European Review for Medical and Pharmacological Sciences 2019; 23: 4980-4987

Metformin with propofol enhances the scavenging ability of free radicals and inhibits lipid peroxidation in mice

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effects of metformin on anesthetic effect and anti-oxidative capacity in mice anesthetized with propofol.

MATERIALS AND METHODS: A total of 150 C57BL/6 mice were randomly assigned into the compatibility of an equivalent threshold dose of metformin with propofol group, and compatibility of different doses of metformin with 80 mg/kg propofol group, with 10 subgroups and 15 mice in each. Metformin and propofol were intraperitoneally injected in mice. The induction time of anesthesia in each mouse was recorded. 1 min after the disappearance of righting reflex, serum samples, and brain tissues were harvested, respectively. Subsequently, the contents of superoxide dismutase (SOD), malondialdehyde (MDA), and lactate dehydrogenase (LDH) in serum and brain homogenate of each group were measured. Furthermore, the protein expressions of nuclear factor-kappaB (NF-κB) and Nuclear erythroid 2-related factor 2 (Nrf2) were analyzed by Western blot.

RESULTS: Metformin supplementation did not influence the induction time of propofol anesthesia in mice, while the dose of propofol was significantly decreased. Besides, no significant correlation was found between an-esthesia induction time and the dose of metformin. Meanwhile, a certain dose of metformin could markedly increase the SOD activity in mouse brain tissues, whereas it could decrease the serum levels of MDA and LDH. In addition, metformin could remarkably inhibit the NF-κB activity and promote the Nrf2 expression.

CONCLUSIONS: Metformin improves the anesthetic effect of a single dose or continuous intraperitoneal injection of propofol in mice. The compatibility of a certain dose of metformin with propofol can enhance the scavenging ability of free radicals and their metabolites. Furthermore, this inhibits lipid peroxidation in mice via NF-κB inhibition and Nrf2 activation.

Key Words:
Propofol, Metformin, Anesthesia, Oxidative stress.

Introduction

Propofol is a short-acting, non-accumulative alkylphenol intravenous anesthetic[1-3], which is widely used in clinical practice[4]. Propofol is not only used in a single dose, but also in continuous or daily administration for a long time. This may remarkably increase the incidence of adverse reactions[5,6]. Studies have shown that propofol has an inhibitory effect on cardiovascular in a dose-dependent manner. More seriously, propofol anesthesia may inhibit the respiratory center and weaken the respiratory amplitude, eventually leading to airway obstruction[7].

Imbalance of cellular redox state in the process of metabolism will result in an oxyradical chain reaction. Such dysfunction further causes damaged biomembrane and its functions. Eventually, it leads to lipid peroxidation and reactive oxygen species (ROS) accumulation[8,9]. The main function of superoxide dismutase (SOD) is to remove superoxide anion free radicals and protect the body from damage induced by oxygen free radicals. Previous researches have demonstrated that the level of SOD indirectly reflects the scavenging ability of ROS[10]. Malondialdehyde (MDA) is a metabolite produced by lipid peroxidation of cell membranes. An elevated level of MDA reflects the imbalance of oxidation and antioxidant homeostasis[11]. Meanwhile, lactate dehydrogenase (LDH) is a kind of cytoplasmic enzyme. When the cell membrane is damaged or the cell membrane permeability is increased

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due to external stimuli, a large amount of cytoplasmic LDH may be released[12].

Nuclear factor-kappaB (NF-κB) is a dimer composed of the Rel protein family[13,14]. NF-κB is widely distributed in eukaryotic cells, which is also an important transcriptional regulator in cells. NF-κB can be activated by multiple stimulatory factors, thereby regulating the expressions of various genes involved in oxidative stress and inflammatory response. Reports have indicated that oxidative stress is an important activator of NF-Kb[15]. Nuclear erythroid 2-related factor 2 (Nrf2) is a member of the Cap-n-collar (CNC) transcription factor family, which acts as a key transcription factor regulating cell resistance to external stimuli. Furthermore, Nrf2 regulates the transcription and expressions of antioxidant enzymes by interacting with antioxidant response elements (ARE)[16,17].

Metformin is the first-line hypoglycemic agent for patients with type 2 diabetes[16,18]. Currently, the specific role of metformin in regulating the anesthetic effect of propofol remains unclear. In this work, metformin and propofol were intraperitoneally injected in mice for a single dose or continuous administration. The anesthesia effect of each mouse was observed and recorded. Meanwhile, the contents of SOD, MDA, and LDH in serum and brain tissues of mice were detected for evaluating the antioxidant capacity of metformin. The aim of our study was to provide a theoretical basis for metformin as an adjuvant drug to enhance the anesthetic effect of propofol.

Materials and Methods

Experimental Animals

A total of 150 male C57BL/6 mice in SPF (specific pathogen free) level (6-8-week-old, weighing 19-21 g) were used in this study. All mice were housed in an environment with a temperature of 21-25°C and humidity of 40-60%. Meanwhile, all mice were with free access to water and food. After 1 week of adaptive feeding, all mice were assigned into 2 groups with 5 subgroups in each. The two groups included compatibility of equivalent threshold dose of metformin with propofol group, and compatibility of different doses of metformin with 80 mg/kg propofol group. In compatibility of equivalent threshold dose of metformin with propofol group, mice were further divided into normal saline group (NS group), 80 mg/kg propofol (PPF 80 group), 70 mg/kg propofol + 50 mg/kg metformin (PPF 70 + Met 50 group), 55 mg/kg propofol + 100 mg/kg metformin (PPF 55 + Met 100 group), and 50 mg/kg propofol + 200 mg/kg metformin (PPF 50 + Met 200 group). In compatibility of different doses of metformin with 80 mg/kg propofol group, mice were further divided into normal saline group (NS group), 80 mg/kg propofol (PPF 80 group), 80 mg/kg propofol + 50 mg/kg metformin (PPF 80 + Met 50 group), 80 mg/kg propofol + 100 mg/kg metformin (PPF 80 + Met 100 group), and 80 mg/kg propofol + 200 mg/kg metformin (PPF 80 + Met 200 group). This study was approved by the Animal Ethics Committee of The First People's Hospital of Lianyungang Animal Center.

Establishment of Anesthetic Hypnotic Induction Model in C57BL/6 Mice

Loss of righting reflex (LORR) was the standard of hypnosis. Subsequently, anesthesia induction time and anesthesia maintenance time were recorded, respectively. Anesthesia induction time was defined as the duration from intraperitoneal injection of an anesthetic drug to the recovery time of righting reflex less than 2 times within 1 min. Meanwhile, anesthesia maintenance time was defined as the duration from LORR to the recovery time of righting reflex. All drugs were immediately mixed before their use. Mice were gently inspected after intraperitoneal injection, and LORR within 30 s was confirmed as successful anesthesia. Finally, anesthetic induction time was observed and recorded in each group except the NC group.

Sample Collection

Blood samples were taken from the right eyeball of each mouse after LORR. Subsequently, the brain tissues were collected after sacrifice. After that, blood samples were centrifuged at 4°C and 5000 rpm/min for 10 min. The upper serum was preserved in -80°C for subsequent experiments. After washing with normal saline, the brain tissues were preserved in liquid nitrogen.

SOD Determination

SOD was determined by using the total superoxide dismutase assay kit with WST-8 (Beyotime, Shanghai, China). Briefly, the samples were hemorrhaged and centrifuged at 4°C, followed by the collection of the supernatant. Subsequently,

the level of SOD at the wavelength of 450 nm was detected by using a microplate reader (Bio-Rad, Hercules, CA, USA). The protein concentration was detected according to the instructions of the BCA (bicinchoninic acid) kit (Beyotime, Shanghai, China).

MDA Determination

MDA determination was performed in strict accordance with the lipid peroxidation MDA assay kit (Beyotime, Shanghai, China). Samples were prepared as previously described. The level of MDA at the wavelength of 532 nm was measured by using a microplate reader.

LDH Determination

LDH determination was performed according to the instructions of the cytotoxicity LDH assay kit (Beyotime, Shanghai, China). Specific procedures were similar to those of MDA determination.

Western Blot

Total protein in treated cells was extracted by the radioimmunoprecipitation assay (RIPA) solution (Invitrogen, Carlsbad, CA, USA). The extracted protein samples were separated by electrophoresis on 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking with skimmed milk, the membranes were incubated with primary antibodies (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. On the next day, the membranes were incubated with the corresponding secondary antibody at room temperature for 1 h. Finally, the protein blot on the membrane was exposed by chemiluminescence.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 statistical software (IBM, Armonk, NY, USA) was used for all statistical analysis. The chi-square test was used for the comparison of classification data, while the *t*-test was used for the comparison of measurement data. Data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

Comparison of Anesthesia Induction Time

No significant difference in anesthesia induction time was observed among the 5 compatibilities of an equivalent threshold dose of metformin with propofol groups ($p>0.05$, Figure 1A). Similarly, no significant difference was found in anesthesia induction time among the 5 compatibilities of different doses of metformin with 80 mg/kg propofol groups as well ($p>0.05$, Figure 1B).

Contents of SOD, MDA, and LDH in Compatibility of Equivalent Threshold Dose of Metformin With Propofol Group

Results showed that no significant differences in the levels of SOD and MDA were found among the 5 compatibilities of an equivalent threshold

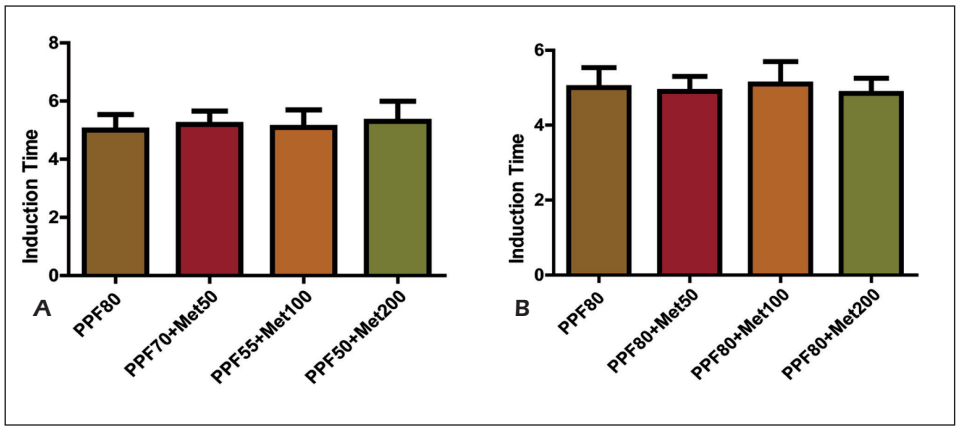

Figure 1. Anesthesia induction time. **A**, Anesthesia induction time in compatibility of an equivalent threshold dose of metformin with propofol groups. **B**, Anesthesia induction time in compatibility of different doses of metformin with 80 mg/kg propofol groups.

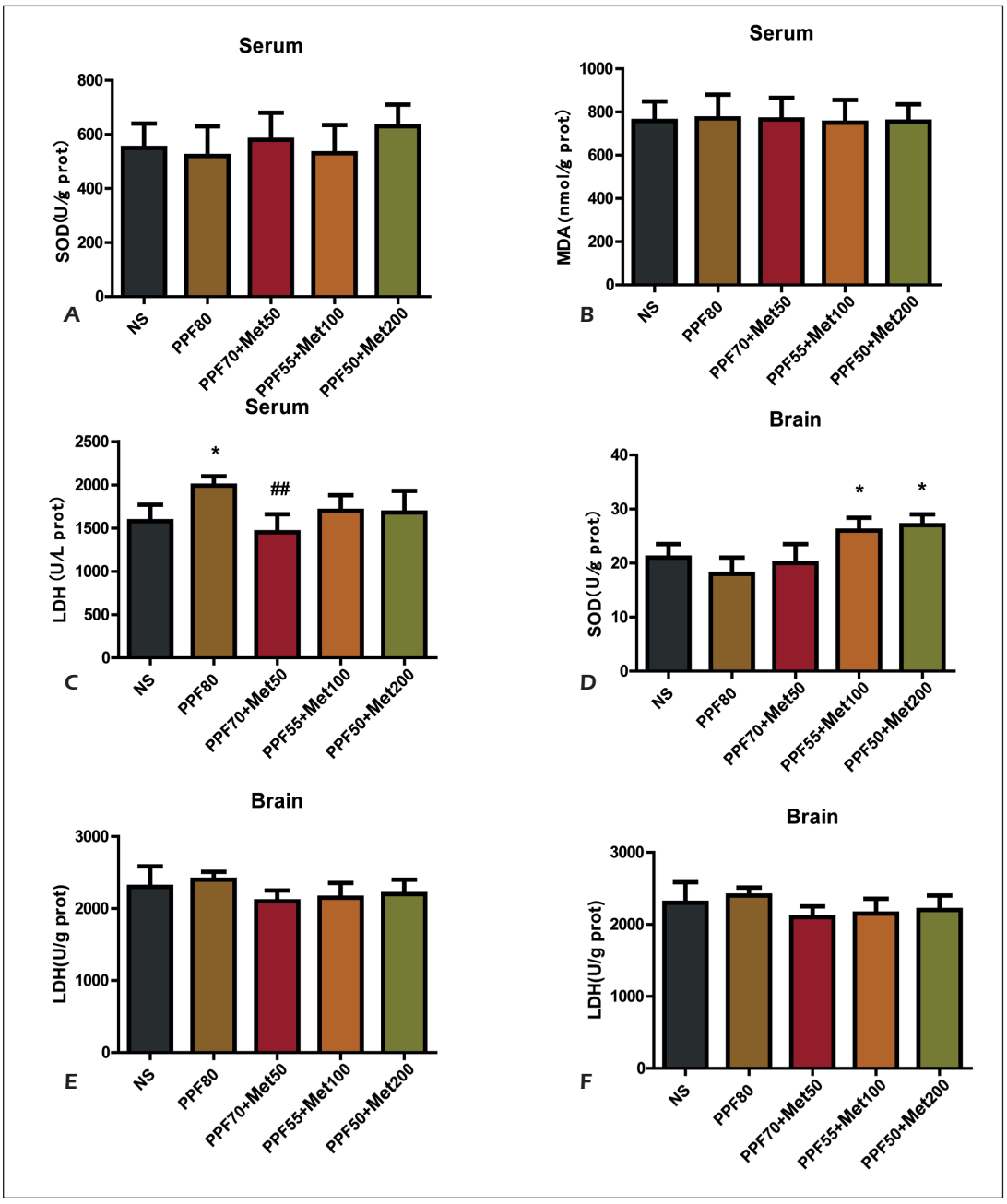

Figure 2. Contents of SOD, MDA, and LDH in compatibility of an equivalent threshold dose of metformin with propofol group. **A**, Serum level of SOD in each group. **B**, Serum level of MDA in each group. **C**, Serum level of LDH in each group. **D**, SOD level in brain tissue of each group. **E**, MDA level in brain tissue of each group. **F**, LDH level in brain tissue of each group (*p<0.05: compared with PPF 80 group; $^{\#}p$<0.05, $^{\#\#}p$<0.01).

dose of metformin with propofol groups (p>0.05, Figure 2A). Compared with NS group, the serum level of LDH in PPF 80 group was significantly higher (p<0.05, Figure 2B). However, the serum level of LDH was remarkably decreased in PPF 70 + Met 50 group when compared with that of PPF 80 group (p<0.05, Figure 2C). Moreover, a significantly higher level of SOD was found in brain tissues of mice in PPF 55 + Met 100 group and PPF 50 + Met 200 group than PPF 80 group (p<0.05, Figure 2D). However, we did not observe significant differences in MDA and LDH levels in brain tissues of mice among the 5 groups (p>0.05, Figure 2E and 2F).

Contents of SOD, MDA, and LDH in Compatibility of Different Doses of Metformin With 80 mg/kg Propofol Group

No significant difference in the serum level of SOD was observed among the 5 compatibilities of different doses of metformin with 80 mg/kg propofol groups ($p>0.05$, Figure 3A). Compared with NS group and PPF 80 group, a significantly lower serum level of MDA was found in PPF 80 + Met 200 group ($p<0.05$, Figure 3B). Meanwhile, the serum level of LDH in PPF 80 group was remarkably higher than that of NC group ($p<0.05$). However, the serum level of LDH was significantly decreased in PPF 80 group when compared with PPF 80 + Met 100 group ($p<0.05$, Figure 3C).

Subsequently, the level of SOD in brain tissue homogenate of each group was compared. The results showed that a significantly higher SOD level was found in brain tissue homogenate of PPF 80 + Met 100 group than that of NS group and PPF 80 group ($p<0.05$, Figure 3D). However, no significant differences were found in MDA and LDH levels in brain tissues among the 5 groups ($p>0.05$, Figure 3E and 3F).

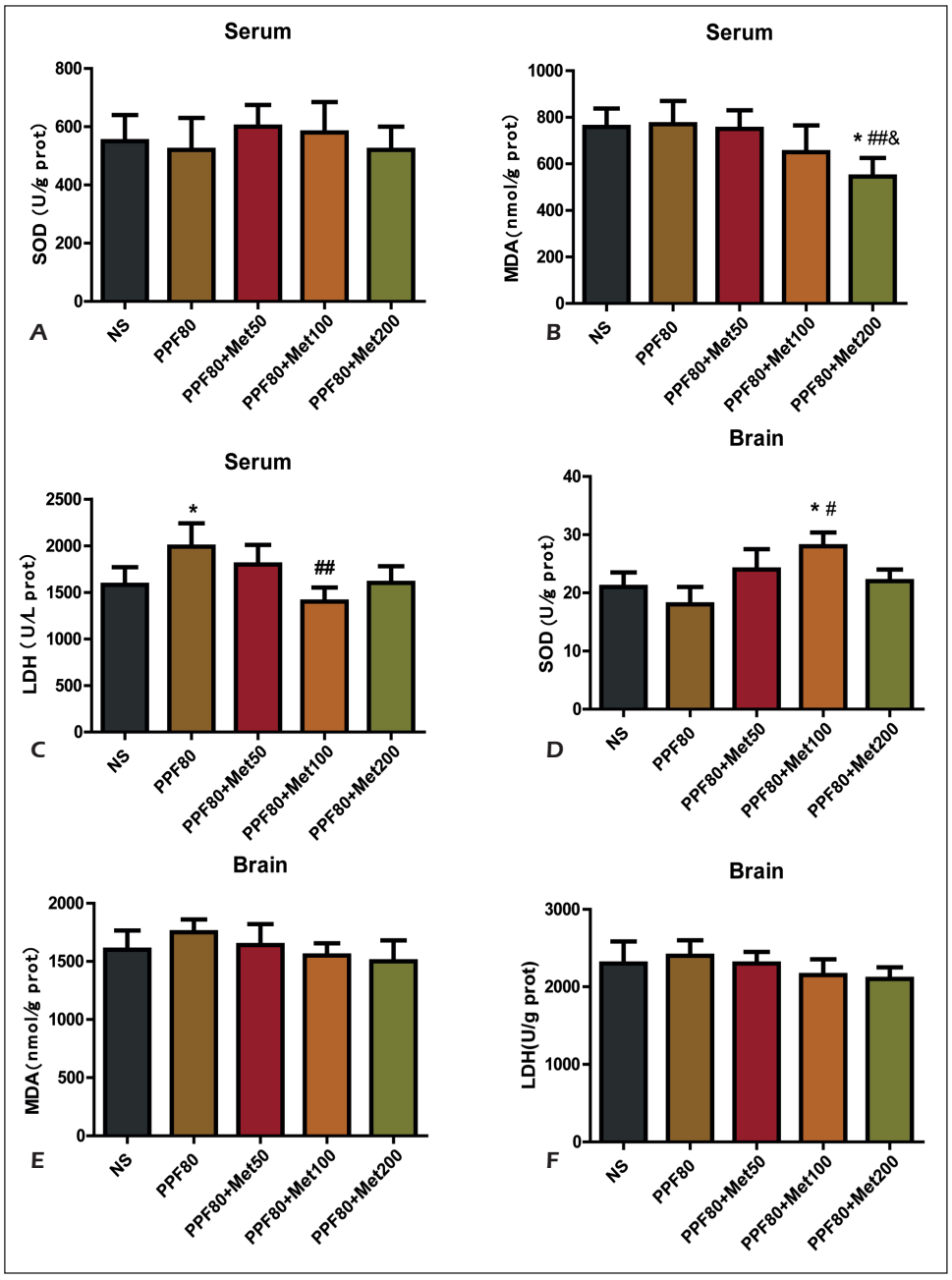

Figure 3. Contents of SOD, MDA, and LDH in compatibility of different doses of metformin with 80 mg/kg propofol group. **A**, Serum level of SOD in each group. **B**, Serum level of MDA in each group. **C**, Serum level of LDH in each group. **D**, SOD level in brain tissue of each group. **E**, MDA level in brain tissue of each group. **F**, LDH level in brain tissue of each group (*$p<0.05$: compared with PPF 80 group; $^{\&}p<0.05$: compared with PPF 80 + Met 50 group; $^{\#}p<0.05$, $^{\#\#}p<0.01$).

Protein Expressions of NF-κB and Nrf2 in Mouse Brain Tissue

Western blotting results indicated that high protein expression of NF-κB was observed in all groups except for NS group ($p<0.05$, Figure 4A and 4D). Compared with PPF 80 group, significantly down-regulated NF-κB and up-regulated Nrf2 were found in brain tissues of mice in another compatibility of an equivalent threshold dose of metformin with propofol groups ($p<0.05$, Figure 4B and 4C). Furthermore, similar results were observed in the 5 compatibilities of different doses of metformin with 80 mg/kg propofol groups as well ($p<0.05$, Figure 4E and 4F).

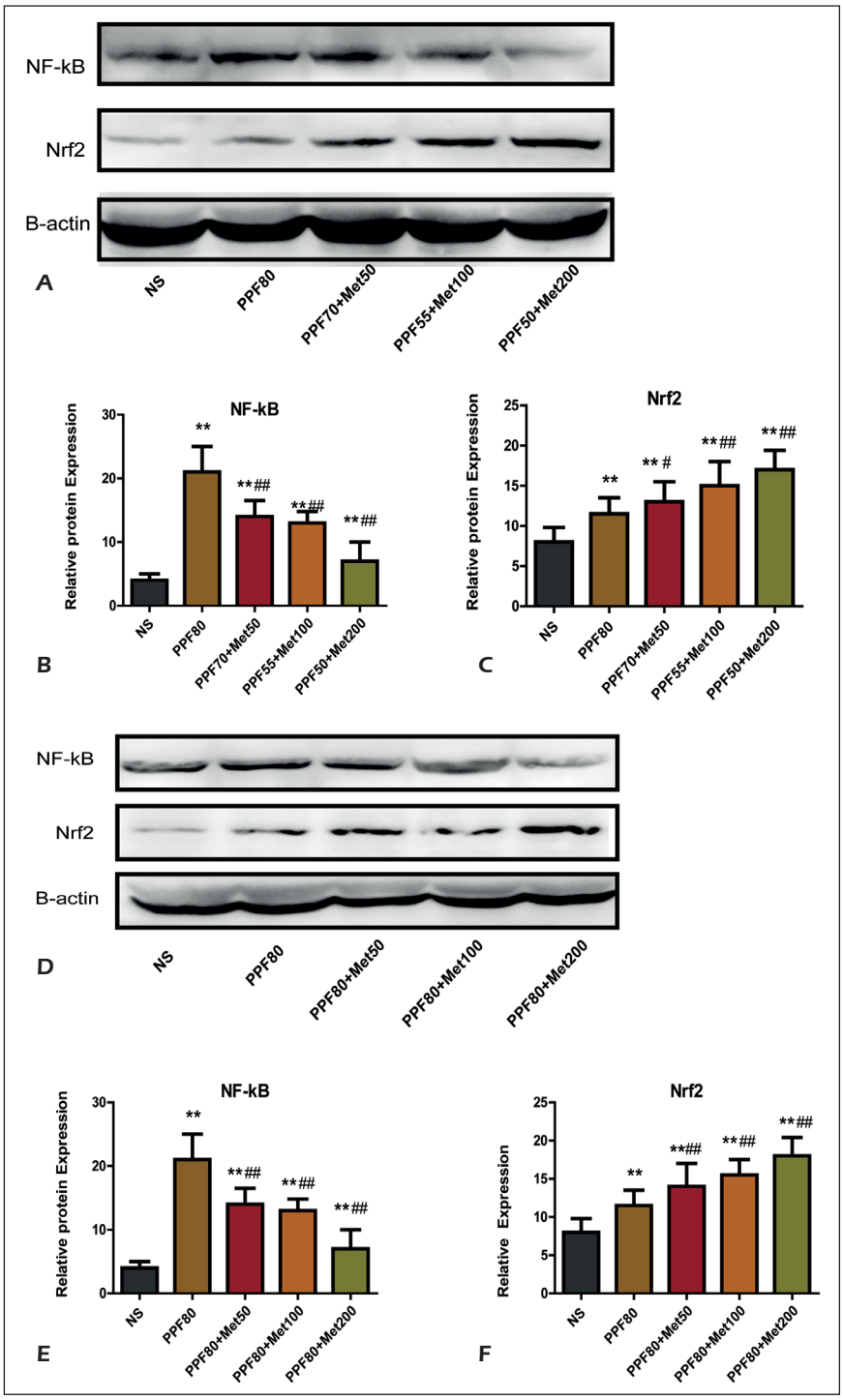

Figure 4. Protein expressions of NF-κB and Nrf2 in mouse brain tissues. **A-C**, Protein expressions of NF-κB and Nrf2 in compatibility of an equivalent threshold dose of metformin with propofol groups. **D-F**, Protein expressions of NF-κB and Nrf2 in compatibility of different doses of metformin with 80 mg/kg propofol groups (*$p<0.05$: compared with NS group; $^{\#}p<0.05$, $^{\#\#}p<0.01$: compared with PPF 80 group).

Discussion

Propofol is commonly used for continuous administration of anesthesia maintenance drugs, or sedation in ICU patients. Long-term and high-dose propofol infusion increases the incidence of adverse reactions[19]. Previous studies have indicated that adverse reactions of propofol mainly influence the vascular system, respiratory system, nervous system, and impair lipid metabolism and coagulation. Propofol may even lead to propofol infusion syndrome (PRIS). Meanwhile, the inhibitory effect of propofol on cardiovascular function is characterized by decreased arterial blood pressure, weakened cardiac contractility, decreased peripheral vascular resistance, cardiac output decline, and reduced left ventricular filling pressure[20].

Metformin is a kind of biguanide compound derived from anthraquinones found in *Galega officinalis*. Metformin is sensitive to insulin, which is also capable of regulating blood glucose. Currently, it is the most widely used drug for patients with type 2 diabetes[21]. Metformin is an inhibitor of the mitochondrial electron transport chain complex I. Researches have suggested that metformin down-regulates ROS level in rats. Other studies[22,23] have demonstrated that metformin reduces the endogenous level of ROS in mouse embryonic fibroblasts. In high-fat diet-induced rats, metformin resists oxidative stress by increasing antioxidant enzyme activity and reducing mitochondrial ROS production[24,25].

In the present investigation, no significant difference in anesthesia induction time was observed between the compatibility of an equivalent threshold dose of metformin with propofol group, and the compatibility of different doses of metformin with 80 mg/kg propofol group. However, we observed a decreased trend of anesthesia induction time after metformin compatibility. Therefore, we speculated that metformin contributed to the dose decline of anesthetic *via* prolonging anesthesia maintenance time. Furthermore, metformin supplementation did not influence the anesthetic induction effect of propofol in mice, whereas the dose of propofol was significantly decreased.

Besides, metformin increased SOD activity in brain tissue of mice, decreased serum levels of MDA and LDH, thereby reducing the degree of lipid peroxidation. Our findings suggested that metformin could inhibit NF-κB activity and promote Nrf2 expression.

Conclusions

We found that metformin improved the anesthetic effect on a single dose or continuous intraperitoneal injection of propofol in mice. The compatibility of a certain dose of metformin with propofol can enhance the scavenging ability of free radicals and their metabolites. Moreover, this inhibits lipid peroxidation in mice *via* NF-κB inhibition and Nrf2 activation.

Conflict of Interests

The authors declare that they have no conflict of interest.

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