.5
MCV (fL)	44.7±4.3	44.7±4.3	44.7±4.3	45.8±5.7
MCH (pg)	14.2±1.3	14.5±1.1	14.7±1.6	14.4±1.9
MCHC (g/L)	335.7±34.9	337.9±33.8	338.5±32.6	331.2±31.8

Note: Compared with group A (isoflurane anesthesia group), * P<0.05; compared with group B (ether inhalation anesthesia group), $^{△}$ P<0.05.

3.2. Effect of different anesthesia methods on erythrocyte SOD activity, MDA content and whole blood GSH-Px activity to mice

The difference in erythrocyte SOD activity, MDA content and whole blood GSH-Px activity between group A and group B was not significant (P>0.05). SOD and GSH-Px of group C and group D were significantly lower than group A and group B (P<0.05). The erythrocytes MDA in group D was significantly lower than group A, B, C, the difference had statistically significant (P<0.05) (Table 2).

Table 2

Comparison of SOD activity, MDA content and whole blood GSH-Px activity of mice in different anesthetized groups.

Group	SOD (U/gHb)	MDA (nmol/mgHb)	GSH-Px (U)
A Group	11 298.5±1542.2	0.5±0.1	1 132.7±174.5
B Group	10 889.2±1336.8	0.5±0.1	1 086.5±163.2
C Group	9 362.4±1263.4$^{*△}$	0.6±0.1	867.2±84.6$^{*△}$
D Group	8 638.3±1167.5$^{*△}$	0.7±0.2$^{*△▲}$	844.9±69.7$^{*△}$

Note: Compared with group A (isoflurane anesthesia group), * P<0.05; compared with group B (ether inhalation anesthesia group), $^{△}$ P<0.05; compared with group C (chloral hydrate intraperitoneal injection group), $^{▲}$$P$ <0.05.

3.3. Effect of different anesthesia methods on mouse erythrocytes-C 3bR and RBC-ICR

The difference in RBC-C 3bRR and erythrocyte-ICR were not significant ($P>0.05$). The erythrocyte 3bRR and erythrocyte-ICR of group C were significantly lower than group A, B ($P<0.05$).The erythrocytes-C 3bRR and erythrocyte-ICR were significantly lower than group A, B, C ($P<0.05$).

Table 3

Comparison of the erythrocytes C3b receptor rosette rate and red blood cell immune complex rosette rate in different anesthetic experimental groups.

Groups	*n*	Erythrocytes -C 3bR (%)	Erythrocytes -ICR (%)
Group A	40	15.5±2.3	25.7±6.8
Group B	40	14.9±2.1	24.8±6.3
Group C	40	12.1±2.2$^{*\triangle}$	21.6±5.9$^{*\triangle}$
Group D	40	8.2±1.7$^{*\triangle\blacktriangle}$	17.9±5.6$^{*\triangle\blacktriangle}$

Note: Compared with group A (isoflurane anesthesia group), $^{*}P<0.05$; compared with group B (ether inhalation anesthesia group), $^{\triangle}$ $P<0.05$; compared with group C (chloral hydrate intraperitoneal injection group), $^{\blacktriangle}$ $P<0.05$.

4. Discussion

Erythrocyte has immune function, which includes recognition, adhesion, anti-antigen, clear immune complexes and participate in controlling immunity. Erythrocyte also has its own complete self-regulating control system, which provide the harmful substances to specific immune system and guide it to clear in an appropriate manner, thus improve the body´s immune function. The change of erythrocyte number and morphological parameters are the important factors which can impact the immune fuction. Anesthesia can not only relieve pain but also affect the body´s immune function[3-6]. Accordingly, this study investigate whether the influence of different experimental methods can cause the different animal´s body stress response.

The experimental results show that compared with isoflurane anesthesia group (group A), ether inhalation anesthesia group (group B) and sodium pentobarbital intraperitoneal injection group (group D), the Hct level in chloral hydrate intraperitoneal injection group (group C) has the highest percentage. Therefore, we hypothesized that the reasons may as follows: Compared with the inhalation anesthesia,the intravenous anesthesia is deeper,because the adaptive changes of plasma volume can induced the relative increasion of Hct levels; The respiration of mice was inhibited after deeply anesthetize. Wang *et al*[7] think that the inhalation anesthetics can maintain good tissue oxygen supply by the study of different methods of anesthesia, while the injectable anesthetic drugs can affect the tissue oxygen supply then induce cell damage. Some studies consider that the SOD activity, MDA content and GSH-Px activity of whole blood were important indicators which indirectly determine the tissue oxidative damage and lipid peroxidation[8-10]. In this study, the MDA content increased significantly of mice injected with chloral hydrate and sodium pentobarbital, while the SOD and GSH-Px were significantly lower. So we think as injections hypnotics, it is difficult to effectively control the amount and depth of anesthesia during the process of chloral hydrate and sodium pentobarbital injection, which easily lead to overdose of anesthetic, thus affect respiratory and cause the damage of cells and tissues. So it will cause smaller influence to the vital signs of mice if we use the inhalation anesthetics during the experiment, which can also maintain the experimental results effectively[11-16].

Traditional studies suggest that red blood cells only participate respiratory function, but in recent years it is found that red blood cells not only have respiratory function, but also have immune function[17]. This study also found that the erythrocytes-C3bR and erythrocyte-ICR in the chloral hydrate intraperitoneal injection group were significantly lower than the inhalation group, and the erythrocytes-C3bR and erythrocyte-ICR in the sodium pentobarbital intraperitoneal injection group were even lower than the chloral hydrate intraperitoneal injection group. Some studies showed that erythrocytes-C3bR and erythrocyte-ICR were commonly indicators to evaluate the immune function. So this study showed that as injections hypnotics, although the chloral hydrate and sodium pentobarbital can maintain the shape of red blood cells, it will affect the oxygenation state of RBC and form a relatively hypoxic state. Compared the two injections hypnotics, pentobarbital sodium has a greater impact on the erythrocyte oxygenation. The main mechanism may be that the immune function of RBC was dominated by the activity of red cell surface C3b receptor and the ability of receptor binding immune complexes. The injection anesthetics have greater impact on the activity of surface receptor of red blood cell membrane, thereby affecting the immune function. While the inhalational anesthetics have less impact on the activity of surface receptor of red blood cell membrane, so that the immune function is relatively stable[18-25].

In summary, the injection anesthetic can cause the damage abnormal changes in red blood cell and abnormal changes of the immune function parameters. So when we conduct animal experiments, we have to consider the impact of different anesthesia methods on the project after we take a good control of the various factors.

Conflict of interest statement

We declare that we have no conflict of interest.

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