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Effects of sufentanil on human gastric cancer cell line SGC-7901 *in vitro*

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Abstract

Sufentanil is a new kind of opioid analgesic and acts on μ opioid receptor. In this study, we aim to investigate the effects of sufentanil on gastric cancer cell line SGC-7901, after being exposed to different concentrations of sufentanil. Gastric cancer cells were exposed to sufentanil for a predetermined time at concentrations of 0, 0.5, 5, 50 and 500 nmol/l, respectively. Cell viability at different time points after exposure to sufentanil was tested by CCK-8 assay. FDA-PI staining was used to observe membrane integrity of gastric cancer SGC7901 cells. The apoptosis of gastric cancer cells was analyzed by Annexin V-FITC/PI Flow Cytometry and the changes of the cell cycle was determined by a detection kit. As a result, cell viability decreased in a dose- and time-dependent manner. Furthermore, with the concentration of sufentanil increased, the proportion of dead and apoptotic SGC-7901 cells increased, and more cells were arrested in G2/M phase. In a word, sufentanil can inhibit the cell viability and induce the apoptosis of gastric cancer SGC-7901 cells in vitro.

Key words: *sufentanil, gastric cancer cell, cell cycle arrest, apoptosis.*

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Introduction

Sufentanil is a new kind of potent opioid analgesic and acts on μ opioid receptor. Due to its strong liposolubility and high adherence ratio to plasma protein in human, sufentanil displays its powerful analgesic effects, which is 5-10 times higher than fentanyl and much higher than morphine.

Some researches [1-11] had found that some opioid can partially inhibit the growth of the tumor cells. For instance, morphine inhibited tumor cell proliferation at the concentration of less than 10 μM [1] and exogenous morphine can inhibit the growth of human gastric cancer cell line MGC-803 [12]. On the contrary, other studies found that the opioid could promote the survival of tumor cells [13-18]. In a clinically relevant dose, morphine could promote tumor neovascularization in a breast tumor xenograft model in mice, which led to tumor progression [16]. Morphine also displayed the effects that can trigger the proliferation of glioblastoma T98G cell [17]. In addition, as another opioid receptor agonist, remifentanil possesses the capability of increasing cellular respiration of human hepatocytes and inhibiting tumor necrosis factor α (TNF-α) induced mitochondrial dysfunction [18].

However, so far, the underlying exact mechanisms how the opioid affects tumor cells has still not been clearly understood. In the present study, as an opioid analgesic, sufentanil was selected to analyze its effects on human gastric cancer cell line SGC-7901 *in vitro*. After exposure to human gastric cancer cells, sufentanil displayed some characteristics that can inhibit the cell viability and induce the apoptosis of gastric cancer SGC-7901 cells *in vitro*.

Material and methods

Chemicals

Sufentanil was purchased from Yichang Humanwell Pharmaceutical Co., Ltd. (Yichang, Hubei, China). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin sulfate, trypsin, fluorescein diacetate (FDA), propidium iodide (PI) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Cell counting-8 kit (CCK-8), Annexin V-FITC/PI apoptosis detection kit, and Cell cycle and apoptosis analysis kit were obtained from Vazyme Biotech Co., Ltd. (Nanjing, Jiangsu, China). Human gastric cancer cell line SGC-7901 was kindly presented by the Jiangsu Key Laboratory of Molecular Medicine, Medical School of Nanjing University. All reagents were of analytical grade.

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Cell culture

Human gastric cancer cell line SGC-7901 was cultured in an incubator with 5% CO_2 at 37°C, and DMEM medium was supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin sulfate (100 μg/ml). The cells in the logarithmic growth phase were collected and prepared for the study.

Cell viability assay

After sufentanil exposure, human gastric cancer cell line SGC-7901 was analyzed by Cell Counting Kit-8 (CCK-8) test for evaluating the changes in cell viability. Briefly, cells were put into a 96-well culture plate at a density of 2×10^4 cells/well with 100 μl of culture medium. After incubation for 24 h, the cells were divided into four processing time groups, then exposed to different levels of sufentanil at concentrations of 0, 0.5, 5, 50, and 500 nM respectively. After exposure to sufentanil for 6, 12, 24, 48 h for different groups respectively in 5% CO_2 at 37°C, the assay was operated in accordance with instructions of the CCK-8 kit. Finally, the absorbance was measured at 450 nm using an automated microplate reader (Bio-Rad, Japan).

FDA and PI staining for morphologic evaluation

FDA-PI staining can be employed to distinguish viable (FDA^+PI^-) from apoptotic or dead cells (FDA^-PI^+). Fluorescein diacetate (FDA) can penetrate the cell membrane, then hydrolyzed by nonspecific esterases in viable cells, displaying green fluorescence that is retained in the cytoplasm of intact cells. However, if the cells are apoptotic or dead, fluorescence is decreased due to the lack of esterases and the leakage of FDA from the cells for poor membrane integrity. Propidium iodide (PI), a nucleic acid-binding dye, can be excluded from viable cells but possesses the ability to enter dead or dying cells readily for the loss of membrane integrity. In our research, cells were inoculated at a density of 2×10^5 ml^{-1} into 96-well plates. After being incubated for 24 h, cells were treated with 0, 0.5, 5, 50, 500 nM sufentanil respectively for 24 h, then stained with 5 μg/ml PI and 4 μg/ml FDA. Finally, the cells were observed using a fluorescent microscope (Nikon, Chiyoda-ku, Tokyo, Japan).

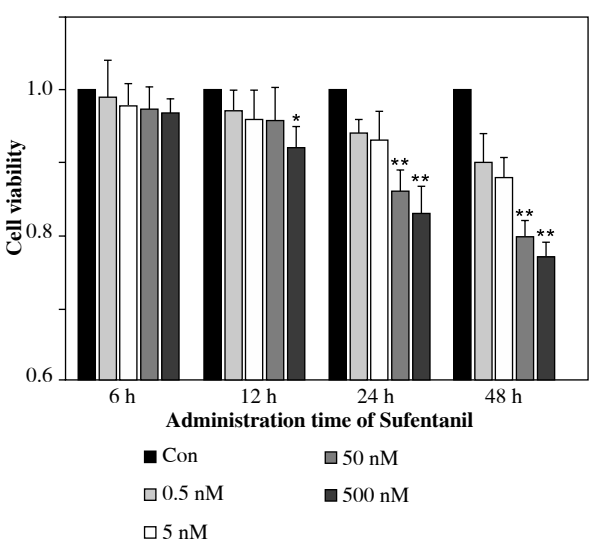

Fig. 1. Effect of different concentrations of sufentanil on cell viability of cultured SGC-7901 gastric cancer cells after being treated for 6 h, 12 h, 24 h and 48 h. Data are shown as mean ± S.E. ($n = 6$). Asterisk denotes a response that is significantly different from the control ($^*p < 0.05$, $^{**}p < 0.01$)

Measurement of apoptosis

After exposure to sufentanil, apoptotic cells were identified by the Annexin V-FITC Apoptosis Detection Kit according to the manufacturer's instructions. SGC-7901 cells were treated with different concentrations of sufentanil for 24 h, then a FACS calibur flow cytometer (BD, USA) was employed. About 1×10^6 cells for each group were stained with Annexin V-FITC for 30 min at 4°C in the dark, and then with propidium iodide for 10 min before flow cytometric analysis.

Cell cycle analysis

Briefly, gastric cancer cells were divided into five groups with about 1×10^6 cells for each, processed with the aforementioned different concentrations of sufentanil for 24 h, then washed twice with ice-cold PBS, treated with trypsin and finally fixed in cold 70% ethanol for 30 min at 4°C. The following operations were carried out in accordance with the manufacturer's instructions of the Cell Cycle and Apoptosis Analysis Kit. Flow cytometry was used for analyzing the treated cells.

Statistical analysis

Each experiment was performed in sextuplicate and repeated at least three times to ensure the replicability. A separate cell preparation was used for each experiment. The data were expressed as the mean ± standard error (S.E.). All calculations and statistical analysis were performed using SPSS for windows version 17.0 (SPSS Inc., Chicago, IL, USA) and Origin pro 8.0 software programs (Origin Lab Co., Northampton, MA, USA). One-way analysis of variance (ANOVA) was used to analyze the difference between groups, followed by Dunnett's *t*-test. $P < 0.05$ was regarded as statistically significant.

Results

Cell viability assay

It could be found that, at different time points after treatment with sufentanil, the cell viability of all groups de-

creased as the concentration of sufentanil elevated (Fig. 1). For the 12 h-treated group, cell viability significantly decreased as the concentration of sufentanil reached 500 nM. When the processing time was 24 h or 48 h, compared with the control group, the differences in cell viability are extremely significant when the concentration of sufentanil reached 50 nM or above. The results indicated that the growth and proliferation of cancer cells was inhibited after being exposed to sufentanil. Especially, for SGC-7901 cells, the lowest effective concentration of sufentanil may be only 50 nmol/l.

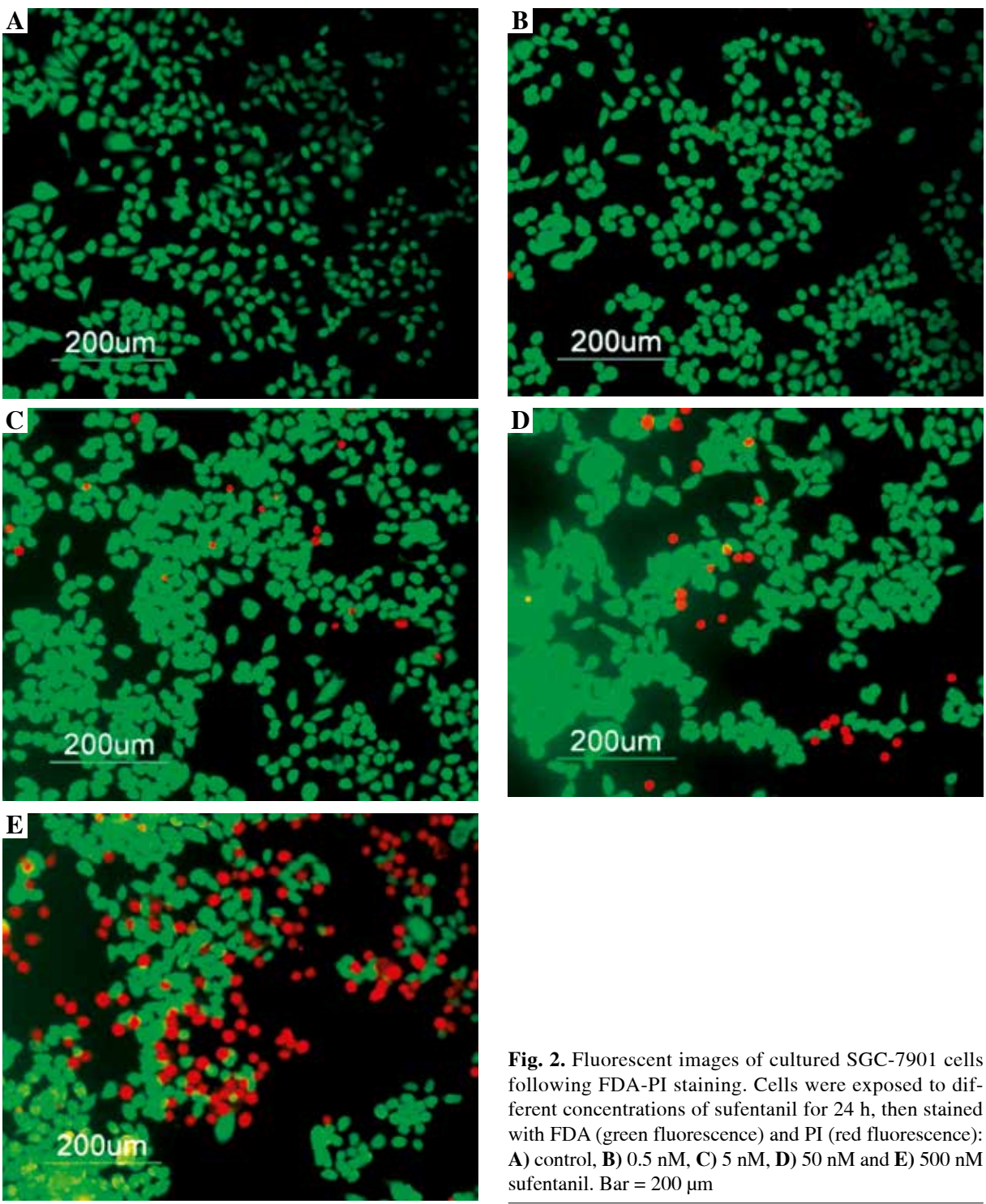

Fig. 2. Fluorescent images of cultured SGC-7901 cells following FDA-PI staining. Cells were exposed to different concentrations of sufentanil for 24 h, then stained with FDA (green fluorescence) and PI (red fluorescence): **A)** control, **B)** 0.5 nM, **C)** 5 nM, **D)** 50 nM and **E)** 500 nM sufentanil. Bar = 200 μm

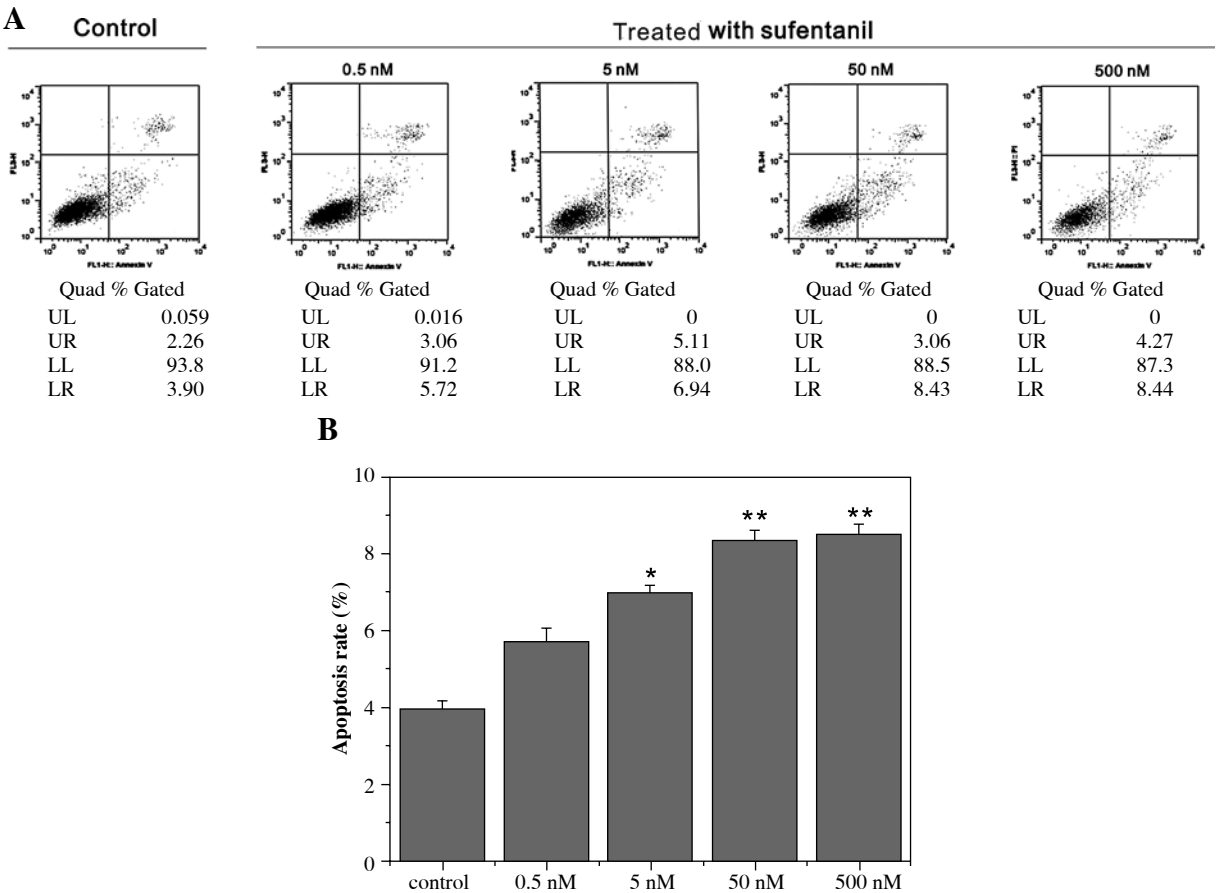

Fig. 3. A) Flow cytometry dot plots showing the binding of Annexin V-FITC (FL-1) and PI uptake (FL-3) of SGC-7901 cells treated with different concentrations of sufentanil for 24 h, followed by percentages of four quadrants respectively below. **B)** Apoptosis rates of SGC-7901 gastric cancer cells after being treated with different concentrations of sufentanil for 24 h. Data are shown as mean ± S.E. ($n = 3$). Asterisk denotes a response that is significantly different from the control ($^{*}p < 0.05$, $^{**}p < 0.01$)

FDA and PI staining

As the concentration of sufentanil increased, the proportion of dead cells that display red fluorescence increased, however viable cells that show green fluorescence gradually reduced (Fig. 2), which could be clearly observed by fluorescence microscopy. These findings suggested that the membrane integrity in part of gastric cancer cells had been destroyed when exposed to sufentanil, and the number of dead cells displayed a dose-dependent effect.

The analysis of apoptosis

To further study the possible inducing apoptosis effect of sufentanil on SGC-7901 gastric cancer cells, cells were stained with Annexin V-FITC and PI, and subsequently analyzed by flow cytometry. The dual parameter fluorescent dot plots showed that the viable cells (Annexin V^-/PI^-) were in the lower left quadrant, the apoptotic cells were in the lower right quadrant (Annexin V^+/PI^-). As indicated in Fig. 3A and 3B, with the concentration of sufentanil elevated, the percentages of apoptotic cells increased. When treated with 50 nmol/l sufentanil or above, compared with the control group, the percentages of apoptotic cells were significantly higher ($p < 0.01$). Obviously, these results indicated that sufentanil could induce apoptosis in gastric cancer SGC-7901 cells.

Effect of sufentanil on cell cycle

Flow cytometry was used to determine whether the inhibitory effects of sufentanil on the proliferation of SGC-7901 cells is mediated, at least in part, through affecting cell cycle progression. As indicated in Fig. 4A, the green peak represented the cells in G1 phase, the yellow peak illustrated the cells in S phase and the blue peak displayed the cells in G2 phase. When treated with sufentanil at the concentration of 5 nM or above, the percentages of SGC-7901 cells in S phase and G2 phase were significantly different from the control (Fig. 4B). These findings indicated

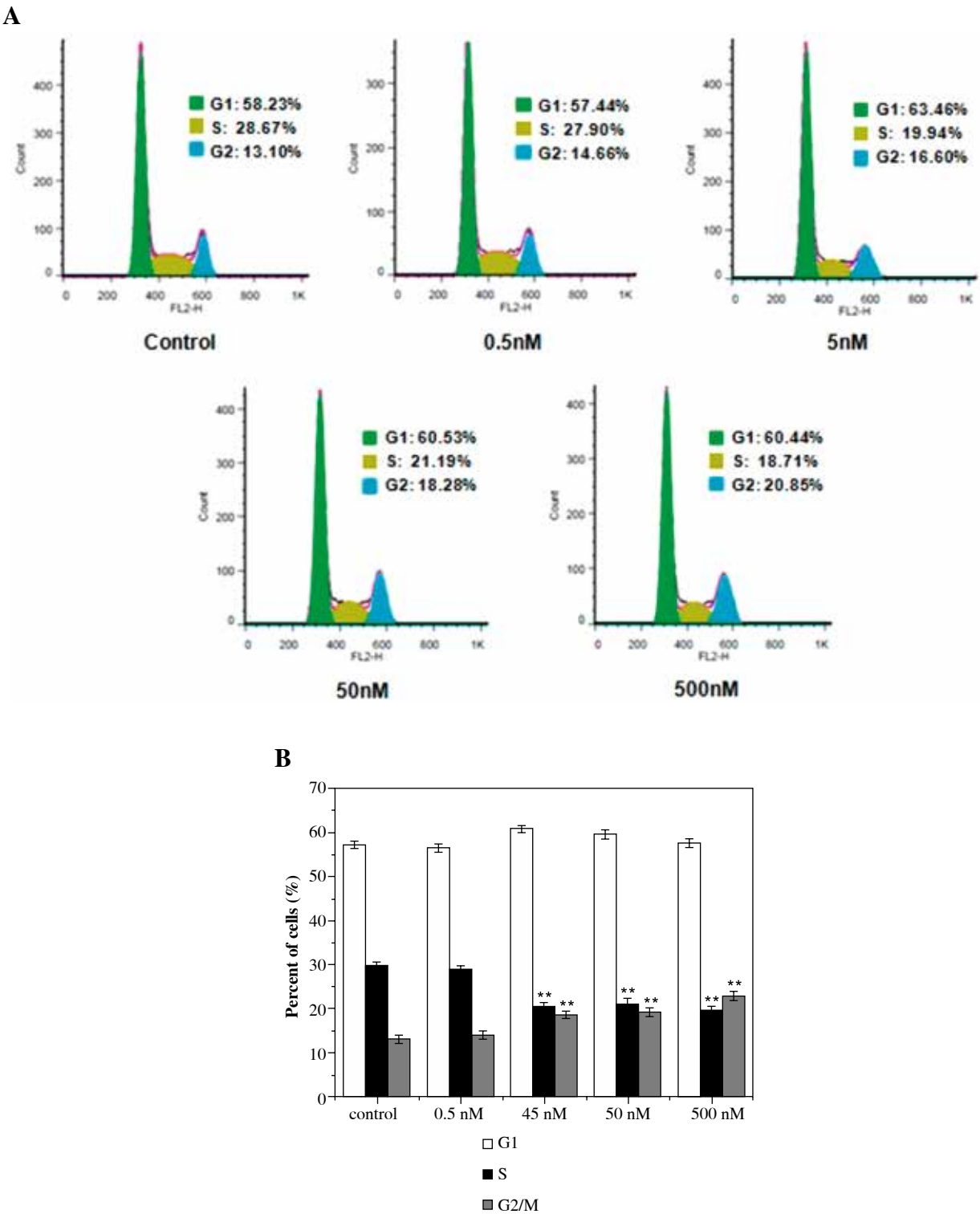

Fig. 4. A) Cell cycle analysis of SGC-7901 cells treated with different concentrations of sufentanil for 24 h. Phases of G1, S and G2 are displayed by three different colors in each chart. **B)** Percents of SGC-7901 gastric cancer cells in G1, S and G2 phase respectively after being treated with different concentrations of sufentanil for 24 h. Data are shown as mean ± S.E. ($n = 3$). Asterisk denotes a response that is significantly different from the control ($^{*}p < 0.05$, $^{**}p < 0.01$)

that sufentanil could lead to cell cycle arrest at the G2/M phase, suggesting that sufentanil may inhibit cell proliferation through controlling the G2/M checkpoint and inducing a specific block in cell cycle progression.

Discussion

As a potent analgesic which is widely used in the clinical management of pain, sufentanil is much better than morphine and fentanyl in the analgesic effect, although all these three analgesics play a role through μ opioid receptors. In recent years, the inhibitory effects of opioid analgesics on the tumor cell growth have been increasingly reported. However, the underlying specific mechanisms wherein are still unknown.

In the present study, through FDA and PI staining and the assay of CCK-8, some facts could be found that with the increasing of the concentration of sufentanil, cell viability declined obviously. Many studies have indicated that morphine, also known as an opioid analgesic, may down-regulate the development and spread of tumor cells. It has been found that morphine inhibited the growth of liver cancer cells in mice [19]. Moreover, morphine has been demonstrated to decrease the development of human breast cancer cells *in vitro* [6]. So far, the complexity of opioid-tumor cells interactions has been found in many studies, but how they influence the growth of cancer cells is far from being clearly understood.

Apoptosis is an active intracellular programmed death process that is caused by physiological and pathological changes to remove redundant and damaged cells. In our research, gastric cancer SGC-7901 cells were arrested in G2/M phase increasingly and showed more apoptotic characteristics with the increase of the concentration of sufentanil. In the process of apoptosis, the key point may be the triggering of caspase cascade [20]. For instance, caspase 3 is an well-known main executor of apoptosis and can lead to morphological changes of cells, such as fragmentations of chromosomal DNA and nucleus and the forming of many apoptotic bodies [21]. In addition, NF-κB is a specific nuclear transcription factor that plays an important role in regulation of many genes which closely related to apoptosis and tumorigenesis [22]. A large number of researches suggested that inhibition of NF-κB can suppress the growth of cancer cells [23]. It has been reported that the anticancer effects of opioid analgesic might be mediated through the suppression of NF-κB [24]. Therefore, we believe that the triggering of caspase cascade and inhibition of NF-κB may be involved in the process of the inhibitory effects on tumor cells growth from sufentanil. However, the specific mechanism remains to be explored in future studies.

In conclusion, sufentanil, one of the potent opioid analgesics, can inhibit the cell viability and induce apoptotic effects when directly touched with gastric cancer SGC-7901 cells *in vitro*. Further studies will be needed to explore the exact underlying mechanisms.

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Authors declare no conflict of interest.

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