ORIGINAL PAPER

Nagoya J. Med. Sci. **85**. 579–591, 2023
doi:10.18999/nagjms.85.3.579

Propofol elicits apoptosis and attenuates cell growth in esophageal cancer cell lines

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ABSTRACT

Propofol is a pharmaceutical agent commonly used as an intravenous anesthetic in surgical treatments and a sedative in intensive care. However, it is largely unknown how exposure to propofol affects the proliferation, invasion, and apoptosis of neoplastic cells in esophageal cancer. In this study, we sought to elucidate the impact of propofol exposure on the growth properties of human esophageal cancer cell lines in vitro. We treated two human esophageal cancer cell lines, KYSE30 and KYSE960, with up to 10 μg/mL of propofol for 12–36 h. The treated cells were then analyzed by cell proliferation assay, Matrigel invasion assay, quantification of caspase-3/7 and -9 activities, and cell staining with Annexin V and 7-aminoactinomycin D to detect early apoptosis and cell death, respectively, via flow cytometry. We found that 3–5 μg/mL propofol reduced the growth and Matrigel invasion of both cell lines in a dose-dependent manner. Executioner caspase-3/7, but not caspase-9 involved in intrinsic apoptosis pathway, was activated by cell exposure to 3–5 μg/mL propofol. In addition, 3–5 μg/mL propofol augmented early apoptosis in both cell lines and increased cell death in the KYSE30 cell line. In summary, exposure to propofol, at concentrations up to 5 μg/mL, led to the reduction of cell growth and Matrigel invasion, as well as the augmentation of apoptosis in esophageal cancer cell lines. These data will help define a methodology to safely utilize propofol, a common general anesthetic and sedative, with esophageal cancer patients.

Keywords: apoptosis, esophageal cancer, proliferation, propofol

Abbreviation:
7-AAD: 7-aminoactinomycin D

INTRODUCTION

Propofol is a lipophilic intravenous anesthetic agent commonly used in surgical operation and intensive care owing to its rapid and controllable sedative effect.[1,2] Propofol creates diverse impacts on cell biological functions, including positive modulation of γ-aminobutyric acid A ($GABA_A$) receptor.[3] Propofol also binds to cellular and mitochondrial membranes, disrupts their

Received: July 13, 2022; accepted: October 24, 2022
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lipid bilayer structure, and causes cellular and mitochondrial dysfunction.[4-6] In clinical practice, propofol is known to protect various organs including neuron, brain, and heart from ischemia-reperfusion injury.[7-9]

Propofol has been shown to confer negative effects on the proliferation and invasion of cultured neoplastic cells and elicit cellular apoptosis in vitro.[10] A study demonstrated that proliferation was suppressed by cell exposure to propofol in two breast cancer cell lines.[11] Propofol also attenuated cell invasion and induced caspase-3-mediated apoptosis by suppressing HOST2/JAK2/STAT3 signaling in ovarian cancer cell lines.[12]

Conversely, evasion from cell death and the enhancement of proliferation in cancer cell lines caused by propofol exposure has also been demonstrated in previous studies.[2,13] Exposure to propofol promoted cell migration and invasion by upregulating the transcription repressor protein SNAI1 in two oral squamous cell carcinoma cell lines.[14] Propofol augmented cell growth and invasion through the upregulation of an oxidative stress responder NRF2 in a gallbladder cancer cell line.[15] Collectively, reported evidence suggests that propofol likely exerts varied effects on oncogenic properties of cancer cells, depending on cellular context or the types of cancer.

Esophageal cancer has the highest incidence rates in East Asian male populations, and is known by its rapid progression and poor prognosis.[16,17] Surgical resection is one of primary treatments against esophageal cancer, where propofol is frequently employed for anesthesia and post-operative sedation. However, to date, there have been rare studies documenting how propofol affects the growth properties of esophageal cancer cells. In this study, we thus exposed two human esophageal cancer cell lines to propofol, and examined the exposed cells for alteration in growth properties including proliferation, invasion to Matrigel, and cellular apoptosis, in comparison to those in non-exposed controls.

METHODS

Cell lines and culture reagents

Human esophageal cancer cell lines KYSE30 and KYSE960 were obtained from the Japanese Collection of Research Bioresources Cell Bank (JCRB; Osaka, Japan).[18,19] The cell lines were grown in Dulbecco's modified Eagle's medium (D-MEM; Thermo Fischer Scientific, Tokyo, Japan) supplemented with 5% fetal bovine serum (Atlas Biologicals, Ft. Collins, CO, USA) and 1% penicillin and streptomycin (Nacalai Tesque, Kyoto, Japan), and were cultured under 5% CO_2 at 37 °C in a SCA Direct Heat Incubator (ASTEC, Fukuoka, Japan).

Cell exposure to propofol

Cell lines were seeded in regular medium either in 6-well, 24-well, or 96-well tissue culture plates, depending on assays to be performed after propofol exposure. Expected cell confluency between 40% and 80% was confirmed via microscopy at 24 h post-seeding. The cells were then exposed to propofol (Sigma-Aldrich, Taufkirchen, Germany) for a predetermined duration of time in a humidified incubator. Propofol was in advance diluted with 95% (v/v) ethanol at the concentration of 2 mg/mL, and the resulting solution was contained in culture medium at concentrations of 0.1%–0.5% (v/v) to give final propofol concentrations between 2 μg/mL and 10 μg/mL. In all experiments, culture medium for non-exposed controls contained 0.5% (v/v) ethanol without propofol. The duration of cell exposure to propofol in each experiment is schematically shown in the corresponding figure. Propofol-containing media were refreshed every 12 h during the exposure periods.[20-22] After exposure to propofol, the cells were transferred to an incubator where they were further incubated under standard culturing conditions until they

were examined in assays.

Cell proliferation assay

Cells were seeded in 24-well tissue culture plates (5–10 × 10^4 cells/well), and propofol-containing ethanol was added to the well for predetermined periods of time. After cessation of propofol exposure, the cells were incubated under the standard condition to allow recovery for the time periods indicated in respective schematics. To assess cell proliferation, cells were either dissociated with trypsin (Nacalai Tesque) and counted using a Beckman Coulter Z Series (Beckman Coulter, Brea, CA, USA) at the end of cell incubation, or photographed and analyzed using IncuCyte ZOOM (Essen Bioscience, Birmingham, UK) over time during cell incubation.

Matrigel invasion assay

Cell invasion assays were performed using Transwell Permeable Supports with 8.0-μm diameter pores (Corning, Oneonta, NY, USA). The Transwell inserts were coated with 200 μl/well of 300 μg/mL Matrigel Basement Membrane Matrix (Corning) and were dried in air for 2 h at room temperature in tissue culture hood. The Matrigel in the Transwell inserts were immersed into serum-free DMEM immediately before use.

Cells were seeded into 24-well tissue culture plates at a density of 5–10 × 10^4 cells/well, exposed to the indicated concentrations of propofol for 24 h, dissociated with trypsin, and reseeded in triplicate into the Transwell inserts (2.5 × 10^4 cells/well) prepared as mentioned above. After 24 h of incubation at 37 °C under 5% CO_2, the filters were fixed using 0.5% paraformaldehyde and stained with 0.1% crystal violet in phosphate-buffered saline (PBS). Cells that migrated through Matrigel in four randomly selected microscopic fields were counted by two experienced researchers using the Axio Vert. A1 fluorescence microscopy (Carl Zeiss Microscopy, Oberkochen, Germany) at a magnification of 40×, and mean cell numbers per visual field were determined.

Quantification of caspase-3/7 and caspase-9 activities

Cells were seeded into black opaque 96-well culture plates at a density of 4 × 10^3 cells/well, incubated for 1 h with or without a pan-caspase inhibitor Z-VAD-FMK (20 μM; AdooQ BioScience, Irvine, CA, USA), and exposed to propofol for 24 h. The cells were then subjected to the quantification of caspase-3/7 and caspase-9 activities performed using Caspase-Glo 3/7 and Caspase-Glo 9 Assay Systems (Promega, Madison, WI, USA) according to the manufacturer's instructions. Chemiluminescence intensity was measured using a SpectraMax M3 plate reader (Molecular Devices, San Jose, CA, USA).

Detection of early apoptosis and cell death

To detect early apoptosis, cells attached to 6-well tissue culture plates (1–2× 10^5 cells/well) were exposed to propofol for 24 h and incubated for 6 h or 24 h after the completion of exposure. The cells were then collected by trypsinization, washed with PBS once, and stained for 20 min with fluorescein isothiocyanate-conjugated Annexin V (Medical & Biological Laboratories, Aichi, Japan) 25-fold diluted in Annexin-binding buffer (0.01M HEPES, 0.14M NaCl, 3mM $CaCl_2$, pH 7.4). Apoptotic cells were detected as Annexin-V-positive cells via fluorescence flow cytometry.

For cell death detection, cells to be stained were prepared in the same fashion as above, except that the cell-incubation time after propofol exposure was fixed to 24 h. The incubated cells were stained with Annexin V for 20 min as described above and then with 7-aminoactinomycin D (7-AAD; AAT Bioquest, Sunnyvale, CA, USA) diluted in PBS (25-fold) for 15 min. Cells positive for 7-AAD were determined under fluorescence flow cytometry and defined as a population of

dying cells. Positivity for Annexin V in the stained cells was not addressed in the context of cell death detection in this study.

A Gallios Flow Cytometer (Beckman Coulter) was used for all flow cytometric analyses, and data acquired were analyzed using Kaluza software (Beckman Coulter).

Statistical analyses

Data are reported as mean ± SD, and 'n' represents the number of times independent assays were performed. One-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was performed to analyze data obtained in cell proliferation assay, cell invasion assay, and Annexin V staining assay performed after 6-h post-exposure incubation. Two-sided Student's *t*-tests were applied to analyze data acquired in the assessment of caspase activities, Annexin V staining assay performed after 24-h post-exposure incubation, and 7-AAD staining assay. IBM SPSS Statistics version 24 (IBM, Chicago, IL, USA) was used for all statistical analyses. *p*-values less than 0.05 were considered statistically significant.

RESULTS

Propofol attenuates cell growth in esophageal cancer cell lines in a dose- and time-dependent fashion

We initially assessed the effects of ethanol and dimethyl sulfoxide (DMSO), two diluents commonly used in cell biological assays, on the proliferation of esophageal cancer cell lines. Two human esophageal cancer cell lines, KYSE30 and KYSE960, were treated with 0.5% (v/v) ethanol or 0.5% (v/v) DMSO for 24 h, and cell numbers in these samples were compared with those in non-treated controls at 48 h after the completion of the treatments. We then found a significant attenuation of cell growth in KYSE30 cells treated with 0.5% (v/v) DMSO but not with 0.5% (v/v) ethanol, compared with non-treated controls (Supplementary Figure 1a). Neither 0.5% (v/v) ethanol nor 0.5% (v/v) DMSO affected the growth of KYSE960 cells (Supplementary Figure 1b). From these results, we hereafter chose to use ethanol as a diluent, and performed a series of assays supplementing propofol-containing ethanol to culture medium in such a way that the dose of ethanol does not exceed 0.5% (v/v).

To understand the impact of propofol exposure on esophageal cancer cell growth, we next conducted proliferation assays using KYSE30 and KYSE960 cell lines that had been exposed to 2–5 μg/mL propofol in regular medium for 24 h (Figure 1a). Counting of the cells demonstrated a significant dose-dependent attenuation of cell growth in propofol-exposed samples for both cell lines (Figure 1b). We also investigated the growth-suppressive effects of 12, 24, and 36 h propofol exposure in the KYSE30 (3 μg/mL) and KYSE960 (5 μg/mL) cell lines (Figure 1c). Cell counting performed at 72 h after the initiation of propofol exposure indicated that the attenuation of cell growth is dependent on the duration of propofol exposure and, among the durations that we assessed in this assay, 24 h is the minimal exposure duration required to significantly attenuate cell proliferation in both cell lines (Figure 1d). Thus, hereafter, the duration of cell exposure to propofol in this study was fixed to 24 h for both of the KYSE30 and KYSE 960 cell lines. We subsequently assessed the attenuation of cell growth at multiple time points within the period of post-propofol exposure. Phase-contrast cell images were captured every 24 h throughout 3-days post-exposure incubation, and confluency in the cell cultures was determined using an IncuCyte Zoom live cell imaging system (Figure 1e). The results of this assay indicated that the suppression of cell growth upon propofol exposure becomes more apparent over time during 3-days post-exposure incubation period in both esophageal cancer cell lines (Figure 1f).

The differences in cell proliferation between propofol-exposed and non-exposed samples seemed less remarkable in this assay than in the previous cell counting-based assays (Figure 1b and 1d); this is presumably because these assays employ distinct strategies for measuring cell numbers. Collectively, the above-described experimental results indicated that propofol suppresses the proliferation of two human esophageal cancer cell lines, KYSE30 and KYSE960, in a dose- and time-dependent fashion.

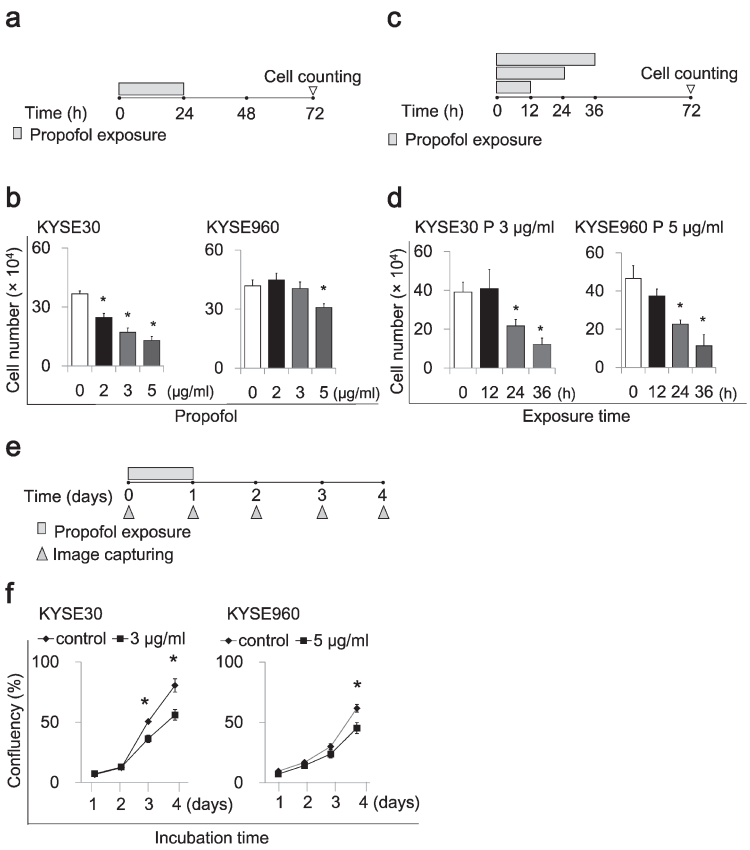


Fig. 1 Dose- and time-dependent attenuation of cell growth in propofol-exposed human esophageal cancer cell lines KYSE30 and KYSE960

Fig. 1a: Experimental design to address the dose effect of propofol exposure. Propofol was used at concentrations of 2–5 μg/mL.

Fig. 1b: Cell numbers counted in the assays delineated in (a) (n = 4).

Fig. 1c: Experimental design to assess the impact of propofol-exposure time. The concentrations of propofol applied are denoted in (d).

Fig. 1d: Cell numbers counted in the assays delineated in (c) (n = 4).

Fig. 1e: Experimental design to conduct a time-course assessment for cell growth upon propofol exposure. Images were obtained using an IncuCyte Zoom live cell camera. The concentrations of propofol applied are denoted in (f).

Fig. 1f: Confluency of cells determined using the IncuCyte Zoom software at the time points indicated in (e) (n = 4).

In (b), (d), and (f), data are expressed as the mean ± SD values of those acquired in multiple independent experiments. The numbers of times experiments were repeatedly performed are shown in the legends for individual panels. $*p < 0.01$.

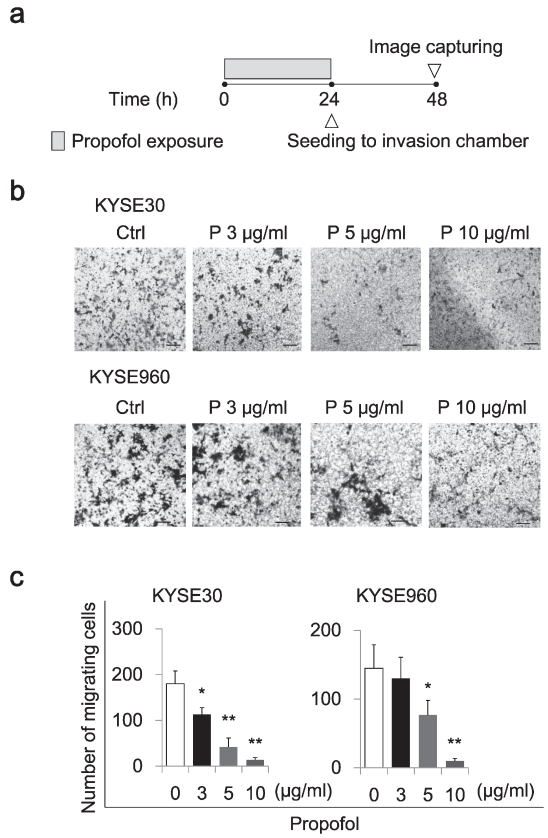


Fig. 2 Reduced invasion into Matrigel in propofol-exposed KYSE30 and KYSE960 cells

Fig. 2a: Experimental design of the Boyden chamber-based invasion assay. Cells exposed to propofol were seeded into Transwell inserts coated with Matrigel and allowed to migrate into Matrigel for 24 h. Then the cells that migrated through Matrigel were imaged.

Fig. 2b: Representative images of crystal violet-stained cells that have migrated through Matrigel. Original magnification, 40×; scale bar, 50 μm. Ctrl indicates non-propofol exposure control; P, propofol.

Fig. 2c: Numbers of cells that migrated through Matrigel per microscopic visual field (mean ± SD; n = 3). $*p < 0.05$; $**p < 0.01$.

Propofol reduces Matrigel invasion in esophageal cancer cell lines

Invasion into extracellular matrix is a critical property for tumor cells required to achieve distant metastasis. We therefore performed a Boyden chamber-based assay to explore whether cell exposure to propofol affects the capacity of Matrigel invasion in esophageal cancer cell lines. The KYSE30 and KYSE 960 esophageal cancer cell lines were exposed to different concentrations of propofol for 24 h, spread into Matrigel-filled Boyden-chamber inserts and incubated for 24 h. Cells that penetrated through Matrigel were then stained and counted. As shown in Figure 2, cell exposure to propofol resulted in a dose-dependent suppression of Matrigel invasion by the KYSE30 and KYSE960 cell lines.

Propofol activates executioner caspase-3/7 in esophageal cancer cell lines

To address whether propofol induces apoptosis in esophageal cancer cell lines, we initially examined the activity of caspases, key molecules involved in apoptosis signaling pathways, in propofol-exposed KYSE30 and KYSE960 cell lines. Six hours after the completion of propofol

exposure, the activities of caspase-3/7 and caspase-9 in the exposed cells were quantified and compared with those in non-exposed controls (Figure 3a). We found that propofol exposure upregulates the activity of apoptosis-inducing executioner caspases (caspase-3/7) by 1.37 ± 0.09-fold (p = 0.04) in KYSE30 cells and by 1.23 ± 0.09-fold (p < 0.001) in KYSE960 cells. This upregulation in caspase-3/7 activities was diminished by the supplementation of a pan-caspase inhibitor Z-VAD-FMK to culture medium at 1 h prior to the initiation of propofol exposure (Figure 3b). In contrast, caspase-9, a caspase playing a critical role in intrinsic apoptosis signaling pathway, was not activated by propofol exposure in either cell lines (Figure 3c). These data suggest that propofol triggers apoptosis in esophageal cancer cell lines but does not activate intrinsic apoptosis signaling pathway.

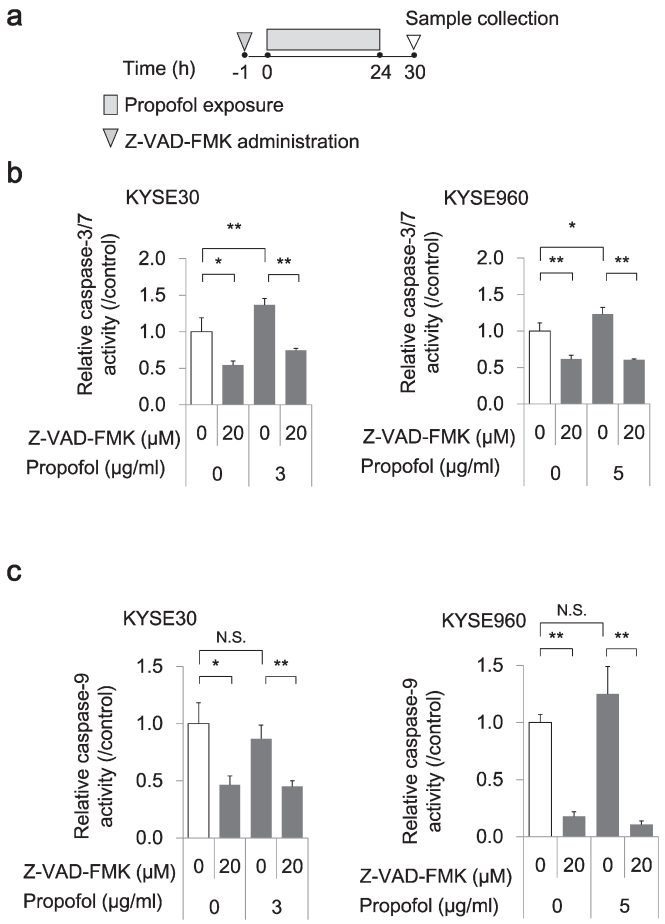


Fig. 3 Activation of executioner caspase-3/7 in propofol-exposed KYSE30 and KYSE960 cells

Fig. 3a: Experimental design. Cells collected at the indicated time point were processed for chemiluminescence-based measurement of caspase-3/7 and -9 activities. The doses of Z-VAD-FMK and propofol applied are denoted in panels (b) and (c).

Fig. 3b: Quantification of caspase-3/7 activities. Data for respective samples are shown relative to those for non-exposure controls.

Fig. 3c: Quantification of caspase-9 activities. Data for respective samples are shown relative to those for non-exposure controls.

In (b) and (c), data are expressed as the mean ± SD values of those acquired in four independent experiments. *p < 0.05; **p < 0.01.

Propofol elicits early apoptosis in esophageal cancer cell lines

To confirm the induction of cellular apoptosis by propofol exposure using a distinct assay system, we next immunostained KYSE30 and KYSE960 cells with Annexin V at 6 h after the completion of propofol exposure (Figure 4a). Subsequent flow cytometric analyses demonstrated

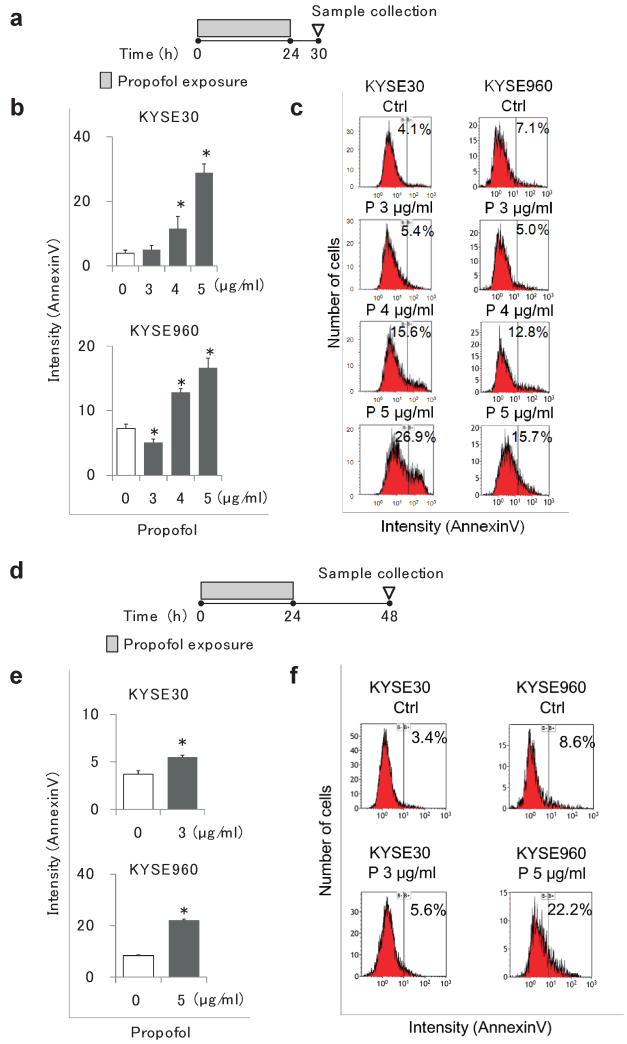


Fig. 4 Induction of early apoptosis in propofol-exposed KYSE30 and KYSE960 cells

Fig. 4a: Experimental design to analyze propofol-exposed cells after 6-h-long post-exposure incubation. Cells collected at the indicated time point were processed for Annexin V staining. The concentrations of propofol applied are denoted in (b) and (c).

Fig. 4b: Ratios of Annexin V-positive cells quantified by fluorescence flow cytometry (n = 4). $*p < 0.01$.

Fig. 4c: Representative histograms. Percentages noted in the histograms represent the ratios of Annexin V-positive cells.

Fig. 4d–f: Flow cytometric analyses of propofol-exposed cells after 24-h-long post-exposure incubation (n = 3). Experimental design and data are shown in the same fashion as (a)–(c).

In (b) and (e), data are expressed as the mean ± SD values of those acquired in multiple independent experiments. The numbers of times experiments were repeatedly performed are shown in the legends for individual panels. In (c) and (f), Ctrl indicates non-exposure control; P, propofol.

a significant, dose-dependent increase in Annexin V-positive cells upon cell exposure to propofol in both esophageal cancer cell lines (Figure 4b and 4c). We also immunostained the cells with Annexin V at 24 h after the completion of propofol exposure (Figure 4d). Both cell lines again exhibited a statistically significant increase in Annexin V-positive cells upon propofol exposure (Figure 4e and 4f). These results are consistent with elevated caspase-3/7 activity in KYSE30 and KYSE960 cells exposed to propofol, and provide further evidence that propofol promotes cellular apoptosis in esophageal cancer cell lines.

Propofol triggers cell death in the KYSE30 cell line

7-AAD is a fluorescent dye that penetrates the nuclear membrane of cells and intercalates into genomic DNA in cells undergoing irreversible necrosis or cell death.[23] We next utilized 7-AAD to study whether propofol induces an irreversible state of late apoptosis, or cell death, in esophageal cancer cell lines. KYSE30 and KYSE960 cells were exposed to propofol for 24 h, stained with 7-AAD at 24 h after the completion of propofol exposure, and analyzed by flow cytometry (Figure 5a). As shown in Figures 5b and 5c, cell exposure to propofol resulted in a notable increase (3.62 ± 0.06-fold; $p < 0.001$) of 7-AAD positive cells in the KYSE30 cell line, while no significant change was detected in the frequency of 7-AAD-positive KYSE960 cells. These data collectively suggest that exposure of esophageal cancer cell lines to propofol enhances early apoptosis as indicated by elevated Annexin V positivity, which results in an increase in cell death as marked by elevated 7-AAD positivity at least in a subset of cell lines.

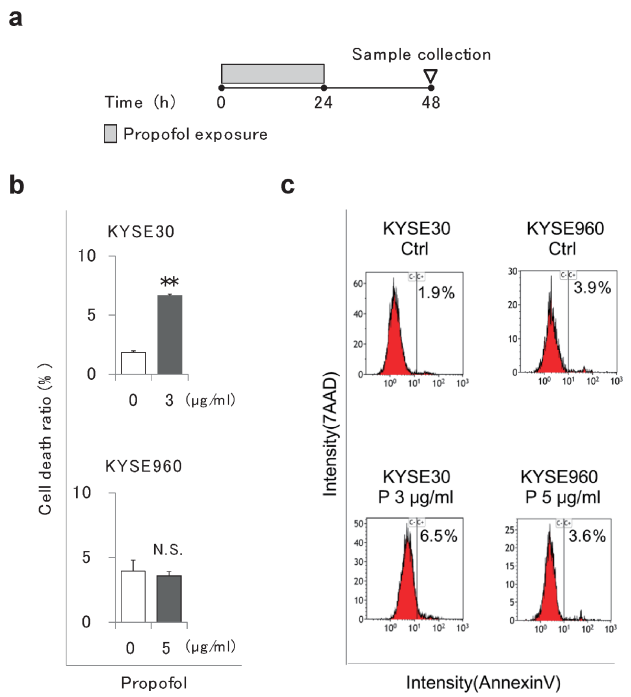


Fig. 5 Induction of cell death in propofol-exposed KYSE30 cells

Fig. 5a: Experimental design. Cells collected at the indicated time point were processed for 7-AAD staining. The concentrations of propofol applied are denoted in (b) and (c).

Fig. 5b: Ratios of 7-AAD-positive cells determined by fluorescence flow cytometry (mean ± SD; n = 3). *$p < 0.01$.

Fig. 5c: Representative histograms. Percentages noted in the graphs represent the ratios of 7-AAD-positive cells. Ctrl indicates non-exposure control; P, propofol.

DISCUSSION

In this study, we showed that exposure to propofol (3–5 μg/mL for 24 h) elicited a dose- and time-dependent attenuation of cell growth in the human esophageal cancer cell lines KYSE30 and KYSE960. We also found that Matrigel invasion is reduced upon propofol exposure in both of these cell lines. In addition, propofol elicited the activation of executing caspase-3/7 but not caspase-9, a key player in the intrinsic apoptosis pathway. Lastly, propofol exposure enhanced early apoptosis in both cell lines and accelerated cell death in the KYSE30 cell line. Although previous studies have reported propofol-triggered growth suppression, reduced invasion, caspase activation, and cellular apoptosis in other types of cancer cell lines,[24-27] to the best of our knowledge, this is the first report providing a detailed assessment for the impact of propofol exposure on the growth properties of esophageal cancer cell lines. Our experimental data suggest that cell exposure to propofol activates caspase cascade and thereby promotes cellular apoptosis, which results in an increase in cell death at least in a subset of esophageal cancer cell lines. It is further speculated that the observed attenuation of cell proliferation in propofol-exposed esophageal cancer cell lines may not be entirely atributable to the genuin suppression of cell growth; it might be attributable, at least partly, to the increased cellular apoptosis elicited by propofol exposure.

In our experiments, propofol was found to consequences of propofol exposure in thse cell lines is unclear; however, previous studies have documented several differences in genetic backgrounds between the KYSE30 and KYSE960 cell lines, including the status of the *TP53* tumor suppressor gene and the *HRAS* oncogene (both mutated only in KYSE30 cells).[28,29] It is thus possible that different cellular properties, including different genetic profiles, of these cell lines were responsible for the observed distinct survival of the cell lines after propofol exposure. Another possible explanation may be that incubation periods longer than 24 h after the completion of propofol exposure is required to allow the detection of propofol-induced cell death in the KYSE960 cell line.

In our study, propofol exposure augmented cellular apoptosis as indicated by the activation of executioner caspase-3/7 and an increase in Annexin V-positive cells. However, propofol seemed not to significantly activate intrinsic apoptosis pathway which is initiated by the impairment of cellular integrity, such as severe DNA damage and ischemia. It is unknown how the excutioner caspases in esophageal cancer cell lines were activated upon propofol exposure; however, the possibility remains that the extrinsic apoptosis pathway is activated by propofol and stimulates caspase cascade, or the executioner caspases have an as-yet-unknown upstream signaling pathway(s) and propofol stimulates that pathway.

Propofol has generally been used within the concentration range of 1.6–8.9 μg/mL in most in vitro studies exploring the biological effects of propofol on cultured cells including cancer cell lines,[13,25,30,31] and this study was also conducted primarily using 3–5 μg/mL propofol. Thus, when comparing the biological consequences of propofol exposure among multiple types of cancer, experimental data obtained in this study should be directly comparable to those obtained in other in vitro studies. As a caveat, however, it has been established that the vast majority of propofol injected into a blood vessel is conjugated with erythrocytes or serum albumin, and thus it is unclear which concentration of propofol should be used in in vitro cell biological studies to precisely recapitulate in vivo propofol administration in clinical settings. To circumvent this issue in addressing the pharmacological action of propofol on cancer cells, it will be beneficial to conduct future studies employing animal models or clinical specimens derived from patients treated with propofol. Such efforts will eventually enable us to establish a safe usage of propofol with patients suffering from neoplastic diseases including esophageal cancer.

AUTHOR CONTRIBUTION

R.Z. and A.Z. contributed equally to the acquisition and analysis of data.

ACKNOWLEDGEMENTS

We wish to thank Ms Eri Yorifuji in the Division of Medical Research Engineering, Nagoya University Graduate School of Medicine, for assistance with live imaging and Ms Yukino Konishi in the Faculty of Pharmacy, Meijo University, for assistance with cell maintenance and collecting data.

FUNDING/SUPPORT

This study was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS) [21K08990, 19K09349, 19K18291 to Y.K.], as well as the Program Supporting Research Activities of Female Researchers from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan, 2016–2018 (to Y.K.).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

REFERENCES

1 Pavel MA, Petersen EN, Wang H, Lerner RA, Hansen SB. Studies on the mechanism of general anesthesia. *Proc Natl Acad Sci U S A.* 2020;117(24):13757–13766. doi:10.1073/pnas.2004259117.

2 McKeage K, Perry CM. Propofol: a review of its use in intensive care sedation of adults. *CNS Drugs.* 2003;17(4):235–272. doi:10.2165/00023210-200317040-00003.

3 Sahinovic MM, Struys MMRF, Absalom AR. Clinical Pharmacokinetics and Pharmacodynamics of Propofol. *Clin Pharmacokinet.* 2018;57(12):1539–1558. doi:10.1007/s40262-018-0672-3.

4 Sumi C, Okamoto A, Tanaka H, et al. Propofol induces a metabolic switch to glycolysis and cell death in a mitochondrial electron transport chain-dependent manner. *PLoS One.* 2018;13(2):e0192796. doi:10.1371/journal.pone.0192796.

5 Urabe T, Yanase Y, Motoike S, et al. Propofol induces the elevation of intracellular calcium via morphological changes in intracellular organelles, including the endoplasmic reticulum and mitochondria. *Eur J Pharmacol.* 2020;884:173303. doi:10.1016/j.ejphar.2020.173303.

6 Tsuchiya M, Asada A, Arita K, et al. Induction and mechanism of apoptotic cell death by propofol in HL-60 cells. *Acta Anaesthesiol Scand.* 2002;46(9):1068–1074. doi:10.1034/j.1399-6576.2002.460903.x.

7 Bunte S, Behmenburg F, Eckelskemper F, et al. Cardioprotection by Humoral Factors Released after Remote Ischemic Preconditioning Depends on Anesthetic Regimen. *Crit Care Med.* 2019;47(3):e250-e255. doi:10.1097/CCM.0000000000003629.

8 Hausburg MA, Banton KL, Roman PE, et al. Effects of propofol on ischemia-reperfusion and traumatic brain injury. *J Crit Care.* 2020;56:281–287. doi:10.1016/j.jcrc.2019.12.021.

9 Ulbrich F, Eisert L, Buerkle H, Goebel U, Schallner N. Propofol, but not ketamine or midazolam, exerts neuroprotection after ischaemic injury by inhibition of Toll-like receptor 4 and nuclear factor kappa-light-chain-enhancer of activated B-cell signalling: A combined in vitro and animal study. *Eur J Anaesthesiol.* 2016;33(9):670–680. doi:10.1097/EJA.0000000000000449.

10 Li R, Huang Y, Lin J. Distinct effects of general anesthetics on lung metastasis mediated by IL-6/JAK/STAT3 pathway in mouse models. *Nat Commun.* 2020;11(1):642. doi:10.1038/s41467-019-14065-6.

11 Tian D, Tian M, Ma ZM, Zhang LL, Cui YF, Li JL. Anesthetic propofol epigenetically regulates breast cancer trastuzumab resistance through IL-6/miR-149-5p axis. *Sci Rep.* 2020;10(1):8858. doi:10.1038/s41598-020-65649-y.

12 Shen X, Wang D, Chen X, Peng J. Propofol inhibits proliferation, migration, invasion and promotes apoptosis by regulating $HOST_2/JAK_2/STAT_3$ signaling pathway in ovarian cancer cells. *Cytotechnology.* 2021;73(2):243–252. doi:10.1007/s10616-021-00462-7.

13 Zhong H, Song R, Pang Q, et al. Propofol inhibits parthanatos via ROS–ER–calcium–mitochondria signal pathway in vivo and vitro. *Cell Death Dis.* 2018;9(10):932. doi:10.1038/s41419-018-0996-9.

14 Li C, Xia M, Wang H, Li W, Peng J, Jiang H. Propofol facilitates migration and invasion of oral squamous cell carcinoma cells by upregulating SNAI1 expression. *Life Sci.* 2020;241:117143. doi:10.1016/j.lfs.2019.117143.

15 Zhang L, Wang N, Zhou S, Ye W, Jing G, Zhang M. Propofol induces proliferation and invasion of gallbladder cancer cells through activation of Nrf_2. *J Exp Clin Cancer Res.* 2012;31(1):66. doi:10.1186/1756-9966-31-66.

16 Malhotra GK, Yanala U, Ravipati A, Follet M, Vijayakumar M, Are C. Global trends in esophageal cancer. *J Surg Oncol.* 2017;115(5):564–579. doi:10.1002/jso.24592.

17 Abbas G, Krasna M. Overview of esophageal cancer. *Ann Cardiothorac Surg.* 2017;6(2):131–136. doi:10.21037/acs.2017.03.03.

18 Shimada Y, Imamura M, Wagata T, Yamaguchi N, Tobe T. Characterization of 21 newly established esophageal cancer cell lines. *Cancer.* 1992;69(2):277–284. doi:10.1002/1097-0142(19920115)69:2<277::AID-CNCR2820690202>3.0.CO;2-C.

19 Tanaka H, Shibagaki I, Shimada Y, Wagata T, Imamura M, Ishizaki K. Characterization of p53 gene mutations in esophageal squamous cell carcinoma cell lines: increased frequency and different spectrum of mutations from primary tumors. *Int J Cancer.* 1996;65(3):372–376. doi:10.1002/(SICI)1097-0215(19960126)65:3<372::AID-IJC16>3.0.CO;2-C.

20 Barann M, Friederich P, Retzmann K, Dybek A, Urban BW. Loss of Propofol during In Vitro Experiments. *Anesthesiology.* 2000;93(1):310–311. doi:10.1097/00000542-200007000-00067.

21 Husna M, Munawiroh SZ, Puji Ekawati R, Hanifah S. Systematic review of the stability and compatibility of propofol injection. *Anaesthesiol Intensive Ther.* 2021;53(1):79–88. doi:10.5114/ait.2021.103542.

22 Sall JW, Leong J. Technical communication: stability of propofol in polystyrene-based tissue culture plates. *Anesth Analg.* 2013;117(1):65–67. doi:10.1213/ANE.0b013e318292f32e.

23 Jiang L, Tixeira R, Caruso S, et al. Monitoring the progression of cell death and the disassembly of dying cells by flow cytometry. *Nat Protoc.* 2016;11(4):655–663. doi:10.1038/nprot.2016.028.

24 Zhang F, Wang C, Cui Y, et al. Effects of Propofol on Several Membrane Characteristics of Cervical Cancer Cell Lines. *Cell Physiol Biochem.* 2016;40(1–2):172–182. doi:10.1159/000452535.

25 Tatsumi K, Hirotsu A, Daijo H, Matsuyama T, Terada N, Tanaka T. Effect of propofol on androgen receptor activity in prostate cancer cells. *Eur J Pharmacol.* 2017;809:242–252. doi:10.1016/j.ejphar.2017.05.046.

26 Liu F, Qiu F, Fu M, Chen H, Wang H. Propofol Reduces Epithelial to Mesenchymal Transition, Invasion and Migration of Gastric Cancer Cells through the MicroRNA-195-5p/Snail Axis. *Med Sci Monit.* 2020;26:e920981. doi:10.12659/MSM.920981.

27 Li H, Lu Y, Pang Y, Li M, Cheng X, Chen J. Propofol enhances the cisplatin-induced apoptosis on cervical cancer cells via EGFR/JAK2/STAT3 pathway. *Biomed Pharmacother.* 2017;86:324–333. doi:10.1016/j.biopha.2016.12.036.

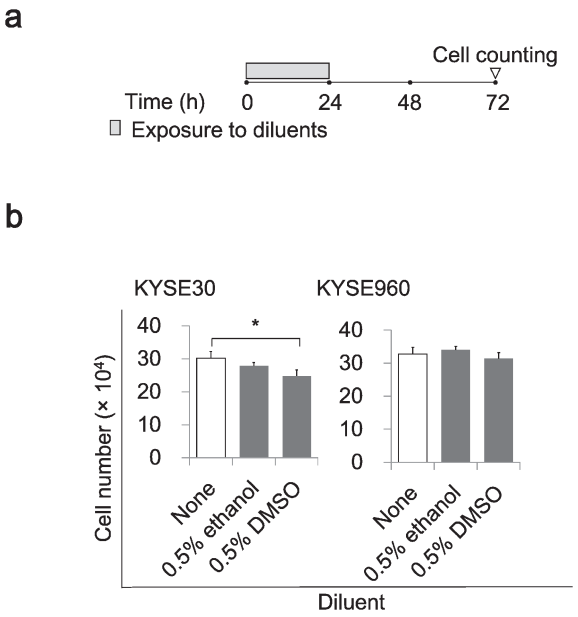
28 Rigberg DA, Kim FS, Blinman TA, et al. p21 Expression Is Increased by Irradiation in Esophageal Squamous Cell Carcinoma. *J Surg Res*. 1998;76(2):137–142. doi:10.1006/jsre.1998.5308.

29 Fukuoka S, Kojima T, Koga Y, et al. Preclinical efficacy of Sym004, novel anti-EGFR antibody mixture, in esophageal squamous cell carcinoma cell lines. *Oncotarget*. 2017;8(7):11020–11029. doi:10.18632/oncotarget.14209.

30 Takaishi K, Kudo Y, Kawahito S, Kitahata H. Clinically relevant concentration of propofol and benzodiazepines did not affect in vitro angiogenesis. *J Anesth.* 2021;35(6):870–878. doi:10.1007/s00540-021-02993-x.

31 Deng F, Ouyang M, Wang X, et al. Differential role of intravenous anesthetics in colorectal cancer progression: implications for clinical application. *Oncotarget.* 2016;7(47):77087–77095. doi:10.18632/oncotarget.12800.

SUPPLEMENTARY MATERIALS



Supplementary Fig. 1 Proliferation of KYSE30 cells was suppressed by 0.5% (v/v) DMSO but not by 0.5% (v/v) ethanol

Supplementary Fig. 1a: Experimental design to assess the effects of 0.5% (v/v) diluents (ethanol and DMSO) on the proliferation of esophageal cancer cell lines KYSE30 and KYSE960.

Supplementary Fig. 1b: Cell numbers counted in the assay delineated in (a) (mean ± SD; n = 5). DMSO indicates dimethyl sulfoxide. $*p < 0.05$.