J Anesth (2014) 28:298–301
DOI 10.1007/s00540-013-1717-2

SHORT COMMUNICATION

Effects of morphine and fentanyl on 5-fluorouracil sensitivity in human colon cancer HCT116 cells

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Received: 22 August 2013 / Accepted: 11 September 2013 / Published online: 5 October 2013

Abstract Opioids are widely used for perioperative pain management in cancer surgery patients. It has been reported that opioids may alter cancer recurrence or progression; however, there are no published reports regarding the effects of opioids on chemotherapy after cancer surgery. Here we investigated the effects of opioids (morphine or fentanyl) on cell proliferation and 5-fluorouracil sensitivity in the human colon cancer cell line, HCT116. First, we exposed cancer cells to the opioid at various concentrations for 6 or 24 h and evaluated cell proliferation using a MTT assay. Next, to simulate the potential postoperative situation in which anticancer drugs are administered after cancer surgery, cancer cells were incubated with the opioid for 6 or 24 h, followed by treatment with 5-fluorouracil for 48 h. Although fentanyl did not affect cell proliferation, morphine exposure for 6 h enhanced the proliferation. However, sensitivity of HCT116 cells to 5-fluorouracil was not altered in all treatment groups. The current study demonstrated that the opioids commonly used during postoperative periods do not affect 5-fluorouracil sensitivity in human colon cancer HCT116 cells.

Keywords Opioid · Colon cancer · 5-Fluorouracil

There is a growing recognition of the potential for some anesthetics or anesthetic techniques to influence long-term outcomes in cancer surgery patients. Several retrospective clinical studies have shown a reduced incidence of cancer recurrence after regional anesthesia and reduced doses of opioids after surgery [1–3]. These studies have largely focused on the potential beneficial effects of regional anesthetics and analgesia; however, basic research data demonstrate that opioids induce tumor growth [4, 5], inhibit apoptosis [6, 7], and promote angiogenesis [8, 9]. Thus, the differences in cancer recurrence rates may result from the effects of opioids on tumor cell growth. Therefore, concerns have been raised regarding the safety of perioperative opioid analgesia in cancer patients. Furthermore, although the benefit of postoperative systemic adjuvant chemotherapy for patients who have undergone potentially curative surgical tumor resection has been established, the direct effects of opioids on cancer cell sensitivity to postoperative chemotherapy have not been examined adequately. Therefore, in the present study, we investigated whether opioids commonly used during general anesthesia alter sensitivity to cancer chemotherapy in the human colon cancer cell line, HCT116.

HCT116 cells, purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), were cultured in McCoy's 5A medium supplemented with 10 % fetal bovine serum and 1 % penicillin–streptomycin. Cells were incubated at 37 °C in humidified atmospheric air with 5 % CO_2. The medium was changed every 3 days and passaged every 5–7 days. For experiments, cells were plated in 96-well plates at a density of 5×10^3 cells/well. Twenty-four hours later, cells were cultured for 6 or 24 h in fresh medium containing an opioid at clinically relevant concentrations. Twenty-four hours after incubation, we investigated cell proliferative activity. Next, to examine the effects of opioids on chemotherapy sensitivity, HCT116 cells were incubated with various opioids for 6 h followed by treatment with 5-fluorouracil for 48 h. Cellular proliferative activity was determined by the MTT (3-[4,5-

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dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's protocol. The absorbance of the formazan product was then measured at a wavelength of 570 using 650 nm as the reference. MTT assay values for labeled cells were expressed as the percentage of the corresponding average values in control cells. Each experiment was performed in triplicate and repeated four or five times. GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis. Statistical analyses were performed using unpaired Student's *t* test (two-tailed) and one-way analysis of variance (ANOVA) test followed by the Dunnett post hoc test. All data are expressed as the mean ± SD (standard deviation). Statistical significance was defined as $P < 0.05$.

As shown in Fig. 1a, treatment with clinical relevant doses of fentanyl for 6 and 24 h had no effect on the proliferative activity of HCT116 cells. In contrast, morphine exposure for 6 h significantly enhanced proliferative activity; however, these effects disappeared in HCT116 cells treated with morphine for 24 h (Fig. 1b). Next, we confirmed that the proliferative activity was inhibited by 5-fluorouracil in a dose-dependent manner (Fig. 2a). As shown in Fig. 2b, morphine exposure for 6 h did not affect the sensitivity of HCT116 cells to 5-fluorouracil (5 ng/ml).

In the current in vitro study, we demonstrated that exposure of HCT116 cells to morphine for 6 h resulted in enhanced proliferation. However, morphine exposure for 6 h did not alter the sensitivity to 5-fluorouracil. This is the first study to investigate the effects of opioids on subsequent chemotherapy.

It is likely that opioids directly regulate the growth of cancer cells by modulating cell proliferation and/or apoptosis. Recent laboratory studies have demonstrated that the μ-opioid receptor regulates cancer progression [10–12], and morphine was shown to trigger human glioblastoma T98G cell proliferation [5]. Gupta et al. [8] reported in an animal study that morphine administered in clinically relevant doses increased tumor progression. These reports support the hypothesis that μ-opioid receptor activation promotes tumor progression.

In contrast, Tegeder et al. [13] reported that high concentrations of morphine (>10 μM) inhibited tumor cell

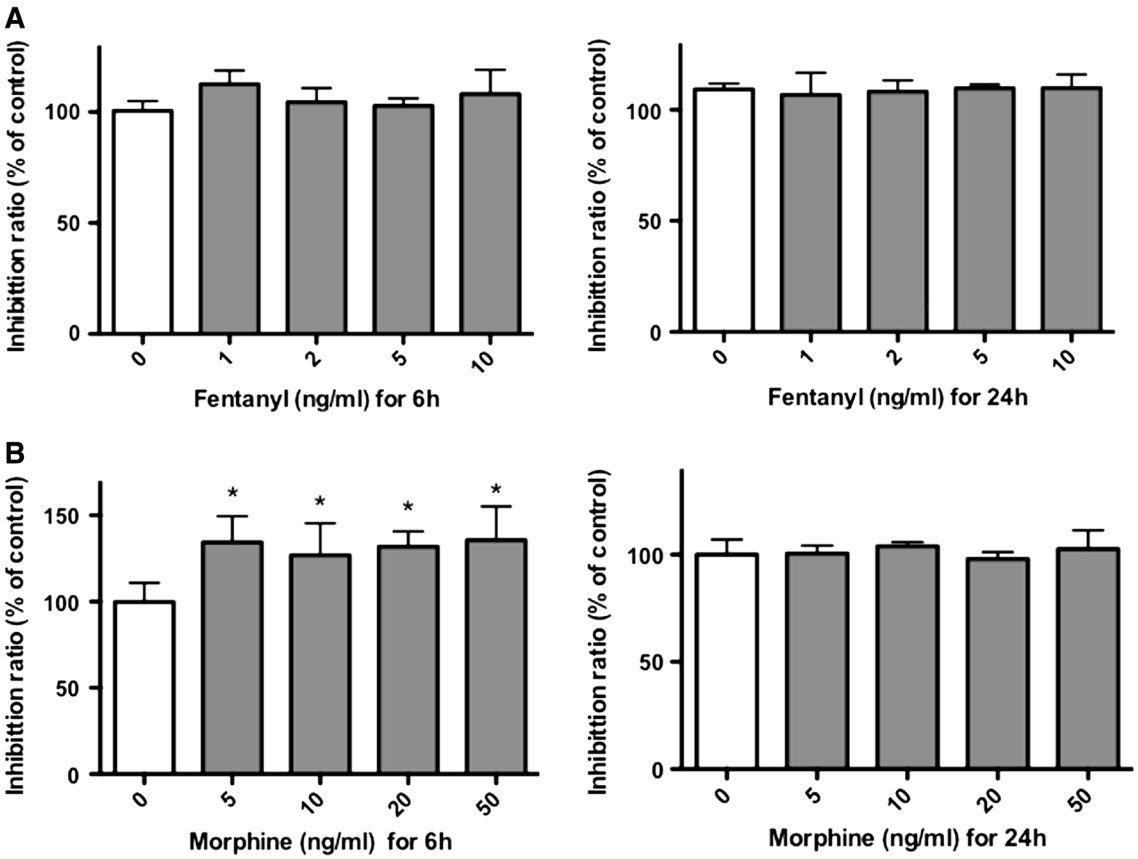

Fig. 1 Effects of opioids on proliferative activity in HCT116 cells. **a** Fentanyl (1–10 ng/ml) did not affect the proliferative activity of HCT116 cells. **b** Morphine exposure for 6 h, but not for 24 h, enhanced cell proliferation. MTT assay values were expressed as the percentage of the corresponding average values in control cells. All data are expressed as mean ± SD in four or five independent experiments. *$P < 0.05$ versus control

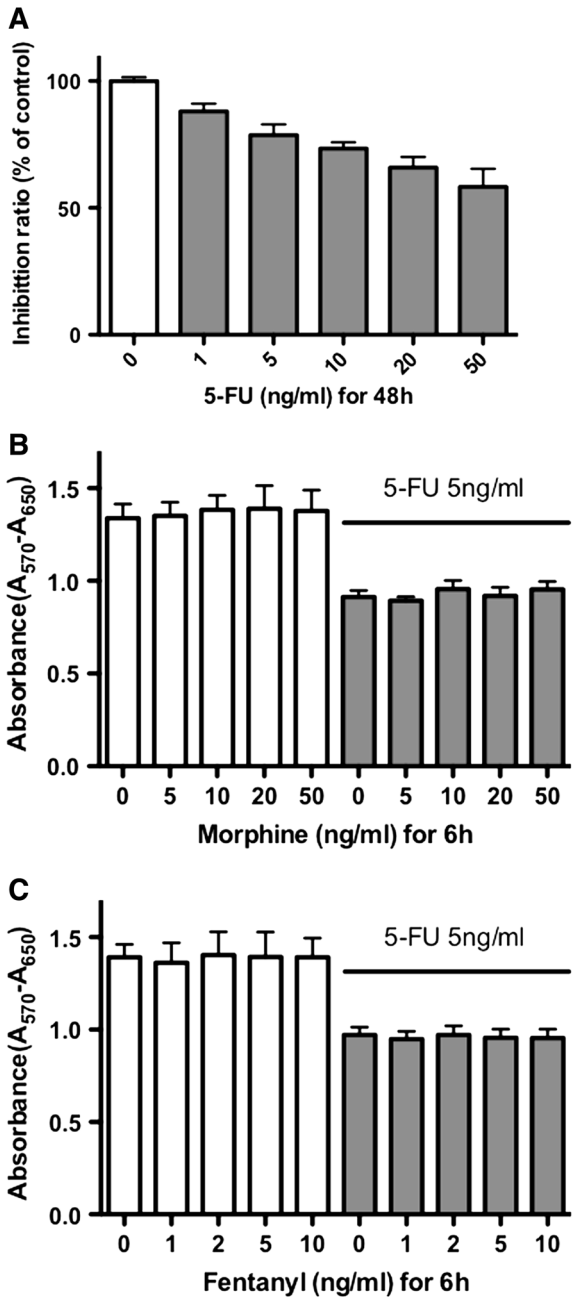

Fig. 2 HCT116 cell sensitivity to 5 ng/ml 5-fluorouracil after opioid treatments. **a** Proliferative activity was inhibited by 5-fluorouracil in a dose-dependent manner. **b** Neither fentanyl nor morphine affected HCT116 cell sensitivity to 5-fluorouracil (5 ng/ml for 48 h). All data are expressed as mean ± SD in four independent experiments

proliferation. In another study, morphine significantly reduced the growth of MCF-7 and MDA-MB-231 tumors, and intermittent injections of morphine decreased the growth of tumors in a rat model of metastasizing colon cancer [14]. Furthermore, several studies have documented a pro-apoptotic effect of opioids on cancer cells in vitro [15–18]. A possible explanation for the discrepancies among these previous studies is that tumor suppression occurs after chronic high doses of morphine, whereas tumor-enhancing effects with morphine occur after a single dose or low doses. Consistent with this hypothesis, 6-h exposure to morphine in HCT116 cells enhanced proliferation in our study. Thus, it remains poorly understood whether morphine itself modifies the growth of tumor cells.

On the other hand, remifentanil, a potent μ-opioid receptor agonist, is commonly used during general anesthesia. However, there are no published reports regarding the effects of remifentanil on proliferation in cancer cells. In our preliminary study, remifentanil itself did not affect the proliferative activity (data not shown). This finding suggests that different receptors other than μ-opioid receptors can be involved in the enhanced proliferation induced by morphine.

Our findings should be interpreted within the constraints of the study's potential limitations. Some researchers postulate that morphine can promote tumor growth as a result of immunosuppression, as the negative effects of morphine and other opioids on the immune system are well established [4, 19–21]. However, clinical and animal studies on the immunosuppressive effects of opioids during surgery are complex because pain itself may suppress immunity. Therefore, we first explored the direct effects of opioids on cancer cells by conducting in vitro cell culture experiments. In future animal studies, it will be necessary to investigate whether opioids affect cancer cell sensitivity to chemotherapeutic drugs.

In conclusion, the current study suggests that the opioids commonly used during perioperative periods do not affect 5-fluorouracil sensitivity in human colon cancer HCT116 cells.

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