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ORIGINAL RESEARCH

Impact of different analgesic depths and abdominal trauma of different severities on stress and recovery of rats undergoing total intravenous anesthesia

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Abstract: A number of animal models have been developed to examine the pathophysiological consequences of surgical procedures, but anesthetic methods, monitoring, and management measures in these models are very different from those used in humans. This study was designed to create a rat model of abdominal surgery using anesthetic methods and perioperative treatment similar to those used in the clinic and to investigate the effects of different injury severities and depths of anesthesia and analgesia on surgical stress and postoperative recovery. Abdominal skin/muscle incision was compared with exploratory laparotomy in rats under propofol intravenous anesthesia, accompanied by perioperative measures such as oxygen inhalation, fluid infusion, warmth, blood gas analysis, and infection prevention. Stress indices (mean arterial pressure, heart rate, blood glucose, and plasma corticosterone) were monitored during anesthesia and surgery, and recovery indicators (body weight, food consumption, and pain) were measured after surgery. In addition, animals undergoing laparotomy were subjected to low and high dosages of propofol and sufentanil, in order to examine the relationship between anesthetic and analgesic depth and stress on recovery. Exploratory laparotomy induced a greater stress response and caused slower postoperative recovery as measured than somatic injury. High-dose sufentanil downregulated plasma corticosterone and improved postoperative recovery more effectively than high-dose propofol ($P<0.05$). Taken together, a rat model of abdominal surgery using anesthetic methods and perioperative treatment similar to those used in the clinic was successfully developed. It showed a positive correlation between severity of surgical trauma and stress response and postoperative recovery and a significant role of adequate analgesia in reducing surgical stress and improving postoperative recovery.

Keywords: animal model, injury severity, stress response, postoperative recovery, analgesic depth, abdominal trauma

Introduction

Surgical procedures induce tissue damage, inflammation, and a stress response,[1–3] and inflammatory reactions, a high catabolic state, and pain in the postoperative period will delay wound healing and recovery of physiological function. The features and pathogenesis of postoperative pain are different from those seen in selective pain types such as inflammatory, chemical, and neuropathic pain.[4–6] In addition, the postoperative pain reaction to treatments using nonsteroidal anti-inflammatory drugs (NSAIDs), N-methyl-D-aspartate (NMDA) or non-NMDA receptor antagonists, and selective P2X3 or P2X2/3 receptor antagonists is different.[7–9]

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A number of animal models have been developed to examine the pathophysiological consequences of surgical procedures. However, anesthetic methods, monitoring, and management measures in these models are so different from those used in humans that it is difficult to apply the knowledge gained from these studies to clinical practice.[2,10–12]

In this study, a model that could be used as a research platform for study of surgical stress and pain, which might be useful in translational medicine, was established. In doing so, a surgery protocol was devised using the same anesthetic and similar monitoring procedures as those seen in humans. In addition, the responses seen in this protocol to different intensities of surgical stress, pain management, and different combinations of propofol and sufentanil, anesthetic and analgesic drugs commonly used in clinical practice, were assessed to examine postsurgical recovery from pain after exploratory laparotomy. Laparotomy, an operation causing severe postsurgical pain,[13] was selected to provide the most intense surgical stress.

This model was used to compare the effects of severity of surgery (skin/muscle incision vs exploratory laparotomy) on the stress response and postoperative recovery and the effects of increasing anesthesia or analgesia levels on surgical stress and postoperative recovery after exploratory laparotomy.

Materials and methods

Animals

Male Sprague Dawley rats (12–15 weeks old, 270–320 g) were used in this study. They were housed individually in cages on a 12-hour light/dark cycle under a temperature of 20°C–24°C and given free access to food and water. All procedures were approved by the Animal Care and Use Committee of Central South University and were performed according to the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health in 1996.

Two protocols were used, the first to determine the effect of surgery severity and the second to compare the effect of increased anesthesia or increased analgesia on surgical stress and postoperative recovery. The postoperative pain score (as assessed by the open field test) was the primary outcome.

Experiment 1: surgery severity – incision into abdominal muscle versus incision plus exploratory laparotomy

Grouping

Thirty-six rats were randomly divided into a control group, an incision group, and a laparotomy group (12 rats in each group).

Preliminary experiments

Total intravenous anesthesia was selected for induction and maintenance of anesthesia during surgery, because it is one of the most common clinical methods used for general anesthesia in humans. Tracheal intubation was not performed in order to simplify interpretation of the results by eliminating the possibility of a stress reaction following tracheal intubation. Instead, a mask was used for delivery of oxygen. The oxygen flow was 2 L/min. In order to reduce the effect of factors related to anesthesia itself on outcome, the depth of anesthesia was adjusted to the superficial anesthesia or sedation level when examining the effect of surgical severity. In our preliminary studies, the effect of different anesthetic concentrations on depth of anesthesia was evaluated by observing respiratory and circulatory reactions to tail pinch. Based on the results of others[4,14,15] and on the results of preliminary experiments, propofol was selected with an induction dose of 10 mg/kg and a maintenance dose of 30 mg/kg/h. This dosage put the rats into sedation rather than anesthesia. Rats were quiet with a stable heart rate and breathing frequency when no stimulus was applied. When medium pain was given by tail pinch, they showed a slight body movement (retraction of limbs) and a mild increase in heart rate (5–10 beats per minute (bpm)) and breathing frequency (5–10 bpm), and they recovered from the pain stimulus within 3 minutes. After surgery, the rats with this level of anesthesia needed only 4–5 minutes for complete resuscitation and had good mobility.

Anesthesia and analgesia

Anesthesia and surgery were performed in fasted rats at 9 AM daily. The fasting period was 12 hours, from 9 PM the previous night to 9 AM. They had free access to water during this period. For anesthesia, a 24 G catheter filled with heparinized saline was inserted into the tail vein of the conscious rat for infusion. The rats were placed in a fixator for this procedure. After 30 minutes, anesthesia was induced intravenously with 10 mg/kg propofol and then maintained at the rate of 30 mg/kg/h. The depth of anesthesia was controlled at the level of sedation with no added analgesia, that is, only a slight limb retraction, a mild increase in heart rate (5–10 bpm) or respiratory rate, and no visible fluctuation of blood pressure in response to a moderate tail pinch were seen. Heart and respiratory rates were recorded through a needle electrode connected to a multifunctional electrocardiographic monitor (Solar 8000M; GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). Blood pressure was measured through a femoral catheter connected to a pressure transducer connected to the same monitor. Local

anesthesia with bupivacaine (0.25%) was used for femoral artery catheterization.

Surgical procedure

When the righting reflex had disappeared, the rat was fixed upon the operating table and a catheter was inserted in a sterile manner into the right femoral artery under local anesthesia in order to monitor blood pressure. For this procedure, the skin was incised, the subcutaneous tissue and fascia femoral sheath bluntly dissected, with avoidance of the femoral nerve and vein. Abdominal surgery began 3 minutes after disappearance of the pain response to the level described previously. The control group received propofol intravenous anesthesia and blood pressure monitoring, but no surgery; the incision group received a 2 cm longitudinal incision, 0.5 cm below the xiphoid, through the abdominal musculature; the laparotomy group received the same incision as the incision group and an additional exploratory laparotomy. For the exploratory laparotomy, the surgeon's index finger was inserted into the peritoneal cavity for gut exploration for 2 minutes and a 10 cm long section of small intestine was externalized, kneaded moderately for 5 minutes, and then returned.[2,15] Surgical wounds were wiped with sterile gauze and the peritoneum, muscle layer, and skin layer were sutured separately with 4.0 Mersilk thread (Johnson & Johnson, New Brunswick, NJ, USA).[16] The duration of surgery was ~30 minutes and anesthesia lasted ~1 hour.

During the surgery and anesthesia, rats underwent fluid infusion through the tail vein (3 mL/hour), inhaled oxygen with masks (2 L/minute), and had their temperature maintained between 36°C–37°C using a heating lamp. Intramuscular penicillin injection (80,000 IU) through the femoral triceps was given before and for 3 days after surgery to prevent infection.[17] Rats with hypoxia, hypercarbia, or electrolyte disturbances during the surgery were excluded. Animals with abnormal preoperative blood glucose or severe postoperative wound infection were also rejected. At the end of the experiment, all the rats were euthanized by injecting 450 mg/kg chloral hydrate intraperitoneally.

Data collection

Stress-related parameters including mean invasive arterial pressure (MAP), heart rate, blood glucose, and plasma corticosterone were measured. MAP and heart rate were recorded at the following times: presurgery (T0), skin incision (T1), muscle incision (T2), gut exploration (T3), muscle suture (T4), skin suture (T5), 1 minute after surgery (T6), 3 minutes after surgery (T7), and 5 minutes after surgery (T8). Arterial blood gas and blood glucose were measured before and after surgery. Plasma corticosterone from femoral arterial blood was measured by using an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc., Minneapolis, MN, USA) from blood samples taken before and after surgery (both samples being taken before 12 AM). The recovery indicators of changes in body weight, food consumption, and pain behavior were measured postoperatively. Body weight and food consumption were measured 3 days before and daily between 10 and 12 AM for 7 days after surgery. Bedding, food, and water supply were changed every day in order to avoid contamination and infection of the surgical wounds. Water was tap water boiled and cooled to room temperature before being loaded into the water bottle. Change in body weight was recorded as the difference between current body weight and preoperative body weight. Change in food consumption was recorded as the difference between previous day's food weight and food weight remaining 24 hours later. Postoperative ambulation and pain behavior were assessed using the open field test before and 6 and 24 hours after surgery, as previously described.[18,19] Briefly, the animals were tested using a square box (100×100×100 cm) with the top open and the bottom separated into 25 identical grids. They were placed in the middle of the open field, acclimated for 10 minutes, and the subsequent behavior frequencies were recorded. More than 50% of the body spanning into the adjacent grid was judged as a score of one and, for scoring, the total scores within 1 minute were aggregated.

Experiment 2 – effect on stress and recovery of increasing anesthesia (propofol) or analgesia (sufentanil) in rats with exploratory laparotomy

Grouping

Twenty-four rats were randomly divided into a low-sufentanil/high-propofol group and a high-sufentanil/low-propofol group (12 rats in each group).

Preliminary experiments

The infusion dosage of the anesthetic propofol is known to be highly related to anesthetic depth as assessed by the bispectral analysis. The analgesic sufentanil is a highly selective μ-opioid receptor agonist providing stronger analgesia (high correlation between analgesic intensity and its dosage) with less inhibition of the respiratory and circulatory systems than fentanyl and remifentanil, and is commonly used in clinical anesthesia.[20] Therefore, total propofol–sufentanil intravenous

anesthesia was selected to assess the respective effects of anesthetic and analgesic depth.

High and low dosages of propofol and sufentanil were infused to compare the effects of anesthetic or analgesic depth on both surgical stress and postoperative recovery. In the preliminary experiments, it was found that infusing propofol at the rate of 30 mg/kg/h provided only sedation. Infusing propofol at the rate of 50 mg/kg/h caused a light stress response with a blood pressure and heart rate decrease of <10% without noxious stimulation and no limb retraction in response to a medium tail pinch. Propofol infused at the rate of 100 mg/kg/h without accompanying sufentanil produced a deep anesthetic state, with a blood pressure and heart rate decrease of >30% of the preoperative values. However, when infused with 2 μg/kg/h sufentanil, blood pressure and heart rate remained at normal levels. Therefore, to investigate the effect of anesthetic depth, propofol doses of 50 or 100 mg/kg/h were selected. To select appropriate doses of sufentanil for analgesia, five dosages (1, 2, 3, 4, and 5 μg/kg/h, eight animals at each dosage) were tried initially and their impact on hemodynamics during surgery was observed. On the basis of the results, which showed the following doses to produce minimal changes in blood pressure and heart rate, 2 μg/kg/h sufentanil was selected for low analgesic dose and 4 μg/kg/h sufentanil was selected for high analgesic dose.

Anesthesia and analgesia

The high-propofol/low-sufentanil group (P100S2) was given an intravenous infusion of propofol 100 mg/kg/h plus sufentanil 2 μg/kg/h. The high-sufentanil/low-propofol group (P50S4) was given an infusion of propofol 50 mg/kg/h plus sufentanil 4 μg/kg/h, following an initial injection of 1 μg/kg sufentanil. The infusion of sufentanil was terminated in both the groups at the end of surgery. The dose of propofol was not terminated at that time, but was reduced to a sedative dose, 30 mg/kg/h, in order to provide adequate sedation for obtaining blood samples for corticosterone measurement. The 150 mL blood sample for each measurement was collected in a heparinized tube, stored overnight at 4°C, and then centrifuged to remove blood cells and obtain plasma. The plasma corticosterone concentration was measured using the ELISA kit, according to the manufacturer's instructions.

Surgical procedure

Exploratory laparotomy was performed in rats under total intravenous anesthesia using the propofol and sufentanil concentrations assigned to each group. The surgical procedure was the same as that used in the laparotomy group in Experiment 1. Perioperative monitoring and supportive procedures were the same as those of Experiment 1.

Data collection

Plasma corticosterone was measured every hour during the 6 hours after surgery. Body weight and food consumption were measured daily for 7 days after surgery. The open field test was given presurgery and 8 and 24 hours after surgery.

Animal surgery and anesthesia were performed by the same two people, including anesthesia, arteriovenous puncture, abdominal surgery, and arterial blood collection. Postoperative indicator measurements (open field test, body weight, and food intake) were completed independently by a third person. Analysis of the data was done by statisticians who were not otherwise involved in the study.

Statistical analysis

Data for MAP, heart rate, blood glucose, plasma corticosterone, body weight, food consumption, and open field test scores are presented as mean and standard deviation. A linear mixed model was used to investigate the effect of experimental groups (denoted as group effect), time after experiment (denoted as time effect), and their interaction (denoted as group × time effect), because the factors in the study were all repeated measurements across time and group. When these main effects showed significance, further post hoc multiple comparisons were conducted using the Bonferroni correction to control overall type I error rates. A power analysis was not done to determine the sample number. Instead, the sample number was selected based on the postoperative pain score as the primary outcome and on the results of others.[21]

Statistical analyses were performed with SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) and the figures were drawn with IBM SPSS statistical software version 22 for Windows (IBM Corporation, Armonk, NY, USA). Two-tailed $P<0.05$ indicated statistical significance, and the P-value less than corrected type I error indicated statistical significance in post hoc tests.

Results

No significant differences were found among groups in preoperative MAP, heart rate, blood glucose, plasma corticosterone, body weight, food consumption, open field test scores, or duration of anesthesia and surgery (all $P>0.05$). Basal values (for all rats) were the following: MAP, 110.4±2.5 mmHg; heart rate, 365.9±3.5 bpm; blood glucose, 4.1±0.4 mmol/L; body weight, 286.1±13.5 g; and food consumption, 24.8±1.4 g/day.

During the operation, no animals underwent hypoxia or hypothermia, and all blood gas analyses were normal, so no rats were excluded. No animals died from anesthetic or surgical complications, and all wounds healed well. However, four rats had severe postoperative wound infections and were rejected.

Surgical severity-related changes

Stress response

Figure 1A and B shows MAP and heart rate changes during surgery for the control, incision, and laparotomy groups in Experiment 1. MAP and heart rate rose significantly during the incision period in both the incision and laparotomy groups, stabilized in the incision group but increased further in the laparotomy group during gut exploration, and decreased to presurgery levels in both the incision and laparotomy groups as muscle and skin were sutured and surgery ended. MAP was higher and heart rate was faster in the laparotomy group compared to the incision group from the initiation of gut exploration onward, and this difference reached statistical significance 3 and 5 minutes after the end of surgery for blood pressure, and 5 minutes after the end of surgery for heart rate. Time, group, and time × group effects were significant for both MAP and heart rate (all $P<0.001$).

Figure 1C and D shows blood glucose and plasma corticosterone levels in the control, incision, and laparotomy groups before and after surgery. Blood glucose and plasma corticosterone levels rose significantly after surgery in both the incision and laparotomy groups, and these increases

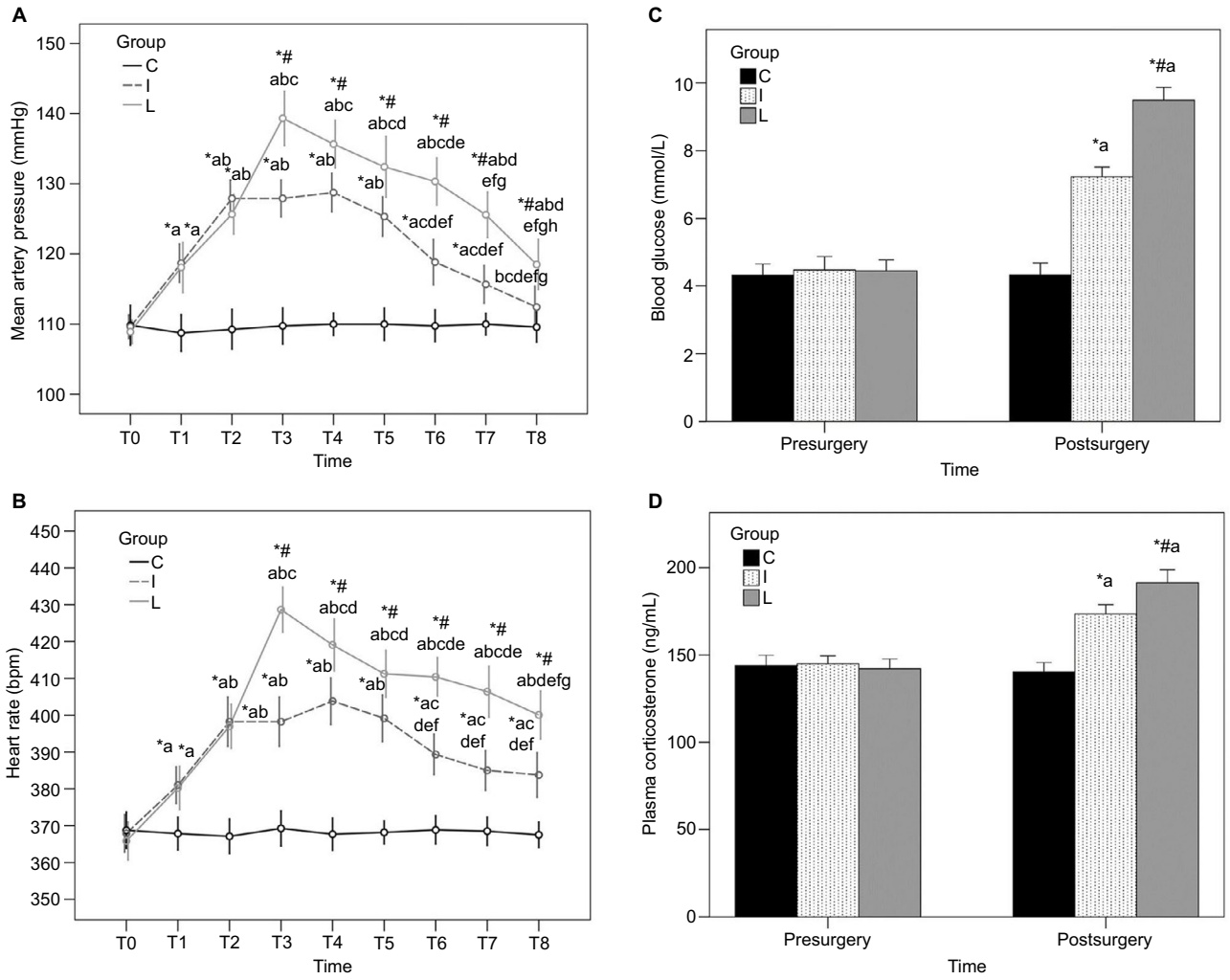

Figure 1 Changes in Experiment 1: surgery severity across time and group in (**A**) mean arterial blood pressure, (**B**) heart rate, (**C**) blood glucose, and (**D**) plasma corticosterone.
Notes: Corrected α-levels by Bonferroni post hoc tests were used for group comparisons and for time comparisons. For (**A**), (**B**), and (**C**), *significantly different from group C; #significantly different from group I; a, significantly different from T0; b, significantly different from T1; c, significantly different from T2; d, significantly different from T3; e, significantly different from T4; f, significantly different from T5; g, significantly different from T6; h, significantly different from T7. For (**D**), *significantly different from group C; #significantly different from group I; a, significantly different from presurgery.
Abbreviations: C, control group; I, incision group; L, laparotomy group; T0, presurgery; T1, skin incision; T2, muscle incision; T3, gut exploration; T4, muscle suture; T5, skin suture; T6, 1 minute after surgery; T7, 3 minutes after surgery; T8, 5 minutes after surgery.

were significantly higher in the laparotomy group than in the incision group. Group, time, and group × time effects were significant for both glucose and corticosterone (all $P<0.001$).

Postoperative recovery

Figure 2A and B shows changes in body weight and food consumption during the 7 days after surgery. Body weight decreased significantly compared to control in the incision and laparotomy groups during the first 2 days after surgery and then increased steadily during the next 4 days. The laparotomy group had a greater weight loss than the incision group at all times, and this difference was statistically significant on days 1 through 4. Food consumption was significantly lower compared to control for 3 days in the incision group and 6 days in the laparotomy group. In the incision group, food consumption decreased for the first 2 days and returned back to control values by day 4. In the laparotomy group, food consumption decreased for 3 days and returned to control levels by day 7. From day 1–7, food consumption was significantly lower in the laparotomy group than in the incision group. Group, time, and group × time effects were significant for both body weight and food consumption (all $P<0.001$).

Figure 2C shows postsurgery results of the open field test. Upon emergence from anesthesia, the rats showed pain behaviors such as licking the wound, bucking, twitching, and torsion. The open field test scores were significantly lower in the incision and laparotomy groups compared to those in the control group 8 and 24 hours after the operation, and this

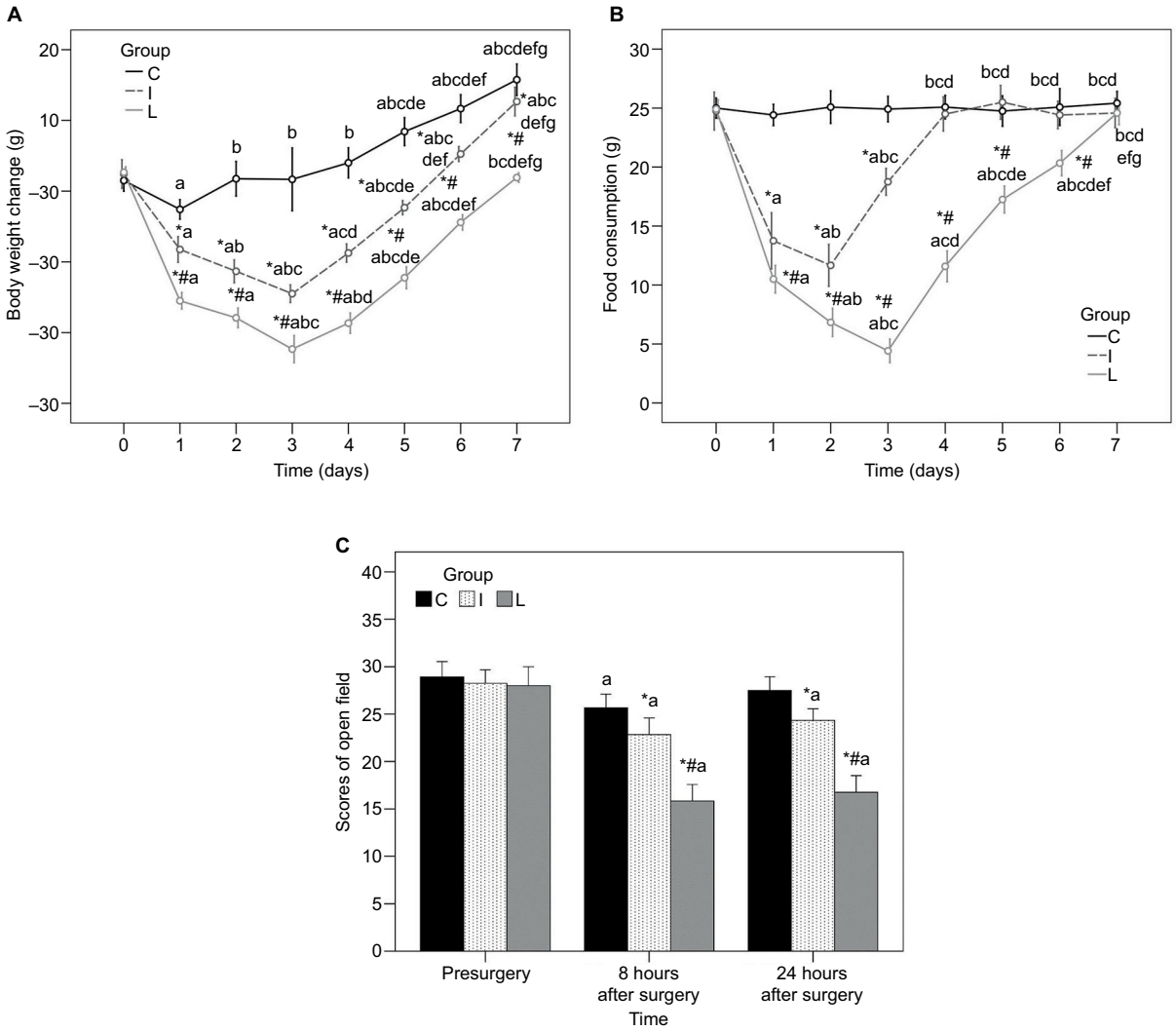

Figure 2 Changes in Experiment 1: surgery severity across time and group in (**A**) body weight, (**B**) food consumption, and (**C**) open field test score.
Notes: Corrected α-levels by Bonferroni post hoc tests were used for group comparisons and for time comparisons. For **(A)** and **(B),** *significantly different from group C; #significantly different from group I; a, significantly different from baseline; b, significantly different from 1 day after surgery; c, significantly different from 2 days after surgery; d, significantly different from 3 days after surgery; e, significantly different from 4 days after surgery; f, significantly different from 5 days after surgery; g, significantly different from 6 days after surgery. For **(C),** *significantly different from group C; #significantly different from group I; a, significantly different from baseline.
Abbreviations: C, control group; I, incision group; L, laparotomy group.

decrease was significantly greater in the laparotomy group than in the incision group. Group, time, and group × time effects were significant ($P<0.001$) for this test.

Anesthetic and analgesic depth-related differences during and after exploratory laparotomy

Stress response

When the stress-related effects during exploratory laparotomy with high-propofol and high-sufentanil anesthesia were compared, no significant differences among the groups in MAP or heart rate were seen during the 6 hours after surgery (data not shown), but postsurgical plasma corticosterone levels differed significantly (Figure 3) during this time period.

In the high-propofol group, corticosterone levels increased at the end of surgery, decreased somewhat during the first hour after surgery, and then increased steadily over the next 6 hours. Corticosterone levels were significantly higher than the presurgery levels at all time points. In the high-sufentanil group, corticosterone levels increased slightly but significantly compared to presurgery levels at the end of surgery, decreased to presurgery levels during the first 2 hours after surgery, and then increased steadily for the next 4 hours. Corticosterone levels were significantly higher in high propofol-treated rats than in high sufentanil-treated rats at the end of surgery and all subsequent times. Group, time, and group × time effects were significant between the two groups (all $P<0.001$)

Postsurgical recovery

Analgesia played an important role in improving recovery. Changes in body weight and food consumption during recovery are shown in Figure 4A and B. Body weight and food consumption decreased in the high-propofol and high-sufentanil groups during the first 2 days after surgery and then increased until reaching presurgical levels on day 6. Rats anesthetized with high propofol had significantly lower body weight and food consumption than those anesthetized with high sufentanil at all times during the first 5 days.

Figure 4C compares the open field test scores between the two groups 8 and 24 hours after surgery. The postsurgery scores were significantly lower than the presurgery scores, and the high-propofol group scores were significantly lower than the high-sufentanil group scores at both the 8- and 24-hour time periods. Group, time, and group × time effects were statistically significant ($P<0.001$).

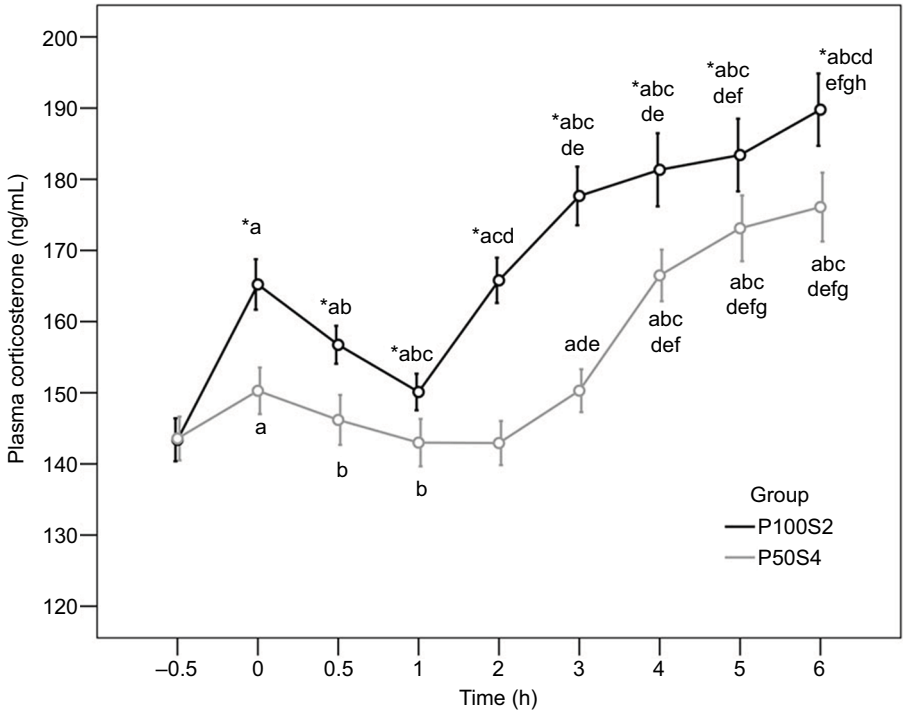

Figure 3 Changes in Experiment 2: anesthetic and analgesic depth across time and group in plasma corticosterone.

Notes: Corrected α-levels by Bonferroni post hoc tests were used for group comparisons and for time comparisons. *Significantly different from high-sufentanil group; a, significantly different from baseline (–0.5 hours); b, significantly different from 0 hour after surgery; c, significantly different from 0.5 hours after surgery; d, significantly different from 1 hour after surgery; e, significantly different from 2 hours after surgery; f, significantly different from 3 hours after surgery; g, significantly different from 4 hours after surgery; h, significantly different from 5 hours after surgery. P100S2, high-propofol group; P50S4, high-sufentanil group.

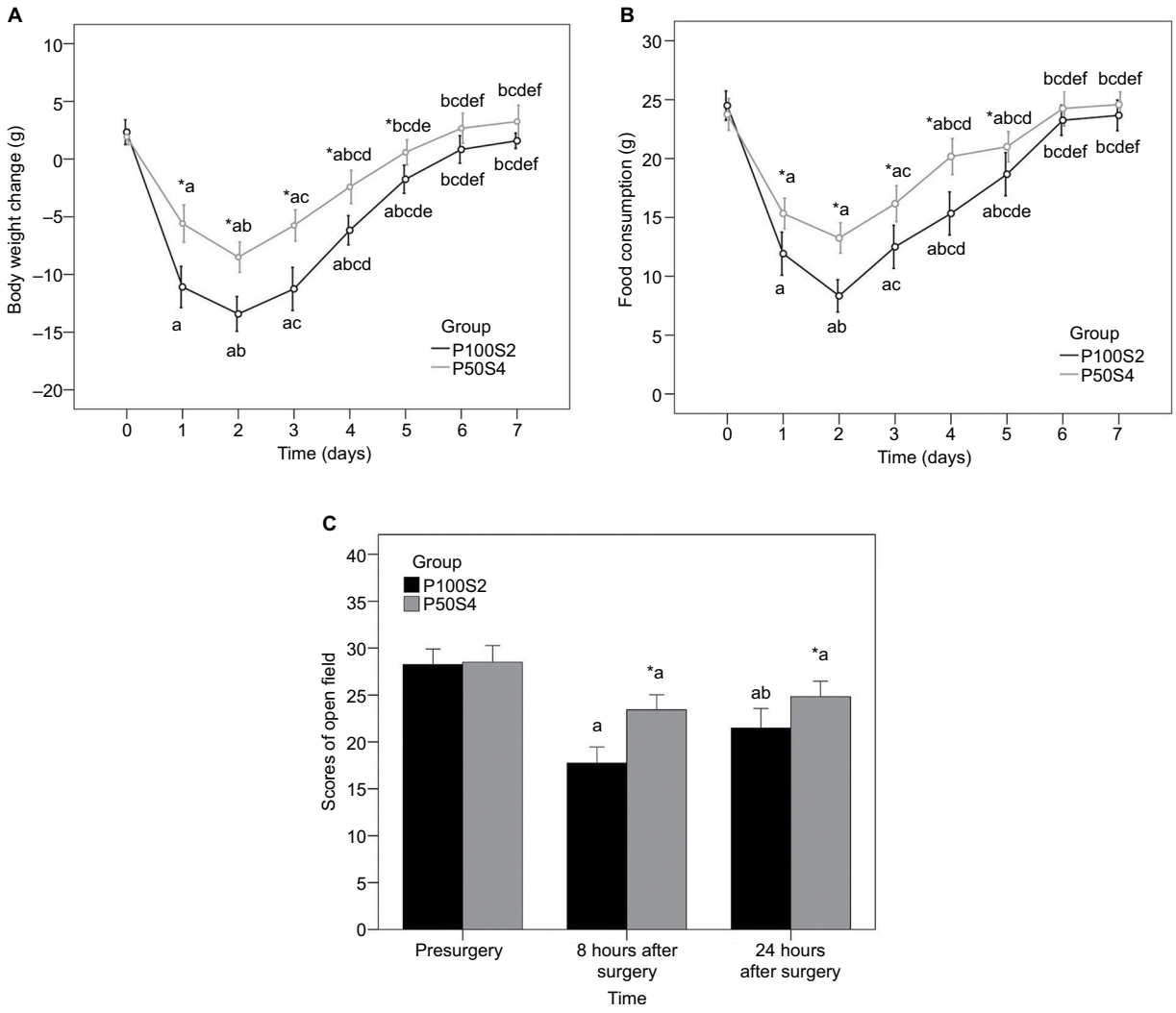

Figure 4. Changes in Experiment 2: anesthetic and analgesic depth across time and group in (**A**) body weight, (**B**) food consumption, and (**C**) open field test scores.
Notes: Corrected α-levels by Bonferroni post hoc tests were used for group comparisons and for time comparisons. For (**A**) and (**B**), *significantly different from high-propofol group; a, significantly different from baseline; b, significantly different from 1 day after surgery; c, significantly different from 2 days after surgery; d, significantly different from 3 days after surgery; e, different from 4 days after surgery; f, different from 5 days after. For (**C**), *significantly different from high-propofol group; a, different from presurgery; b, different from 8 hours after surgery. P100S2, high-propofol group; P50S4, high-sufentanil group.

Discussion

In this rat model of abdominal surgery using anesthesia methods commonly used in the clinic, increasing the severity of the surgery significantly increased the surgical stress response and slowed the postoperative recovery. Increasing perioperative analgesia with sufentanil decreased postoperative corticosterone levels, lessened postoperative pain behaviors, and increased recovery compared to the results seen after increasing the depth of anesthesia with propofol.

The model developed in this study was a rat abdominal surgery model that used anesthetic methods and surgical care similar to those used clinically. Increased blood pressure, heart rate, blood glucose, and plasma corticosterone (cortisol in humans) are classical markers of stress that are sensitive and easy to measure. Therefore these markers were used to assess the stress level. The high catabolic state, surgical stress, and postoperative pain caused by surgical trauma can result in insufficient food intake and significant weight loss,[2,3] and postoperative changes in these parameters have been reported to reflect recovery of physiological function with high specificity and sensitivity.[17,18] The open field test can be used to monitor pain behavior and recovery of physical function. Therefore, these three parameters were used to measure postoperative recovery. Although postoperative analgesia is used in humans, it was not used in the rat studies because the goal was to observe the effects of the procedures on postsurgical recovery, and recovery from pain is one of the indicators of postsurgical recovery.

In this model, visceral damage induced a much more severe cardiovascular reaction and a larger corticosterone

increase than somatic damage. The degree and duration of postoperative pain and impairment of physical function were also worse and more prolonged in visceral damage. This suggests that surgical stress and postoperative pain after exploratory laparotomy are mainly related to visceral tissue damage, an interpretation consistent with the results of others.[11,22] The results of this study, therefore, indicate that a successful animal model, using anesthetic and perioperative techniques similar to those used clinically, of abdominal surgery induced by different injury severities has been made.

The results showed that the low-propofol/high-sufentanil and high-propofol/low-sufentanil protocols each provided satisfactory anesthesia for exploratory laparotomy, with blood pressure and heart rate fluctuating within 10% of the basal value in both protocols. However, the two protocols had different effects on stress hormone levels and postoperative physical functions. The higher dose of the analgesic inhibited the stress reaction, reduced postoperative pain, and promoted postoperative recovery and early mobility to a greater degree than those seen with the higher dose of anesthetic. Also, both protocols inhibited the stress reaction and promoted postoperative recovery to a greater degree than those seen in the laparotomy group of Experiment 1, a group that received no analgesic and only a sedative dose of anesthetic. Opioids are known to decrease the stress response caused by surgery by suppressing the release of cortisol. A previous study in humans has shown that fentanyl given during induction of anesthesia in lower abdominal surgery decreases cortisol levels, but fentanyl given 60 minutes after the start of surgery has no effect, because the stress response has already occurred.[23] These results are compatible with the results of the present study that perioperative sufentanil decreases postoperative corticosterone. The results indicate that optimal dosages of propofol and sufentanil should be considered carefully during total intravenous anesthesia, in order to provide the greatest protective effect during surgical procedures.

Animal models of incisional pain have shown incisional pain to cause mechanical and heat hyperalgesia, and deep tissue injury to increase mechanical, but not heat, hyperalgesia.[1,24,25] Animal models involving greater surgical injury, such as bone injury,[26] gastrocnemius incision,[27] thoracotomy,[28] and laparotomy,[17] have also been developed. Although these models simulated different kinds of clinical surgery, the potential interference with results of factors such as type and depth of anesthesia, hypoxia, blood loss, abnormal temperature, infection, and psychological stress was not fully considered. There was no proper method to monitor vital signs during surgery; anesthetic was administered peritoneally or intramuscularly, methods unused in clinical practice; the focus was mostly on postsurgical pain with less attention paid to the stress response; and there was no comparison of reactions caused by different levels of surgical damage or different tissues under the same conditions. So the results were variable and hard to interpret in a way that would be useful clinically.

The protocol was designed to simulate clinical conditions in the animal model used in this study. Therefore, total intravenous anesthesia was used and vital signs were monitored, and perioperative management included oxygen supplementation and monitoring of arterial blood oxygen levels, fluid infusion, and maintenance of body temperature, so that the experimental outcomes could be translated into clinical use more effectively and easily. In this study, the postsurgical outcome measurements of food intake, weight gain, and performance in the open field test did not, of course, simulate the clinical conditions. But by using these parameters, total recovery could be assessed, that is, return to normal, which of course is the ultimate clinical goal.

This model had certain limitations. Although designed to simulate conditions used in clinical practice, this model differed from these conditions in that tracheal intubation was not performed and laparoscopy was performed during spontaneous breathing and without muscle relaxants. Also, a limitation of rat models of pain and surgical stress is that rat physiology (including drug metabolism) is not as similar to human physiology as is the physiology of some larger animals, especially the pig.[29] Also, because of the rat's small size, some procedures, such as taking more than a limited number of blood samples for assay, cannot be done. However, the cost of experiments using large animals is higher and the time to complete them is longer than in experiments using rats as a model, and rats have an additional advantage that there are many previous experiments on surgery in rats to use for guidance and comparison.

In this study, analgesia was not provided and anesthesia was induced only to a sedative level in Experiment 1 so that the stress response to surgery itself could be clearly separated from superimposed anesthetic and analgesic effects. However, this protocol had the limitation that surgery in humans is generally performed in the presence of analgesia and full anesthesia, and so this difference must be considered when interpreting the results in terms of their relevance to translational medicine. Another limitation is that, although the effects of high propofol/low sufentanil and low propofol/high sufentanil on perioperative and postoperative parameters were studied, a group with low propofol/low sufentanil was not included for comparison.

In summary, a successful animal model of abdominal surgery induced by different injury severities has been made, using anesthetic methods and perioperative manage-

ment similar to those in clinical use. It may serve as a future experimental platform for studies about perioperative stress, organ damage, and acute and chronic surgical pain because it is easy to perform and has good repeatability.

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Author contributions

Hai-ming Huang contributed to study design, definition of intellectual content, literature research, clinical studies, experimental studies, data acquisition, and manuscript preparation. Jun Cao helped in literature research, clinical studies, experimental studies, and manuscript editing. Lin-mei Zhu was involved in literature research, experimental studies, data acquisition, and manuscript preparation. Yu-qing Chen contributed to experimental studies, data acquisition, and manuscript preparation. Fu-ding Lu contributed to data analysis. Hong-wei Cai was the guarantor of integrity of the entire study and contributed to study concepts, study design, definition of intellectual content, data analysis, and manuscript editing. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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