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The effect of different anesthetics on tumor cytotoxicity by natural killer cells

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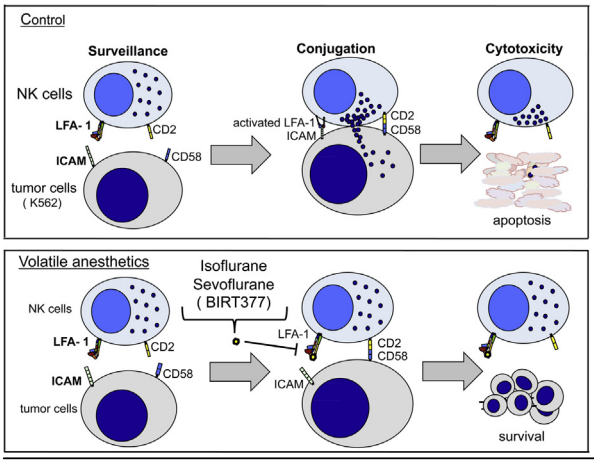
HIGHLIGHTS

- LFA-1 is involved in NK cell-mediated conjugation, polarization and tumor cytotoxicity.
- Volatile anesthetics isoflurane and sevoflurane are LFA-1 inhibitors.
- Volatile anesthetics attenuated NK-cell mediated conjugation, polarization and cytotoxicity.
- Intravenous anesthetics did not affect these NK cell functions.

GRAPHICAL ABSTRACT



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ABSTRACT

A number of retrospective studies have suggested that choice of anesthetic drugs during surgical tumor resection might affect tumor recurrence/metastasis, or outcome of patients. The recent study showed that volatile anesthetics-based general anesthesia was associated with the worse outcomes than intravenous anesthetics-based general anesthesia. However, the underlying mechanism is yet to be determined. Because natural killer (NK) cells are implicated as important immune cells for tumor recurrence/metastasis in the perioperative period, we examined the effect of different anesthetics on NK cell-mediated tumor cytotoxicity. Because adhesion molecule leukocyte function-associated antigen-1 (LFA-1) is functionally important in NK cells and is inhibited by commonly used volatile anesthetics isoflurane and sevoflurane, we hypothesized that these anesthetics would attenuate NK cell-mediated cytotoxicity. Using human NK cell line NK92-MI cells and tumor cell line K562 cells as a model system, we performed cytotoxicity, proliferation, conjugation and degranulation assays. Lytic granule polarization was also assessed. We showed that isoflurane, sevoflurane and LFA-1 inhibitor BIRT377 attenuated cytotoxicity, and reduced conjugation and polarization, but not degranulation of NK cells. Our data suggest that isoflurane and sevoflurane attenuated NK cell-mediated cytotoxicity at least partly by their

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LFA-1 inhibition in vitro. Whether or not isoflurane and sevoflurane attenuate NK cell-mediated tumor cytotoxicity in patients needs to be determined in the future.

1. Introduction

Local tumor recurrence and/or distal metastasis after surgical resection remain to be the main cause of morbidities and mortalities in solid tumors despite significant advancement in tumor therapies over years (Gottschalk et al., 2010). Causes of recurrence and/or metastasis are potentially multifactorial, including the dissemination of tumor cells during surgical resection and the functional suppression of immune cells in the perioperative period (Tavare et al., 2012). Various retrospective studies have demonstrated the association between anesthetic regimens and tumor recurrence/metastasis and/or patient survival, suggesting that anesthesia regimens might play an important role in tumor metastasis and/or recurrence after surgery (Schlagenhauff et al., 2000; Exadaktylos et al., 2006; Biki et al., 2008; Christopherson et al., 2008; Lin et al., 2011). Overall, these studies reported that the anesthetic regimens sparing general anesthesia by incorporating regional anesthesia or no use of general anesthesia were associated with better survival, or less recurrence/metastasis. Furthermore, a recent retrospective study showed an association of poorer outcomes with patients who received volatile anesthetics-based general anesthesia for tumor resection surgery than patients who received total intravenous anesthetics-based general anesthesia (Wigmore et al., 2016). The foundation of these associations remains unclear, however.

Natural killer (NK) cells are a phenotypically distinct population of lymphocytes ($CD56^+/CD3^-$) that lyse tumor cells using constitutively expressed lytic machinery independent of prior immunization. NK cells survey and conjugate with tumor cells devoid of major histocompatibility complex (MHC) class I, and polarize lytic granules toward them. Subsequent degranulation of lytic proteins such as perforin, granzyme and Fas ligands leads tumor cells to apoptosis (Fig. 1). The correlation of perioperative NK cell suppression with tumor recurrence and mortality after surgical resection of colorectal and lung tumors suggests that adequate, perioperative NK cell function is critical to minimize post-resection tumor recurrence (Tartter et al., 1987; Fujisawa and Yamaguchi, 1997). NK cells express a number of activating and inhibitory receptors on their cell surfaces to recognize stress ligands as well as MHC class I (Pegram et al., 2011). Leukocyte function-associated antigen-1 (LFA-1) is one of the activating receptors and a major adhesion molecule on NK cells. It binds to intercellular adhesion molecule 1 (ICAM-1) on tumor cells, assisting the conjugation of NK cells with tumor cells. The binding of LFA-1 to ICAM-1 then reorganizes cytoskeletal structures within NK cells and induces lytic granule polarization (Fig. 1). And LFA-1 inhibition or deficiency was found to impair NK cell-mediated cytolysis (Kohl et al., 1984; Weitz-Schmidt et al., 2009).

Previously, we demonstrated that volatile anesthetics isoflurane and sevoflurane allosterically blocked LFA-1 (Yuki et al., 2008, 2010, 2012; Zhang et al., 2009). LFA-1 is a structurally dynamic protein consisting of a large, extracellular component, and small transmembrane and cytoplasmic domains. It is a heterodimeric molecule with the α and β subunits. The α subunit contains the ligand-binding domain on its top. During conformational changes of LFA-1 as a whole, this ligand-binding domain also undergoes significant changes. And the pocket called 'lovastatin site' in the ligand-binding domain allosterically regulates the exposure of the ligand-binding site. A small molecule BIRT377 binds to 'lovastatin site' and prevents the exposure of ICAM-1 binding site (Kelly et al., 1999; Woska et al., 2001). Overall, LFA-1 transforms itself to bind to ICAM-1 only when it is fully activated. Isoflurane and sevoflurane also bind to this 'lovastatin site' and stabilize LFA-1 in a conformation that is not favorable for ICAM-1 binding. Here, we hypothesized that isoflurane and sevoflurane would impair NK cell function through their LFA-1 inhibition. We tested this hypothesis using NK cell line NK92-MI cells and tumor cell line K562 cells. We also used BIRT377 as a reference of LFA-1 inhibition via occupancy of 'lovastatin site'.

2. Methods

2.1. Cell cultures

NK92-MI cells, K562 cells and Jurkat cells were purchased from ATCC (Manassas, VA, USA). NK92-MI cells were cultured in alpha Minimum Essential Medium without ribonucleosides and deoxyribonucleosides (Sigma Aldrich, St. Louis, MO, USA), with 0.2 mM inositol (Alfa Aesar, Haverhill, MA, USA), 0.1 mM 2-mercaptoethanol (Gibco, Waltham, MA, USA), 0.02 mM folic acid (Sigma Aldrich), 12.5% fetal bovine serum (FBS) (Atlantic Biologicals, Miami, FL, USA) and 12.5% horse serum (Gibco). K562 cells and Jurkat cells were grown in RPMI1640 with 10% FBS. All the cells were cultured at 37 °C, 5% CO_2.

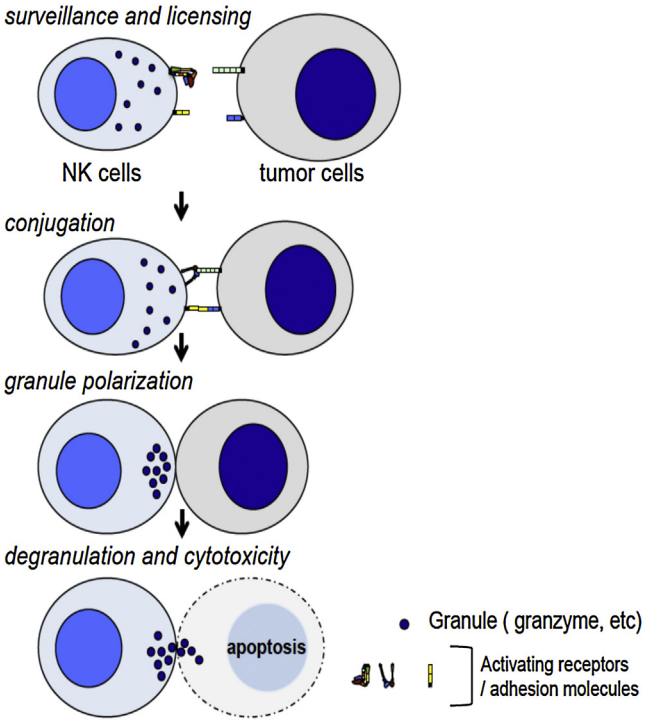

Fig. 1. Steps of natural killer (NK) cell-mediated cytotoxicity.
NK cells survey and are educated by their surrounding cells (surveillance and licensing), and conjugate with cells devoid of MHC I (conjugation). The lytic granules in NK cells are polarized (granule polarization) and released (degranulation). Tumor cells undergo apoptosis subsequently (cytotoxicity).