Effect of Halothane on Intercellular Adhesion Molecule-1 (ICAM-1) in Melanoma Cells

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There have only been a few reports relating to the effect of inhalational anesthetics on the tumor cell morphology in cancer patients undergoing surgery. We hypothesized that some anesthetic agents might influence the spread of unresectable cancer cells and might additionally worsen the condition of the patient due to depressed host immune surveillance. We therefore evaluated the influence of halothane on tumor cell adhesion, which is closely linked to tumor cell metastasis. Human melanoma cells from SK-MEL-37 cell-line were exposed to 4% halothane for 3, 6, 12 or 24 hours, respectively. Furthermore, after 24 hours halothane exposure, they were incubated in a 5% CO_2 atmosphere for 12 or 24 hours. The cells were then analyzed using a fluorescence flowcytometer and intercellular adhesion molecule-1 (ICAM-1) expression in SK-MEL-37 cells was quantified as the intensity of fluorescence of ICAM-1 expressed in 10,000 cells. ICAM-1 expression in cells exposed to halothane for 3, 6, 12 or 24 hours was lower than that of non-exposed cells and returned to control level after further incubation in 5% CO_2 atmosphere for either 12 or 24 hours. We conclude that halothane might affect the progression of tumor cell metastasis *in vitro*. (Key words: halothane, ICAM-1, immune surveillance)

(Azuma K, Mike N, Fujiwara Y, et al.: Effect of halothane on intercellular adhesion molecule-1 (ICAM-1) in melanoma cells. J Anesth 7: 442–446, 1993)

Anesthetic agents affect human immune responses[1–6] but few reports have explored the possible effect of inhalational anesthetics on tumor cells. We hypothesized that anesthetic agents might influence the growth and proliferation of unresectable cancer cells and could further deteriorate cancer patients due to host immune surveillance suppression.

We studied the relationship between halothane and cell-surface antigens in melanoma cells and found that expression of HLA-DR antigen in melanoma cells was increased by halothane.[7] We noted that halothane could modify anti-tumor immunity related to changes in HLA-DR expression. In the present study, we focused on Intercellular Adhesion Molecule-1 (ICAM-1), which plays an important role in immune surveillance.

The aims of this study were to evaluate the relationship between

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halothane and ICAM-1 expression in tumor cells, and to study the influence of anesthetic agents on immune surveillance.

Materials and Methods

Cells

The human melanoma cell line SK-MEL-37 was used. Cells were grown in a culture flask (Sumitomo Bakelite, Japan) containing 5 ml of minimum essential medium (MEM: Nissui, Japan) supplemented with 10% fetal bovine serum (FBS: Gibco, USA), 2 mM L-glutamine (Nissui, Japan), 10 mM non essential amino acids solution (Gibco, USA) and 100 $U \cdot ml^{-1}$ penicillin-100 $\mu g \cdot ml^{-1}$ streptomycin (Gibco, USA) in a 5% CO_2 atomosphere at 37°C. The medium was changed twice weekly.

Experimental protocol

At the start of experiment, cells were washed in 0.25% typsin (Chiba Kessei, Japan). Then they were incubated with 5 ml of 0.25% typsin in a 5% CO_2 atmosphere at 37°C for 5 minutes and were harvested by vigorous pipetting. Cells were then centrifuged at 1000 rpm for 5 minutes with 5 ml of fresh medium and 3×10^5 cells were cultured in a 60 mm diameter dish (Corning, USA) containing 5 ml of the medium at 37°C in a 5% CO_2 atmosphere for 48 hours. The cells were then placed in an air-tight exposure chamber warmed to 37°C. The chamber (16 × 30 × 15 cm) was connected to a modified anesthesia machine (AIKA, Ichikawa Shiseido & Co., Ltd, Japan), and a mixture of gases (5% CO_2, 20% O_2, balanced N_2 and 4% halothane) was supplied through a vaporizer (Fluotec, Cyprane Ltd, England). The concentration of each gas was analyzed with an infrared gas analyzer (Capnomac, Datex, Finland) at the outlet of the exposure chamber. After the concentration of each gas was confirmed, the chamber was sealed tightly. Cells were exposed to halothane for 3, 6, 12 or 24 hours. Some dishes of cells were incubated in a 5% CO_2 atmosphere for a further 12 or 24 hours after 24 hours exposure to anesthesia, in order to investigate the recovery from anesthesia.

Cells were washed in 0.25% trypsin and harvested as described. They were centrifuged at 1000 rpm for 5 minutes with 5 ml of fresh medium, and the pellet was washed with phosphate-buffered saline (PBS: Nissui, Japan) twice. Then the cells were resuspended in 50 μl of PBS with 10 μl of anti-human ICAM-1 mouse monoclonal antibody (clone 84H10, Cosmo, Japan) and 10 μl of human γ-globulin (Organon Teknika Corporation, Netherlands), and incubated at 4°C for 30 minutes. The cells were washed with PBS twice, again. Next, they were treated with 10 μl of fluorescein isothiocyanate (FITC)-rabbit antimouse IgG (MBL, Japan) and 50 μl of PBS and incubated at 4°C for 30 minutes. They were washed with PBS twice, fixed with PBS supplemented with 0.1% paraformaldehyde, and stored at 4°C. The cells were analyzed using a fluorescence flowcytometer (Epics Profile, Coulter, USA), and the ICAM-1 expression in SK-MEL-37 cells was quantified as the intensity of fluorescence of the net ICAM-1 expressed per 10,000 SK-MEL-37 cells.

Results

Figure 1 represents the histograms of fluorescence intensity in 10,000 non-exposed cells (solid line) and 10,000-halothane exposed cells (dotted line), respectively. The X axis shows the logarithm of fluorescence intensity, and Y axis, the cell number. ICAM-1 expression in cells exposed to halothane for 3, 6, 12 or 24 hours was lower than that of non-exposed cells (fig. 1. a, b, c, d), and returned to control level after further incubation in 5% CO_2 atmosphere for either 12 or 24 hours (fig. 1. e, f).

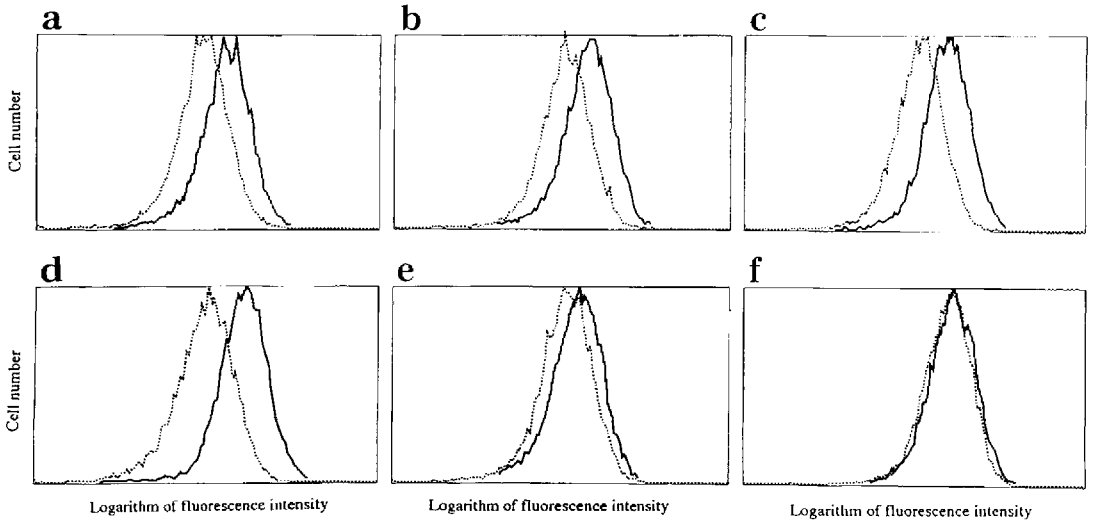

Fig. 1. Histograms of fluorescence intensity of ICAM-1 expression in 10,000 melanoma cells.
X axis: Logarithm of fluorescence intensity, Y axis: Cell number
Solid line: Non-exposed cells to halothane, Dotted line: 4% halothane exposed cells Halothane exposure, duration
a: 3 hours, b: 6 hours, c: 12 hours, d: 24 hours
e: 12 hours exposure to standard culture conditions after 24 hours halothane exposure
f: 24 hours exposure to standard culture conditions after 24 hours halothane exposure

Discussion

Relationship between morphological changes in host cells following anesthesia has been reported earlier.[1–6,9,10] T-lymphocytes exposed to halothane *in vitro* showed decreased interferon production[8], and natural killer cell cytotoxicity was depressed.[11] However, only a few studies report the effects of inhalational anesthetics on the tumor cell itself.[8] Shapiro et al. suggested that some anesthetics have the potential to accelerate tumor metastasis attributable to tumor cell membrane changes.[12] We earlier reported that the expression of HLA-DR antigen, located on the melanoma cell membrane was increased after 12 hours of 2% halothane exposure and after 24 hours of 4% halothane exposure compared to that of nonexposed cells[7] and hypothesized that some anesthetic agents could affect tumor cell membrane and therefore could potentiate tumor metastasis. This study is an extension of that concept, designed to further support our earlier observations and to investigate the changes of cell membrane antigens under inhalational anesthesia *in vitro*.

Some accessory molecules which cooperate with specific receptors and play an important role in cellular interactions of the immune system have been defined.[13] Lymphocyte function associated antigen-1 (LFA-1) is one of them.[13–15] LFA-1 is expressed in cytotoxic cells, such as natural killer cells (NK), cytotoxic T-lymphocytes (CTL) and lymphokine activated killer cells (LAK). These cells play a pivotal role in immune surveillance. They kill tumor cells and virus-infected cells, and ICAM-1, a glycoprotein and a ligand for LFA-1[13–15], plays a crucial role in immune surveillance.[13–15] ICAM-1 is expressed on the surface of both nonhematopoietic and hematopoietic cells and some tumor cells.[16] The expression of ICAM-1 in tumor cells is also

closely linked to tumor metastasis.[17] Cytotoxic cell–mediated tumor cell and virus-infected cell destruction occurs after LFA-1 binds to ICAM-1. Therefore, the decrease observed in ICAM-1 expression in melanoma cells during halothane exposure in our studies suggests that this anesthetic agent accelerates tumor cell metastasis by suppression of tumor cell 'recognition' mediated via interaction between LFA-1 and ICAM-1 molecules.

Poste and Fidler reported that the process of metastasis is composed of sequential steps as follows[18]: 1) malignant cells are released from the primary tumor and invade the surrounding host tissue; 2) invading tumor cells enter into lymphatics or blood vessels and are transported to distant sites in the body; 3) they lodge, arrest and adhere to the capillary beds of various organs; 4) they exit from capillaries into the surrounding host tissue; and 5) they proliferate to form metastasis. The decrease in ICAM-1 expression could make melanoma cells easily detached from the primary lesion and the recovery of ICAM-1 expression that our results demonstrated after returning cells to standard culture conditions (fig. 1, e and f), could render the cells adherent again, aiding their attachment to the vascular endothelium of other organs. Hence, a fluctuation in ICAM-1 expression could affect progression of tumor metastasis, *in vitro*.

Interleukin 1 (IL-1) and interferon-γ (IFN-γ) augment ICAM-1 expression in melanoma cells[17] and are effective therapeutic anti-cancer agents; our findings showed that halothane decreases ICAM-1 expression on melanoma cells. It is interesting that both IL-1 and IFN-γ and halothane seem to share a common cell surface molecular mechanism pathway, however, their effects are diametrically opposite, since they have opposite effects on ICAM-1 expression. It is therefore highly likely that inhalational anesthetics which are 'common drugs' used during cancer surgery could accelerate tumor metastasis secondary to reduced ICAM-1 expression though this supposition remains to be established *in vivo*.

The mechanism that alters ICAM-1 expression is not known. The increase in surface antigen expression might not simply mean an increase in the ratio of cell surface to total ICAM-1.[16] ICAM-1 is a glycoprotein expressing on the cell membrane.[14] Halothane reportedly causes a dose-dependent and reversible inhibition of protein synthesis, which is not associated with cell damage or with ATP depletion.[19] In contrast, the decrease in surface ICAM-1 expression may be the result of shedding. Tsujisaki et al. showed[20] that the incidence of positivity for ICAM-1 antigen in malignant disease was higher than that in benign disease or in healthy controls, and that the sera of cancer patients with liver metastasis showed higher levels of circulating ICAM-1 antigen. Shedding ICAM-1 antigen may also block the attachment of cytotoxic T cell and/or NK cells to cancer cells, since LFA-1 could be blocked with soluble ICAM-1. This could be one of the escape mechanisms of cancer cells from the immunosurveillance system of the host[20].

We conclude that halothane might affect progression of tumor cell metastasis *in vitro*. Therefore, anesthesiologists should be aware that the use of certain anesthetics carries with it the risk of promoting tumor cell metastasis.

We are making further investigations into the various concentration of halothane and other anesthetics to characterize the effect of anesthetics on tumor metastasis.

(Received Aug. 3, 1992, accepted for publication Jan. 29, 1993)

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