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SHORT COMMUNICATION

Effect of sevoflurane on human hepatocellular carcinoma HepG2 cells under conditions of high glucose and insulin

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Abstract Diabetes mellitus is associated with morbidity and progression of some cancers, such as hepatocellular carcinoma. It has been reported that sevoflurane, a volatile anesthetic agent commonly used in cancer surgery, can lead to lower overall survival rates than those observed when propofol is used to treat cancer patients, and sevoflurane increases cancer cell proliferation in in vitro studies. It has been also reported that glucose levels in rats anesthetized with sevoflurane were higher than those in rats anesthetized with propofol. We investigated the effect of sevoflurane, under conditions of high glucose and insulin, on cell proliferation in the human hepatocellular carcinoma cell line, HepG2. First, we exposed HepG2 cells to sevoflurane at 1 or 2 % concentration for 6 h in various glucose concentrations and then evaluated cell proliferation using the MTT assay. Subsequently, to mimic diabetic conditions observed during surgery, HepG2 cells were exposed to sevoflurane at 1 or 2 % concentration in high glucose concentrations at various concentrations of insulin for 6 h. One-percent sevoflurane exposure enhanced cell proliferation under conditions of high glucose, treated with 0.05 mg/l insulin. Our study implies that sevoflurane may affect cell proliferation in human hepatocellular carcinoma cells in a physiological situation mimicking that of diabetes.

Keywords Sevoflurane · Hepatocellular carcinoma · Diabetes · Proliferation

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Diabetes and cancer are common diseases whose incidence is increasing globally. For years, researchers have focused on the incidence of patients with concurrent diabetes and cancer. Recently, diabetes has been shown to be associated with the development of cancer. In particular, epidemiologic data provide evidence to demonstrate that there is an association between diabetes and increased morbidity and progression of liver [1, 2] and pancreatic [3] cancers.

However, it has been suggested that some general anesthetics affect the long-term outcomes in patients who undergo cancer surgery. In a retrospective study, sevoflurane, one of the most commonly used volatile general anesthetics for cancer surgery patients, leads to lower overall survival rates than propofol [4]. Furthermore, several in vitro studies have revealed that sevoflurane increases cancer cell proliferation [5, 6]. In a rodent study, it has been reported that there were different effects on glucose metabolism between sevoflurane and propofol [7], and glucose levels in rats anesthetized with sevoflurane were higher than those in propofol-anesthetized rats [8]. Based on the finding that cancer cells mainly depend on anaerobic glycolysis for producing ATP, even in the presence of oxygen, it is possible that exposure to sevoflurane in those with diabetic conditions could result in enhanced cancer cell proliferation. Thus, in the present study, we investigated whether sevoflurane can alter the proliferation of cancer cells under conditions of high glucose and insulin in the human hepatocellular carcinoma cell line, HepG2.

HepG2 cells, purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), were cultured in Eagle's minimum essential medium supplemented with 10 % fetal bovine serum (FBS) 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 1×10^4 cells/well. The medium was changed 24 h later with a fresh medium of clinically relevant glucose concentrations. To maintain osmotic pressure, mannitol was added to the same amount as that corresponding to the control. Cells from the sevoflurane treatment group were placed in the sevoflurane exposure chamber, and 1 or 2 % sevoflurane was introduced into the chamber with 21 % O_2, 5 % CO_2, and 74 % N_2 for 6 h. The concentrations of sevoflurane dissolved in the media were measured by gas chromatography (1.6 μg/ml at 1 % sevoflurane and 18.8 μg/ml at 2 % sevoflurane, respectively). Subsequently, the media were replaced with media of the same contents, and 48 h after incubation we investigated cell proliferative activity. Next, to examine the effects of sevoflurane on proliferation of HepG2 under conditions of high glucose concentration and high insulin concentration, HepG2 cells were exposed to 1 or 2 % sevoflurane under conditions of standard glucose concentration (100 mg/dl) or high glucose concentration (200 or 300 mg/dl) at various concentrations (0.0005, 0.05, and 5 mg/l) of human recombinant insulin (Wako Pure Chemical Industries, Osaka, Japan) for 6 h. Subsequently, media were replaced with media of the same contents, and 48 h after incubation, we investigated cell proliferative activity. Cellular proliferative activity was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's protocol. Absorbance of the formazan product was then measured at a wavelength of 570 nm using 650 nm as the reference. Each experiment was performed in triplicate and repeated 5 times. GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis. Statistical analyses were performed using the one-way analysis of variance (ANOVA) test and unpaired Student's *t*-test (two-tailed) with Bonferroni correction. All data are expressed as mean ± SD (standard deviation). Statistical significance was defined as $p < 0.05$.

As shown in Fig. 1a, treatment with a clinically relevant dose of glucose had no effect on the proliferative activity of HepG2 cells. In addition, we confirmed that insulin did not affect the cell proliferation at both standard (100 mg/dl) and high (300 mg/dl) glucose concentrations (Fig. 1b, c). Exposure to either 1 or 2 % sevoflurane for 6 h did not show a significant difference in the proliferative activity, even under high glucose conditions (300 mg/dl, Fig. 2a, b). However, exposure to 1 % sevoflurane with the same concentration of glucose (300 mg/dl) and 0.05 mg/l insulin significantly enhanced cell proliferation of HepG2 cells.

To our knowledge, this is the first study to investigate the direct effects of sevoflurane on cancer proliferation during hyperglycemia with insulin. According to our data, a clinically relevant dose of sevoflurane is likely to enhance cancer cell proliferation under conditions of high glucose concentration with insulin in HepG2 cells.

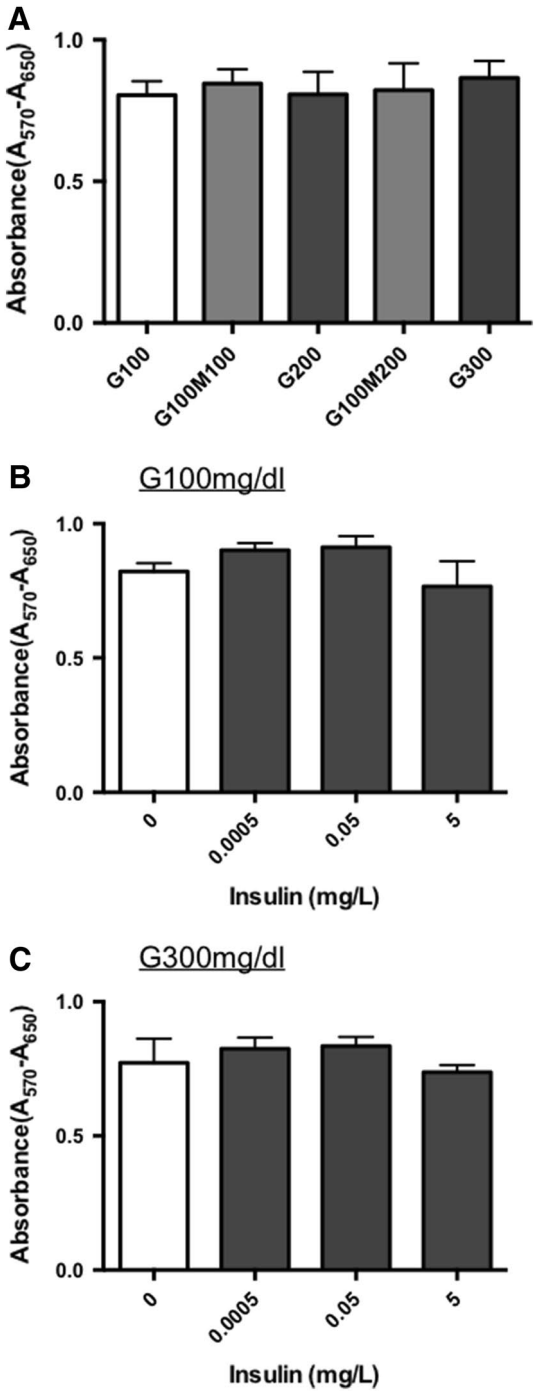

Fig. 1 Effects of both high glucose (**a**) and insulin (**b**, **c**) on proliferative activity in HepG2 cells. Neither condition affected the proliferative activity of HepG2 cells (one-way ANOVA). All data are expressed as mean ± SD in five independent experiments. *G* glucose, *M* mannitol

Enlund et al. [4] reported in a previous clinical study that patients anesthetized with sevoflurane may have shorter overall survival rates than patients anesthetized with propofol during cancer surgery. Several recent in vitro studies

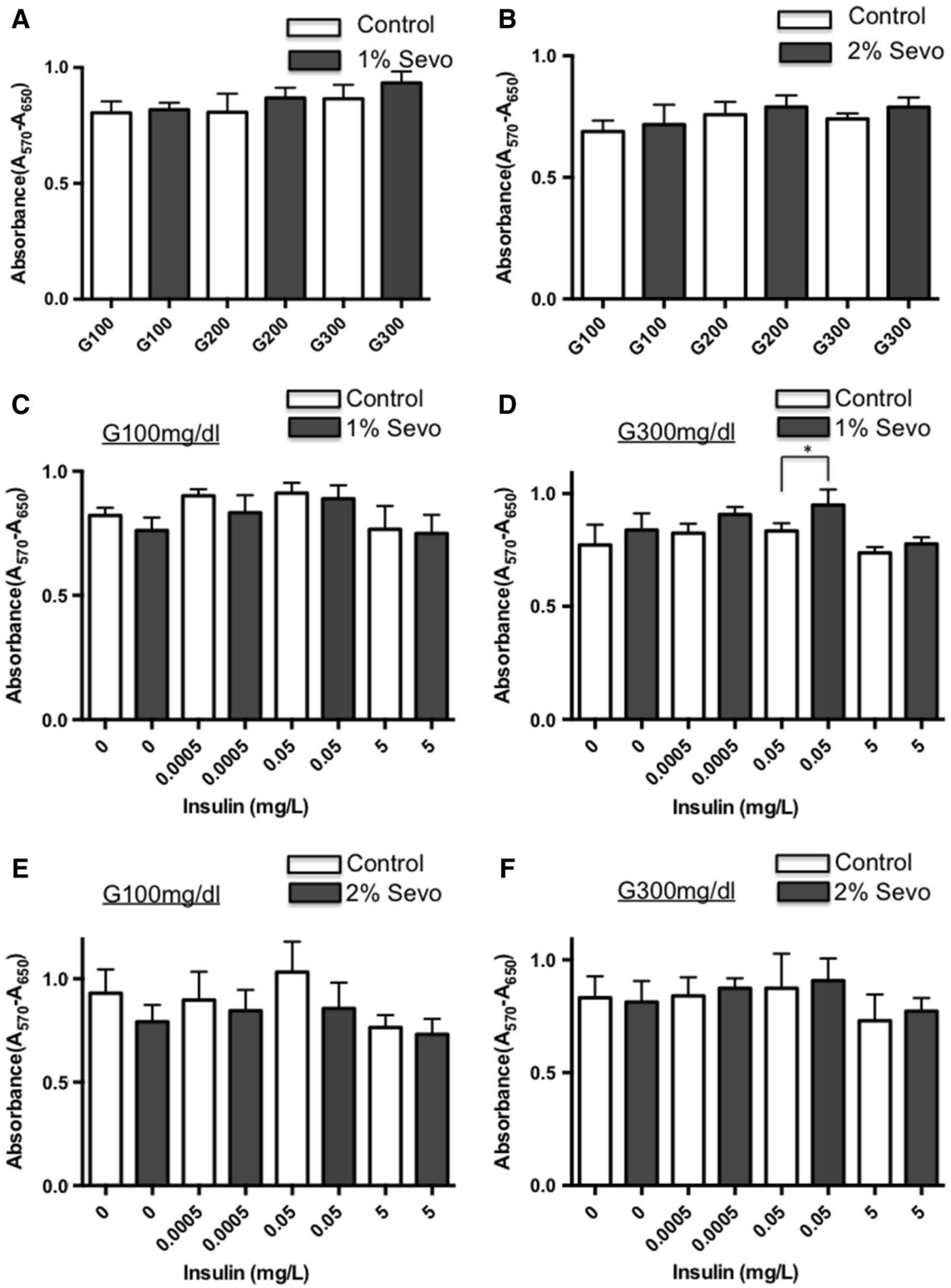

Fig. 2 Effects of sevoflurane on proliferative activity in HepG2 cells from a diabetic patient. **a**, **b** One or 2 % of sevoflurane at various glucose concentrations. **c**, **d** One percent sevoflurane at 100 or 300 mg/dl of glucose concentration with various insulin concentrations. **e**, **f** Two percent sevoflurane at 100 or 300 mg/dl of glucose concentration with various insulin concentrations. One percent sevoflurane was used for 6 h to enhance cell proliferation at 300 mg/dl of glucose with 0.05 mg/l of insulin. All data are expressed as mean ± SD in five independent experiments. *G* glucose, *Sevo* sevoflurane; **P* <0.0125 versus control samples

have demonstrated that volatile anesthetic agents enhance cancer progression [5, 9, 10].

However, it has been reported that diabetes mellitus contributes to cancer development and poor prognosis of hepatocellular carcinoma in several clinical studies [1, 2]. Feng et al. [11] reported that insulin, but not glucose, significantly enhanced the proliferation of HepG2 in an in vitro study. In another study, sevoflurane increased glucose uptake in skeletal muscle cells through the glucose transporter (GLUT) [12]. According to these reports, we speculated that HepG2 cell exposure to sevoflurane in the presence of high concentrations of insulin could potentially augment cell proliferation by increasing glucose uptake. According to this hypothesis, our study showed that 1 % sevoflurane for 6 h enhanced cell proliferation under conditions of high glucose with 0.05 mg/l insulin. Although this observed phenomenon is limited to a specific condition, it is possible that sevoflurane could enhance cancer cell proliferation in vivo and consequently have an adverse effect on survival outcomes in cancer patients. Additionally, we investigated glucose uptake in HepG2 cells after 1 % sevoflurane exposure and found that there was no difference in glucose uptake between sevoflurane and control groups (data not shown). Thus, the underlying mechanisms remain obscure.

Our findings should be interpreted within the constraints of the study's potential limitations. Some researchers postulate that sevoflurane can promote tumor growth as a result of negative effects on the immune system, specifically monocytes, natural killer cells, macrophages, cytotoxic T cells, and T helper cells [13–17]. However, clinical and animal studies assessing the immunosuppressive effects of sevoflurane during surgery are complex because pain may suppress immunity and opioids use may also suppress immunity by abrogating IL-1 and prostaglandins [18–20]. Therefore, we have explored the direct effects of sevoflurane on HepG2 cells under conditions of high insulin and high glucose in vitro. In future studies, it will be necessary to investigate whether sevoflurane can affect cancer growth under conditions of high glucose and high insulin in vivo.

In conclusion, the current study suggests that sevoflurane may affect cell proliferation in human hepatocellular carcinoma HepG2 cells under conditions of insulin and high glucose.

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