Suppression of Natural Killer Cell Activity and Promotion of Tumor Metastasis by Ketamine, Thiopental, and Halothane, but Not by Propofol: Mediating Mechanisms and Prophylactic Measures

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Postoperative immunosuppression is partly ascribed to anesthesia and has been suggested to compromise patients' resistance to infection and tumor metastasis. We compared the effects of various anesthetics on natural killer (NK) cell activity and on resistance to experimental metastasis, and studied mediating mechanisms and prophylactic measures. Fischer 344 rats served as controls or were anesthetized for 1 h with ketamine, thiopental, halothane, or propofol. Anesthetized rats were either maintained in normothermia or left to spontaneously reach 33°C–35°C. Rats were then injected IV with MADB106 tumor cells, and 24 h later lung tumor retention was assessed, or 3 wk later, lung metastases were counted. Additionally, the number and activity of circulating NK cells were assessed after anesthesia. All anesthetics, except propofol, significantly reduced NK activity and increased MADB106 lung tumor retention or lung metastases. Hypothermia had no significant effects. Ketamine increased metastasis most potently, and this effect was markedly reduced in rats pretreated with a β-adrenergic antagonist (nadolol) or with chronic small doses of an immunostimulator (polyriboinosinic:polyribocytidylic acid). Overall, the marked variation in the NK-suppressive effects of anesthetics seems to underlie their differential promotion of MADB106 metastasis. Prophylactic measures may include perioperative immunostimulation and the use of β-blockers.

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A large proportion of cancer-related deaths are due to metastatic development, rather than directly related to the primary tumor, as surgeons can successfully remove primary tumors in most cases. Although indispensable, surgical excision of the primary tumor has long been suspected to facilitate metastatic development. This facilitation has been ascribed to various mechanisms acting in synergy, including shedding of tumor cells into the circulation, a decrease in levels of antiangiogenic factors (factors that prevent the development of new blood vessels), and the suppression of antimetastatic immunity (1). It is becoming apparent that the perioperative period is characterized by pronounced immunosuppression (2), and animal studies have implicated this suppression in increased susceptibility to postoperative infection and tumor recurrence (3,4).

Several factors have been proposed to underlie the suppression of immunity by surgery. These include the neuroendocrine stress responses to surgery (1), hypothermia (5), blood transfusion (6), and, central to the current study, the use of anesthetics (7,8) that may directly interact with leukocytes or may act via some of the aforementioned mechanisms.

One immune function shown to be suppressed by anesthetics is natural killer (NK) cell activity (9). NK cells are a subpopulation of lymphocytes that spontaneously recognize and kill virally infected cells, as well as a variety of tumor cells during the metastatic process (10). Large doses of opioids used for general anesthesia in patients or in rats have been shown to suppress NK activity for several days, and animal studies have implicated the neuroendocrine response produced by opioids in mediating such effects (9).

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This study examines drugs from different pharmaceutical categories that are often used in anesthesia, compares their effect on the susceptibility to metastasis in nonoperated rats, and seeks the physiological and immunological mechanisms that mediate their effects. To this end, we used an animal model that we have previously used to study the promotion of metastasis by surgery (3,11).

Methods

All experiments were approved by the Institutional Animal Care and Use Committee of Tel Aviv University. Fischer 344 male and female rats, 13–16 wk old (age-matched within each experiment), were purchased from Harlan Laboratories (Jerusalem, Israel) and housed four per cage on a 12:12-h reverse light/dark schedule (darkness during daytime, the period of anesthesia). All animals had free access to food and water and were handled daily for a week before each experiment.

Anesthetics, Doses, and Routes of Administration. Doses of the different anesthetics were individually adjusted for each rat to reduce the respiratory rate to approximately 40 breaths/min. In pilot studies for each drug, this rate of respiration characterized rats that were unresponsive to noxious stimuli (surgical incision).

Anesthesia was initiated by placing each rat in a jar with a halothane-soaked (Rhone-Poulenc, Bristol, UK) pad for 15–30 s, until the rat was down. Rats were then maintained at 2%–3% halothane in room air via a vaporizer (flow-through system; FLUOTEC MK III; annually calibrated). Rats breathed spontaneously throughout anesthesia, and the halothane concentration was adjusted according to the animal's respiratory pattern (concentrations usually decreased toward 2% with time).

In Experiments 1, 3, and 4 (see below), ketamine was injected intraperitoneally (IP) (80 mg/kg) immediately after subcutaneous (SC) administration of diazepam (12 mg/kg). The doses of ketamine and diazepam used in these experiments were selected on the basis of pilot studies achieving similar levels of anesthesia preventing muscle tension and spasm (diazepam) as in the other anesthetics.

Thiopental and propofol were administered IV in all experiments, and ketamine was administered IV in Experiment 2. For IV injection, a 22-gauge catheter was inserted into the tail vein, and the anesthetic was given intermittently according to the animal's respiratory pattern. The cumulative average doses used during the 1-h anesthesia were 92.5, 83.3, and 74 mg/kg, respectively.

We changed the route of ketamine administration from IP in Experiment 1 to IV in Experiment 2 and back to IP in Experiments 3 and 4. In the first experiment, ketamine had much greater effect than thiopental and propofol, which were administered IV. In the second experiment, we administered all three drugs IV to ascertain that it was not the route of administration (of ketamine) that underlay its larger effects. Indeed, in Experiment 2, ketamine still had very large effects—much greater than those of the other drugs. In Experiments 3 and 4, in which only ketamine was used, we returned to the IP administration route, which is often used and is more easily implemented.

β-Adrenergic Blockade. In Experiment 4, the nonselective β-adrenergic antagonist nadolol was administered, first simultaneously with the induction of anesthesia (SC, 0.4 mg/kg) and then again 5 h later with tumor inoculation (SC, 0.2 mg/kg).

Immunostimulation with Poly I-C. We used very small doses of a common biological response modifier—polyriboinosinic:polyribocytidylic acid (poly I-C). Specifically, poly I-C 0.2 mg/kg was injected IP 5 days, 3 days, and 1 day before anesthesia with ketamine. Control rats were similarly injected with saline.

MADB106 Tumor Cells: Maintenance and Radiolabeling. MADB106 is a selected variant cell line obtained from a pulmonary metastasis of a mammary adenocarcinoma (MADB100) chemically induced in the inbred Fischer 344 rat (12). This syngeneic tumor metastasizes only to the lungs after IV inoculation, is maintained in monolayer cell culture in complete medium, and is detached from the flask by using 0.25% trypsin. For DNA radiolabeling of MADB106 tumor cells, 0.4 μCi/mL of ^{125}iododeoxyuridine (Rotem Taasiot, Dimona, Israel) was added to the growing cell culture 1 day before cell harvesting.

Assessment of Lung Tumor Retention (LTR). Rats were lightly anesthetized with halothane, and 4×10^5/kg ^{125}iododeoxyuridine-labeled MADB106 tumor cells in 2 mL/kg of phosphate-buffered saline were injected into their tail veins. Light anesthesia was achieved by placing each rat in a jar with a halothane-soaked pad for approximately 15–30 s (until the rat loses the righting reflex) and maintaining it thereafter under 3% halothane until the end of the injection procedure. The entire injection procedure (from removing a rat from its home cage to returning it after awakening) lasted 2–3 min. Twenty-four hours later, rats were killed with an overdose of halothane, and their lungs were removed and placed in a gamma counter for assessment of radioactive content. LTR was calculated as the ratio of radioactivity measured in the lungs to radioactivity in the injected tumor cell suspension. Our previous studies have indicated that the level of lung radioactivity reflects the number of viable tumor cells in the lungs (5,13,14).

Induction and Counting of Tumor Metastases. Rats were lightly anesthetized with halothane (see above),

and 10^5 MADB106 tumor cells (approximately 4×10^5/kg) were injected into their tail veins in 0.5 mL of phosphate-buffered saline supplemented with 0.1% bovine serum albumin. Three weeks later, rats were killed with an overdose of halothane, and their lungs were removed and placed for 24 h in Bouin solution (72% saturated picric acid solution, 23% formaldehyde [37% solution], and 5% glacial acetic acid). After the lungs were washed in ethanol, visible surface metastases were counted.

Whole Blood NK Cytotoxicity Assay. The activity of NK cells was assessed by using the standard whole-blood cytotoxicity assay (14). This procedure assesses the *in vitro* antitumor cytotoxicity of NK cells per milliliter of blood without exclusion of any cell type from the blood but after replacement of serum with complete medium (washing of blood). Blood (1.5 mL) was drawn by cardiac puncture under light halothane anesthesia into heparinized syringes (45 U of preservative-free heparin). To assess NK cytotoxicity (NK killing of YAC-1 target cells) at seven different effector/target (E:T) ratios (different ratios of NK to target cells), "washed" blood was successively diluted twofold in complete medium (starting with 150 μL in the highest E:T ratio). Six-thousand chromium-51-radiolabeled YAC-1 lymphoma cells were added on top of the blood in a microtiter plate. After a 4-h incubation period, samples of supernatant were recovered for the assessment of radioactivity. Specific killing was calculated as [(sample release − spontaneous release)/(maximal release − spontaneous release)] × 100.

Earlier studies indicated that cytotoxicity measured with this procedure is attributable to NK cells, rather than other cell types or soluble factors (14). For more details, see Shakhar and Ben-Eliyahu (14).

Flow Cytometry. Fluorescence-activated cell sorter analysis was used to assess the number of NK cells in the blood. NK cells were identified as NKR-P1^{bright} (CD 161^{bright}) lymphocytes by using fluorescein isothiocyanate-conjugated anti-NKR-P1 monoclonal antibodies (PharMingen, San Diego, CA). To assess the absolute number of NK cells per microliter of blood, a fixed number of polystyrene microbeads (20 μm; Duke Scientific, Palo Alto, CA) were added to the blood samples before they were prepared for cytometric analysis. For further details, see Shakhar and Ben-Eliyahu (14).

Statistical Analysis. Analysis of variance (ANOVA) was used to analyze LTR. Provided that significant group differences were indicated by ANOVA, Fisher planned contrast was used to conduct pairwise comparisons. In Experiment 2, the ketamine group had much larger variation than all other groups, violating ANOVA assumptions. Thus, unpaired Student's *t*-tests were used to compare each anesthetic group with the control group, with Bonferroni's correction for multiple comparisons. NK cell activity was analyzed with repeated-measures ANOVA (with the different E:T ratios as the repeated measures). A value of $P < 0.05$ was considered significant in all studies. StatView (Version 4.0; SAS Institute, Cary, NC) was used for all statistical analysis.

Because of the large number of animals required for Experiments 1 and 2, these experiments were each conducted over a few sessions. Each session included animals from all experimental groups (i.e., each group was represented evenly in all sessions). The time and order of the induction of anesthesia and of tumor inoculation were counterbalanced across all groups in each experiment.

Results

Experiment 1: The Effects of Anesthesia with Halothane, Ketamine, Propofol, and Thiopental on LTR of the MADB106

In Experiment 1, to investigate the effects of anesthesia induced by halothane, ketamine, propofol, and thiopental on susceptibility to metastatic development, we used the NK-sensitive MADB106 tumor line and assessed LTR 24 h after tumor inoculation.

Rats were divided into six groups: a control group that was injected with saline and five experimental groups that were treated with halothane (2%–3%), diazepam (12 mg/kg SC), ketamine (80 mg/kg IP) plus diazepam (12 mg/kg SC), propofol (75 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} IV), or thiopental (67.5 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} IV) (also see the dosing regimen described above). Each of the last four experimental groups was subdivided into a hypothermia group (rats spontaneously reaching 33°C–35°C) and a normothermia group (rats maintained at 37°C–38°C by using warming pads). Temperature was measured with a rectal probe thermometer. Anesthesia was induced either 1 or 5 h before IV inoculation with MADB106 tumor cells and was maintained for 1 h in all experimental groups. Overall, 269 (184 male and 85 female) rats were evenly divided among the different groups. The experiment was conducted over five sessions (a week apart along 5 consecutive weeks), and each included all subgroups. Female rats were used in three of the five sessions.

ANOVA revealed a significant effect of the anesthetics on LTR ($F_{5,263} = 50.3$; $P < 0.0001$). Fisher planned contrast indicated that the ketamine and thiopental groups had significantly larger LTR compared with the control group and the propofol group. Ketamine had larger LTR compared with all anesthetic groups (thiopental, halothane, and propofol). Ketamine caused a 5.5-fold increase in LTR, and thiopental caused a 2-fold increase. Diazepam, halothane, and propofol did not cause significant effects on LTR in this study (Fig. 1).

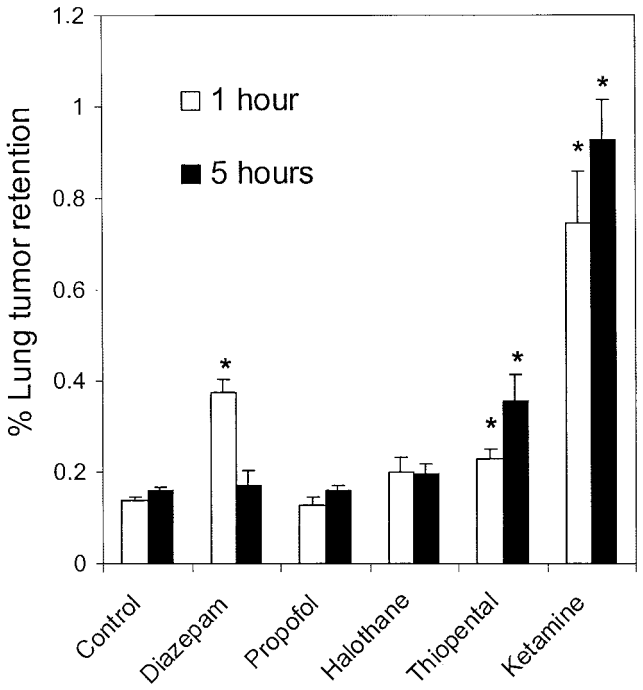

Figure 1. Percentage of lung tumor retention (LTR) of radiolabeled MADB106 tumor cells (mean ± SEM). Animals were treated with halothane (2%–3%), diazepam (12 mg/kg subcutaneously [SC]), ketamine (80 mg/kg intraperitoneally) plus diazepam (12 mg/kg SC), propofol (10 mg/kg IV), or thiopental (50 mg/kg IV). Anesthesia was maintained for 1 h in all experimental groups but was induced either 1 or 5 h before IV inoculation with tumor cells. Control rats were injected with saline. LTR was significantly larger in the ketamine- and thiopental-treated groups. Diazepam caused a significant increase only at the 1-h time point, halothane caused a nonsignificant increase, and propofol caused a nonsignificant decrease in LTR. *Significant difference from control. A total of 269 male and female rats were used.

The effect of anesthesia was dependent on the interval between anesthesia and tumor inoculation (Fig. 1). The increase in LTR was larger at 5 h than at 1 h after anesthesia in the thiopental ($P = 0.056$) and ketamine ($P = 0.23$) groups. In respect to diazepam, an opposite effect of time was evident: at 1 h, diazepam caused significantly larger levels of LTR compared with 5 h (2.2-fold; $P = 0.003$) and compared with the control groups (both 1 and 5 h; $P < 0.01$).

Body temperature spontaneously decreased to 33°C–35°C in all rats assigned to the hypothermia condition. No significant effects of hypothermia on LTR were observed under any of the anesthetics used (halothane, thiopental, propofol, and ketamine) or when the effect of hypothermia was assessed in all anesthetic groups together. Specifically, ANOVA did not reveal a significant effect of hypothermia or a significant interaction of hypothermia with anesthetics. In fact, the hypothermic and normothermic groups were very similar.

The effects of sex and the interaction between sex and anesthetics or hypothermia on LTR were investigated over 3 sessions, in which 85 females and 109 males were used. ANOVA did not reveal a significant sex effect or an interaction of sex with any other condition.

Experiment 2: The Effects of Anesthesia with Halothane, Ketamine, Propofol, and Thiopental on Number and Activity of NK Cells, and Its Within Animal Correlation with MADB106 LTR

Experiment 2 involved the effects of anesthesia with halothane, ketamine, propofol, and thiopental on the number and activity of NK cells and its within-animal correlation with MADB106 LTR. In this experiment we studied, within each rat, indices of NK cells and resistance to metastasis of the NK-sensitive MADB106 tumor. Rats were randomly assigned to one of five groups: a control group and four experimental groups that were treated with halothane via a vaporizer (2%–3%) or infused IV with ketamine, propofol, or thiopental (see Methods). These experimental groups were further divided into two subgroups of hypothermia and normothermia, as described in Experiment 1. Anesthesia was maintained for 1 h. Three and one-half hours after its induction, blood was drawn from all animals and was used to count NK cells and to assess their cytotoxicity in a chromium-51-release assay. Immediately after blood withdrawal, animals were inoculated with MADB106 tumor cells, and LTR was assessed 24 h later. Overall, 76 male rats were used in this experiment, which was conducted over 2 sessions, each including approximately even numbers of rats from each subgroup.

The Effects of Anesthetics on Activity and Numbers of NK Cells. Regarding NK activity, all anesthetics except propofol suppressed NK activity (Fig. 2B). Specifically, repeated-measures ANOVA indicated significant group differences ($F_{4,79} = 2.975$; $P = 0.0242$), and planned contrasts indicated that ketamine, halothane, and thiopental, but not propofol, significantly suppressed NK activity compared with control levels. No significant differences between hypothermal and normothermal conditions were revealed.

Flow cytometric analysis was used to record the number of NK cells per microliter of blood. ANOVA indicated significant group differences ($F_{4,41} = 6.201$; $P < 0.0005$), and Fisher planned contrast indicated that thiopental, ketamine, and propofol caused a significant decrease in NK cells compared with the control group (Fig. 2A). The reduction caused by halothane did not reach statistically significant levels. Thiopental caused the largest decrease (a reduction of 55.13% of control levels), followed by ketamine (29.25%), propofol (23.5%), and halothane (21.29%). Thus, we also

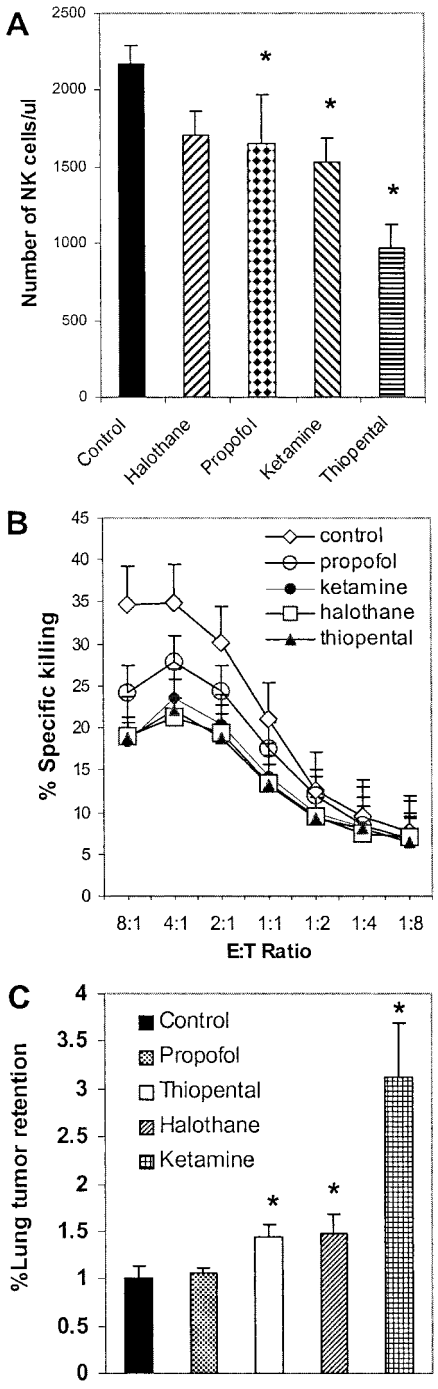

Figure 2. Rats were anesthetized for 1 h with halothane via a vaporizer (2%–3%) or via a tail-vein intermittent administration of ketamine (total of 74 mg · kg^{-1} · h^{-1}), propofol (83.3 mg · kg^{-1} · h^{-1}), or thiopental (92.5 mg · kg^{-1} · h^{-1}) (20, 22.5, and 25 mg per animal, respectively). Controls rats were injected subcutaneously with saline. Three and one-half hours after the induction of anesthesia, 1 mL of blood was drawn from all animals by cardiac puncture under light halothane anesthesia, and the number (A) and activity (B) of natural killer (NK) cells were assessed. Immediately after blood withdrawal, animals were inoculated IV with radiolabeled MADB106 tumor cells, and lung tumor retention (LTR) was assessed 24 h later (C). All data are presented as mean ± SEM. *Significant difference from control. (A) Numbers of NK cells (NKR-P1^{bright}) per microliter of blood. Thiopental, ketamine, and propofol caused a significant decrease in the number of NK cells compared with the control group. (B) Percentage of specific killing per milliliter of blood at different effector to target (E:T) ratios (NK: YAC-1). Ketamine, halothane, and thiopental, but not propofol, significantly suppressed NK activity per milliliter of blood compared with control levels. (C) Percentage of LTR of radiolabeled MADB106 tumor cells. Ketamine-, halothane-, and thiopental-treated, but not propofol-treated, rats had significantly larger LTR compared with control levels. A total of 76 male rats were used.

conducted unpaired Student's *t*-tests to individually compare each anesthetic group with thiopental. There was a significantly larger reduction in the number of NK cells per microliter relative to halothane (t_{17} = 3.229; P = 0.0049), ketamine (t_{15} = 2.322; P = 0.0347), and propofol (t_{18} = 2.075; P = 0.05).

In Vivo *Effects of Anesthesia on LTR.* The different anesthetics induced different levels of LTR, and ANOVA revealed significant group differences ($F_{4,71}$ = 11.684; $P < 0.0001$) (Fig. 2C). Because the ketamine group had significantly larger variance than all other groups (violating a prerequisite for conducting ANOVA-based planed contrast), pairwise Student's *t*-test comparisons were used to assess specific differences between anesthetic groups and the control group. Significantly larger LTR was revealed in respect to ketamine (t_{10} = −5.082; $P < 0.0001$), thiopental (t_{15} = −2.350; P = 0.0242), and halothane (t_{18} = −2.091; P = 0.0434), but not to propofol (t_{12} = −0.243; P = 0.8095). Hypothermia did not cause a significant effect.

Correlation Between LTR and NK Activity. A significant Pearson correlation of r = −0.38 was found between the levels of LTR and the levels of NK activity (average levels of the largest two E:T ratios) when all experimental groups were combined (approximately 14% explained variance). When this correlation was assessed separately within each group, the levels of correlations observed were −0.196 for halothane, −0.467 for ketamine, −0.293 for propofol, −0.422 for thiopental, and −0.289 for control, but none reached a significant level (probably because of the small number of rats in each group).

Experiment 3: The Effects of Anesthesia with Ketamine on MADB106 Metastases

In Experiment 3, the effects of anesthesia with ketamine on MADB106 metastases, rats were randomly assigned to one of three groups (n = 10–12 per group): a control group that was undisturbed, a control group injected with saline, and an experimental group that was treated with ketamine (80 mg/kg IP) and diazepam (12 mg/kg SC). Anesthesia was induced 5 h before IV inoculation with MADB106 tumor cells and lasted for approximately 1 h. Three weeks later, rats were killed, and pulmonary metastases were counted.

The average number of metastases in the ketamine group was more than 2 times larger than in the control and control saline groups. ANOVA revealed significant

group differences in the number of metastases ($F_{2,28}$ = 6.255; $P < 0.0057$), and Fisher planned contrast indicated that the ketamine group had significantly more metastases compared with each of the control groups (Fig. 3).

Experiment 4: The Involovement of β-Adrenergic Mechanism in the Metastasis-Promoting Effects of Ketamine, and the Use of Immunostimulation as a Prophylactic Measure

In Experiment 4, we studied the involvement of β-adrenergic mechanisms in the metastasis-promoting effects of ketamine and the use of immunostimulation as a prophylactic measure. Because the effects of ketamine evident in Experiments 1 and 2 were the largest, we chose to continue our investigation of mediating mechanisms with respect to this anesthetic. In the following study, we tested the hypothesis that the metastasis-promoting effects of ketamine are mediated by β-adrenoceptor stimulation and that these effects can be attenuated by chronic immunostimulation.

Rats were pretreated with a β-adrenergic antagonist (nadolol), with an immunostimulator (poly I-C), with both nadolol and poly I-C, or with saline. The exact schedules of nadolol/poly I-C treatments are detailed in the Methods section. Each of these four groups was subdivided, and rats either were anesthetized with ketamine (80 mg/kg IP) plus diazepam (12 mg/kg SC) for 1 h or were undisturbed in their home cages (n = 5–10 per group). Five hours after anesthesia initiation, all rats were inoculated with radiolabeled MADB106 tumor cells, and 24 h later, LTR was assessed.

In nonanesthetized rats, nadolol, poly I-C, or both had no significant effects on MADB106 LTR. Ketamine caused a 7.4-fold increase in LTR, and both nadolol and poly I-C significantly reduced the effect of ketamine when used alone (Fisher planned contrast). Their combined use completely abolished the effects of ketamine, yielding a significantly greater blockade than each treatment alone (Fisher planned contrast) (Fig. 4).

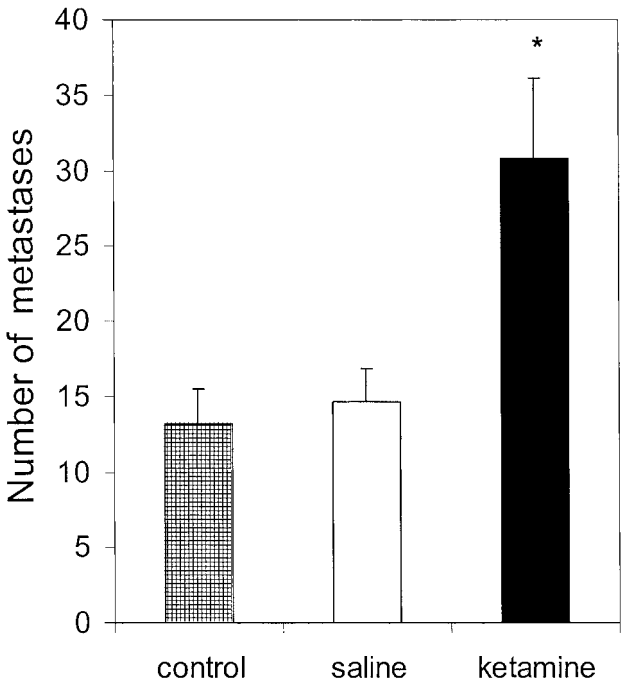

Figure 3. Number of MADB106 metastases (mean ± SEM) in control and ketamine-treated animals. Rats were randomly assigned to an undisturbed control group, to a control group injected with saline (saline group), or to an experimental group treated with ketamine (80 mg/kg intraperitoneally) plus diazepam (12 mg/kg subcutaneously) (ketamine group). Anesthesia lasted for approximately an hour and was induced 5 h before IV inoculation with MADB106 tumor cells. Three weeks later, rats were killed, and pulmonary metastases were counted. The ketamine group had significantly more metastases than each of the control groups (*). A total of 35 male rats were used.

Discussion

This study in a rat model of breast cancer metastasis demonstrates that thiopental, ketamine, and halothane, but not propofol, can significantly promote LTR or the number of MADB106 lung metastases. Ketamine had the largest effects, increasing LTR and lung metastases more than 2.5-fold in all experiments. It is noteworthy that the 24-hour LTR index was predictive of the effects on the number of metastases that would have developed weeks later, had the rat not been killed: previous studies have demonstrated this association with respect to different manipulations that either increased or decreased LTR and correspondingly affected numbers of lung metastases (11,13–15). Thus, our findings that certain anesthetics increase LTR suggest that these results may have clinical significance with respect to metastatic development, as was indeed indicated herein with respect to ketamine.

As a preliminary inquiry of immunological mechanisms mediating these effects of anesthetics, we studied NK cells at the time of tumor inoculation. Again, all anesthetics except propofol significantly reduced the activity of circulating NK cells. The number of circulating NK cells per milliliter of blood was reduced by all anesthetics, although the effects of halothane did not reach statistical significance. Thus, the evident suppression of NK activity is attributed, to some degree, to a decrease in the number of circulating NK cells.

Although many mechanisms, immunological and nonimmunological, can potentially mediate the effects of anesthetics on resistance to tumor development, it seems that in this study, reduced NK activity was a major underlying factor. Previous research points to NK cells as a pivotal factor controlling both LTR and lung metastases of the MADB106 tumor (12): pulmonary NK cells were observed interacting with MADB106 cells *in situ* (within the lung tissue) (16);

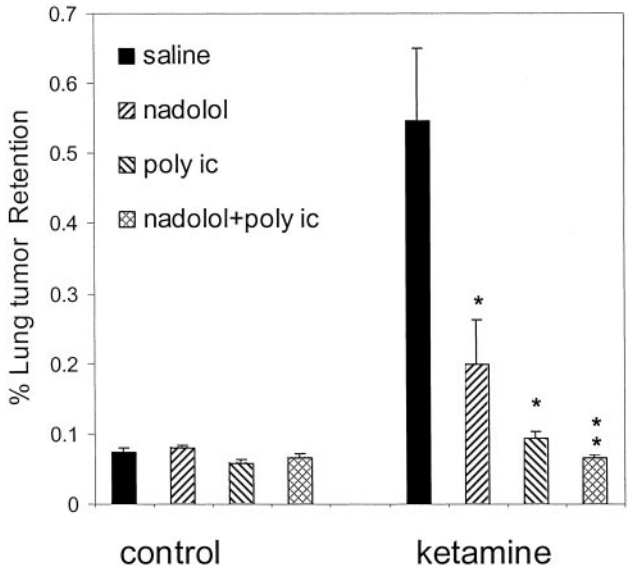

Figure 4. The attenuating effects of β-adrenergic blockade (by nadolol) and of immunostimulation (by poly I-C) on the promotion of MADB106 lung tumor retention (LTR) by ketamine (mean ± SEM). Rats were pretreated with nadolol, poly I-C, both nadolol and poly I-C, or saline. Each of these four groups was subdivided, and rats were either anesthetized with ketamine (80 mg/kg intraperitoneally) plus diazepam (12 mg/kg subcutaneously) for approximately an hour (ketamine) or were undisturbed in their home cages (control). Five hours after anesthesia induction, all rats were inoculated with radiolabeled MADB106 tumor cells, and 24 h later, LTR was assessed. Both nadolol and poly I-C significantly reduced the metastasis-promoting effects of ketamine, and their combined use completely abolished it, yielding a significantly greater blockade than each treatment alone. *Significant difference from the ketamine-saline group; **significant difference from each of the ketamine-treated groups. A total of 68 male rats were used.

selective *in vivo* depletion of NK cells typically increases MADB106 metastasis more than 100-fold (14); adoptive transfer of NK cells, but not other types of leukocytes, restores resistance to metastasis (12); substances that enhance NK cell activity (e.g., lipopolysaccharide and poly I-C) improve resistance to metastasis; and manipulations that compromise NK activity (e.g., prolonged hypothermia, alcohol consumption, swim stress, and surgery) interfere with it (3,5,13). Provided this significance of NK activity in controlling MADB106 metastasis, our current findings that some anesthetics, but not others, suppressed NK activity and correspondingly suppressed resistance to MADB106 metastasis support our suggestion of NK cell mediation of the effects of anesthetics. Further support for this suggestion is derived from the protective effects of immunostimulation and of the β-adrenergic antagonist against the deleterious effects of ketamine. As discussed below, these protective effects were most likely mediated by protecting NK cells from *in vivo* suppression by ketamine.

In this study, we focused on ketamine when attempting to elucidate mechanisms of action, because it had the largest effect. Our findings suggest the involvement of peripheral β-adrenoceptor activation in mediating the effects of ketamine on MADB106 LTR: nadolol, a β-adrenergic antagonist with low permeability to the blood-brain barrier, attenuated this effect when administered peripherally in relatively small doses (14). Importantly, ketamine was previously reported to interact with α_1 and β_2 adrenoceptors and to inhibit the reuptake of catecholamines (17), and we have previously shown that peripheral β-adrenergic stimulation (induced either pharmacologically or through stressful stimuli) suppresses NK activity and promotes MADB106 metastasis (14). In a previous study using the same tumor model, nadolol also attenuated an increase in LTR caused by thiopental (5). Adrenoceptor stimulation by anesthetics may be induced via their direct interaction with the receptors, as may be the case with ketamine, or via central mechanisms that activate the sympathetic nervous system, as may be the case with thiopental (5). Propofol, which did not increase MADB106 LTR and may have even reduced it (−20% in the first study), was reported to be a weak β-adrenergic antagonist (18). Perhaps related to this is the fact that patients anesthetized with propofol showed smaller postoperative responses of catecholamines, interleukin (IL)-6, and cortisol and smaller reductions in the number of T and B cells compared with patients receiving isoflurane (19).

Systemic administration of poly I-C was reported to elicit an immune response resembling the response to viral infections. Specifically, this response involves the release of various cytokines (including interferon [IFN]-α, -β, and -γ; IL-1, -2, -6, and -12; and tumor necrosis factor-α), facilitation of cellular immune functions (such as cytotoxicity by NK, T, and macrophage cells), and altered expression of various cellular adhesion molecules (20). Here, we used our recently developed regimen of poly I-C, which is based on chronic administration of small doses of this biological response modifier. Implementing such an approach during the perioperative period seems clinically feasible because a clinical trial has already used a regimen of chronic small doses of slow-absorbed poly I-C in otherwise untreated patients with malignant gliomas (21). In the current study, poly I-C markedly and significantly attenuated the effects of ketamine on LTR. Importantly, poly I-C exerted no effect on baseline levels of LTR in nonanesthetized rats. This suggests that poly I-C protected against the effects of ketamine, rather than acting independently of ketamine by merely increasing baseline resistance to MADB106 metastasis. Indeed, several studies have shown that *in vitro* activation of NK cells with poly I-C or with the cytokines it releases (e.g., IFNs or IL-2) can induce a state of relative resistance of NK cells to suppression by catecholamines and by other compounds that suppress NK activity by increasing intracellular cyclic

adenosine monophosphate (22) (e.g., prostaglandin E_2). Moreover, we have reported that the same *in vivo* regimen of poly I-C protected pulmonary NK cells from *in vitro* suppression by prostaglandin E_2 (23). Thus, we suggest that the beneficial effects of poly I-C evident in this study are mediated by reduced susceptibility of NK cells to suppression by ketamine-induced β-adrenoceptor activation.

Some levels of intraoperative hypothermia occur in most operations. In a previous study (5), we exposed rats under thiopental anesthesia to severe hypothermia (i.e., 30°C for four hours). This condition markedly suppressed NK activity and resistance to MADB106 metastasis. In this study, we assessed the influence of mild levels of hypothermia (33°C–35°C for one hour) and did not observe significant effects on NK activity or MADB106 metastasis. This indicates that the anesthetics that did promote metastasis in this study have done so independently of hypothermia. Notwithstanding, some regimens of anesthesia (e.g., spinal block) and some perioperative interventions (e.g., ketorolac) can reduce the stress response to surgery, as well as the postsurgical susceptibility to infections and metastasis (8,11).

One drawback of our study is that the translation from doses used in rats to doses used in humans is not straightforward. In this study, anesthesia levels were adjusted to those needed for surgical procedures, yet some of the anesthetics may have induced deeper anesthesia than others. Another drawback is that in clinical practice it is common to use combinations of drugs (e.g., induction with thiopental and maintenance with volatile anesthetics), rather than one anesthetic. This study, however, aimed at evaluating and comparing the effect of various anesthetics independently of each other and of other surgical-related procedures to start disentangling the overall effect of the perioperative settings. Clearly, more studies are needed to clarify mechanisms of immunosuppression by anesthetics and the clinical ramification of their effects on host susceptibility to dormant and opportunistic diseases.

It is becoming apparent that the perioperative period is characterized by pronounced immunosuppression, including NK activity, and that anesthesia contributes to this condition (7,8). Animal studies demonstrated a key role for cell-mediated immunity (e.g., NK, macrophage, and cytotoxic T-lymphocyte activity) in controlling metastasis (10,24), as well as an association between surgery-induced immunosuppression and host susceptibility to metastasis (4,5). Human studies show that small perioperative levels of NK activity are associated with increased cancer-related morbidity and mortality in patients with colorectal, breast, lung, and head and neck cancers (10,24). Thus, anesthesia and hypothermia should be carefully considered when there is a high risk of metastatic spread. Indeed, in melanoma patients, a large-scale study (25) reported that substituting general anesthesia with local anesthesia is an independent favorable prognostic factor that results in less distant recurrence. Of particular clinical relevance is the removal of potentially metastasizing tumors in procedures that involve immunosuppressive anesthetics and that tend to be accompanied by hypothermia (e.g., open-abdominal surgeries). Because surgery is an important aspect of cancer treatment, anesthetics that are less immunosuppressive may be advantageous. Prophylactic measures, such as preoperative stimulation of the immune system or the perioperative use of specific blockers, may be considered.

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