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Surgical pain attenuates acute morphine tolerance in rats

S. T. Ho[1]*, J. J. Wang[2], W. J. Liaw[3], H. K. Lee[4] and S. C. Lee[5]

[1]Department of Anaesthesiology, National Defence Medical Centre/Tri-Service General Hospital, Taipei, Taiwan. [2]Department of Anaesthesiology, National Defence Medical Centre/Tri-Service General Hospital and Cathay General Hospital, Taipei, Taiwan. [3]Department of Anaesthesiology, Tri-Service General Hospital and Graduate Institute of Medical Sciences, National Defence Medical Centre, Taipei, Taiwan. [4]Department of Pharmacology, National Defence Medical Centre and [5]Department of Surgery, National Defence Medical Centre/Tri-Service General Hospital, Taipei, Taiwan

**To whom correspondence should be addressed at: Department of Anaesthesiology, National Defence Medical Centre/ Tri-Service General Hospital, No. 8, Sec. 3, Tingchow Rd., Taipei, Taiwan*

Nociceptive stimuli, such as formalin-induced pain and adjuvant-induced arthritis, attenuate tolerance to morphine antinociception. In this study, we have explored the effect of upper and lower abdominal surgical pain on the prevention of acute tolerance to morphine antinociception in Sprague–Dawley rats. Group I received lower abdominal surgery (LAS) and i.v. morphine infusion; group II received LAS and i.v. saline infusion; group III received upper abdominal surgery (UAS) and i.v. morphine infusion; group IV received UAS and i.v. saline infusion; group V received i.v. morphine infusion; and group VI received i.v. saline infusion. The antinociceptive effects of morphine were measured by an infrared thermal tail flick test. We also measured plasma concentrations of morphine in rats receiving morphine infusions with or without surgical treatment. We found that acute tolerance to morphine antinociception developed after 2 h following i.v. infusion of morphine alone. However, both UAS and LAS significantly slowed the rate of development of acute tolerance to morphine. The area under the time–response curves (AUC) of groups I and III were mean 34 556 (SD 5607) and 32 548 (9783), respectively, which were significantly different from that of group V (18 759 (8225)) ($P<0.01$). Also, there were no significant differences between groups I and III. There were no significant differences between groups for plasma morphine concentrations during the 8-h study (e.g. groups I, III and V: 179.9 (22.6), 182.7 (14.4) and 170.9 (15.8) ng ml^{-1} at 8 h, respectively) and we suggest that the appearance of acute morphine tolerance after morphine infusion is not pharmacokinetic in nature.

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After long-term administration or continuous infusion of morphine in animals, tolerance to morphine antinociception develops rapidly,[1–3] however, this phenomenon is rarely observed in patients.[4 5] The mechanism of this discrepancy between patients and animals is not clear. Some have suggested that the presence of pain during morphine administration may contribute to this difference.[6–8] Using animal models of formalin paw injection and adjuvant-induced arthritis, it has been found that the appearance of noxious stimuli during morphine administration may disturb the development of tolerance to morphine antinociception.[9–11] This finding is valuable in determining the correlation between acute tolerance to morphine antinociception and painful stimuli. However, the nature of this pain is not quite similar to clinical pain as formalin and adjuvant are not the causative agent of clinical pain; also, these two animal models can only represent in part, clinical pain (i.e. not incisional pain). In clinical practice, postoperative pain is a major problem for the acute pain service; therefore, it is important to develop an animal model of pain to study the relationship between clinical acute pain and morphine tolerance. In our animal study, two clinical procedures, upper abdominal and lower abdominal surgery, were developed. Using these two surgical procedures, we have explored the effect of postoperative pain on the prevention of acute tolerance to morphine antinociception in Sprague–Dawley rats.

Materials and methods

All testing was performed in accordance with the recommendations and policies of the International Association

for the Study of Pain[12] and the study was approved by our Institutional Animal Investigation Committee. Male Sprague–Dawley rats (250–300 g) obtained from the National Lab Animal Breeding and Research Centre, National Science Council, Taipei, Taiwan, were housed in cages with controlled room temperature (22±1°C), humidity (50±10%) and a 12-h light–dark cycle (from 06:00 to 18:00). Food pellets and water were available *ad libitum* throughout the experiment. Tests were performed only after the rats had acclimatized to the above environment for at least 7 days. Experiments were performed between 08:00 and 17:00 in random order.

All animals received i.v. catheterization 24 h before the experiment under pentobarbital (pentobarbitone) 45 mg kg^{-1} i.p. anaesthesia. Briefly, after local skin infiltration with 2% xylocaine, a polyethylene cannula (PE-50) filled with heparinized saline 50 u. ml^{-1} was inserted into the right external jugular vein. The free end was tunnelled subcutaneously, exteriorized through a stab wound, and then fixed to the back of the neck. After catheterization, animals were housed separately to avoid cannula dislodgement.

Twenty-four hours after i.v. catheterization, the rats were allocated randomly to one of the two studies: pharmacodynamic or pharmacokinetic.

Pharmacodynamic study

Rats were allocated randomly to one of six groups (n=12 in each group): group I received lower abdominal surgery (LAS) and i.v. morphine infusion; group II received LAS and i.v. saline infusion; group III received upper abdominal surgery (UAS) and i.v. morphine infusion; group IV received UAS and i.v. saline infusion; group V received i.v. morphine infusion; and group VI received i.v. saline infusion. Surgery was performed under diethyl ether anaesthesia and the types of surgical incisions used are shown in Figure 1 (A, B). In UAS, a skin incision was made from the midline of the abdomen (linea alba), starting 1 cm below the xiphoid cartilage and running parallel to the subcostal line, approximately 2 cm in length. The wound was deepened to the peritoneal cavity to expose the underlying liver and intestine. The wound was then sutured in layers with 3-O silk. Finally, the skin was closed with four stitches, approximately 0.3–0.5 cm apart (3-O silk). In LAS, a transverse skin incision was made at the level of the inguinal region, perpendicular to the midline of the abdomen, and extended approximately 1 cm from the midline (linea alba) on each side. The incision wound was deepened to the peritoneal cavity to expose the underlying bladder and intestine. Finally, the wound was sutured in layers with 3-O silk; the skin was sutured as described above. All surgical procedures were completed within 15 min. Rats in groups V and VI did not undergo surgery but diethyl ether anaesthesia was given to these rats.

After anaesthesia or surgery, or both, the rats were allowed 30 min to recover. Morphine (dissolved in 0.9% (w/v) sodium chloride solution) or saline was infused into the conscious animals from a continuous drug infusion balloon catheter (40 ml/24 h, Mitsuya, Osaka, Japan) connected to the jugular vein cannula by a PE-50 tube. The rate of morphine infusion was 2 mg kg^{-1} h^{-1} and the duration of infusion was 8 h.

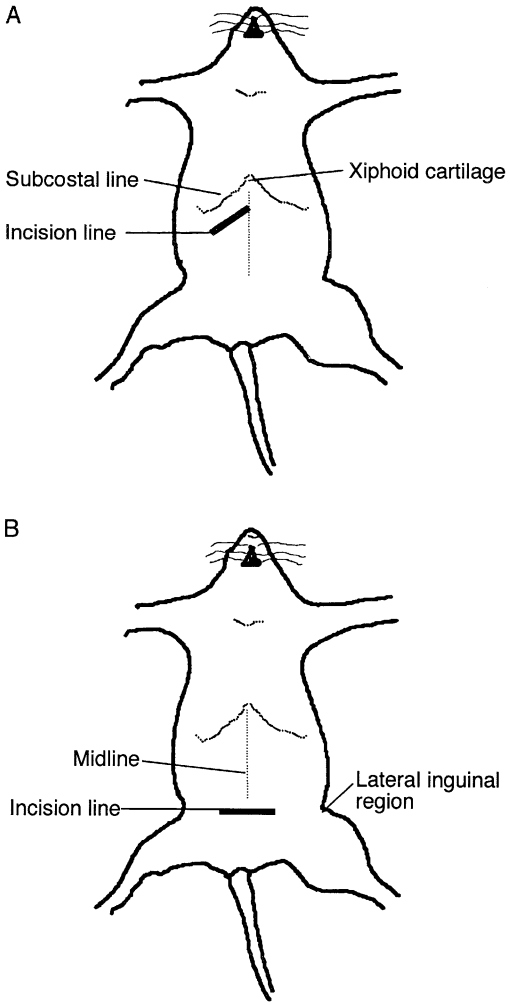

Fig 1 A: Upper abdominal surgery (UAS). A skin incision was made from the midline of the abdomen (linea alba), starting 1 cm below the xiphoid cartilage and running parallel to the subcostal line, approximately 2 cm in length. The wound was deepened to the peritoneal cavity to expose the underlying liver and intestine. The wound was later sutured in layers with 3-O silk. Finally, the skin was closed with four stitches, approximately 0.3–0.5 cm apart, with 3-O silk. B: Lower abdominal surgery (LAS). A transverse skin incision was made at the level of the inguinal region, perpendicular to the midline of the abdomen, extending approximately 1 cm from the midline (linea alba) on each side. The incision wound was deepened to the peritoneal cavity to expose the underlying bladder and intestine. Finally, the wound was sutured in layers with 3-O silk and the skin sutured as described above.

The antinociceptive effect was measured using an infrared thermal tail flick test (7371, Ugo Basile, Italy). Latency from the time of stimulus to tail flick (TF) was assigned as response latency. Radiant heat was set to provide a pre-drug latency of 2–4 s. To prevent tissue damage, a 10-s cut-off time was set. A tail flick test was performed before

catheterization and was repeated the day after catheterization to exclude the effect of catheterization on the nociceptive response. On the day of the experiment, tail flick latencies were measured 10 min before and 30 min after medication, and then every hour for 8 h. Effects were expressed as percentage of the maximal possible analgesia (%MPA):

%MPA=((response latency–baseline latency)/ (cut-off latency–baseline latency))×100%.

After testing, time–response curves were constructed and the antinociceptive effect was expressed as area under the time–response curve (AUC). The AUC above pre-test baseline values was calculated by trapezoidal approximation for each rat from 0 to 8 h.

Pharmacokinetic study

Rats were allocated randomly to one of three groups (n= 15 in each group): group 1 received LAS and i.v. morphine infusion; group 2 received UAS and i.v. morphine infusion; and group 3 received i.v. morphine infusion alone. The surgical procedures and morphine infusion were the same as described in the pharmacodynamic study (groups I, III, V). All rats received diethyl ether anaesthesia. Thirty minutes after the end of anaesthesia, morphine was infused.

Blood (1 ml) was collected from rats in each group by direct cardiac puncture at 1 min before infusion of morphine and at 1, 2, 4 and 8 h after the start of infusion. During the study, each rat received only one puncture and three rats in each group were used for blood collection time. Plasma was obtained by centrifugation and frozen immediately to –20°C until assay. Plasma concentrations of morphine were measured using a modification of the method of Svensson.[13] This method uses high pressure liquid chromatography with electrochemical detection. The detection limit of the method was 100 pg ml^{-1} with coefficients of variation of 7.4%, 9.6%, 8.0% (within-day) and 10.7%, 9.8%, 5.7% (between-day) at 0.25, 2.5 and 25 ng ml^{-1}, respectively. In this assay, morphine can be distinguished from its metabolites, morphine-3-glucuronide and morphine-6-glucuronide.

Statistical analysis

Results are expressed as mean (SD). AUC betweeen groups and differences in plasma concentrations of morphine between groups were analysed by the Kruskal–Wallis test. $P<0.05$ was considered significant and a significant decrease in AUC was considered as tolerance.

Results

In the pharmacodynamic part of the study, tail flick latencies were not significantly different before and after catheterization in all groups on the day before morphine administration (Table 1). On the day of the experiment, rats that received saline infusion (groups II, IV and VI) did not show any analgesic effects (Fig. 2A). Rats that received i.v. morphine infusion demonstrated a significant analgesic effect which reached its maximum, on average, 2 h after i.v. infusion (groups I, III and V) (Fig. 2A). In group V, the analgesic effects of morphine decayed gradually for 2 h after infusion. In groups I and III, the analgesic effects of morphine decreased more slowly after reaching a maximum level and were present for longer than in group V (Fig.

Table 1 Tail-flick (TF) latencies (s) measured in rats before (BIC) and after (AIC) i.v. catheterization (mean (SD))

	TF (BIC)	TF (AIC)
Control	2.42 (0.69)	2.62 (1.04)
Upper abdominal surgery	2.58 (1.00)	2.47 (0.48)
Lower abdominal surgery	2.68 (0.31)	2.33 (0.83)

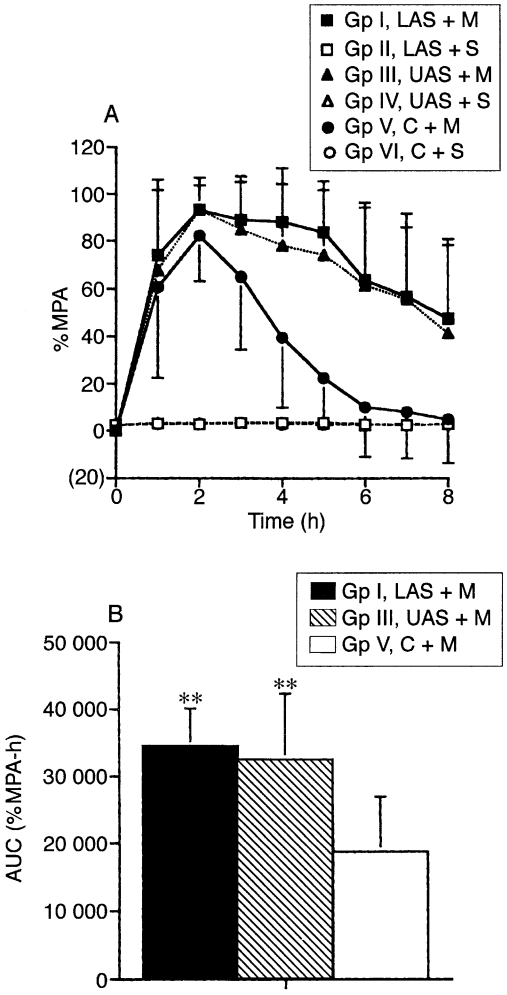

Fig 2 Analgesic effect of morphine or saline in surgically treated or control rats (n=12 in each group). Animals receiving upper (UAS) or lower (LAS) abdominal surgery were treated with morphine (M) 2 mg kg^{-1} h^{-1} or saline (S) i.v. infusion. Control (C) animals did not undergo surgery but were treated with morphine or saline infusion. A: Data are expressed as percentage of maximal possible analgesia (%MPA). B: Data are expressed as area under the response time curve (AUC), which was obtained from A (groups I, III and V). Values are mean (SD). Significant differences from control group: **$P<0.01$. In A, Gp II, LAS+S; Gp IV, UAS+S; and Gp VI, C+S are superimposed at approximately 0% (bottom straight line).

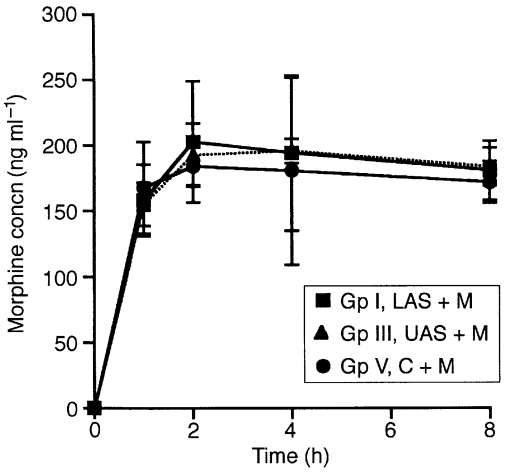

Fig 3 Plasma concentrations of morphine. After i.v. infusion of morphine (M) 2 mg kg^{-1} h^{-1}, the plasma concentration of morphine reached a pseudo-steady state between 2 and 8 h in all animals. Each point indicates mean (SD). There were no significant differences between the morphine and control groups (C) (using one-way analysis of variance). UAS and LAS = Upper and lower abdominal surgery, respectively.

2A). AUC values in groups I and III were larger than that in group V and AUC values in groups I and III were not significantly different (Fig. 2B).

In the pharmacokinetic part of the study, plasma concentrations of morphine reached a pseudo-steady state after i.v. infusion from 2 to 8 h (Fig. 3). There were no significant differences between groups in plasma morphine concentrations. This pharmacokinetic result suggests that the appearance of acute morphine tolerance after i.v. morphine infusion is not pharmacokinetic in nature.

Discussion

Nociceptive stimuli, such as formalin-induced pain and adjuvant-induced arthritis, attenuate tolerance to morphine antinociception.[9–11 14–18] In our study, we also demonstrated that the appearance of surgical pain during administration of morphine significantly attenuated the development of acute tolerance to morphine antinociception.

Acute tolerance to the antinociceptive effect of morphine may occur after a short period of morphine administration in animals. For example, Abdelhamid and colleagues demonstrated a three-fold increase in the ED_{50} of the antinociceptive effect of morphine, 4 h after s.c. injection of morphine 100 mg kg^{-1} in mice.[19] Cox, Ginsburg and Osman[20], Ling and colleagues[3] and Kissin and colleagues[21 22] also found that acute tolerance to morphine antinociception may develop within 8 h after i.v. infusion in rats. We also demonstrated that the antinociceptive effect of morphine decayed rapidly after a 2-h morphine infusion. In contrast, morphine tolerance may not occur in rats treated with formalin injections into a paw[9 10] or adjuvant injection into a joint model.[9 11 15] Vaccarino and colleagues examined the development of tolerance to morphine antinociception in rats in the presence or absence of pain induced by s.c. injection of formalin in paws.[9] They found that tolerance to morphine did not occur after repeated injections of a high dose of morphine in the presence of formalin-induced pain. Using an animal model of Freund's adjuvant or vehicle-injected rats allowed to self-administer i.v. morphine 5 mg kg^{-1} on a 24 h/day schedule, Lyness, Smith and Heavner demonstrated that arthritic rats self-injected significantly less morphine than pain-free animals.[11]

Clinically, opioids are used widely in the treatment of acute and chronic pain. However, the development of tolerance to the analgesic effect of opioids rarely occurs. We found only one report on the possible occurrence of acute opioid tolerance in humans after a large dose of fentanyl (25 μg kg^{-1}) given before surgery.[23] Other reports stated that patients who receive morphine for persistent pain do not develop marked tolerance.[4 5 8 24–26] Also, increased morphine doses during chronic pain management may be caused by treatment events (such as surgery, invasive exploration, etc.) or disease progression.[8 24–26] In our study, we found that surgical pain significantly slowed the development of morphine tolerance. This result is clinically valuable as these animal models are directly analogous to clinical postoperative patients; therefore, our results may also be valuable in explaining some clinical phenomena.

In previous studies, there was no suitable animal model to represent the natural course of clinical pain, especially postoperative pain. Postoperative pain management is the major work of the acute pain service; therefore, it is important to develop suitable animal models which can show the characteristics of clinical postoperative pain and allow clinical, pain-related problems to be studied. As animal models, such as formalin-induced pain and adjuvant-induced arthritis, inadequately represent clinical pain, they are not as well correlated with clinical conditions as our animal model. First, formalin and adjuvant are not clinically used and also they are not the causative agents of clinical pain. Second, the natural course of formalin-induced pain is only chemical-related and is not relevant to surgical pain. Also, adjuvant-induced arthritis may only partly represent clinical arthritis. Third, nociception of adjuvant-induced arthritis is not strong enough and did not consistently attenuate morphine tolerance in previous reports.[10 27] Our animal models of surgical pain are analogous to the clinical patient with postoperative pain; therefore, they are different from those of previous animal models and are more suitable for use in studies of clinical pain-related phenomena.

The reason why surgical pain attenuates acute morphine tolerance is not known. However, activation of the hypothalamic–pituitary–adrenal (HPA) axis by stressful stimuli, or direct treatment with ACTH (adrenocorticotropic hormone) or corticosterone may attenuate the development of morphine tolerance.[28–31] Surgical pain is a type of stressful stimulus and, therefore, we speculate that it may attenuate acute morphine tolerance via activation of the HPA axis. The intensity of surgical pain may decrease with time and, therefore, the endocrine responses to these painful stimuli may also decrease. In our study, we also speculate that

morphine tolerance may eventually develop completely in all groups in a time-dependent manner, despite different treatments. But within the 8-h observation period, we found that surgical pain significantly attenuated morphine tolerance.

In summary, using two animal models, upper and lower abdominal surgery, we have explored the effect of postoperative pain on the prevention of acute tolerance to morphine antinociception in rats. We found that acute morphine tolerance developed rapidly after i.v. infusion of morphine alone and that co-treatment with upper or lower abdominal surgery during morphine infusion significantly attenuated the development of tolerance to morphine antinociception.

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