主論文の要約

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

（プロポフォールは食道がん細胞株のアポトーシスを誘発し増殖を低下する）

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【Introduction】

The lipophilic intravenous anesthetic agent propofol is commonly used in surgical operations and intensive care owing to its rapid and controllable sedative effects. Propofol has diverse effects on cellular biological functions, including positive modulation of the γ-aminobutyric acid A (GABAA) receptor. Propofol also binds to cellular and mitochondrial membranes, disrupts their lipid bilayer structure, and causes cellular and mitochondrial dysfunctions. Propofol has been shown to confer negative effects on the proliferation and invasion of cultured neoplastic cells and elicit cellular apoptosis in vitro. Conversely, evasion from cell death and enhancement of proliferation in cancer cell lines caused by propofol exposure have also been demonstrated in previous studies. Several reports suggest that propofol likely exerts varied effects on the oncogenic properties of cancer cells, depending on the cellular context or type of cancer. However, it is largely unknown how exposure to propofol affects the proliferation, invasion, and apoptosis of neoplastic cells in esophageal cancer. This study was conducted to elucidate the impact of propofol exposure on the growth properties of human esophageal cancer cell lines in vitro.

【Material and Methods】

Two Japanese human esophageal cancer cell lines, KYSE30 and KYSE960, were treated with up to 10 μg/mL propofol for 12–36 hours (h). The cells were then analyzed by cell proliferation assay. To explore the effect of propofol exposure on invasive tumorigenesis, a Matrigel invasion assay using a Boyden chamber-based assay was performed. The quantification of caspase-3/7 and -9 activities was evaluated using chemiluminescence-conjugated caspase-3/7 and -9 antibodies. Cell staining with Annexin V and 7-aminoactinomycin D (7-AAD) was performed to detect early apoptosis and cell death, respectively, via flow cytometry.

【Results】

To understand the impact of propofol exposure on esophageal cancer cell growth, two proliferation assays were carried out using KYSE30 and KYSE960 cells that had been exposed to 2–5 μg/mL propofol in a regular medium for 12–36 h. Cell counts demonstrated a significant dose- and time-dependent attenuation of cell growth in the KYSE30 and KYSE960 cell lines. Cell exposure to propofol resulted in a dose-dependent suppression of Matrigel invasion in the KYSE30 and KYSE960 cell lines. To address whether propofol induces apoptosis in esophageal cancer cell lines, caspase activity was examined in propofol-exposed KYSE30 and KYSE960 cell lines. Propofol-exposed esophageal cell lines upregulated the activity of apoptosis-inducing executioner caspases (caspase-3/7), whereas caspase-9 was not activated by propofol exposure in either cell line. These data suggest that propofol triggers apoptosis in esophageal cancer cell lines, but does not activate the intrinsic apoptotic signaling pathway. To confirm the induction of cellular apoptosis by propofol exposure, cells were immunostained

with Annexin V at 6 h or 24h after the completion of propofol exposure. Both cell lines exhibited a statistically significant increase in Annexin V-positive cells after propofol exposure. 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. Further analysis was conducted to detect irreversible necrosis or cell death by staining KYSE30 and KYSE960 cells exposed to propofol with 7-AAD. The results demonstrated a significant increase in the frequency of 7-AAD-positive cells in the KYSE30 cell line, whereas no significant change was detected in the 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 indicated by elevated 7-AAD positivity, at least in a subset of cell lines.

【Discussion】

In this study, cells exposed to propofol (3–5 μg/mL for 24 h) elicited a dose- and time-dependent attenuation of cell growth and dose-dependent attenuation of Matrigel invasion in the human esophageal cancer cell lines KYSE30 and KYSE960. In addition, propofol elicited the activation of caspase-3/7, but not caspase-9, which is a key player in the intrinsic apoptosis pathway. Finally, propofol exposure enhanced early apoptosis in both cell lines and accelerated cell death in KYSE30 cells. Although previous studies have reported propofol-triggered growth suppression, reduced invasion, caspase activation, and cellular apoptosis in other types of cancer cell lines, to the best of my knowledge, this is the first report to provide a detailed assessment of the impact of propofol exposure on the growth properties of esophageal cancer cell lines.

This study suggests that cell exposure to propofol activates the 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 attributable to the suppression of cell growth; it might be attributable, at least partly, to the activated cellular apoptosis elicited by propofol exposure.

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; this study was also conducted primarily using 3–5 μg/mL propofol. Thus, when comparing the biological consequences of propofol exposure in multiple types of cancer, the experimental data obtained in this study should be directly comparable to those obtained in other in vitro studies. However, it has been established that the vast majority of propofol injected into blood vessels is conjugated with erythrocytes or serum albumin; thus, it is unclear which concentration of propofol should be used in in vitro cellular 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 using animal models or clinical specimens derived from patients treated with propofol. Such efforts will eventually enable us to establish the safe usage of propofol in patients suffering from neoplastic diseases, including esophageal cancer.

【Conclusion】

In summary, exposure to propofol at concentrations up to 5 μg/mL led to a reduction in 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, in patients with esophageal cancer.