THE EFFECT OF PROP OFOl ON THE PROLIFERATION OF NEPHROBLASTOMA CELLS AND THE UNDERLYING MECHANISM

JUNJIANG LIU 1#, HONGMEI WANG 1#, XIN ZHAO 1, LIN LI 2, HONGLIAN XIA 2, YAN LIU 2,3*

1First Clinical Medical College, Mudanjiang Medical University, Mudanjiang, 157011, China
2Department of Anesthesiology, Affiliated Hongqi Hospital of Mudanjiang Medical University, Mudanjiang, 157011, China
3Department of Anesthesiology, Hengqin Branch of Zuhai People’s Hospital, Zuhai, 519000, China

*corresponding author: liuyangseal@163.com
#Authors with equal contribution.

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Abstract

This study aimed to evaluate propofol’s effect on nephroblastoma cells proliferation and its underlying mechanism. The treatment with 10, 20 and 40 μg/mL propofol significantly reduced the proliferation of SK-NPE-1 cells in a dose-dependent manner compared to the control group and the groups treated with 1 and 5 μg/mL propofol. In groups treated with 10, 20 and 40 μg/mL propofol, a time-dependent effect of exposure to propofol on the SK-NPE-1 cell proliferation was observed. The apoptotic effect of propofol compared with the control group was evaluated by the PI/Annexin V-FITC double staining method. The effect was dose-dependent till the concentration of 20 μg/mL, showing that 20 μg/mL is the optimum apoptotic concentration. The treatment with 10, 20 and 40 μg/mL propofol significantly decreased FASN and P-mTOR protein levels and increased the expression of AMPKα and P-AMPKα compared to the control group. Real-time PCR test results showed that FASN mRNA expression levels in the groups treated with 5, 10, 20 and 40 μg/mL propofol significantly decreased compared to the control group. In conclusion, propofol can inhibit the proliferation and promote the apoptosis of nephroblastoma cells. This mechanism is associated with the inhibition of FASN expression by the AMPKα/mTOR signalling pathway.

Rezumat

Studiul și-a propus evaluarea efectului propofolului asupra proliferării celulelor de nefroblastom și a mecanismului său. Tratamentul cu 10, 20 și 40 μg/mL propofol a redus semnificativ proliferarea celulelor SK-NPE-1 într-o manieră doză-dependentă, comparativ cu grupul control și cu grupurile tratate cu 1 și 5 μg/mL propofol. În grupurile tratate cu 10, 20 și 40 μg/mL propofol, s-a observat un efect dependent de timp al expunerii la propofol asupra proliferării celulelor SK-NPE-1. Efectul apoptotic al propofolului comparativ cu grupul control a fost evaluat prin metoda colorației duble PI/Annexin V-FITC. Efectul a fost doză-dependent până la concentrația de 20 μg/mL, demonstrând că aceasta este concentrația apoptotică optimă. Tratamentul cu 10, 20 și 40 μg/mL propofol a scăzut semnificativ nivelurile proteinelor FASN și P-mTOR și a crescut expresia AMPKα și P-AMPKα, comparativ cu grupul control. Rezultatele testului PCR au arătat că nivelurile expresiei ARNm FASN în grupurile tratate cu 5, 10, 20 și 40 μg/mL propofol, au scăzut semnificativ în comparație cu grupul control. În concluzie, propofolul poate inhiba proliferarea și poate promova apoptoza celulelor de nefroblastom. Acest mecanism este asociat cu inhibarea expresiei FASN de către calea de semnalizare AMPKα/mTOR.

Keywords: propofol, nephroblastoma, proliferation, apoptosis, AMPK α/mTOR

Introduction

Nephroblastoma, also known as a renal embryonal tumour, is a malignant tumour that originates from the embryonic cells of the kidney and is the second most common abdominal malignant tumour in infants and young children [1]. This tumour accounts for 6% of all paediatric tumours, and the combined incidence in children and adolescents younger than 15 years is about 0.1% [2]. The main peak age of nephroblastoma is 1 to 5 years old, and the average age of onset is 3.5 years [3]. Tumours are mostly singular, but they can also be of unilateral multicentric origin or bilateral. Abdominal masses are common clinical manifestations. Due to the continuous development of comprehensive treatment levels, the disease’s survival rate has been greatly improved in recent years. The current global overall survival rate is as high as 70% to 80%, and it may even be higher [4]. The possibility of malignant metastasis still exists in the terminal stage of nephroblastoma, and the mortality rate is still as high as 5% [5]. Therefore, looking for the mechanism of the occurrence and development of nephroblastoma is of great significance to the occurrence and development of the intervention treatment of the disease and the improvement of the cure of the disease. Propofol is currently the most widely used intravenous anaesthetic in clinical practice, mainly for anaesthesia induction, anaesthesia maintenance, post anaesthesia care unit (PACU) and intensive care unit (ICU) [6]. The drug is characterized by a fast onset, intense action and fast metabolism. It is also the most commonly
used drug for target-controlled infusion of tumour resection surgery under general anaesthesia. In addition to anaesthesia induction, anaesthesia maintenance and intensive care unit sedation, propofol is also an anaesthetic agent against cerebral ischemia-reperfusion, myocardial ischemia/reperfusion injury, in addition to myocardial protection, neuroprotection and intestinal tract mucosal protection [7]. Propofol is a calcium channel antagonist and glutamate antagonist at the level of N-methyl-D-aspartate (NMDA) receptors. It not only has GABAergic activity and antioxidant effect, but also can inhibit cell apoptosis and reduce cytotoxicity [8]. In recent years, more and more studies have confirmed that propofol can inhibit the invasion of tumour cells [9]. Related studies have shown that propofol inhibits the growth, proliferation and migration of human colon cancer cells Caco-2 and LoVo in a dose- and time-dependent manner [10]. Studies have also shown that propofol may inhibit cell proliferation and invasion by up-regulating the expression of miRNA in breast cancer MCF-7 cells [11]. However, the role of propofol in nephroblastoma is still unclear, therefore this study explores the effect and the underlying mechanism through which propofol influences the nephroblastoma cell proliferation and provides a valuable reference for clinical nephroblastoma treatment.

Materials and Methods

Cell culture

Human Ewing Sarcoma Cell line (SK-NEP-1) was purchased from Wuhan Punuosai Life Technology Co., Ltd., Wuhan, China. The cells were kept in 12% foetal bovine serum (US Hyclone company) in RPMI-1640 (Roswell Park Memorial Institute) medium (Thermo Fisher Scientific Co., Ltd., Waltham, MA USA) with 1% penicillin and streptomycin (Hanzhou Jinuo Biological Co., Ltd., China) and incubated in an incubator at 37°C in 5% CO₂ atmosphere. The culture medium was replaced every 24 hours. The cell passage treatment was done by aspirating the original medium, rinsing thoroughly with phosphate-buffered saline (PBS) and then digesting with 0.25% trypsin (Hanzhou Jinuo Biological Co., Ltd., China). The digestion was stopped when the cells became round and tapped off the wall. Follow-up experiments were performed on SK-NEP-1 cells in the growth phase.

Experiment grouping

The SK-NEP-1 cells were randomly divided into a blank group (group C1), propofol 1 µg/mL (P1 group), propofol 5 µg/mL (P2 group), propofol 10 µg/mL (P3 group), propofol 20 µg/mL (P4 group) and propofol 40 µg/mL (P5 group). The cells in the C1 group were cultured normally without any treatment, while the cells in the P1 group, P2 Group, P3 group, P4 group and P5 group were treated with 1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL and 40 µg/mL propofol (Sigma-Aldrich, MA, USA), respectively.

Cell proliferation ability detected by CCK-8 assay

Cell counting kit-8 (CCK-8) (Beijing Soleibiao Tech. Co., Ltd., China) was used to detect the proliferation ability of SK-NEP-1 cells without or with treatment. The cells in the logarithmic growth phase are digested with 0.1% trypsin to prepare a single-cell suspension, and the cell concentration is adjusted to 5 × 10⁴ cells per mL. A 96-well plate was inoculated with 10,000 cells per well, incubated with the experimental media containing different propofol concentrations. CCK-8 solution was added to each well in a 5% CO₂ atmosphere at 37°C. The absorbance (OD) was measured at 450 nm after 12 h, 24 h, 36 h, 48 h, 60 h and 72 h, respectively, to detect cell proliferation.

Apoptosis rate detected by flow cytometry

SK-NEP-1 cells in the logarithmic growth phase were inoculated in a 6-well plate at the concentration of 1.0 × 10⁵ cells/well. Each group had 3 multiple wells, 2 mL per well. When the cells adhered to the wall, the experimental media containing different propofol concentrations were added and placed in an incubator to continue culturing. After 48 hours, the cells were collected in a centrifuge tube, mixed with 100 µL of 1 Binding Buffer to reselect the cells, and then added 5 µL of Annexin V-FITC (Nanjing Novazin Biotechnology Co., Ltd., China) and propidium iodide (PI) were added. The cell apoptosis rate was determined by flow cytometry (BD Bioscience, Accuro C6).

Expression of related proteins detected by western blotting

The used antibodies were purchased from Shanghai Biyuntian Biotechnology Co., Ltd., China. The cells from each group were collected, and 100 µL of samples were lysed on ice for 30 min. After centrifugation, the supernatant was used for protein detection. Total protein quantification was performed following the instructions of the bicinechinonic acid (BCA) kit (Shanghai Biyuntian Biotechnology Co., Ltd., China) using sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE). ImageJ software (National Institute of Health, USA) was used to analyse the relative expression of AMPKα, P-AMPKα, FASN, P-mTOR, TOR proteins.

FASN mRNA expression level detected by Real-time PCR

Real-time PCR was used to detect the expression level of FASN. The SK-NEP-1 cells in the logarithmic growth phase were moved in a 6-well culture plate. After 24 h, the experimental culture media containing different propofol concentrations was added. After 72 hours of intervention, all cells were collected, and the total RNA of each group was extracted by the TRIzol method. Five µL of RNA sample was mixed 495 µL 1 TE Buffer, and the absorbance was measured at 260 nm and 280 nm to determine the concentration of RNA in each sample RNA. Four µg of each extracted RNA sample was used for reverse transcription into cDNA, and the cDNA product obtained was used as a template to detect the expression level of FASN.
mRNA. Quant Studio 6 Flex PCR (Thermo Fisher, USA) amplification conditions were: denaturation at 95°C for 10 s, annealing at 62°C for the 30 s, extension at 72°C for 15 s, a total of 40 cycles. Data processing was performed using the 2-ΔΔCT method to calculate the relative expression level of the target gene. FASN primer: 5'-AAGGACCTGTCTAGGTGATGC-3'; reverse primer: 5'-TGGCTTCATAGGTGACTTCCA-3'; product length is 106 bp, with GAPDH as a reference, GAPDH forward primer: 5'-TGTCTAGGACCGTTACTGAC-3'; reverse primer: 5'-GTCGGTTACCTGACTCCA-3'.

Statistical analysis
SPSS23.0 software (IBM, USA) was used for the statistical analysis and the results were expressed as mean ± standard deviation. The comparison between the two groups was performed by Student's t-test and the comparison between multiple groups was performed by one-way analysis of variance. p < 0.05 was used as the statistical difference.

Results and Discussion
Effects of different concentrations of propofol on the proliferation of nephroblastoma cells
After 48 h, the proliferation ability of SK-NEP-1 cells in the P3, P4 and P5 groups were significantly lower compared with the C1 groups (p < 0.01, Figure 1). The effect was dose-dependent till the concentration of 20 µg/mL (P4 group). Compared with the P4 group, the proliferation ability of SK-NEP-1 cells in the P5 group was significantly increased (p < 0.05).

Effect of propofol on the apoptosis of nephroblastoma cells
After culturing for 48 hours, the PI/Annexin V-FITC double staining method was used to detect cell apoptosis. The results showed that compared with the C1 group, the apoptotic ability of the P1, P2, P3, P4 and P5 groups was significantly increased. The effect was dose-dependent till 20 µg/mL (Figure 3A-3G) (p < 0.05). In the P5 group, the proportion of apoptosis in the P5 group was significantly decreased compared with the P4 group (p < 0.05).
Effects of different propofol concentrations on apoptosis of nephroblastoma cells at different times. A-F: effects of C1, P1, P2, P3, P4 and P5 groups on apoptosis of SK-NEP-1 cells at different time points. G: apoptosis rate

**p < 0.01 compared with C1 group, # p < 0.05 compared with P4 group

Effect of propofol on proteins associated with nephroblastoma cells

Western blotting test results showed (Figure 4) that the protein levels of AMPKα and p-AMPKα in groups P3, P4 and P5 were significantly increased compared with the C1 group (p < 0.01). The protein levels of FASN and p-mTOR in groups P2, P3, P4 and P5 were significantly decreased compared with the C1 group (p < 0.01). There was no significant difference in mTOR protein levels among groups P1, P2, P2, P3, P4 and P5 (p > 0.05).

Influence of different concentrations of propofol on FASN mRNA expression level

Real-time PCR test results showed that FASN mRNA expression levels in P2, P3, P4 and P5 groups were significantly reduced compared with C1 groups (p < 0.05 and p < 0.01, Figure 5). The effect was dose-dependent till 20 µg/L (group P4). Compared with the P4 group, the expression level of FASN protein in the P5 group was significantly increased (p < 0.05). The above results indicate that propofol can reduce the expression level of FASN mRNA.

The occurrence of nephroblastoma results from a combination of many factors and is related to genetic factors. The disease is recognized as the best malignant solid tumour in children with modern comprehensive therapy [12].
It protein synthesis by 20 µg/mL, the effect is dose-dependent. The results showed that the longer the time of propofol action, the stronger the inhibition effect on nephroblastoma cells, this study used flow cytometry to detect the apoptosis rate. The effects of propofol on the proliferation of nephroblastoma cells at different doses and different times are shown in Figure 5.

**Figure 5.** Effects of different concentrations of propofol on FASN mRNA expression

*p < 0.05, ** p < 0.01 compared with C1 group

# p < 0.05 compared with the P4 group

Therefore, clear staging and diagnosis of the exact case classification, the use of individualized comprehensive treatment, and the search for effective therapeutic drugs are effective means to improve the prognosis of children with Wilms' tumour and increase the survival rate. Propofol is one of the most commonly used intravenous anaesthetics that can produce smooth induction and can wake up quickly. It has a fast onset, short action time, quick wake-up, few side effects and strong controllability [13]. In addition to the advantages of various anaesthetics, the drug can also exert some non-anaesthetic effects. Relevant studies have shown that propofol has the ability to inhibit tumour proliferation and has a positive effect on the prognosis of surgically treated tumour patients [14]. In *vitro*, experimental studies have proved that propofol inhibits the invasion and migration of liver cancer cells in liver cancer cells [15]. Studies have also shown that propofol can inhibit the proliferation and metastasis of human ovarian cancer cells by inhibiting the HOST2/JAK2/STAT3 signalling pathway [16]. However, the effect of propofol on the proliferation of nephroblastoma cells and its mechanism is still unclear, so this study mainly explores the effect of propofol on the proliferation of nephroblastoma cells at different doses and different times, which is the clinical nephroblastoma.

This study found that the higher is the propofol concentration, the stronger is the inhibitory effect on the proliferation ability of SK-NEP-1 cells. By verifying the proliferation ability of SK-NEP-1 cells at different times, it was found that the proliferation ability of SK-NEP-1 cells in the P3, P4 and P5 groups at 48 h, 60 h and 72 h was significantly reduced, indicating that the longer the time of propofol action, the stronger the inhibitory effect on SK-NEP-1 cell proliferation. In order to further verify the effect of propofol on nephroblastoma cells, this study used flow cytometry to detect the apoptosis rate. The results showed that till 20 µg/mL, the effect is dose-dependent. The effect of propofol on tumour cell proliferation belongs to the category of non-anaesthetic effects. The mechanism of the effect on tumour biological activity is not fully elucidated. Except for cell proliferation inhibition, propofol stimulates tumour cell apoptosis. Relevant studies have shown that propofol can inhibit the proliferation of oesophageal cancer cells and promote apoptosis [17], similar to this study. Propofol may affect the expression of tumour cell-related molecules through immune regulation. AMPK is a trimeric active enzyme composed of a catalytic subunit (α) bound to two regulatory subunits (p and Y), which participate in various biological processes such as cell proliferation and cell differentiation [18]. Activating AMPKα can inhibit protein synthesis by regulating the phosphorylation of multiple proteins. mTOR is a member of the protein kinase family. There are many signalling pathways that regulate mTOR. The negative regulation is mainly the AMPK/mTOR signalling pathway [19]. When cells lack nutrients, activating AMPKα can inhibit the activity of mTOR [20]. Fatty acid synthase (FASN) is not only over-expressed in liver cancer and prostate cancer, but the increased expression of FASN is closely related to abnormal tumour proliferation and poor prognosis [21]. Relevant studies have shown that inhibiting FASN can inhibit the development of various tumours [22]. This study found that propofol can promote the protein expression of AMPKα and P-AMPKα in SK-NEP-1 cells and reduce the protein expression of FASN and P-mTOR. As the concentration of propofol increases, the protein expression level of AMPKα gradually increases, while the protein expression levels of FASN and P-mTOR decrease significantly.

**Conclusions**

This study confirmed that propofol could inhibit the proliferation of Nephroblastoma cells and promote the apoptosis of nephroblastoma cells. The mechanism of action may be to inhibit FASN expression by activating AMPKα/mTOR signalling pathway, thereby achieving inhibition. Nephroblastoma cell proliferation and promote cell apoptosis.

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**Conflict of interest**

The authors declare no conflict of interest.

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