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Research Report

Midazolam exacerbates morphine tolerance and morphine-induced hyperactive behaviors in young rats with burn injury

Li Song[a,b,1], Shuxing Wang[a,1], Yunxia Zuo[b], Lucy Chen[a], Jeevendra A. Martyn[a], Jianren Mao[a,*]

[a]MGH Center for Translational Pain Research, Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[b]Department of Anesthesia, West China Hospital, Sichuan University, Chengdu, China

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ABSTRACT

Midazolam and morphine are often used in pediatric intensive care unit (ICU) for analgesia and sedation. However, how these two drugs interact behaviorally remains unclear. Here, we examined whether (1) co-administration of midazolam with morphine would exacerbate morphine tolerance and morphine-induced hyperactive behaviors, and (2) protein kinase C (PKC) would contribute to these behavioral changes. Male rats of 3–4 weeks old were exposed to a hindpaw burn injury. In Experiment 1, burn-injured young rats received once daily saline or morphine (10 mg/kg, subcutaneous, s.c.), followed 30 min later by either saline or midazolam (2 mg/kg, intraperitoneal, i.p.), for 14 days beginning 3 days after burn injury. In Experiment 2, young rats with burn injury were administered with morphine (10 mg/kg, s.c.), midazolam (2 mg/kg, i.p.), and chelerythrine chloride (a non-specific PKC inhibitor, 10 nmol, intrathecal) for 14 days. For both experiments, cumulative morphine anti-nociceptive dose–response (ED50) was tested and hyperactive behaviors such as jumping and scratching were recorded. Following 2 weeks of each treatment, ED50 dose was significantly increased in rats receiving morphine alone as compared with rats receiving saline or midazolam alone. The ED50 dose was further increased in rats receiving both morphine and midazolam. Co-administration of morphine and midazolam also exacerbated morphine-induced hyperactive behaviors. Expression of the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor and PKCγ in the spinal cord dorsal horn (immunohistochemistry; Western blot) was upregulated in burn-injured young rats receiving morphine alone or in combination with midazolam, and chelerythrine prevented the development of morphine tolerance. These results indicate that midazolam exacerbated morphine tolerance through a spinal NMDA/PKC-mediated mechanism.

*Corresponding author. Fax: +1 617 724 2719.
E-mail address: jmao@partners.org (J. Mao).
[1]Contributed equally to this work.

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1. Introduction

Over 30% of people who experience burn injury in the United States are children (Borse and Sleet, 2009). Clinically, opioids are among the first-line drugs used to treat severe pain resulting from burn injury particularly in the intensive care unit (ICU) setting (Stoddard et al., 2006). However, the development of opioid analgesic tolerance, a diminished opioid anti-nociceptive effect following repeated exposure to opioid, could significantly hamper the clinical effectiveness of opioid therapy.

Many pediatric patients with burn injury have a prolonged stay in ICU and require both analgesia and sedation. Benzodiazepines are the most frequently used sedatives in ICU settings. At the cellular level, benzodiazepines potentiate γ-amino butyric acid (GABA) actions by increasing the frequency of chloride channel opening and by prolonging its open state (Matsumoto, 1989; Reynolds et al., 1992). Midazolam is a water-soluble and short-acting benzodiazepine. Acting on a benzodiazepine receptor-$GABA_A$ ionophore complex, midazolam reduces excitability of second-order neurons in the spinal cord dorsal horn and brain stem (Haefely, 1988; Richards et al., 1986). However, intraperitoneal or intrathecal application of midazolam alone has been shown to induce both hyperalgesia and antinociception (Clavier et al., 1992; Kontinen and Dickenso, 2000; Lim et al., 2006; Niv et al., 1988; Rattan et al., 1991;Shih et al., 2008; Tatsuo et al., 1999; Yanez et al., 1990). Clinically, midazolam and morphine are the most frequently used drugs in ICU, including pediatric ICU, to achieve sedation and analgesia (Chamorro et al., 2010; Soliman et al., 2001). To date, it remains unclear as to how morphine and midazolam might interact at the behavioral and cellular level, particularly in pediatric populations.

Our previous studies indicate that burn injury itself may have a differential effect on the development of morphine tolerance in adult versus young rats (Wang et al., 2005, 2011). In this study we used a burn injury model of young rats to examine whether (1) co-administration of midazolam with morphine would exacerbate morphine tolerance and morphine-induced hyperactive behaviors, and (2) protein kinase C (PKC) would contribute to the tolerance and behavioral hyperactivity. In this study, we found that co-administration of morphine with midazolam reduced the analgesic effect of morphine and increased hyperactive behaviors such as scratching and jumping in the absence of naloxone-precipitated withdrawal. Co-administration of morphine and midazolam upregulated the expression of the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor and PKC within the spinal cord dorsal horn.

2. Results

2.1. Burn injury induced hindpaw hyperalgesia

There were no differences in baseline nociceptive threshold to thermal stimulation between sham and burn-injured groups (Fig. 1A). Burn injury induced hyperalgesia in the ipsilateral hindpaw when compared to sham injured rats (Fig. 1A). No changes in baseline nociceptive threshold in the contralateral hindpaw in burn-injured or sham rats. Moreover, in burn injured rats treated with 1 ml/kg saline (SAL–SAL), 1 ml/kg saline plus 2 mg/kg midazolam (SAL–MID), 10 mg/kg morphine plus 1 ml/kg saline (MOR–SAL), or10 mg/kg morphine plus 2 mg/kg midazolam (MOR–MID), there was no significant interaction between groups and days after burn injury ($P=0.145$), indicating that midazolam per se did not further exacerbate burn injury-induced hyperalgesia. Fig. 1B shows the time course of hyperalgesia in various groups of rats after burn injury. All groups of rats in Fig. 1B were burn-injured rats and all groups were tested on a designated day of behavioral testing before a drug administration.

2.2. Midazolam exacerbated morphine tolerance

Morphine tolerance was developed in the MOR–SAL group and further exacerbated in the MOR–MID group in burn-injured young rats over a 2-week period (Fig. 2). As shown in Table 1

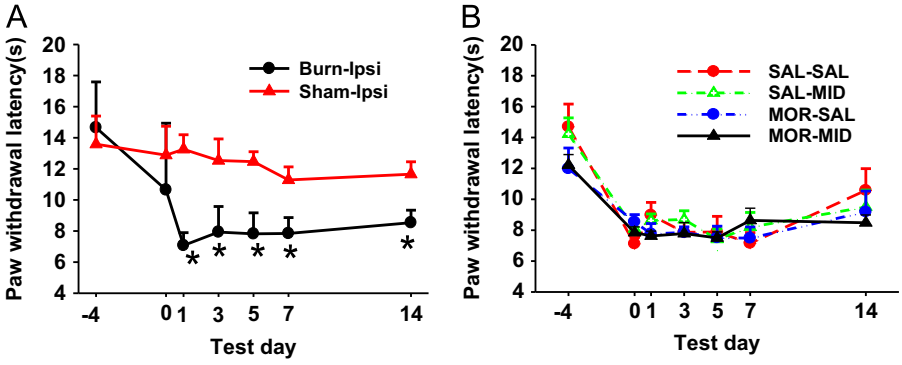

Fig. 1 – Burn injury-induced hyperalgesia. Hindpaw withdrawal latency to thermal stimulation in burn-injured young rats was determined on days-4, 0, 1, 3, 5, 7, and 14. Nociceptive threshold was decreased on the ipsilateral side of burn-injured rats. There were significant differences ($P<0.0001$) between pre-injury baseline (day-4) and the remaining time points (A). However, the ipsilateral withdrawal latency to thermal stimulation were not significantly different in all groups of burn-injured rats ($P=0.145$) treated with morphine, midazolam, or both (B). The time point day 0 (post-injury baseline, day 0) represents 3 days after burn injury but before the first morphine or midazolam administration. These behavioral tests were made before each injection on the designated days. Data are presented as mean ± SD for 6 rats per group. *$P<0.05$, as compared with the Sham–Ipsi (ipsilateral) group.