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Effect of Different Propofol Concentrations on Rat Lung Mesenchymal Stem Cells

Propofolün Farklı Konsantrasyonlarının Rat Akciğer Mezenkimal Kök Hücreler Üzerindeki Etkisi

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ABSTRACT

Objective: Lung mesenchymal stem cells (LMSCs) play important roles in the maintenance of organ integrity and healing processes. It is important not to harm or decrease LMSCs, especially in patients with lung diseases. Propofol may be administered to patients with acute or chronic lung diseases due to the need for anesthesia or sedation. We aimed to investigate the cytotoxic/proliferative effects of propofol on LMSCs.

Methods: The mesenchymal stem cells were subjected to propofol at 25 µM (Group P25), 50 µM (Group P50), 100 µM (Group P100), or 0 µM (Group P0). The values of the cell proliferation curve and the normalized cell index at the 6th, 24th, 48th, and 72nd hours after propofol administration were obtained with the xCELLigence Real-Time Cell Analyzer system.

Results: The normalized cell index values were lower in Group P100 compared to Group P25 at the 6th, 24th, 48th, and 72nd hours. They were also lower in Group P100 compared to Group P0 (p=0.001) at the 24th hour, but this effect was not seen at the following time points.

Conclusion: The cytotoxic effect of 100 µM propofol was observed only after 24 hours and this effect was temporary. Therefore, propofol can be used during anesthesia practices, but we recommend using the lowest dose possible.

Keywords: Lung, propofol, stem cell

ÖZ

Amaç: Akciğer mezenkimal kök hücreleri (AMKH), organ bütünlüğünün korunmasında ve iyileşme süreçlerinde önemli rol oynamaktadır. Özellikle akciğer hastalığı olan hastalarda bu hücrelerin sayı ve fonksiyonuna zarar verilmemesi son derece önemlidir. Akut veya kronik akciğer hastalığı olan hastalarda anestezi veya sedasyon ihtiyacı nedeniyle propofol uygulanabilir. Bu çalışmada, propofolün AMKH üzerindeki sitotoksik/proliferatif etkisinin araştırılması amaçlanmıştır.

Yöntem: İn vitro ortamda üretilen mezenkimal kök hücrelere 25 µM (Grup P25), 50 µM (Grup P50), 100 µM propofol (Grup P100) veya 0 µM propofol (Grup P0) eklendi. Propofol eklendikten sonraki 6., 24., 48. ve 72. saatlerde hücre proliferasyon eğrisi ve normalize hücre indeksi değerleri xCELLigence real-time cell analyzer cihazından elde edildi.

Bulgular: Normalize hücre indeksi değerleri 6., 24., 48. ve 72. saatlerde Grup P100'de P25'e göre daha düşüktü. Normalize hücre indeksi değerleri, sadece 24. saatte ve Grup P100'de Grup P0'a göre daha düşüktü (p=0,001), ancak bu etkinin sonraki saatlerde devam etmediği görüldü.

Sonuç: Propofol yüksek dozlarda kullanıldığında sitotoksik etkiye sahiptir, ancak bu etkinin kalıcı olmadığı görülmüştür. Bu nedenle propofol anestezi ve yoğun bakım uygulamaları sırasında kullanılabilir ancak mümkün olan en düşük dozun tercih edilmesi gerektiğini düşünüyoruz.

Anahtar sözcükler: Akciğer, propofol, kök hücre

INTRODUCTION

Mesenchymal stem cells (MSCs) are a type of stem cell with capacities for proliferation and self-renewal. These cells have a fibroblastoid morphology in vitro culture (1). The MSCs must be plastic-adherent, express the proper positive (CD44 and CD90) and negative (CD11b/c and CD45) surface antigens, and have multipotent differentiation potential (such as adipogenic, chondrogenic, and osteogenic potential) ac-

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cording to the criteria of the International Society of Gene and Cell Therapy (ISGCT) (2). These cells constitute a progenitor cell population that can be isolated from many tissues, including those of the lungs. Lung mesenchymal stem cells (LMSCs) play important roles in the maintenance of organ integrity and healing processes and have immunomodulator, anti-inflammatory, regenerative, regulative, and repairing effects (3).

Propofol is an intravenous anaesthetic agent commonly used to induce and maintain anesthesia in patients (4). It decreases proinflammatory cytokines, inhibits neutrophil and macrophage chemotaxis, and reduces free oxygen radicals (5). Propofol may be administered to patients with acute or chronic lung disease due to the need for sedation or anesthesia. It is important not to harm or decrease LMSCs, especially in patients with acute or chronic lung disease. To our knowledge, the effects of propofol on LMSCs have not yet been established. Therefore, this study aimed to investigate the effects of different propofol concentrations on LMSCs using the xCELLigence Real-Time Cell Analyzer (RTCA).

MATERIAL and METHODS

This study was approved by the local ethics committee for animal experimentation (Ethical Committee Ref No: G.U.E.T 18.003, date: 16.01.2018). All experimental procedures were performed in accordance with the ethical standards recommended by the relevant institutional review board. Furthermore, this study was conducted according to the Guide for the Care and Use of Laboratory Animals and reported according to ARRIVE guidelines.

Two newborn male Wistar albino rat pups obtained from the Gazi University Animal Laboratory were euthanized after the administration of intramuscular ketamine (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}). Sternotomy was performed carefully to avoid damaging the lungs after sterilization with 70% ethanol. Lung lobes were transferred to 50-mL tubes containing 35 mL of cold 30% citrate-phosphate-dextrose adenine (CPDA-1) anticoagulant (26.30 g trisodium citrate dihydrate, 3.27 g ascorbic acid monohydrate, 2.22 g monosodium dihydrogen phosphate, 31.80 g D-glucose, and 0.275 g adenine in 1 L purified H_2O; this solution was filtered using a sterile 0.22-µm membrane filter before use) in phosphate-buffered saline to remove blood and debris. Because of the risk of needing to repeat the study, some of the rat lung tissue was preserved in glycerol and dimethyl sulfoxide (DMSO) at -80 °C.

Lung tissues were cut into small pieces and placed into cell culture flasks. These pieces were cultured at 37 °C in a sterile incubator (Nuve, Turkey) with 5% CO_2 and >95% humidity. The cell population was expanded in a specific MSC medium with 20% fetal bovine serum (Lonza, Belgium), 2% L-glutamine (Lonza, Belgium), 1% penicillin/streptomycin/amphotericin (Biological Industries, Israel), and 77% low-glucose Dulbecco's modified Eagle medium (Gibco, USA). The medium was changed every 3 days and cells were passaged at 80% confluency. The cells between passage 2 and passage 3 were used for characterization and differentiation.

Surface Marker Detection

The surface antigen markers of MSCs were detected using a flow cytometer (FACSAria III, USA). Cells were stained with CD11b/c, CD90, CD44, and CD45 conjugated fluorescent antibodies (all from BD, USA) and analyzed by flow cytometry with the appropriate software.

Adipogenic, Osteogenic and Chondrogenic Differentiation

When the first cultures reached 80% confluence, cells were incubated in differentiation mediums for 3 weeks. The differentiation capacity of the LMSCs was evaluated using differentiation lineage-specific mediums (Gibco, USA) according to the manufacturer's instructions. Alizarin Red S staining (Sigma, USA) for osteogenic differentiation, Oil Red O staining (Sigma, USA) for adipogenic differentiation, and Alcian Blue staining (Sigma, USA) for chondrogenic differentiation were performed according to the manufacturer's instructions.

Immunophenotyping of the Cell Population

After the cell population showed a predominant fibroblast-like morphology at passage 2, surface antigens were assessed by flow cytometry (>40,000 cells counted in a gated area). The lung cell population expressed several cell surface antigens identified with MSCs. Flow cytometry indicated that the MSCs expressed CD44 and CD90. However, the expressions of CD11b/c and CD45 were generally negative (93.9% CD44+, 89.5% CD90+, 18.7% CD45+, 10.5% CD11b/c+).

The cell population was expanded in differentiation mediums for 3 weeks. Osteogenic, adipogenic, and chondrogenic differentiation potential was thus induced in these cells. Osteogenic differentiation was identified by positive Alizarin Red staining, adipogenic differentiation was identified by positive staining with Oil Red O, and chondrogenic differentiation was identified by staining with Alcian Blue. These results allowed the isolated lung cell population to be defined as LMSCs according to the criteria of the ISGCT (2).

Propofol Cytotoxicity Study

The effect of propofol on LMSCs was investigated with the xCELLigence RTCA (Roche Applied Science, Germany, and ACEA Biosciences, USA). The xCELLigence RTCA is an useful equipment for cytotoxicity analysis and monitors 16-well e-plates to measure electrical impedance. Different numbers of adherent cells in the cell culture change the impedance

values. Thus, this system detects cell adhesion, spreading, proliferation, and apoptosis. The measure of cell adhesion is defined as the cell index (CI), but a normalized CI (NCI) can be used to reduce the influence of inter-experimental variations and to ensure the same baseline. The NCI has been defined as the ratio of the CI value to the initial CI value immediately after injecting a chemical solution into the well. In other words, $NCI_{(a)}=CI_{(a)}/CI_{(0)}$, where $CI_{(0)}$ is the cell index at the time of exposure (6). In the present study, 2×10^4 cells were seeded per well. Propofol was applied to the wells at the 28th hour when cells were in the logarithmic phase. As propofol's solvent, 0.1% DMSO was used.

Cells were subjected to propofol at 25 µM (Group P25), 50 µM (Group P50), or 100 µM (Group P100). The MSC medium containing 0.1% DMSO was applied to the wells in the control group (Group P0). All experiments were repeated at least three times. The cell proliferation curve and NCI values at the 6th, 24th, 48th, and 72nd hours after propofol treatment were obtained using the xCELLigence RTCA.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics 20.0 for Windows (IBM Corp., USA). All data were presented as mean ± standard deviation (SD) and minimum-maximum values. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to evaluate the normality of continuous data. One-way analysis of variance and Kruskal-Wallis H tests were used to evaluate differences between groups. The Bonferroni method as a post hoc test was used to determine the groups between which differences arose in the event of statistically significant differences between the groups. Data were analyzed at a 95% confidence level and results were considered significant at $p<0.05$.

RESULTS

Figure 1 shows the proliferation curves of all groups after propofol treatment. Group P25 showed better proliferation curve results than Groups P0, P50, and P100.

Six hours after propofol treatment, NCI values were 0.978 ± 0.010, 0.990 ± 0.485, 0.897 ± 0.765, and 0.885 ± 0.656 in Groups P0, P25, P50, and P100, respectively (p=0.031). The NCI values were significantly higher in Group P25 than Group P100 (p=0.008).

Twenty-four hours after propofol treatment, NCI values were 1.513 ± 0.735), 1.687 ± 0.108, 1.347 ± 0.140, and 1.315 ± 0.645 in Groups P0, P25, P50, and P100, respectively (p=0.000). The NCI values were significantly higher in Group P0 than Group P100 (p=0.001). The NCI values were also significantly higher in Group P25 compared to Group P100 (p=0.000) and Group P50 (p=0.001).

Forty-eight hours after propofol treatment, NCI values were 1.778 ± 0.129, 2.489 ± 0.212, 1.680 ± 0.150, and 1.645 ± 0.148 in Groups P0, P25, P50, and P100, respectively (p=0.010). The NCI values were significantly higher in Group P25 compared to Group P100 (p=0.005) and Group P50 (p=0.005).

Seventy-two hours after propofol treatment, NCI values were 1.539 ± 0.097, 2.307 ± 0.274, 1.499 ± 0.134, and 1.456 ± 0.157 in Groups P0, P25, P50, and P100, respectively (p=0.014). The NCI values were significantly higher in Group P25 compared to Group P100 (p=0.005) and Group P50 (p=0.005).

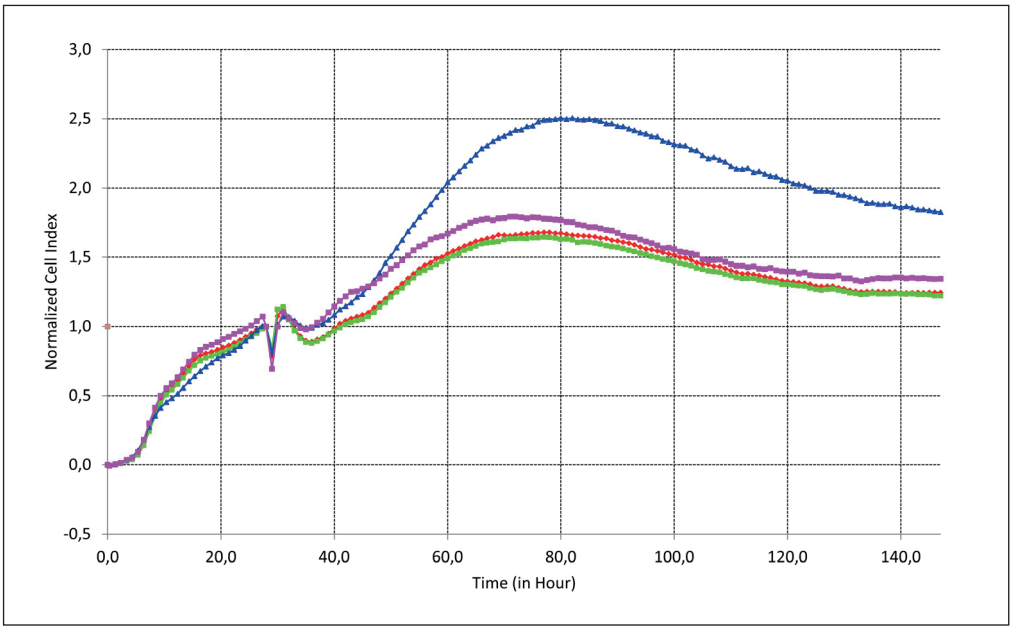

Figure 1: The proliferation curves of all groups following propofol treatment (Blue: Group P25, Purple: Group P0, Red: Group P50, Green: Group P100).

DISCUSSION

In the present study, it was aimed to investigate the effects of different propofol concentrations on LMSC proliferation. According to our results, the highest dose of propofol (100 µM) reduced LMSC proliferation more than the lowest dose (25 µM) at the 6th, 24th, 48th, and 72nd hours.

Propofol is frequently used in anesthesia and intensive care practices (4). It was previously concluded that the propofol blood concentration during sedation is 2 µg mL^{-1} (11.2 µM) while it is 4 µg mL^{-1} (22.4 µM) during general anesthesia (7). However, higher doses of propofol have been applied in previous cell culture experiments (8,9). For this reason, it was decided to use doses of 100 µM and below in our study. In addition, the propofol doses used in the present study were the same as in previous cell culture experiments (8-10).

Propofol is an intravenous, non-water-soluble agent. Dimethyl sulfoxide is a non-cytotoxic propofol solvent commonly used in in vitro studies. It has been shown that DMSO is not cytotoxic to live cells at a concentration of 0.1% (11). Therefore, 0.1% DMSO was used as a propofol solvent in the present study.

Propofol has significant effects on many different tissue-specific MSCs (8,9,12). When sevoflurane or propofol was administered to cultured bone marrow-derived MSCs, sevoflurane was shown to reduce cell viability at concentrations of 2.3% and 3%, while propofol had no effect (8). However, in that study, the exposure time was 4 hours. Zhou et al. added the sera of their patients, obtained after the administration of general anesthesia with sevoflurane or propofol, to cultured neural stem cells (13). They subsequently reported lower cell viability and density and higher superoxide dismutase and glutathione peroxidase levels in the sevoflurane group. However, Li et al. showed that stem cell proliferation and viability decreased after propofol administration (14). In the present study, only 100 µM propofol had decreased cell proliferation compared to the control group at the 24th hour. It was shown that propofol did not significantly affect NCI values at other doses or at other times compared to the control group. This may be due to propofol, which has no lasting effect on the proliferation of LMSCs, being rapidly eliminated such that its effects disappear.

The dose of propofol may also be a parameter affecting cell proliferation. Palanisamy et al. studied the effects of 2.5 and 5.0 µg mL^{-1} propofol on neural progenitor stem cells at the 4th and 24th hours (10). Neither dose of propofol caused cell death after 4 or 24 hours. Neither dose of propofol decreased cell proliferation after 4 hours, but cell proliferation decreased after 24 hours. This effect was temporary, however, and the neural stem cell pool was not affected. The propofol doses used in that study were similar to blood sedation concentration levels, so it is not surprising that cell viability and proliferation were not affected. Tao et al. found that adult rat-derived hippocampal neural stem cell viability and proliferation increased with 50 and 100 µM propofol at 24 hours, while there was no change with 10 µM propofol compared to the control group (9). On the contrary, our study showed that LMSC proliferation decreased with 100 µM propofol after 24 hours. While similar doses were used in our study, the difference in results may be due to the different stem cell types, different environmental conditions of the stem cells, or the use of different viability and proliferation tests.

We demonstrated that LMSCs with 25 µM propofol showed better proliferation patterns than the cells with 0, 50 and 100 µM propofol in this study. A previous study reported that propofol had negative effects on adipose-derived stem cell proliferation, migration ability, invasion ability and cell viability via phosphatidylinositol 3-kinase/protein kinase B and wingless-related integration site pathways (12). These pathways play an important role in the autocrine and paracrine functions of the cell membrane and cell proliferation activities (15,16). Chen et al. concluded that low-dose (10 µM) propofol activated hippocampal neurogenesis, increased the number of viable cells and expressed higher mitochondrial metabolism genes in cultured neural stem cells, while high-dose (100 µM) propofol did not affect these cells (17). However, Lv et al. indicated that 5 and 25 µM propofol inhibited lipopolysaccharide-induced apoptosis in lung epithelial cell cultures, while 1 µM propofol had no effect (18). Another previous study found that 25 µM propofol had been effective, while 50 and 250 µM propofol had negative effects on cardiac myoblasts cell viability (19). Although propofol reduces proinflammatory cytokines, inhibits neutrophil chemotaxis, decreases reactive oxygen species and scavenges free radicals, the differences between experimental systems, protocols and doses may cause these different results.

The xCELLigence RTCA system is a dynamic, real-time, label-free cellular analysis system with high sensitivity and specificity, and it has taken the place of conventional assays for cellular analysis. Conventional viability and proliferation tests use pre-markers to show cell proliferation and pretreat stem cells, and they are highly sensitive to ambient conditions. However, real-time, label-free, quantitative examination of the cells without any pretreatment is optimal. The xCELLigence RTCA offers an ideal system and has been used successfully in many previous cytotoxicity studies (20,21). Therefore, we used the xCELLigence RTCA system in the present study to investigate propofol's cytotoxic effects.

There are some limitations to the present study. This study was conducted in vitro and complementary in vivo studies are needed to support our findings. In addition, the study did

not investigate the pathway through which 100 µM propofol caused temporary cytotoxicity after 24 hours. Previous studies showed that propofol might increase cytotoxicity by upregulation of microRNA-206, increasing mitochondrial fission by increased expression of activated dynamin-related protein 1 and cyclin-dependent kinase 1 and down-regulation of microRNA-21 in other stem cells (14,22,23). Future research should focus on the mechanisms by which high-dose propofol causes cytotoxicity in LMSCs. Furthermore, western blotting or reverse transcription-quantitive polymerase chain reaction analyses were not used to detect proteins related to important signalling pathways for investigating the proliferative effect of 25 µM propofol in our study. This study constitutes an important step in evaluating the efficacy and safety of propofol in patients with lung diseases. Further large-scale prospective studies on this topic will provide us with a better ability to identify the potential advantages or disadvantages of propofol.

CONCLUSION

The present study has confirmed that a high dose (100 µM) of propofol decreased the proliferation of cultured LMSCs compared to a low dose (25 µM) at the 6th, 24th, 48th, and 72nd hours. Cytotoxic effects of propofol were observed only after 24 hours and at a dose of 100 µM, however, this effect was temporary. Therefore, propofol can be used in anesthesia and intensive care practices, but we recommend using the lowest dose possible.

AUTHOR CONTRIBUTIONS

Conception or design of the work: SC, JE
Data collection: SC, FAP, MST
Data analysis and interpretation: SC, FA
Drafting the article: SC
Critical revision of the article: JE, FAP, OO
The author (SC, JE, FAP, MST, OO) reviewed the results and approved the final version of the manuscript.

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