Morphine Induces Sepsis in Mice

Mary E. Hilburger,* Martin W. Adler, Allan L. Truant, Joseph J. Meissler, Jr., Vilas Satishchandran,* Thomas J. Rogers, and Toby K. Eisenstein

Gram-negative sepsis and subsequent endotoxic shock remain major health problems in the United States. The present study examined the role of morphine in inducing sepsis. Mice administered morphine by the subcutaneous implantation of a slow-release pellet developed colonization of the liver, spleen, and peritoneal cavity with gram-negative and other enteric bacteria. In addition, the mice became hypersusceptible to sublethal endotoxin challenge. The effects were blocked by the simultaneous implantation of a pellet containing the opioid antagonist naltrexone. These findings show that morphine pellet implantation in mice results in the escape of gram-negative organisms from the gastrointestinal tract, leading to the hypothesis that morphine used postoperatively or chronically for analgesia may serve as a cofactor in the precipitation of sepsis and shock. In addition, morphine-induced sepsis may provide a physiologically relevant model of gram-negative sepsis and endotoxic shock.

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Veterinary care was provided and experimental procedures involving animals were performed under approved Institutional Animal Care and Use Committee protocols. Animals were housed in an accredited facility (American Association for Accreditation of Laboratory Animal Care).
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Food and fresh water were available ad libitum. All mice were allowed to acclimate for at least 1 week before testing.

**Drug treatment.** Drug pellets were obtained from the National Institute on Drug Abuse (Rockville, MD). Mice were anesthetized with methoxyflurane (Pittman-Moore, Mundelein, IL) and given a subcutaneous (sc) implant of one 75-mg morphine pellet, a 30-mg naltrexone pellet, a placebo pellet, or a morphine pellet plus a naltrexone pellet. The incision through which the pellet was inserted was closed with a 9 mm surgical clip (Autoclip; Clay Adams, Sparks, MD). Mice were observed until they recovered from anesthesia and were housed in groups of 5 or 24 or 48 h.

In three preliminary studies, we observed that when peritoneal macrophages from morphine-treated C57BL/6J mice were cultured in antibiotic-free medium, the culture supernatants were contaminated with gram-negative bacteria, including *Escherichia coli* and *Proteus mirabilis*, while macrophage cultures from placebo- or naltrexone-treated mice were sterile (data not shown). To confirm that the morphine pellets were not contaminated with bacteria, the pellets were dissolved in 1 mL of sterile water, and 200 μL was plated onto each of 5 trypticase soy agar (TSA) plates (Becton Dickinson, Cockeysville, MD). No bacterial growth was observed on any of the plates. Systematic studies were then carried out to determine whether morphine pellet implantation induces a sepsis syndrome and if the effect could be blocked by the opioid receptor antagonist naltrexone.

**Microbial growth.** Animals were sacrificed at 24 or 48 h after pellet implantation. At this time, the peritoneal cavity of individual mice was washed by injecting 5 mL of sterile RPMI medium (GIBCO/BRL, Grand Island, NY) into the cavity using a 10-mL syringe and a 20-gauge needle and aspirating the fluid back into the syringe. The wash fluid was assessed for microbial growth by plating 100 μL onto TSA plates. Individual livers and spleens from the same mice were aseptically removed, weighed, and placed into 5 mL of sterile water. Tissue homogenates were prepared by grinding the organs (SDT Tissumizer with SDT100EN probe; Tekmar, Cincinnati) until completely homogenized. Homogenate (100 μL) was plated onto TSA plates. In one experiment, samples were also plated onto CDC anaerobe blood agar and CDC anaerobe laked blood agar with kanamycin and vancomycin (BBL, Becton Dickinson) and incubated anaerobically. No bacteria were cultured from the livers of placebo- or morphine- plus naltrexone-treated animals at 48 h (data not shown).

We have previously noted differences in the capacities of morphine and other opioids to suppress immune function of spleen cells in different mouse strains [29]. To rule out a possible strain-specific phenomenon, the effect of morphine on colonization of the organs was carried out using C3HeB/FeJ mice. Morphine treatment also induced extraintestinal growth of enteric bacteria in these mice, but maximal colonization of various sites was noted at 48 h after pellet implantation. As shown in figure 1, which represents the pooled results of three experiments, morphine-treated C3HeB/FeJ mice showed evidence of sepsis in the form of enteric flora in the peritoneum, spleen, and liver, whereas placebo- or morphine- plus naltrexone-treated animals did not. In particular, *P. mirabilis* and enterococci were cultured from the organs of morphine-treated mice, whereas cultures from placebo- or morphine- plus naltrexone-treated

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>No. of mice with positive cultures/ total mice tested</th>
<th>No. of cultures with &gt;100 cfu/plate1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo 3/122</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>Morphine 10/12f</td>
<td>9/12</td>
<td></td>
</tr>
<tr>
<td>Morphine + naltrexone 5/12f</td>
<td>1/12</td>
<td></td>
</tr>
<tr>
<td>Naltrexone 0/5</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

* Mice received subcutaneous implant of 75-mg morphine pellet, placebo pellet, morphine pellet plus 30-mg naltrexone pellet, or naltrexone pellet alone, 24 h before sacrifice.

2 See Methods.

1 Single colonies of *Escherichia coli* or diphtheroids.

f *E. coli, Proteus mirabilis, or both.*

P < .01, morphine vs. placebo or morphine vs. naltrexone; P < .05, morphine vs. morphine + naltrexone.

E. coli or diphtheroids.
mice yielded mostly staphylococci or streptococci. From table 2, which summarizes the incidence of colonization with enteric organisms, it is clear that only morphine-treated mice had significant colonization of liver, spleen, and peritoneal cavity with enteric bacteria.

Table 2. Incidence of colonization with enteric organisms in C3HeB/FeJ mice.

<table>
<thead>
<tr>
<th>Site cultured, treatment</th>
<th>Proteus mirabilis</th>
<th>Escherichia coli</th>
<th>Enterococcus faecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal cavity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Morphine</td>
<td>13/32</td>
<td>1/32</td>
<td>3/32</td>
</tr>
<tr>
<td>Morphine + naltrexone</td>
<td>1/17</td>
<td>0/17</td>
<td>0/17</td>
</tr>
<tr>
<td>Spleen</td>
<td>1/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Placebo</td>
<td>1/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Morphine</td>
<td>13/32</td>
<td>0/32</td>
<td>5/32</td>
</tr>
<tr>
<td>Morphine + naltrexone</td>
<td>0/17</td>
<td>0/17</td>
<td>0/17</td>
</tr>
<tr>
<td>Liver</td>
<td>1/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Placebo</td>
<td>1/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>Morphine</td>
<td>18/32</td>
<td>2/32</td>
<td>9/32</td>
</tr>
<tr>
<td>Morphine + naltrexone</td>
<td>1/17</td>
<td>0/17</td>
<td>0/17</td>
</tr>
</tbody>
</table>

* Samples taken 48 h after pellet implantation.

As gram-negative infection sensitizes mice to endotoxin [30], experiments were carried out to determine whether morphine sensitizes mice to LPS-induced lethality. As shown in table 3, mice receiving morphine pellets and a sublethal injection of LPS all died, compared with 33% mortality in mice receiving a morphine pellet and an injection of saline. Implantation of a naltrexone pellet with the morphine pellet reduced the mortality following LPS injection from 100% to 30%.

Discussion

Bacterial sepsis and its complications are important and unsolved clinical problems. These conditions are common in postsurgical patients, but the exact factors leading to sepsis are unknown. The results presented here show that morphine pellet implantation led to a septic state, as animals became colonized with enteric organisms in liver, spleen, and peritoneal cavity. Further, morphine-treated mice had heightened sensitivity to the lethal effects of LPS. These effects were shown to be opioid receptor–mediated, as they were blocked by naltrexone, an opioid receptor antagonist. The implication of these observations is that morphine itself may be a precipitating factor or cofactor for induction of sepsis in postsurgical patients.

Common laboratory practice for research on the effects of morphine in rodents often involves the sc administration of a slow-release pellet [31–33]. The morphine pellet model has similarities to what would occur during and after a simple surgical procedure, in that the animals are given anesthesia by...
inhalation, they receive a surgical incision, they have foreign body implants (i.e., the morphine pellet and the surgical clip), and they receive morphine. The surgical trauma and foreign body implants alone were not sufficient to induce sepsis, as shown by the failure to culture large numbers of enteric organisms from placebo- or naltrexone-treated mice.

In mice given morphine pellets, serum morphine concentrations of ~2 mg/mL were achieved 6–24 h after pellet implantation, which is significantly higher than what has been observed in humans receiving therapeutic doses of morphine. However, this declines to and remains at 0.6 µg/mL from 48 to 96 h [34]. A typical postoperative dose for humans might be 7–10 mg intravenously or 10 mg sc, which for a 70-kg person would result in an initial plasma level in the range of 0.2–0.7 µg/mL [35, 36]. In addition, morphine is used in higher doses for anesthesia. Spontaneous mortality in morphine-treated animals has been reported for rats and mice [32, 37]. Our data support the hypothesis that these deaths may be the result of morphine-induced sepsis. Other observations consonant with this hypothesis are reported by Chao et al. [38], who found that administration of morphine to mice infected with Toxoplasma gondii or pretreated with heat-killed Corynebacterium parvum resulted in a rapid-death syndrome.

A well-known complication of morphine administration is constipation, associated with decreased peristalsis and increased muscle tone and spasms [39]. Morphine acts primarily at µ-opioid receptors, providing a possible mechanism for its effects on gastrointestinal function in the experiments reported here. The role of κ-opioid receptors in the regulation of gastrointestinal motility has been more controversial [45, 50–52]. Of particular interest is a study showing that a κ-agonist can produce analgesia without inhibiting gastrointestinal propulsion in rats [53]. In humans, opioids that exert major actions at the κ receptor, such as meperidine, have much less effect on the gut than opioids acting primarily at µ-opioid receptors.

Morphine may also sensitize to infection with endogenous organisms through mechanisms other than direct effects on the gastrointestinal tract. There is a substantial literature documenting immunosuppressive effects of morphine [54, 55]. Splenic macrophage function [56] and phagocytic activity of peritoneal macrophages [57] is known to be compromised in morphine-treated mice. Human peripheral blood mononuclear cells treated with morphine in vitro have depressed respiratory burst activity and a reduced capacity to elaborate cytokines in response to various stimuli [58, 59]. Chemotactic responses of human neutrophils are inhibited by morphine [60, 61], as is up-regulation of adhesion molecules on these cells [62]. Diminished capacity to mount nonspecific host defenses against organisms that escape from the gastrointestinal tract could contribute to increased microbial burden in the organs and peritoneal cavity of morphine-treated mice.

The results described here present a model for spontaneous sepsis following morphine administration by slow-release pellets in mice. To explore the relevance of these findings for humans, studies using other animal models, including primates, and methods for administering morphine that more closely mimic those used in humans should be carried out. If these findings are applicable to humans, they suggest mechanisms for prevention of sepsis, including the use of nonopioid analgesics or possibly the use of opioid analgesics that have their primary effect on κ-opioid receptors.

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References


