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ORIGINAL ARTICLE

# Remote intrathecal morphine preconditioning is ineffective in the presence of neuraxial blockade with lidocaine

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**Abstract** Remote intrathecal morphine preconditioning (RMPC) induces cardioprotection via a neural pathway. Intrathecal lidocaine (LID) blocks spinal cord nerve transmission. This study examine whether LID prevents the effects of RMPC. Anesthetized, open chest, male Sprague-Dawley rats were assigned to one of seven treatment groups 3 days after intrathecal catheter placement. Rats from both RMPC and LID groups, respectively, received intrathecal morphine (3 μg/kg) and lidocaine (1%, 10 μL); morphine was administered by three cycles of 5-minute infusions interspersed with 5-minute infusion-free periods. The LID + RMPC group received the combination of LID and RMPC. Intrathecal naloxone methiodide (NM) (20 μg/kg) was administered either 15 minutes before RMPC, or 5 minutes before LID + RMPC. Ischemia and reperfusion injury were then induced by 30 minutes of left coronary artery occlusion, followed by 120 minutes of reperfusion. Infarct size, as a percentage of the area at risk (AAR), was determined by 2,3,5-triphenyltetrazolium staining. The RMPC and LID groups markedly reduced the infarct size (IS) compared with controls. LID prevented the effect of RMPC. NM had no effect on control and LID + RMPC treatments. However, NM pretreatment reversed cardioprotection of RMPC treatment. Intrathecal morphine preconditioning is ineffective in the presence of neuraxial blockade with lidocaine.


Conflict of Statement: The authors have no conflicts of interest relevant to this article.

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## Introduction

Recently, several studies have shown that intrathecal or intracerebroventricular administration of morphine confers remote cardioprotection [1—4]. Lidocaine is one of the drugs which has been confirmed to be safe in humans and has the potential to protect the myocardium, not only against ischemia, but also against reperfusion injury in animal models [5,6]. However, lidocaine blocks cardioprotection produced by ischemic preconditioning [7] or sevoflurane postconditioning [8] in isolated perfused rat hearts. Blockade of spinal nerves with epidural anesthesia could attenuate myocardial apoptosis in acute myocardial ischemia and infarction in rats [9]. Addition of bupivacaine to intrathecal opioid could prolong the duration of analgesia [10]. Evidence suggests that bupivacaine potentiates the binding of morphine to opioid receptors, especially the highly dense kappa receptors, as the result of an associated conformational change in opioid receptors [11]. However, whether spinal blockade could interfere with remote morphine preconditioning has not been examined. Since intrathecal morphine, in part, utilizes a neural pathway for transmission [2], we hypothesized that spinal local anesthesia may attenuate this mode of cardioprotection. In this study, we examine whether intrathecal lidocaine (LID) prevents cardioprotective effects of remote central morphine preconditioning in rats.

## Materials and methods

This study protocol was approved by our institutional animal ethics committee and the procedures were conducted in accordance with the NIH Animal Research Advisory Committee guidelines. Male Sprague-Dawley rats, weighing between 280 and 300 g, were used for this study.

Rats were anesthetized by an intraperitoneal injection of pentobarbitone (50 mg/kg). After sterile preparation of the posterior neck, a small polyethylene-10 catheter (4 cm) (Smiths Medical International Ltd, Kent, UK) was inserted into the thoracic spinal cord through an opening in the atlanto-occipital membrane as mentioned before [12]. The wound was closed with deep sutures, followed by cutaneous interrupted sutures. After recovery, these animals were examined for gross motor or sensory deficits. Animals demonstrating any deficits were excluded from further experimentation. In addition, Evans blue dye was injected through the intrathecal catheter to determine catheter location and any damage to the spinal cord after finishing the experiment.

Three days after intrathecal catheter placement, the rats with intrathecal catheters were re-anesthetized by intraperitoneal administration of pentobarbitone (50 mg/kg) and maintained by repeat doses of 25 mg/kg every 60—90 minutes as necessary. All of the animals underwent tracheotomy and tracheal intubation. Mechanical ventilation was provided with a Harvard Apparatus Rodent Respirator (Harvard Apparatus, Boston, MA, USA), and the rats were ventilated with room air at 70—80 breaths/minute. Body temperature was monitored and maintained at 37 ± 1°C [mean ± standard deviation (SD)] using a heating pad. The right femoral artery was cannulated for direct blood pressure monitoring via a pressure transducer and the right femoral vein was cannulated for saline infusion. Subcutaneous stainless steel electrodes were connected to a PowerLab monitoring system (ML750 PowerLab/4sp with MLT0380 Reusable BP Transducer; AD Instruments, Colorado Springs, CO, USA) in order to monitor the lead II ECG and heart rate. A left thoracotomy was performed to expose the heart at the fifth intercostal space, a 6-0 Prolene loop, along with a snare occluder, was placed at the origin of the left coronary artery. Regional ischemia was induced by pulling the snare and securing the threads with a mosquito hemostat. Ischemia was confirmed by electrocardiographic changes, a substantial decrease in mean arterial pressure, and cardiac cyanosis. Rats were omitted from further data analysis if severe hypotension (arterial mean blood pressure <30 mmHg) or intractable ventricular fibrillation occurred. After surgical preparation, the rats were allowed to stabilize for 15 minutes.

Rats were randomly assigned to receive one of seven treatments (Fig. 1). All animals were subjected to 30 minutes of ischemia by occlusion of the left coronary artery, followed by 2 hours of reperfusion by release of the occlusion: the remote intrathecal morphine preconditioning (RMPC) and LID groups, respectively, received intrathecal morphine (3 μg/kg) and lidocaine (1%, 10 μL); morphine was administered by three cycles of consecutive 5-minute infusions, interspersed with 5-minute infusion-free periods. This pattern of alternating drug administration with a drug-free period was done to mimic the pattern of ischemic preconditioning. The LID + RMPC group received the combination of LID and RMPC. A nonspecific opioid-receptor antagonist, naloxone methiodide (NM), which does not cross the blood—brain barrier, was used to evaluate the involvement of opioid receptors of the spinal cord. Intrathecal NM (20 μg/kg) [13] was administered either 15 minutes before RMPC (NM + RMPC), or 5 minutes before LID + RMPC (NM + LID + RMPC). Sole administration of NM intrathecally was also performed to exclude any

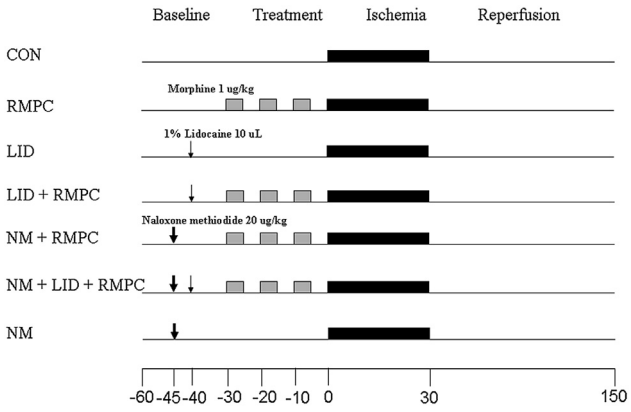


**Figure 1.** Bar graphs depicting the experimental protocol. CON = control; LID = intrathecal lidocaine (1%, 10 μL); NM = intrathecal naloxone methiodide (20 μg/kg); RMPC = three cycles of consecutive 5-minute intrathecal morphine (3 × 1 μg/kg) infusions interspersed with 5-minute infusion-free periods.

cardioprotective effect. As negative controls (CON), one group only received ischemia and reperfusion injury.

The hearts were excised and transferred to a Langendorff apparatus on completion of the reperfusion period and immediately perfused with normal saline for 1 minute, at a pressure of 100 cm $H_2O$, to flush out residual blood. The snare was securely re-tightened and 0.25% Evans blue dye was injected to stain the normally perfused region of the heart. This procedure allowed visualization of the normal, nonischemia region and the area at risk (AAR). The hearts were then frozen and cut into 2 mm slices. Thereafter, the slices were stained by incubation at 37°C for 20 minutes in 1% 2,3,5-triphenyltetrazolium in phosphate buffer at pH 7.4. This was followed by immersion in 10% formalin for 20 minutes to enhance the contrast of the stain. The areas of infarct and risk zone for each slice were traced and digitized using a computerized planimetry technique (SigmaScan 4.0, Systat Software Inc., Richmond, CA, USA). The volumes of the left ventricles, infarct size (IS), and AAR were calculated by multiplying each area with slice thickness and summing the product. The IS was expressed as a percentage of the AAR (IS/AAR) and this ratio was used to compare the differences among the groups (Fig. 2).

Data are expressed as mean ± standard deviation and data analysis was performed with a personal computer statistical software package (Prism v4.0; GraphPad Software, San Diego, CA, USA). The hemodynamic data were analyzed using two-way analysis of variance, with the Bonferroni correction applied for multiple comparisons if significant F ratios were obtained. The IS as expressed as percentage of the AAR (IS/AAR) were analyzed between groups using one-way analysis of variance, with a Student-Newman-Keuls *post hoc* test for multiple comparisons. Statistical differences were considered significant if the $p$ value was $<0.05$.

## Results

A total of 49 rats were used in the study. Four rats were excluded because of neurological damage after intrathecal catheter insertion. A further three did not complete the ischemia reperfusion protocol, because of severe hypotension or ventricular fibrillation. There was one each from the CON, LID, and LID + RMPC groups. A total of 42 rats completed the study; all had the correct position of the intrathecal catheters confirmed at necropsy.

Hemodynamic parameters including heart rate, mean arterial blood pressure, and rate-pressure product (RPP) at baseline, after treatment, 30 minutes after left coronary artery occlusion, and 2 hours after reperfusion, are shown in Table 1. The MAP and RPP were lowered in groups that received LID ($p < 0.05$ vs. CON). There were no significant differences between each of the groups when compared with the CON for each time point. As expected, there was a significant drop of MAP and RPP at 30 minutes of ischemia and 2 hours of reperfusion in all rats, confirming the successful induction of ischemia and reperfusion injury model.

The AAR ranged from 0.41 ± 0.05 $cm^3$ to 0.45 ± 0.04 $cm^3$ and there was no difference in AAR between the control and treatment groups. As shown in Table 2, the IS/AAR of CON was 52.8% ± 5.9%, RMPC and LID markedly reduced IS/AAR to 27.5% ± 3.9% and 43.6% ± 4.9%, respectively ($p < 0.05$ vs. CON). LID reversed the effect of RMPC (IS/AAR: LID + RMPC, 45.6% ± 6.0%; $p < 0.01$ vs. RMPC). NM (20 μg/kg) had no effect on CON and LID + RMPC treatment (IS/AAR: NM, 50.2% ± 5.5%, $p > 0.05$ vs. CON; NM + LID + RMPC, 42.9% ± 7.5%, $p > 0.05$ vs. LID + RMPC). However, NM could reverse the cardioprotective effect produced by RMPC treatment (IS/AAR: NM + RMPC, 51.0% ± 5.6%; $p < 0.01$ vs. RMPC).

## Discussion

The results from this study showed that RMPC and LID both produce a protective effect against myocardial ischemia and reperfusion injury. However, the combination of RMPC and LID induced a weaker cardioprotection, not showing additive effects. NM abolished the protective effect induced by RMPC, but did not alter the effect of the combination of RMPC and LID. The current results indicate that LID prevents the cardioprotective effects of RMPC.

Preconditioning with intrathecal or intracerebroventricular administration of morphine has been shown to protect the heart against ischemia and

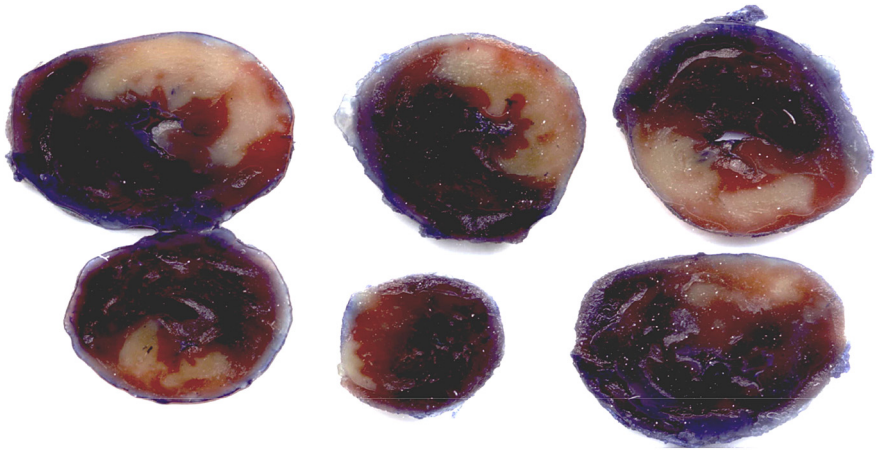

**Figure 2.** Non ischemia region = blue zone; area at risk (AAR) = red zone; infarct size (IS) = white zone.

**Table 1** Hemodynamic parameters in rats for different groups.

| Group | *n* | Baseline | Treatment | Ischemia | Reperfusion |
|---|---|---|---|---|---|
| HR | | | | | |
| CON | 6 | 395 ± 24 | 388 ± 18 | 358 ± 26 | 353 ± 22 |
| RMPC | 6 | 401 ± 18 | 387 ± 12 | 368 ± 11 | 349 ± 19 |
| LID | 6 | 394 ± 13 | 392 ± 14 | 347 ± 15 | 349 ± 17 |
| LID + RMPC | 6 | 390 ± 22 | 400 ± 11 | 357 ± 12 | 362 ± 23 |
| NM + RMPC | 6 | 387 ± 29 | 385 ± 14 | 351 ± 20 | 339 ± 30 |
| NM + LID + RMPC | 6 | 386 ± 41 | 373 ± 39 | 339 ± 37 | 335 ± 31 |
| NM | 6 | 388 ± 26 | 377 ± 23 | 351 ± 18 | 345 ± 25 |
| MAP | | | | | |
| CON | 6 | 113 ± 16 | 104 ± 17 | 83 ± 14 | 91 ± 7 |
| RMPC | 6 | 103 ± 17 | 91 ± 13 | 74 ± 12 | 87 ± 6 |
| LID | 6 | 103 ± 17 | 66 ± 12* | 72 ± 9 | 84 ± 8 |
| LID + RMPC | 6 | 106 ± 15 | 71 ± 10* | 76 ± 13 | 88 ± 11 |
| NM + RMPC | 6 | 100 ± 15 | 100 ± 20 | 71 ± 8 | 82 ± 10 |
| NM + LID + RMPC | 6 | 103 ± 23 | 63 ± 10* | 73 ± 11 | 86 ± 11 |
| NM | 6 | 95 ± 19 | 93 ± 17 | 75 ± 8 | 82 ± 9 |
| RPP | | | | | |
| CON | 6 | 45 ± 8 | 41 ± 8 | 30 ± 7 | 32 ± 2 |
| RMPC | 6 | 41 ± 7 | 35 ± 5 | 27 ± 4 | 30 ± 2 |
| LID | 6 | 40 ± 6 | 26 ± 5* | 25 ± 4 | 29 ± 3 |
| LID + RMPC | 6 | 41 ± 5 | 28 ± 4* | 27 ± 4 | 32 ± 5 |
| NM + RMPC | 6 | 39 ± 6 | 39 ± 9 | 25 ± 3 | 28 ± 4 |
| NM + LID + RMPC | 6 | 40 ± 10 | 23 ± 4* | 25 ± 5 | 29 ± 5 |
| NM | 6 | 37 ± 10 | 35 ± 9 | 26 ± 4 | 29 ± 5 |

Values are presented as mean ± SD. *$p < 0.05$ *versus* control (CON).
Baseline = 15 minutes before surgery; ischemia = 30 minutes after regional ischemia; reperfusion = 2 hours after reperfusion.
CON = control; HR = heart rate (beats per min); LID = intrathecal lidocaine (1%, 10 μL); MAP = mean arterial blood pressure (mmHg); NM = intrathecal naloxone methiodide (20 μg/kg); RPP = rate-pressure product (mmHg/minute per 1000); RMPC = three cycles of consecutive 5-minute intrathecal morphine (3 × 1 μg/kg) infusions interspersed with 5-minute infusion free periods.

reperfusion injury [1–4]. This manifestation of this myocardial adaption is named as "remote morphine preconditioning" [1,2,4]. We have previously demonstrated that the activation of spinal opioid receptors by morphine is an effective means of remotely protecting the heart [3,4]. RMPC similarly can be blocked by hexamethonium, implying that the signals are conveyed along autonomic fibers, as is the case with remote ischemic preconditioning [2]. The signals lead to the release of bradykinin and calcitonin gene related peptide (CGRP) that triggers the cascade of intracellular events that result in the cardioprotective effect [2]. The results from this study confirmed previous findings, showing that RMPC could induce cardioprotection and opioid receptors of the spinal cord are involved in this effect.

**Table 2** Morphometrics in rat hearts for different groups.

| Group | *n* | AAR ($cm^3$) | IS/AAR (%) |
|---|---|---|---|
| CON | 6 | 0.41 ± 0.05 | 52.8 ± 5.9 |
| RMPC | 6 | 0.43 ± 0.02 | 27.5 ± 3.9* |
| LID | 6 | 0.42 ± 0.06 | 43.6 ± 4.9* |
| LID + RMPC | 6 | 0.42 ± 0.07 | 45.6 ± 6.0*,# |
| NM + RMPC | 6 | 0.43 ± 0.04 | 51.0 ± 5.6# |
| NM + LID + RMPC | 6 | 0.45 ± 0.04 | 42.9 ± 7.5*,# |
| NM | 6 | 0.43 ± 0.04 | 50.2 ± 5.5 |
| *P* | | 0.932 | <0.001 |

Values are presented as mean ± SD. *$p < 0.05$ *versus* control (CON); #$p < 0.01$ *versus* RMPC.
AAR = area at risk; CON = control; IS = infarct size; LID = intrathecal lidocaine (1%, 10 μL); NM = intrathecal naloxone methiodide (20 μg/kg); RMPC = three cycles of consecutive 5-minute intrathecal morphine (3 × 1 μg/kg) infusions interspersed with 5-minute infusion-free periods.

Lidocaine is often used for the treatment of ventricular arrhythmias. It has been demonstrated that lidocaine could protect the heart against ischemia injury [5,6]. Hine et al. [14] showed that prophylactic lidocaine administration might increase mortality, due to pump failure or asystole. A randomized, controlled clinical trial has demonstrated that the addition of thoracic epidural to conventional general anesthesia in patients undergoing off-pump coronary artery bypass graft surgery, accounts for a significant reduction in the incidence of postoperative arrhythmias and improvement in overall quality of recovery [15]. LID prevents cardiovascular collapse in a rat model of acute intracranial hypertension [16]. It was reported that thoracic epidural anesthesia could reduce myocardial infarcted size after coronary artery occlusion in dogs [17]. Blockade of spinal nerves could result in the reduction of peripheral resistance of circulation, which may help to improve coronary circulation and attenuate myocardial apoptosis in acute myocardial ischemia and infarction in rats [9]. In this study, we showed that LID also could produce a protective effect.

The opening of $K_{ATP}$ channels has been shown to be an important component of preconditioning, which is the most potent mechanism of protection against myocardial ischemia and reperfusion injury [18]. Thus, ischemic preconditioning and RMPC may share the opening of $K_{ATP}$ channels during cardioprotection [2]. Olschewski et al. [19] found that lidocaine blocked the $K_{ATP}$ channel of rat cardiomyocytes. This probably is the main reason why lidocaine prevents the effects of ischemic preconditioning or sevoflurane postconditioning [7,8].

Interestingly, in this study we observed the preservation of the weaker cardioprotective effect of RMPC, despite the administration of lidocaine. NM did not abolish the effect of the combination of RMPC and lidocaine. This suggests that opioid receptors are not involved in this effect, and that LID prevents activation of spinal cord opioid receptors induced by RMPC.

There are several limitations to this study. First, we did not explain that the specific mechanism underlining the effect of LID prevents the cardioprotective effects of RMPC. Second, lidocaine could cancel cardioprotection produced by ischemic preconditioning in isolated perfused rat hearts [7]. Intrathecal morphine remotely preconditions the heart via a neural pathway [2]. Therefore, we did not show if intravenous lidocaine prevents the effects induced by RMPC in this study. Lastly, whether a higher dose of NM could also abolish the poorer cardioprotective effects of the combination of RMPC and LID was not examined in this study and remains to be demonstrated.

In summary, our findings have demonstrated that LID and intrathecal remote morphine preconditioning both produce protective effects against myocardial ischemia and reperfusion injury in rats. It was further shown that intrathecal morphine preconditioning is ineffective in the presence of neuraxial blockade with lidocaine. These findings strongly remind us that the postoperative analgesia model of simultaneously administered local anesthetic with opioids intrathecally should be cautiously used in patients with ischemia heart disease undergoing surgery.

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