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Evidence that postoperative pain is a mediator of the tumor-promoting effects of surgery in rats ☆

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Abstract

We have previously shown in rats that the provision of analgesic doses of morphine significantly reduces the tumor-promoting effects of undergoing and recovering from surgery. Because morphine had no effect in non-operated animals, and because a single preoperative dose given hours before tumor inoculation was effective, we have suggested that it is the pain-relieving effects of the drug that underlies its beneficial impact. To support and strengthen this suggestion, two different regimens of analgesia were employed, the systemic administration of the more selective μ-agonist, fentanyl, and the intrathecal (i.t.) administration of bupivacaine plus morphine. To assess host resistance against metastasis, we used a lung clearance assay of the MADB106 mammary adenocarcinoma, a natural killer (NK)-sensitive syngeneic cell line that metastasizes only to the lungs. Female and male Fischer 344 rats were randomly assigned to one of four groups using a 2×2 experimental design: experimental laparotomy under halothane anesthesia versus anesthesia alone, by drug treatment versus vehicle. In the first in vivo experiment, fentanyl was administered 20 min before surgery (40 μg/kg subcutaneously (s.c.)), and at the end of surgery in a slow-release suspension (20 μg/kg s.c.). In the second in vivo experiment, bupivacaine (10 μg) plus morphine (20 μg) in 50 μl was administered i.t. before surgery. Surgery resulted in a 3- to 4-fold increase in the lung retention of MADB106 cells in both males and females, and the observed surgery-induced increase in lung tumor retention was reduced by more than 65% in the fentanyl-treated animals and more than 45% in the animals receiving i.t. bupivacaine plus morphine. Neither drug regimen exerted effects in the anesthesia only animals. Surgery also resulted in a significant suppression of whole blood NK activity assessed at 5 h postoperatively, the same time point at which MADB106 tumor cells were inoculated in the in vivo studies. Unlike the in vivo study, fentanyl suppressed NK activity at this time point in non-operated rats, but had no effect in operated rats. Taken together, these findings strengthen the suggestion that the management of perioperative pain is a critical factor in preventing surgery-induced decreases in host resistance against metastasis. If similar relationships between pain and metastasis occur in humans, then pain control must become a priority in the postoperative care of individuals with cancer.

Keywords: Cancer; Natural killer cell; Rat; Fentanyl; Bupivacaine

1. Introduction

Undergoing surgery is well known to result in the suppression of several immune functions including natural killer (NK) cell activity in both animals (Pollock et al., 1987; Sandoval et al., 1996) and humans (Gutman et al., 1993; Koltun et al., 1996; Kutza et al., 1997). Animal studies provide direct evidence indicating a key role played by NK cells in controlling metastasis (Wiltrout et al., 1985; Ben-Eliyahu and Page, 1992) as well as indications that the suppression of NK cell activity by surgery underlies the promotion of metastatic development that has been associated with undergoing surgery (DaCosta et al., 1998; Ben-Eliyahu et al., 1999). Corroborating evidence in humans shows that low NK activity during the perioperative period is associated with higher rates of cancer recurrence and mortality in patients with colorectal (Tartter et al., 1987; Koda et al., 1997), breast (Levy et al., 1985), head and neck (Schantz et al., 1987; Schantz et al., 1989), and lung cancers (Fujisawa and Yamaguchi, 1997). Taken together, these findings suggest that limiting surgery-induced NK suppression might enhance host resistance against metastatic sequelae. Considering that a large portion of individuals with cancer die of metastatic development, such an advancement could prove clinically important given that surgery is often a necessary first-line strategy for cancer treatment.

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Given that acute pain is also known to suppress NK activity (Shavit et al., 1987; Sacerdote et al., 1994) and to promote tumor development in animals (Lewis et al., 1983), our previous studies in rats have explored the possibility that relieving the pain of undergoing and recovering from surgery would reduce its associated tumor-enhancing effects. We have previously shown that the pre- and postoperative administration of an analgesic dose of morphine significantly attenuates surgery-induced increases in both lung metastatic colonization (Page et al., 1993) and lung retention of radiolabeled tumor cells (Page et al., 1994) without affecting the anesthesia control groups. We have also shown that although the provision of morphine at any time relative to surgery significantly reduced surgery-induced increases in tumor cell retention, the preoperative dose of morphine appeared to provide the greatest ameliorative effect (Page et al., 1998). This latter finding is consistent with current theories regarding preemptive analgesia, holding that opiate administration before the surgical insult prevents the establishment of central sensitization and greatly reduces the pain experience (Richmond et al., 1993; Tverskoy et al., 1994), thus supporting the suggestion that pain might be a mediator of the observed surgery-induced increase in susceptibility to metastasis in rats.

The current study further addressed the hypothesis that pain is a significant mediator of surgery-induced decreases in host resistance against metastasis by employing two strategies: (a) using the more selective μ-agonist fentanyl (Adams et al., 1990) in a systemic administration regimen, and (b) administering morphine plus bupivacaine intrathecally (i.t.) to provide pain relief at the spinal level and to avoid systemic drug effects by virtue of the very low doses that are employed. Given previous findings of sex differences in both surgery-induced changes in NK activity (Page and Ben-Eliyahu, 1997; Richardson et al., 1997) and analgesic responses to systemically administered μ-agonists (Baamonde et al., 1989; Candido et al., 1992; Cicero et al., 1997; cf., however, Bartok and Craft, 1997), both males and females were used in these studies.

As for our previous studies, we used the NK-sensitive mammary adenocarcinoma cell line, MADB106, syngeneic to the Fischer 344 rats we study. MADB106 tumor cells seed and colonize only in the lungs following intravenous (i.v.) injection, and the NK sensitivity of these processes has been shown to be limited to the first 24 h after injection in a time dependent and decremental manner (Barlozzari et al., 1985; Ben-Eliyahu and Page, 1992). Thus, the lung retention of MADB106 cells provides an indication of both host susceptibility to metastasis and in vivo levels of NK activity.

2. Materials and methods

2.1. Animals

Male and female mature Fischer 344 (F344) rats, aged 4–5 months (age matched within experiment), were purchased from NIA. Animals arrived a minimum of 4 weeks before each experiment and were maintained on a 12:12-h dark/light cycle in single-sex group housing. Animals had free access to food and water, except for the 8 h prior to undergoing surgery when only water was available. All blood withdrawals and tumor cell injections occurred within the first 8 h after dark onset. All experiments were approved by the Laboratory Care and Use Committee.

After habituation to handling, females underwent daily vaginal cellularity smears for the 10 days prior to all experiments, and males were similarly handled on each occasion. Females were identified as being in proestrus/estrus/metestrus or diestrus days 1 or 2 using the method described by Everett (1989). Female groups were balanced for estrous phases in all studies.

2.2. Surgery

Surgery animals underwent experimental laparotomy while anesthetized with halothane at approximately 2%. Following the achievement of an anesthetic state, both anesthesia only and surgery with anesthesia animals received Penicillin G (25 000 units/kg intramuscularly). Subsequently, the abdomen of the surgery animals was shaved and scrubbed with betadine. The surgical procedure consisted of a 4-cm midline incision, followed by the externalization of 10 cm of the small intestine for a period of 4 min. During the first minute, the small intestine was rubbed between two pieces of gauze in four locations to initiate tissue damage; a saline-soaked gauze pad was placed over the intestines for the remaining 3 min. The intestine was then returned to the abdominal cavity, irrigated with saline, and the muscle and skin layers were sutured with 5-0 monofilament wire. The anesthesia only animals were anesthetized throughout this period at the same dose of halothane as the surgery animals.

2.3. Drugs and administration

2.3.1. Systemic fentanyl

Fentanyl was administered in two preparations. Preoperatively, fentanyl citrate 40 μg/kg was administered subcutaneously (s.c.) 20 min before surgery. This dose was shown by others to provide acute antinociception of 35% maximum possible effect over the first hour after administration using tail withdrawal reaction from 55°C water (10 s cutoff) (De Kock and Meert, 1997). Immediately following the completion of surgery, 20 μg/kg fentanyl was administered s.c. at the dorsal flank in a slow-release suspension (SRS), an oil emulsion comprised of mannide monooleate (Arlacel A, Sigma, St. Louis, MO), light mineral oil, and saline (7, 40, and 53% by volume, respectively) (Frederickson and Smits, 1979; Page et al., 1993, 1994, 1998). The fentanyl concentration was adjusted such that the appropriate dose was administered in 1 ml of the SRS. Vehicle was used for control injections.

2.3.2. Intrathecal bupivacaine and morphine

Bupivacaine (10 μg, 0.25%, Sensorcaine, methylparaben free) plus morphine (20 μg) in 50 μl was injected i.t. via lumbar puncture between the L4 and L5 vertebrae following the establishment of halothane anesthesia and local skin preparation. Saline vehicle was used for control injections. In a pilot study we conducted, trypan blue injections of the same volume with a caudal orientation of the needle bevel were observed to spread cephalically to the mid-thoracic vertebral region upon laminectomy, suggesting the spinal cord was bathed in the injectate to the level of the upper margin of the surgical incision. Additionally, whereas animals receiving the vehicle i.t. injections were responsive to pin prick at the superior aspect of the incision upon awakening from surgery, those receiving bupivacaine plus morphine were unresponsive to this stimulus. Recently, Shahar et al. (submitted) showed analgesic responses to tail immersion (50°C water) for up to 5 h following acute lumbar i.t. injection, employing the same doses and regimens of administration used herein. Similar doses via indwelling i.t. catheter were found by Tejwani et al. (1992) to maintain significant tail-flick antinociception for 6 h following drug administration.

2.4. Tumor cell maintenance and radiolabeling

YAC-1 cells, used for the in vitro whole blood NK cytotoxicity assay, and MADB106 cells, used for the in vivo lung clearance assay were maintained in 5% CO_2 at 37°C in complete medium (RPMI 1640 media (Mediatech) supplemented with 10% heat-inactivated fetal calf serum (FCS), 0.05 mg/ml gentamicin, 2 mM L-glutamine, 0.1 mM non-essential amino acid and 1 mM sodium pyruvate). The adherent MADB106 cells, were separated from the flask (Falcon 3023) using Trypsin 0.25%.

To label the DNA of MADB106 cells, 0.4 μCi [^{125}I]iododeoxyuridine per ml media was added to the growing MADB106 cell culture 24 h before cell harvest. After separation from the flask, cells were washed in phosphate buffered saline (PBS) and reconstituted in PBS.

2.5. Experimental procedures

For all experiments, a $2 \times 2 \times 2$ design was used such that males and females were randomly assigned to undergo either surgery with halothane anesthesia or halothane anesthesia alone, and receive either the drug treatment or its vehicle. Female groups were balanced for estrous phase.

2.5.1. The effects of fentanyl administration on surgery-induced increases in lung tumor retention

Five hours after the completion of surgery, all animals were lightly anesthetized with halothane and injected with 4×10^5 radiolabeled MADB106 cells/kg into the tail vein. Lungs were removed 16 h later and their radioactive content measured in a gamma counter (Packard Cobra 5002). The percent lung retention of radiolabeled tumor cells was calculated using the equation:
(lung count ÷ injectate count) × 100.

2.5.2. The effects of surgery and fentanyl administration on whole blood NK cytotoxicity

Five hours after the completion of surgery, rats were anesthetized with halothane and 1 ml blood was withdrawn into a heparinized syringe (20 units) via cardiac puncture. After washing in 3 ml PBS supplemented with bovine serum albumin (1 g/l), the blood was washed twice again with complete media (15% FCS). Each wash constituted a 10-min centrifugation at $500 \times g$ and aspiration of the supernate down to the original volume. Thus, all the cellular components of the blood remained and the serum was replaced by media.

To label the cytoplasm of YAC-1 target cells, approximately 32×10^6 cells were incubated with 300 μl media, 400 μl FCS, and 0.4 mCi of $^{51}NaCrO_4$ for 60 min. After incubation, the target cells were washed two times in complete media and their concentration adjusted to 8×10^5 cells/ml. Target cells were serially diluted seven times by a factor of 2 to achieve the eight effector to target cell ratios. For plate preparation, 100 μl of the washed blood was placed in each well of a microtiter plate and 150 μl of the ^{51}Cr-labeled YAC-1 cells was placed on top of the blood. Plates were centrifuged for 10 min at $500 \times g$ and incubated for 4 h. After incubation, plates were centrifuged and 100 μl of the supernate was harvested. Percent killing was calculated separately for each tumor cell concentration using the formula: $(0.8 \times \text{experimental} - \text{spontaneous}) \div (\text{maximum} - \text{spontaneous}) \times 100$. The spontaneous and maximum release of ^{51}Cr from the cell cytoplasm was assessed for each effector to target ratio by replacing the blood with media and 1 N HCl, respectively. In addition to using percent specific killing as a measure for NK activity, lytic units (LU) per ml blood were also calculated for each animal at the level of 20% killing. For more details on the whole blood assay and calculation of percent specific killing see Shakhar and Ben-Eliyahu (1998) and Ben-Eliyahu et al. (1999).

2.5.3. The effects of surgery and fentanyl administration on plasma corticosterone levels

At 5 h after surgery, animals were rapidly anesthetized with halothane and 0.5 ml blood was withdrawn via cardiac puncture within 2 min of removing the animal from its cage. Blood samples were centrifuged at $1500 \times g$ for 10 min, and the plasma was collected and stored at −80°C. CS levels were assessed using a commercial double antibody radioimmunoassay (RIA) kit (ICN Biomedical, Costa Mesa CA). All samples were assayed in duplicate in a single RIA.

2.5.4. The effects of surgery and fentanyl administration on rearing behavior

Given our previous findings that morphine administration prevented the activity-suppressing effects of abdominal

surgery (Page et al., 1993, 1998), this experiment assessed whether fentanyl treatment would prevent surgery-induced suppression of activity. At the completion of surgery, animals were given their postoperative dose of fentanyl or vehicle and placed in individual uncovered cages in a room illuminated with a red light. During the latter 30 min of each of the first 4 postoperative hours, rears were enumerated by observers who were unaware of the experimental manipulation. A rear was defined as raising both forepaws off of the cage floor.

2.5.5. The effects of intrathecal administration of bupivacaine plus morphine on surgery-induced increases in lung tumor retention

Given our previous findings using systemic morphine administration, in the current study, we also attempted i.t. anesthesia and analgesia to reduce the total μ-opioid dose and to counter the possibility that systemic opioid mechanisms are involved in the amelioration of surgery-induced increases in lung tumor retention. Following i.t. injection of either bupivacaine plus morphine or saline, surgery animals were prepared and underwent experimental laparotomy. As for the first experiment, 4×10^5 radiolabeled MADB106 tumor cells were injected at 5 h after surgery, and lungs were removed 16 h later for assessment of radioactive content.

2.6. Data analysis

In order to accumulate sufficient numbers of animals and to verify the consistency of the in vivo findings, two replicates of the first experiment (Section 2.5.1), and three replicates of the last experiment (Section 2.5.5) were conducted. Given that all groups were equally represented in each assay, results were combined for statistical analysis by assigning standardized scores within each replication, thus overcoming inter-assay variation in baseline levels of retention. For statistical analysis, repeated measures ANOVA was used to analyze for group differences in number of rears in the behavioral experiment (Section 2.5.4) and factorial ANOVA was used to analyze for group differences in the remaining four experiments, $\alpha < 0.05$. If statistically significant group differences were detected, Bonferroni post-hoc analysis was performed to examine the nature of the interaction.

3. Results

3.1. The effects of fentanyl administration on surgery-induced increases in lung tumor retention

Surgery resulted in a more than 4-fold increase in lung tumor retention which was significantly attenuated by fentanyl administration (Fig. 1). There were no sex differences in these outcomes. Two-way ANOVA indicated a significant interaction between the effects of surgery and fentanyl ($F(1, 87) = 10.965$, $P = 0.001$), such that surgery animals receiving fentanyl retained significantly fewer tumor cells in the lungs than did surgery animals not receiving fentanyl; fentanyl exerted no effects in the anesthesia only animals (Bonferroni post-hoc analysis across sex, $P < 0.001$). Both surgery and fentanyl exerted main effects ($F(1, 87) = 35.942$ and 11.048, respectively, $P = 0.001$). The remaining possible interactions were not significant, including sex by surgery, and sex by fentanyl.

3.2. The effects of surgery and fentanyl administration on whole blood NK cytotoxicity

Surgery resulted in a large and statistically significant decrease in whole blood NK cytotoxicity ($F(1, 123) = 22.227$, $P < 0.001$), and males exhibited significantly greater NK cytotoxicity than the females ($F(1, 123) = 43.938$, $P < 0.001$) (Table 1 and Fig. 2). There was no significant main effect exerted by fentanyl, nor were there any significant interactions.

3.3. The effects of surgery and fentanyl administration on plasma corticosterone levels

Plasma CS levels were affected by surgery, by sex, and by fentanyl treatment; each factor affected CS levels independently of the other two factors. Specifically, surgery resulted in significantly increased CS levels (443 ± 31 (SEM) versus 268 ± 22, surgery versus anesthesia, respectively); fentanyl treatment reduced CS levels significantly (284 ± 27 versus

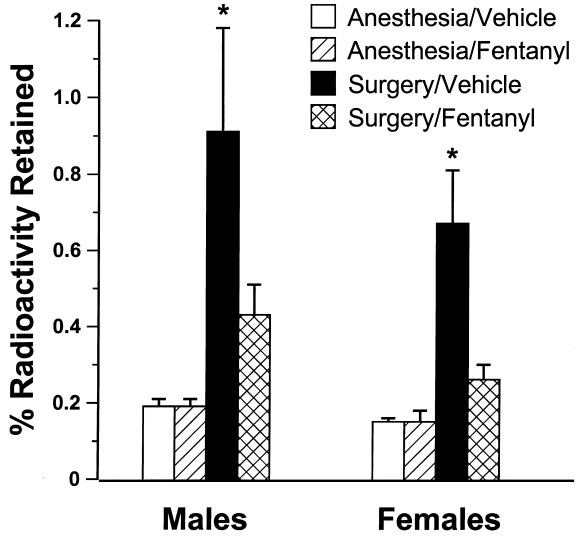

Fig. 1. The effects of surgery and fentanyl administration on the lung retention of radiolabeled MADB106 tumor cells assessed as percent radioactivity retained. All animals were injected with [^{125}I]iododeoxyuridine-labeled MADB106 cells at 5 h after the completion of surgery. Lungs were removed 16 h later and their radioactive content assessed. Error bars represent SEM. *Statistically significant difference versus all other groups within sex ($P < 0.05$) ($n = 7$–8 males and 11–15 females per group).

Table 1
The effects of surgery and fentanyl administration on whole blood NK activity presented as lytic units (±SEM)[a]

Group	Males	Females
Anesthesia/vehicle	89.7 (11.1)	41.8 (9.7)
Anesthesia/fentanyl	72.9 (10.7)	28.1 (5.4)
Surgery/vehicle	41.3 (10.7)	12.1 (6.4)
Surgery/fentanyl	52.0 (13.8)	11.7 (3.6)

[a] Blood was withdrawn via cardiac puncture at 5 h after the completion of surgery and used in the whole blood NK cytotoxicity assay. ($n = 11$–12 males and 19–20 females per group).

508 ± 35, fentanyl versus vehicle, respectively); and females exhibited significantly higher plasma CS levels than males (413 ± 28 and 257 ± 21, respectively) ($F(1, 127) = 21.838$, 21.207, and 15.549, surgery, fentanyl, and sex, respectively, $P < 0.001$). There were no significant interactions among any of the three factors in affecting CS levels.

3.4. The effects of surgery and fentanyl administration on rearing behavior

Compared to non-operated animals, operated animals exhibited a significant reduction in rearing behavior throughout the 4 h postoperative assessment period (Fig. 3) ($F(1, 23) = 43.161$, $P < 0.001$). Among the surgery animals only, fentanyl treatment resulted in a significant increase in activity levels compared to vehicle-treated animals from the second through fourth hour after surgery ($F(1, 15) = 8.751$, $P = 0.01$).

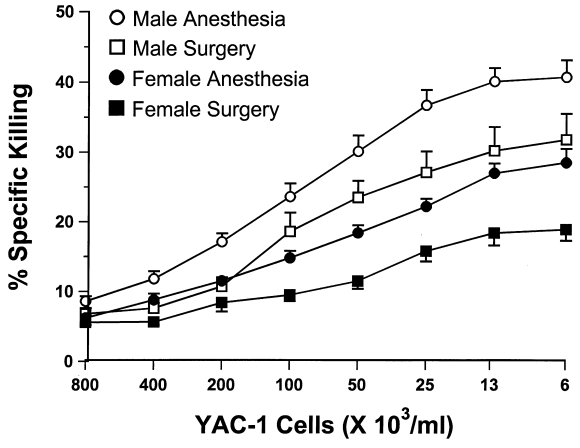

Fig. 2. Female–male differences in the effects of surgery on whole blood NK activity expressed as percent specific killing per ml blood. Blood was withdrawn via cardiac puncture at 5 h after the completion of surgery and used in the whole blood NK cytotoxicity assay. Males exhibited significantly greater NK cytotoxic activity compared to the females ($P < 0.001$). Error bars represent SEM ($n = 11$–12 males and 19–20 females per group).

3.5. The effects of intrathecal administration of bupivacaine plus morphine on surgery-induced increases in lung tumor retention

Surgery resulted in a more than 3-fold increase in the lung retention of radiolabeled MADB106 cells which was significantly attenuated by intrathecal bupivacaine plus morphine administration (Fig. 4). There were no sex differences in these outcomes. Two-way ANOVA indicated a significant interaction between the effects of surgery and i.t. bupivacaine plus morphine ($F(1, 54) = 5.645$, $P < 0.05$) such that surgery animals receiving drug treatment retained significantly fewer tumor cells in the lungs than did surgery animals not receiving the drug treatment. Intrathecal bupivacaine plus morphine exerted no effects in the anesthesia only animals (Bonferroni post-hoc analysis across sex, $P < 0.02$). Surgery exerted a main effect ($F(1, 54) = 40.557$, $P < 0.001$). The i.t. administration of bupivacaine plus morphine exerted no main effect and there were no other significant interactions.

4. Discussion

The results of this study extend our previous findings in rats and supports our hypothesis that the provision of pain relief attenuates surgery-induced increases in metastatic susceptibility. In particular, we have previously shown that a variety of systemic morphine administration regimens improve host resistance against surgery-induced increases

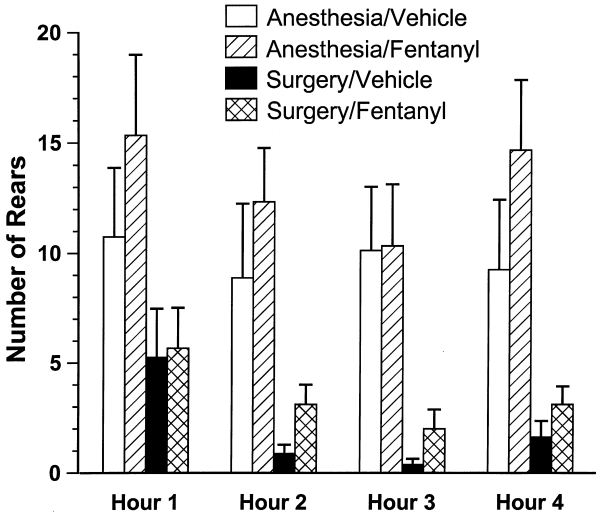

Fig. 3. The effects of surgery and fentanyl administration on exploratory activity assessed as rearing behavior. Animals were observed in a dark room throughout the latter 30 min of each of the first 4 postoperative hours. One rear was scored each time the animal lifted both forepaws off of the cage floor. Surgery resulted in a significant suppression of exploratory behavior ($P < 0.001$); and among the surgery animals, fentanyl treatment resulted in a significant increase in activity levels compared to vehicle-treated animals from the second through fourth hour after operation ($P = 0.01$). Error bars represent SEM ($n = 2$–3 males and 4–6 females per group for a total of 6–9 per group overall).

in susceptibility to metastasis. These regimens include both pre- and postoperative administration (Page et al., 1993, 1994) as well as preoperative only and postoperative only (Page et al., 1998). The current findings show that both the preoperative i.t. administration of bupivacaine plus morphine and the perioperative systemic administration of fentanyl significantly enhance host resistance against surgery-induced increases in lung tumor retention.

The suggestion that it is the pain alleviating effects of these drugs that attenuated the surgery-induced promotion of metastasis, rather than direct effects on immunity, tumor cells or other mechanisms that affect metastasis, is supported by the following arguments. First, the efficacy of the very low doses of bupivacaine plus morphine administered intrathecally argues directly against possible peripheral effects of these drugs. Second, whereas all drug regimens in previous studies as well as the current study were effective in ameliorating surgery-induced increases in lung tumor retention, they exerted no effects in the non-operated rats who were not experiencing pain. This interaction between the effects of the drugs and exposure to surgery indicates a blockade of the impact of surgery, rather than an independent effect of the drugs on tumor metastasis. Third, in behavioral studies, systemic regimens of both morphine and fentanyl administration at least partly restored exploratory behavior following surgery, behavior that was most likely inhibited by surgery-induced abdominal discomfort. Finally, whereas the systemic doses of fentanyl and morphine used in our studies exert similar levels of analgesia (McLaughlin and Dewey, 1994; van den Hoogen and Colpaert, 1987), fentanyl was shown not to exert the same immune and vasoactive effects that are characteristic of morphine (Grossmann et al., 1996; Bilfinger et al., 1998). These findings suggest that the non-pain-alleviating effects of morphine are unlikely to underlie the protective effects of μ-opioids.

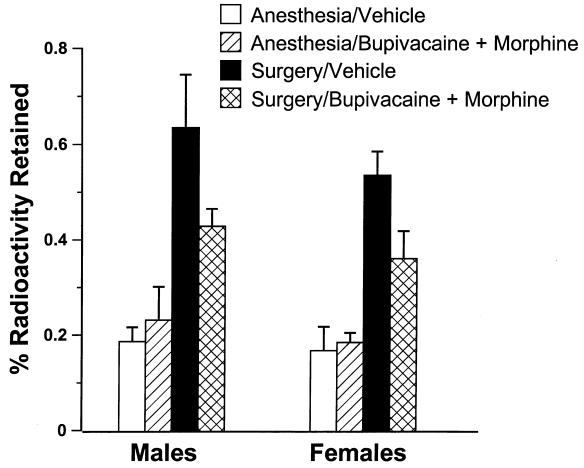

Fig. 4. The effects of intrathecal administration of bupivacaine plus morphine on surgery-induced increases in lung tumor retention assessed as percent radioactivity retained. All animals were injected with [^{125}I]iodo-deoxyuridine-labeled MADB106 cells at 5 h after the completion of surgery. Lungs were removed 16 h later and their radioactive content assessed. There was a statistically significant interaction between the effects of surgery and i.t. drug administration ($P < 0.05$). Error bars represent SEM ($n = 6$–9 males and 5–8 females per group for a total of 11–17 overall).

A mechanism by which opiates might exert their beneficial effect is by preventing spinal cord hyperexcitability induced by C-fiber stimulation. Such protective effects have been shown using systemic administration of both morphine (Woolf and Wall, 1986) and fentanyl (Mazario et al., 1998). Additionally, i.t. administration of μ-agonists has been shown to suppress spinal cord hyperexcitability following subcutaneous formalin injection in rats. These findings are based on the assessment of both dorsal horn neuronal excitatory responses (Dickenson and Sullivan, 1987) and pain behaviors (Yamamoto and Yaksh, 1992; Malmberg and Yaksh, 1993). Finally, intrathecal bupivacaine or morphine administration was shown to block mechanical hyperalgesia following incision on the plantar aspect of the hindpaw in rats (Brennan et al., 1997).

Human studies have shown that improved pain outcomes are achieved with the pre-surgical systemic administration of opiates, including morphine (Richmond et al., 1993) and fentanyl (Tverskoy et al., 1994), as well as by using epidural anesthetics (Gottschalk et al., 1998; Aida et al., 2000), although such effects are not always obvious (Katz, 1995; Kissin, 1996). Further, epidural anesthesia and analgesic techniques have also been shown to ameliorate surgery-induced immunosuppression (Koltun et al., 1996; Le Cras et al., 1998) and to result in a decreased incidence of infection (Cuschieri et al., 1985; Yeager et al., 1987). Although it remains unknown whether the alleviation of perioperative pain will provide similarly beneficial outcomes with respect to tumor development in humans, the current study suggests that it will, specifically in patients with potentially metastasizing cancer.

Surgery resulted in a large and significant reduction in NK activity assessed in vitro. However, unlike the in vivo finding that fentanyl improved the deleterious effects of surgery on the metastasis of the NK-sensitive MADB106 tumor, no beneficial effects of fentanyl were observed when NK activity was assessed in vitro following surgery. Nevertheless, whereas fentanyl administration resulted in a large and significant suppression of NK activity in non-operated rats, it exerted no effect in operated rats. We and others have previously reported such NK-suppressive effects of opiates in naïve rats (Shavit et al., 1987; Bayer et al., 1990; Lysle et al., 1993; Page et al., 1994). Thus, the pattern of interaction between the effects of surgery and of fentanyl evident herein could be interpreted as a summation of two independent effects: one being a suppression of NK activity by fentanyl in both operated and non-operated rats, and second, the prevention of the NK-suppressive effects of surgery in operated rats. Taken together, it could be argued that fentanyl is beneficial in the context of surgery, irrespective of its suppressive effects on NK activity. One factor that might

explain the discrepancy between the in vitro and in vivo findings regarding the effects of fentanyl is the timing at which the two different outcomes were assessed. Whereas in vitro NK activity was assessed at 5 h after surgery, the assessment of lung tumor retention provided a more dynamic measure of in vivo NK activity, beginning at the same time point at which blood was drawn for assessing in vitro NK activity and ending 16 h later when the lungs were removed (Ben-Eliyahu and Page, 1992). During this time, the adverse effects of fentanyl which were evident at 5 h in the in vitro assay may have dissipated, allowing its beneficial impact to become dominant and manifest in the in vivo index. Indeed, Beilin et al. (1996) showed that whereas abdominal surgery patients whose anesthesia was supplemented with a low dose of fentanyl (up to 6 μg/kg) exhibited complete restoration of surgery-induced NK suppression by 48 h after surgery, those treated with high doses of fentanyl (75–100 μg/kg) continued to exhibit significant NK suppression at this time point. A similar trend of more rapid postoperative recovery of NK cytotoxicity is evident in coronary artery bypass grafting patients treated with a low dose of fentanyl (20 μg/kg) prior to cardiopulmonary bypass versus a high dose group (75–100 μg/kg) (Tønnesen et al., 1987). A second factor that might have contributed to the apparent discrepancy between the in vivo and in vitro findings relates to the nature of the in vitro assay itself. Whereas the lung clearance of tumor cells takes place in the whole animal including the complex physiologic environment in which the immune system functions, the in vitro assay necessitates the washing away of this rich hormonal milieu to prepare the blood for co-incubation with the target cells in a neutral environment.

The specific neuroendocrine mediators of the tumor-enhancing effects of surgery are not well understood. Several hormones released during and following surgery have been suggested by us and others to cause suppression of NK activity and to decrease resistance to metastasis. These ligands include catecholamines (Shakhar and Ben-Eliyahu, 1998) and prostaglandins (Lala et al., 1986). Importantly, CS appears not to play a role in the in the context of the current study. Plasma CS levels were significantly increased at 5 h postoperative, indicating surgery-induced activation of the hypothalamic–pituitary–adrenal axis, and were reduced by fentanyl in both operated and non-operated animals. The lack of an association between CS levels and either NK activity or resistance to the NK-sensitive MADB106 line argues against a potential role for CS in mediating the effects of surgery or the protective effects of fentanyl, and is consistent with recent studies, suggesting that physiological levels of corticosteroids do not suppress NK activity in animals (Flores et al., 1990; Page et al., 1998) or humans (Bodner et al., 1998).

The female–male comparisons are important in stressing the similarity of the findings. Although males exhibited higher levels of NK activity than females as we and others have previously reported (Sulke et al., 1985; Page et al., 1995; Page and Ben-Eliyahu, 1997), the NK-suppressive and metastasis promoting effects of surgery were of a similar magnitude in males and females, as were the protective effects of both analgesic regimens. These findings suggest that pain alleviation via these two regimens is equally effective for males and females in this paradigm. To our knowledge, this is the first study to make such direct comparisons.

In conclusion, findings from this study indicate that both the systemic administration of fentanyl and intrathecal administration of bupivacaine plus morphine resulted in a biologically and statistically significant reduction of surgery-induced increases in host susceptibility to metastasis. Importantly, females and males derived similar benefit from these two treatments. These findings, together with our previous studies showing beneficial effects of morphine in this paradigm, strengthen the suggestion that relieving pain may be an important aspect of the surgical care of cancer patients. If such relationships can be extended to humans, this study indicates that adequate pain management may be protective against metastatic sequelae following cancer surgery.

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