Pre-operative versus postoperative administration of morphine: impact on the neuroendocrine, behavioural, and metastatic-enhancing effects of surgery

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Summary

We have previously shown that the pre- and postoperative administration of an analgesic dose of morphine attenuated the tumour-enhancing effects of surgery. This study was undertaken to assess the relative role and exclusive importance of pre- versus postoperative morphine administration on neuroendocrine, metastatic, and behavioural outcomes of surgery in Fischer 344 rats. The natural killer (NK) sensitive mammary adenocarcinoma cell line, MADB106, was used in a lung clearance assay to assess host resistance to metastasis. Either morphine or its vehicle was administered to all rats at three times: (1) 30 min before surgery (8 mg kg-1, in saline); (2) immediately after surgery in a slow release suspension (SRS, 4 mg kg-1); and (3) 5 h after surgery at the time of tumour cell inoculation (2 mg kg-1, in SRS). Five surgery groups underwent an experimental laparotomy with halothane anaesthesia and received either the vehicle at all three times or morphine in one of four different regimens: before surgery only, at all three times, after surgery only at times 2 and 3, and after surgery total at times 2 and 3 with the preoperative dose added at time 2. Two control groups underwent anaesthesia alone and received either morphine or the vehicle at all three times. Surgery resulted in a twofold increase in tumour cell retention, which was significantly attenuated by all four morphine treatment regimens (P<0.05). Furthermore, the two surgery groups that were treated with morphine preoperatively appeared to derive greater benefit than the preoperatively treated groups exhibited a 65–70% attenuation of surgery-induced increases in tumour cell retention, only a 50% attenuation was evident in the two groups treated postoperatively. Surgery significantly reduced rearing behaviour and morphine reversed this effect such that most morphine-treated surgery groups exhibited similar levels of rearing behaviour as was observed in the unoperated animals throughout the 4-h postoperative observation period. Morphine treatment also significantly attenuated surgery-induced increases in plasma corticosterone concentrations assessed at 5 h after surgery. If such relationships hold in humans, these findings support the suggestion that the pre-surgical administration of morphine is key in optimizing its beneficial effects on surgery-induced increases in metastasis. (Br. J. Anaesth. 1998; 81: 216–223)

Keywords: surgery; analgesics opioid, morphine; immune response, natural killer cells; rat

Both human and animal studies have shown surgery to result in a decrease in various measures of immune function, including lymphocyte mitogenic response and natural killer (NK) cell activity.1–3 Animal studies have indicated a significant role for NK cells in controlling tumour development,4 especially the metastatic process,5–7 and human studies have associated low NK cell activity with increased metastatic outcomes.8,10 These would suggest that in the clinical setting, surgery-induced suppression of NK cell activity might render the organism more susceptible to metastatic development. In support of this suggestion, multiple studies in animals have shown surgery and its consequent NK suppression to promote metastatic development.11–13

The role of pain in mediating these immunosuppressive and metastatic-enhancing consequences of undergoing and recovering from surgery have only recently been studied.13–15 We have previously shown that the pre- and postoperative administration of an analgesic dose of morphine significantly attenuated the metastatic-enhancing effects of surgery while exerting no effects in the anaesthesia control groups13,15 Furthermore, morphine treatment reversed surgery-induced decreases in exploratory behaviour,15 suggesting its comfort-enhancing effects in these circumstances. These findings support the suggestion that providing pre- and postoperative pain-relieving medications can ameliorate some aspects of the immune suppressive consequences of undergoing and recovering from surgery, possibly by preventing pain-related neuroendocrine responses.

It is also known that morphine pretreatment can prevent the development of central sensitization from C-fibre stimulation16; thus, providing a rationale for the use of analgesics in conjunction with anaesthetics in individuals undergoing surgery. Indeed, the
administration of opioids before surgery has been shown to provide improved postoperative pain outcomes in humans.\textsuperscript{17–19} Thus, this study was undertaken in an effort to discern the relative importance of the pre- versus postoperative administration of morphine as a means to prevent the detrimental effects of surgery on host resistance against metastasis and exploratory behaviour. Additionally, corticosterone (CS), a stress hormone that is also known to be involved in modulating immune function,\textsuperscript{20} was measured in order to begin characterizing possible mechanisms of morphine’s beneficial effects in the context of surgery.

As for our previous studies, the current study used the MADB106 tumour cell line, syngeneic to the inbred Fischer 344 rat. Following i.v. injection, MADB106 cells are arrested in the lungs and surviving cells grow into lung tumour colonies. Studies have shown these two metastatic outcomes, lung tumour cell retention and the number of lung tumour colonies, to be controlled by NK cells, specifically during the first 24 h after injection.\textsuperscript{37} Therefore, in addition to providing a measure of host susceptibility to metastasis, the retention and colonization of MADB106 cells in the lungs provides an indicator of NK function in the whole animal.

**Materials and methods**

**ANIMALS**

Fischer 344 male rats aged 16 to 19 weeks, age-matched within each experiment, were used in these studies. Animals were maintained in group housing on a 12-h light–dark cycle with free access to food and water except for the 8 h before surgery when only water was available. All experiments were conducted during the first half of the dark cycle. The study design was approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

**SURGERY**

Surgery animals underwent an experimental laparotomy while anaesthetized with halothane (2–2.5%); animals undergoing anaesthesia without surgery were anaesthetized with halothane at the same time and in the same dose as the surgery animals. Once anaesthetized, all animals received penicillin G (25 000 units kg\(^{-1}\) i.m.), and the abdomen of the surgery animals was shaved and scrubbed with betadine solution. Surgery consisted of a 4 cm midline abdominal incision followed by the externalization of 10 cm of small intestine for a period of 4 min. The intestine was rubbed gently between two pieces of gauze in four locations during the first minute and covered with saline-soaked gauze. After returning the intestine to the abdominal cavity and irrigating with saline, the muscle and skin layers were sutured with 5–0 monofilament wire.

**MORPHINE PREPARATION AND ADMINISTRATION**

Morphine sulphate was administered in two different preparations. The preoperative dose, 8 mg kg\(^{-1}\), was administered intraperitoneally (i.p.) in saline 30 min before surgery. Postoperatively, morphine was administered subcutaneously (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 (6.7, 40, and 53.5% by volume, respectively).\textsuperscript{13,15,22} The morphine concentration was adjusted such that the appropriate dose was administered in 1 ml of SRS. The postoperative dose of morphine was administered immediately after the completion of surgery just before discontinuing the halothane (4 mg kg\(^{-1}\) ml\(^{-1}\) in SRS). A second postoperative dose of morphine (2 mg kg\(^{-1}\) ml\(^{-1}\) in SRS) was administered s.c. at the contralateral flank immediately following MADB106 tumour cell injection at 5 h after surgery (Experiment 1 only). Vehicle was used for control injections.

**MADB106 TUMOUR CELL MAINTENANCE AND RADIOLABELLING**

MADB106 cells were maintained in 5% carbon dioxide at 37°C in monolayer culture in complete media (RPMI 1640 media (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS), 0.05 mg ml\(^{-1}\) streptomycin, 2 mmol litre\(^{-1}\) L-glutamine, 0.1 mmol litre\(^{-1}\) non-essential amino acid, and 1 mmol litre\(^{-1}\) sodium pyruvate). Trypsin 0.25% was used for separating the cells from the flask (Falcon 3023).

For DNA radiolabelling of MADB106 cells, 0.4 \(\mu\)Ci\[^{35}\text{S}\]iododeoxyuridine per ml media was added to the growing cell culture one day before cell harvesting. After separation from the flask, cells were washed and resuspended in phosphate buffered saline (PBS).

**EXPERIMENTAL PROCEDURES**

**Experiment 1: The effects of the timing of morphine administration on its attenuation of surgery-induced increases in tumour cell retention**

To assess the role of the pre- versus postoperative morphine treatments, animals were randomly assigned and subjected to either surgery with anaesthesia or anaesthesia alone; and either morphine or its vehicle was administered preoperatively, postoperatively, and at the time of MADB106 tumour cell injection. Table 1 summarizes the constitution of these groups and the morphine treatment regimens.

Five hours after the completion of surgery, all animals were lightly anaesthetized with halothane and injected with 4 \(\times\) \(10^7\) radiolabelled MADB106 tumour cells per kg into the tail vein, followed by the third morphine/vehicle injection. Lungs were removed 14 h later and their radioactive content was measured in a gamma counter (Beckman 5500). In addition, the total radioactive content of the injectate (MAX) was measured, and percent retention was calculated using the formula: (lung radioactivity \(-\) MAX) \times 100.

This experiment was performed in two replicates with all groups represented in each assay. For data analysis, the percent retention for each animal was converted to a percentage of the assay mean, and these normalized scores were used for combined statistical analysis using ANOVA. For graphic illustration, normalized scores were reconverted to percent lung tumour cell retention by multiplying the normalized scores by the overall mean percentage of tumour cells retained across the two replicates.
Experiment 2: The effects of surgery and morphine treatment on rearing behaviour

Given our previous findings that pre- and postoperative morphine treatment prevented the activity-suppressing effects of surgery,13 this experiment assessed the relationship between the timing of morphine administration and its ability to restore surgery-induced decreases in rearing. Animals (n = 32) were randomly assigned to either the Anaesthesia/Vehicle group (n = 6) or to one of five surgery groups (Surgery/Vehicle, Surgery/Morphine, Surgery/Before-only, Surgery/After-only, or Surgery/After-total (n = 7, 6, 4, 3, respectively)). At the completion of surgery the animals received their postoperative morphine/vehicle treatment, and were placed in individual uncovered cages in a room illuminated with a red light. During the latter 30 min of each of the first four postoperative hours, each rear was scored by a blinded observer. One rear was scored each time the animal raised both forepaws off the cage floor. The total number of rears for each 30-min segment for each animal was analysed by repeated measures ANOVA.

Experiment 3: The effects of surgery and morphine on plasma corticosterone levels

Table 2 illustrates the group constitution and morphine regimens for the two assays that comprised this experiment. Assay 2 was undertaken to both verify findings from Assay 1 and to assess the effects of the morphine regimen on animals not experiencing pain associated with surgery.

Five hours after surgery, at the time tumour cells were injected in Experiment 1, each animal was anaesthetized with halothane within 30 s. Approximately 1 ml of blood was obtained from the heart and heparinized (20 ml*). Plasma was removed and stored at -80°C until assayed. Plasma CS concentrations were determined separately for Assays 1 and 2 using radioimmunoassay kits (ICN Biomedicals, Inc., Costa Mesa, California).

To combine results for analysis and to correct for interassay differences, the mean plasma CS concentration was calculated for the three groups common to the two assays (Anaesthesia/Vehicle, Surgery/Vehicle, and Surgery/After-total). The plasma CS concentration for each animal was converted to a percentage score of the three-group mean, and these scores were used for statistical analysis by ANOVA.

Experiment 4: The analgesic effects of the morphine regimens

Standard hot-plate test was used to assess the duration of analgesia produced by two different morphine regimens: the before-only treatment regimen (8 mg kg⁻¹ in saline, n = 8), and the before-and-after treatment regimen (8 mg kg⁻¹ in saline, 4 mg kg⁻¹ in...
SRS at 45 min, and 2 mg kg\(^{-1}\) in SRS at 4 h, \(n = 11\). Control animals received vehicle injections \((n = 9)\).

During each of the five days before the experiment, animals were habituated to handling, including transportation to and handling in the laboratory the day before the experiment. For hot-plate testing \((52^\circ\text{C})\), a 45 s cut-off was used to avoid tissue damage. Baseline latency to hind-paw lick\(^2\) was measured in all animals at time 0, and the first morphine/vehicle injection was given immediately thereafter. Hot-plate testing intervals and additional morphine dosing are indicated in figure 4.

To control for differing baseline latencies and to assess changes in hot-plate latency within animal, hot-plate latencies were converted to percent maximum possible effect \((\%\text{MPE})\) using the formula: \([\text{latency} - \text{baseline latency}] / \left( \text{cut-off} - \text{baseline latency} \right) \times 100\). For both morphine groups, \%\text{MPEs} were compared with the \%\text{MPE} in control animals at the closest time-point tested. ANOVA was used to test the statistical significance of differences in \%\text{MPE} at each time-point.

Results

**EXPERIMENT 1: THE EFFECTS OF THE TIMING OF MORPHINE ADMINISTRATION ON ITS ATTENUATION OF SURGERY-INDUCED INCREASES IN TUMOUR CELL RETENTION**

Surgery resulted in a twofold increase in the retention of tumour cells (Anaesthesia/Vehicle versus Surgery/Vehicle) which was significantly attenuated by all morphine treatment regimens \((F(6,92) = 3.612, P < 0.01; \text{protected } t \text{ tests}, P < 0.05)\) (fig. 1). The morphine regimens that included a preoperative dose of morphine (Surgery/Before-only and Surgery/Morphine) appeared to result in a slightly larger attenuation of the observed surgery-induced increase in tumour cell retention compared with the animals receiving postoperative morphine treatment only (Surgery/After-only and Surgery/After-total). Although there were no significant differences among the morphine-treated surgery groups and the tumour cell retention observed in the preoperatively treated surgery groups was not significantly different from that of the Anaesthesia/Vehicle group, there was a significant difference between the Anaesthesia/Vehicle group and both surgery groups receiving postoperative morphine treatment only \((P < 0.05, \text{protected } t \text{ tests})\).

**EXPERIMENT 2: THE EFFECTS OF SURGERY AND MORPHINE TREATMENT ON RELLING BEHAVIOUR**

Surgery resulted in a significant decrease in the incidence of rearing in the first four postoperative hours which was abolished by all morphine treatment regimens except the Surgery/After-total regimen \((F(5,26) = 2.978, P < 0.05)\) (fig. 2). The animals in the Surgery/After-total group exhibited minimal activity until the third postoperative hour, when their activity level was comparable to the other morphine-treated surgery groups. Except for the Surgery/After-total group, the Surgery/Vehicle group exhibited significantly less rearing behaviour than all other morphine treated groups \((P < 0.05)\).

**EXPERIMENT 3: THE EFFECTS OF SURGERY AND MORPHINE ON PLASMA CORTICOSTERONE CONCENTRATIONS**

Surgery resulted in a twofold increase in plasma CS concentrations (Surgery/Vehicle vs Anaesthesia/Vehicle) which was significantly reduced by all morphine treatment regimens \((F(7,130) = 11.848, P < 0.001; P < 0.01, \text{protected } t \text{ tests})\). In addition, Surgery/After-total animals exhibited significantly lower plasma CS concentrations compared with the Surgery/Morphine and Surgery/Before-only animals \((P < 0.05, \text{protected } t \text{ tests})\). Among the anaesthesia groups, all morphine treatment regimens resulted in a significant reduction in plasma CS compared with the Anaesthesia/Vehicle group \((P < 0.05, \text{protected } t \text{ tests})\). Figure 3 illustrates these findings.

**EXPERIMENT 4: THE ANALGESIC EFFECTS OF THE MORPHINE TREATMENT REGIMEN**

The morphine regimen for both the Before-only and Before-and-after groups resulted in a significant increase in hot-plate latencies for 60 min after the initial morphine dose, evidenced by a significant difference in the \%\text{MPE} of both morphine groups compared with controls \((P < 0.05)\). In the Before-and-after group, no significant additional increase in hot-plate latency was provided by the SRS morphine administration (fig. 4), although the \%\text{MPE} was above control levels for 3 h from the initial injection.

Discussion

The results of this study support our previous findings that the pre- and postoperative administration of
morphine ameliorates the metastatic-enhancing effects of surgery.13 15 The findings show that surgery resulted in approximately a twofold increase in the lung retention of MADB106 cells, and that each of the four morphine regimens significantly attenuated this effect of surgery.

Notably, the current findings also indicate the importance of the preoperative morphine treatment in achieving these beneficial effects. Several findings suggest that, in the current experimental circumstances, the preoperative administration of morphine was the most important of the three injection times. Despite the absence of statistically significant differences in tumour cell retention among the morphine-treated surgery groups, differences are evident in the magnitude of the beneficial effects of morphine. Specifically, in comparison with the Anaesthesia/Vehicle group, surgery did not cause a significant increase in the tumour cell retention exhibited by the surgery groups treated preoperatively with morphine but it did cause a significant increase in the groups treated only postoperatively. For example, the metastatic-enhancing effects of surgery were attenuated by only 50% in both of the Surgery/After-only groups, in contrast with 65% in the Surgery/Morphine group, and 70% in the Surgery/Before-only group. Finally, levels of tumour cell retention in the Surgery/Before-only and Surgery/Morphine groups were equivalent; the additional 6 mg kg\(^{-1}\) of morphine administered postoperatively to the Surgery/Morphine group did not provide further protection against the tumour-enhancing effects of surgery in this paradigm. On the other hand, the two postoperative morphine regimens provided significant protection. Taken together, these findings suggest that preoperative morphine treatment is important, and in the current setting, the provision of additional doses of morphine after surgery did not further improve host resistance to surgery-induced increases in metastasis.

These findings should not be extended to the clinical setting as justification for withholding postoperative analgesic treatments for several reasons. First, postoperative morphine administration was clearly...
beneficial in this paradigm. Second, not only surgery-induced tissue damage but the resulting local inflammatory response can contribute to the initiation and maintenance of central sensitization; therefore, continuing postoperative analgesia would be necessary to maintain both adequate pain control and the suppression of such a hyperexcitable state. This suggestion is supported by clinical findings that aggressive postoperative morphine treatment may reduce late-developing hyperalgesia. Third, the use of the MADB106 tumour model might reflect some but not other aspects of host resistance to metastasis. For example, this model is very sensitive to NK activity during the first 24 h after tumour cell injection; thus, resistance against the retention of MADB106 cells reflects only the earliest events involved in the establishment of metastasis, events that are NK-dependent. Finally, surgery has also been shown to suppress other immune functions such as macrophage phagocytic activity, and macrophages have been shown to play a role in the development of a tumour colony after tumour cells have seeded in the lungs. Therefore, the prevention of ongoing postoperative pain and stress may prove to be important in different experimental conditions.

The morphine dose used in this experimental paradigm is also important. In pilot studies to establish the optimum doses of morphine for the Surgery/Morphine regimen, we found that too little morphine exerted virtually no beneficial effects and too much resulted in tumour cell retention levels exceeding those observed in untreated surgery animals (data not shown). Others have shown that much higher doses of morphine (25–50 mg/kg) than were used in these studies are detrimental to both immunity and tumour resistance.

The preoperative morphine administration regimen provided significant hot-plate analgesia for 90 min ($P<0.05$), and the addition of the postoperative dose extended the analgesia by 60 min. From these data, it could be suggested that morphine exerted no measurable antinociceptive effects at the time tumour cells were injected at 5 h after surgery. However, caution is warranted in drawing inferences from the analgesic effects of morphine as evident in the hot-plate test to its pain alleviating ability in the context of undergoing and recovering from major abdominal surgery. First, the neural mechanisms by which morphine analgesia suppresses the phasic pain brought about by hot-plate testing compared with the tonic pain associated with such stimuli as surgery have been shown to differ. Second, whereas the animals used in the metastatic, behavioural and CS experiments had undergone major surgery, those in the hot-plate study were otherwise normal. Therefore, we submit that the absence of demonstrable hot-plate analgesia at the time tumour cells were injected does not undermine the suggestion that it is the analgesic properties of morphine mediating its beneficial effects in these studies. Moreover, these findings support the suggestion that the prevention of central sensitization is a key effect of the preoperative morphine treatment.

The plasma CS results indicate that undergoing surgery activates the hypothalamic-pituitary-adrenal (HPA) axis, and this activation is reduced by morphine treatment. Given that CS has also been shown to suppress immune function, reducing postoperative levels of CS may be beneficial. However, with regard to NK activity, these findings refute a causal relationship between CS levels and host resistance to metastasis. First, whereas morphine significantly

Figure 4  The effects of the before-only and before-and-after schedules of morphine administration on hot-plate analgesia (32°C) assessed as % maximum possible effect (%MPE, 45 s cut-off) above control levels. Morphine was administered after baseline hot-plate latency measurement (Time 0) to the Before-only and Before-and-after groups (mg kg$^{-1}$ in saline), and to the Before-and-after group also at 45 min (4 mg kg$^{-1}$ in SRS) and 4 h (2 mg kg$^{-1}$ in SRS) later. The Before-only and Before-and-after groups were tested as indicated on the graph. The Control group was tested at time 0, 1, 2, 3, and 4.5 h. Error bars represent SEM. * Indicates a statistically significant difference vs the control %MPE ($P<0.05$), and † indicates $P<0.07$. 

The plasma CS results indicate that undergoing surgery activates the hypothalamic-pituitary-adrenal (HPA) axis, and this activation is reduced by morphine treatment. Given that CS has also been shown to suppress immune function, reducing postoperative levels of CS may be beneficial. However, with regard to NK activity, these findings refute a causal relationship between CS levels and host resistance to metastasis. First, whereas morphine significantly
reduced CS to 50% of control levels in the unoperated animals, morphine increased lung tumour cell retention in this group. Second, whereas morphine completely abrogated the surgery-induced increase in CS levels to below the baseline levels exhibited by the unoperated rats, it only partially reduced tumour cell retention in this group. Figure 5 illustrates these dissociations. This argument should be tempered, however, given that CS levels were assessed only at the time tumour cells were injected. Alternatively, pain and stress may induce their tumour-enhancing effects by activating the sympathetic nervous system.34 In previous studies, our findings have indicated that various stress paradigms, for example, social confrontation and swim stress, suppressed NK activity and increased the lung retention of MADB106 cells via activating the sympathetic nervous system and the release of adrenal catecholamines.35 36 In addition, this mechanism may play a role in mediating the effects of surgery.

In the behavioural study, morphine treatment restored to control levels the markedly reduced rearing behaviour observed in animals undergoing surgery without morphine treatment. Quantitatively, the morphine-treated surgery animals exhibited levels of rearing behaviour that were comparable to those exhibited by the unoperated animals. These results are consistent with our previous behavioural findings15 and support our contention that this morphine regimen provided pain relief. The single exception is the virtual absence of rearing behaviour exhibited by the Surgery/After-total group. These animals were lying on their bellies in the sawdust for the first two postoperative hours, characteristic of animals receiving higher doses of morphine.37 Although it did not appear to affect metastasis, these results suggest that the Surgery/After-total animals may have been overmedicated at the conclusion of surgery.

In conclusion, in the current paradigm, it appears that the preoperative dose of morphine provided the most significant contribution to improving host resistance against the observed endocrine, metastatic, and behavioural consequences of surgery. The preoperative dose alone was at least as potent as the complete regimen in affecting these measures, although its direct analgesic effects ended long before these outcomes were assessed. Nevertheless, postoperative morphine treatment alone was effective and may prove to be a more significant intervention in other circumstances. These findings support the efficacy of providing opioid medication before surgery commences as well as postoperatively. Although the current study cannot claim to prove that the observed beneficial effects of morphine are mediated by its analgesic properties, it provides ample support for this suggestion. If such findings are applicable to humans, these results provide additional evidence that the pain of undergoing and recovering from surgery poses significant risk, a risk that can be ameliorated by simply providing effective analgesia.

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