Effects of Propofol on Oligodendrocyte Myelin Proteins in Sprague-Dawley Rats at Different Developmental Stages: Postprint

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Abstract

Objective To investigate the effect of propofol on myelin basic protein (MBP) in oligodendrocytes of SD rats at different developmental stages. Methods Forty SD rats at postnatal days 3, 7, 14, and 21 were randomly divided into a control group and an experimental group, with 20 rats in each group. The control group (medium and long-chain fat emulsion group) and the experimental group (propofol medium and long-chain fat emulsion injection group) received intraperitoneal injections of fat emulsion 25 mg/kg or propofol 25 mg/kg, respectively, with an additional half of the initial dose administered after 20 minutes, for a total duration of 8 hours. The expression of MBP mRNA and Caspase-3 mRNA was detected by fluorescence quantitative RT-qPCR, and the expression of MBP protein was detected by Western blot and immunohistochemistry. Results Compared with the control group, the expression of MBP mRNA in rats of all ages in the experimental group was significantly downregulated ($P<0.05$), while the expression of Caspase-3 mRNA in 3-, 7-, and 14-day-old rats was significantly upregulated ($P<0.05$). The expression of MBP in 7- and 14-day-old rats in the experimental group was significantly lower compared with the control group ($P<0.05$). There was no statistically significant difference in the expression of Caspase-3 mRNA and MBP between the two groups in 21-day-old rats ($P>0.05$). Conclusion Propofol can inhibit the expression of MBP gene and protein in SD rats, with the most significant effect observed at postnatal days 7 and 14.

Full Text

Preamble

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Abstract

Objective: To investigate the effect of propofol on myelin basic protein (MBP) expression in oligodendrocytes of SD rats at different developmental stages.

Methods: This study examined 3-, 7-, 14-, and 21-day-old SD rats (40 animals per age group). Each age cohort was randomly divided into a control group and an experimental group (20 rats each). The control group received intraperitoneal injections of 25 mg/kg medium-long-chain fat emulsion, while the experimental group received 25 mg/kg propofol medium-long-chain fat emulsion injection. Both groups received supplemental injections of half the initial dose every 20 minutes for 8 hours. Expression of MBP mRNA and caspase-3 mRNA was detected by fluorescence-based quantitative RT-qPCR, while MBP protein expression was assessed via Western blotting and immunohistochemistry.

Results: Compared with control groups, MBP mRNA expression was significantly down-regulated in experimental groups across all ages ($P<0.05$), while caspase-3 mRNA expression was significantly up-regulated in 3-, 7-, and 14-day-old rats ($P<0.05$). MBP protein expression in 7- and 14-day-old rats was significantly decreased in experimental groups compared with controls ($P<0.05$). No statistically significant differences in caspase-3 mRNA or MBP protein expression were observed between groups in 21-day-old rats ($P>0.05$).

Conclusion: Propofol can down-regulate MBP expression at both the mRNA and protein levels in SD rats, with the most pronounced effects observed at 7 and 14 days of age.

Keywords: oligodendrocytes; myelin basic protein; neurotoxicity; propofol

Introduction

Propofol is a widely used intravenous anesthetic for anesthesia induction and maintenance, as well as for sedation in ICU settings, characterized by rapid onset and quick recovery with complete functional restoration. Studies have shown that propofol promotes apoptosis of oligodendrocytes in late-gestation fetal and newborn monkeys [**?**], and our previous research found that propofol exposure during embryonic stages also down-regulates MBP expression in oligodendrocytes of zebrafish [**?**].

Oligodendrocytes are a type of glial cell that wrap around axons to form insulating myelin sheaths, primarily functioning to facilitate efficient saltatory transmission of neural electrical signals and protect neurons [**?**]. MBP is a protein specifically expressed in oligodendrocytes, comprising one-third of myelin proteins [**?**]. Whether propofol administration down-regulates MBP expression in oligodendrocytes of SD rats at different postnatal stages, whether this MBP down-regulation is associated with apoptosis, and at which stage in SD rats

these effects are most pronounced, have not been reported in the literature. This study investigated the effects of propofol on MBP and caspase-3 mRNA expression in oligodendrocytes at different developmental stages (postnatal days 3, 7, 14, and 21) to further elucidate the neurotoxic effects and mechanisms of propofol.

Methods

1.1 Experimental Animal Grouping

A total of 40 SD rats at each of four developmental stages—postnatal day 3 (P3), day 7 (P7), day 14 (P14), and day 21 (P21)—were used. Body weights ranged from 8–10 g (P3), 18–21 g (P7), 32–34 g (P14), to 44–46 g (P21). Animals of both sexes were obtained from the Laboratory Animal Center of Southern Medical University and housed at 18–22 °C under normal light-dark cycles with free access to food and water. Rats were randomly divided into control and experimental groups. Both groups received intraperitoneal injections of either fat emulsion or propofol at 25 mg/kg. The fat emulsion was supplemented with normal saline to match the volume of propofol. Additional doses of half the initial amount were administered every 20 minutes for 8 hours. Following drug treatment, SD rats were placed in a 37 °C oxygen incubator until anesthetic recovery. Two hours later, both groups were euthanized via intraperitoneal injection of 30 mg/kg 1% pentobarbital sodium, and brain tissues were collected and preserved. Brain tissues for protein and RNA analysis were stored at -80 °C, while tissues for sectioning were temporarily stored in 4% paraformaldehyde.

1.2 Materials and Instruments

The following materials were used: propofol medium/long-chain fat emulsion injection (batch No. 16KL_5866, Fresenius Kabi Beijing Pharmaceutical Co., Ltd.), medium-long-chain fat emulsion (batch No. 80GG051, Sino-Swed Pharmaceutical Co., Ltd.), rabbit anti-MBP polyclonal antibody (Abcam ab40390), mouse anti- -actin polyclonal antibody (Nanjing Novizan L/N804051), SP kit (Beijing Zhongshan Golden Bridge SP9000), DAB chromogenic reagent kit (Wuhan Google Biotechnology), PCR primers synthesized by Shanghai Sangon Biotech Co., Ltd., reverse transcription kit (TaKaRa), RNA extraction kit (TaKaRa), and RT-PCR kit (TaKaRa). Equipment included a PCR system (LightCycle480, Roche, Switzerland) and an OLYMPUS DIGITAL CAMERA DP70 (Japan).

1.3 qRT-PCR Measurement of MBP/caspase-3 mRNA Transcription Levels

Total RNA was extracted from brain tissues using an RNA extraction kit. One microgram of RNA was reverse transcribed at 42 °C for 2 minutes. Appropriate amounts of cDNA products were subjected to fluorescence quantitative PCR under the following amplification conditions: 95 °C for 5 seconds, 60 °C for 60

seconds, for 40 cycles. PCR primer sequences were obtained from the GeneBank database: MBP forward primer 5' -TGATGTGTTTGGGGAGGCAGA-3' , reverse primer 5' -AACCCATAGTTCCTCTACGCC-3' ; caspase-3 forward primer 5' -CTGGACTGCGGTATTGAG-3' , reverse primer 5' -CGGGTGCGGTAGAGTAAGC-3'; -actin forward primer 5'-TGACAGGATGCAGAAGGAGA-3' , reverse primer 5' -TAGAGCCACCAATCCACACA-3' . -actin served as the internal reference gene, and relative expression levels of MBP and caspase-3 mRNA were calculated using the 2^{-ΔΔCT} method to evaluate target mRNA expression.

1.4 Western Blotting for MBP Protein

Total protein was extracted from brain tissues, and protein concentration was measured using the BCA method to calculate loading amounts. Twelve percent SDS-PAGE gels were prepared, and 50 g of protein sample was loaded per well for electrophoresis. After membrane transfer, blots were blocked with 5% skim milk for 1 hour and washed with TBST three times. Primary antibody dilutions were added: rabbit anti-MBP polyclonal antibody (1:1000) or mouse anti- -actin polyclonal antibody (1:1000), and incubated overnight at 4 °C on a shaker. After washing, blots were incubated with secondary antibody for 2 hours at room temperature on a shaker (goat anti-rabbit antibody 1:3000 or goat anti-mouse antibody 1:1000). The ECL chemiluminescence reagent kit was used for development, and gel image analysis systems were used to capture images. Image J software was employed to analyze target band gray values.

1.5 Immunohistochemistry

SD rats were anesthetized with pentobarbital sodium, the chest was opened, and perfusion was performed via the left ventricle with a small incision made in the right atrium. After flushing with 0.9% normal saline until the blood became clear, perfusion was switched to 4% paraformaldehyde solution (prepared with 0.1 mol/L PBS, pH 7.4) until the liver became visibly pale and the lungs showed obvious edema, after which the skull was opened to retrieve brain tissue. Brain tissues were fixed in 4% paraformaldehyde solution for 24 hours, dehydrated through graded alcohols, cleared with xylene, and embedded in paraffin. Continuous coronal sections (5–6 m) were cut posterior to the optic chiasm. Using the SP method, paraffin sections were routinely deparaffinized, subjected to microwave antigen retrieval in citrate buffer, incubated with 10% goat serum at 37 °C for 30 minutes, then incubated with rabbit anti-MBP polyclonal antibody (1:100) at 4 °C overnight. Biotin-labeled goat anti-rabbit/mouse IgG was added and incubated at 37 °C for 30 minutes, followed by horseradish peroxidase-labeled streptavidin working solution at 37 °C for 30 minutes. Sections were developed using the DAB chromogenic reagent kit, counterstained with hematoxylin, routinely dehydrated, cleared, and mounted. Mounted sections were observed under a microscope.

1.6 Statistical Analysis

SPSS 20.0 software was used for statistical analysis. Measurement data are expressed as mean ± standard deviation, and comparisons between two groups were performed using independent samples t-test. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of Propofol on MBP mRNA and Caspase-3 mRNA Synthesis in Oligodendrocytes

Following 8 hours of propofol exposure, P3, P7, P14, and P21 SD rats in the experimental groups showed significantly down-regulated MBP mRNA expression compared with their respective control groups ($P<0.05$, Figure 1 [Figure 1: see original paper]). Caspase-3 mRNA expression was significantly up-regulated in P3, P7, and P14 SD rats in the experimental groups ($P<0.05$, Figure 2 [Figure 2: see original paper]).

Figure 1. MBP mRNA expression in SD rats at different developmental stages. P3: Postnatal day 3; P7: Postnatal day 7; P14: Postnatal day 14; P21: Postnatal day 21. *$P<0.05$ vs control group.

Figure 2. Caspase-3 mRNA expression in SD rats at different developmental stages. *$P<0.05$ vs control group.

2.2 Effects of Propofol on MBP Protein Levels in Oligodendrocytes

After intraperitoneal injection of 25 mg/kg propofol for 8 hours followed by anesthetic recovery, brain tissues were collected from SD rats at different developmental stages. Total protein was extracted and MBP protein expression was detected by Western blotting. The results showed that MBP protein was not expressed in either group of P3 SD rats. In P7 and P14 SD rats, MBP protein expression was significantly reduced in the experimental group compared with the control group ($P<0.05$, Figure 3 [Figure 3: see original paper]). In P21 SD rats, no significant difference in MBP protein expression was observed between the experimental and control groups.

Figure 3. MBP expression in SD rats at different developmental stages. *$P<0.05$ vs control group.

2.3 Immunohistochemical Detection of MBP Synthesis in Oligodendrocytes After Propofol Exposure

Immunohistochemical detection of MBP on oligodendrocytes revealed no MBP-labeled positive cells in P3 SD rats. Positive MBP staining with brown granules began to appear at P7. Typical MBP-positive cells were observed in brain sections from P14 and P21 SD rats. Compared with their respective control

groups, the number of positive cells was reduced in brain sections from P7 and P14 SD rats in the experimental group (Figure 4 [Figure 4: see original paper]).

Figure 4. Immunohistochemical detection (SP method) of propofol' s effects on MBP expression in SD rats at different developmental stages (Original magnification: ×200). Black arrows indicate MBP-stained positive cells.

Discussion

Current clinical research remains controversial regarding whether general anesthesia or anesthetic agents cause cognitive decline and intellectual impairment in children. Some studies have reported that general anesthesia in children leads to cognitive dysfunction and intellectual decline [**?**], while others have found no correlation [**?**]. The primary reasons for these contradictory conclusions are: (1) all are retrospective analyses with non-uniform anesthesia protocols and evaluation systems; (2) data loss, loss to follow-up, and incomplete datasets; and (3) underlying diseases and surgical factors in children who underwent early surgery. Rodent studies have confirmed that isoflurane exposure in fetal and newborn rats leads to subsequent cognitive dysfunction [**?**]. Similar phenomena have been reported for propofol in recent years [**?**], though the specific mechanisms remain unclear.

MBP constitutes an important component of myelin protein in vertebrates, accounting for one-third of total myelin protein, and is synthesized and secreted by oligodendrocytes in the central nervous system. The MBP gene shows high conservation between humans and rodents, with homologous sequences reaching 93% identity in the coding region [**?**]. The specificity of MBP expression and its high conservation across species make it an ideal marker protein for studying oligodendrocytes. MBP forms myelin sheaths around neuronal axons, primarily functioning in insulation and saltatory conduction of neural signals. Abnormal MBP expression can impair, reduce, or eliminate myelin formation, affecting not only neural information transmission but also contributing to sclerosis-related diseases. Clinically, this manifests as cognitive dysfunction, language and vision impairment, tremor, muscle spasm, and other symptoms [**?**]. Studies have shown that both isoflurane and ketamine can increase oligodendrocyte apoptosis [**?**], and subsequent experiments in fetal monkeys and zebrafish verified that propofol reduces MBP expression [**?**]. However, no studies have reported on propofol' s inhibition of MBP expression in SD rats. Our experimental findings demonstrate that propofol can down-regulate MBP at both the gene and protein levels in SD rats, with the most pronounced effects observed at 7 and 14 days of age. This is primarily because the peak period of MBP expression in SD rats occurs around postnatal days 8–20, making it susceptible to external interference [**?**]. In 3-day-old SD rats, although propofol down-regulated MBP mRNA expression, its effects on MBP protein levels were not clearly demonstrated, likely because changes at the mRNA level occur earlier than those at the protein level, consistent with the temporal sequence from transcription to translation. Immunohistochemical labeling of MBP in brain sections from SD

rats further confirmed that MBP is barely expressed at P3, while propofol reduced MBP expression at P7 and P14. In 21-day-old SD rats, oligodendrocytes are developmentally mature and largely resistant to external interference.

The absorption rate of intraperitoneal injection is 80–85% that of intravenous injection, making the 25 mg/kg intraperitoneal propofol dose used in this study appropriate for SD rats.

Cell apoptosis is initiated primarily through two pathways: extracellular signals activating intracellular apoptotic enzymes (caspases) and mitochondria releasing apoptotic enzyme activators to activate caspases. Caspase-8, -9, and -3 play crucial roles in apoptotic signal transduction. In most studies of apoptotic mechanisms, caspase-3 is a key molecule, and recent research has revealed an even closer relationship between cell apoptosis and caspase-3 activation [**?**]. Propofol induces neuronal apoptosis, inhibits neuronal proliferation, and affects neurobehavioral function in adulthood [**?**]. Brain development is a long and continuous process, with a rapid developmental phase occurring from 6 months post-conception through several years after birth in humans. Postnatal days 7 and 14 represent the peak brain development period reported in most rodent studies, during which the nervous system is sensitive to drug effects, and propofol-induced increased apoptosis occurs primarily during this window. Our experimental study demonstrated that propofol significantly up-regulated caspase-3 mRNA expression in 3-, 7-, and 14-day-old SD rats. Research indicates that the degree of nervous system development in SD rats corresponds to different gestational ages in human newborns: 2–4-day-old rats correspond to premature infants born at 23–32 weeks gestation, 5–7-day-old rats correspond to premature infants at 33–36 weeks, 8–12-day-old rats correspond to term infants at 37–42 weeks, and 21-day-old rats correspond to the toddler period [**?**]. Our study further confirms that propofol-induced apoptosis promotion is associated with the peak period of brain development.

Milanović et al. [**?**] found that intraperitoneal injection of 25 mg/kg propofol for 8 hours in 7-day-old SD rats promoted neuronal apoptosis, with a righting reflex recovery time of approximately 20±2 minutes. Our study referenced this protocol and adopted an 8-hour exposure duration. Allegaert et al. [**?**] suggested that a single intravenous bolus of 3 mg/kg propofol is an appropriate anesthetic induction dose for term and preterm infants. Since the drug distribution volume in animals is substantially larger than in humans, and according to drug concentration conversion formulas, the dose used in rats is approximately 6.3 times that in humans, with intraperitoneal absorption at 80–85% of intravenous injection, the 25 mg/kg intraperitoneal propofol dose used in this study is appropriate for SD rats.

This study is the first to demonstrate that repeated propofol administration can down-regulate both MBP gene and protein levels in 7- and 14-day-old SD rats. The specific mechanism underlying this down-regulation remains unclear but may be related to increased oligodendrocyte apoptosis. Our experiments also found that propofol up-regulated caspase-3 gene expression, and re-

lated literature has confirmed that caspase-3 up-regulation is closely associated with increased apoptosis, suggesting that propofol-induced down-regulation of MBP gene and protein levels in SD rats at different developmental stages may be related to increased oligodendrocyte apoptosis, though further cellular experiments are needed for confirmation [**?**]. Whether propofol-induced down-regulation of MBP expression in oligodendrocytes leads to cognitive dysfunction and whether this effect recovers over time require further experimental validation.

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Note: Figure translations are in progress. See original paper for figures.