Enhanced antinociceptive efficacy of epidural compared with i.v. methadone in a rat model of thermal nociception

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Editor’s key points
- Methadone has several properties that might favour epidural administration for analgesia.
- The analgesic and pharmacokinetic properties of methadone given by i.v. or epidural routes were compared in a rat model of thermal nociception.
- Although pharmacokinetically similar, epidural methadone was a more effective analgesic than i.v. methadone.

Background. The properties of methadone suggest a potential advantage for epidural over i.v. administration for pain relief, but little supportive evidence exists.

Methods. To investigate the pharmacokinetic and the pharmacodynamic properties of epidural and i.v. methadone, four doses of methadone (0.1, 0.25, 0.5, and 0.75 mg kg⁻¹) were investigated by each route in a rat model. The tail-flick and hot water tail immersion test were used for thermal nociception. The magnitude of antinociceptive efficacy was expressed as per cent maximal possible effect (%MPE) of tail withdrawal latency, and the area under the %MPE vs time curve indicated the cumulative antinociceptive effect. A pharmacokinetic model describing the disposition and elimination of methadone was established.

Results. The pharmacokinetic profiles of methadone were not significantly different after epidural and i.v. administration. A two-compartment model with saturable elimination provided a good fit of the experimental data. At equivalent doses, epidural methadone produced higher cumulative antinociceptive effect in both thermal models. Supraspinal opioid effect, assessed by pinna reflex presence, was significantly lower with epidural methadone at equivalent doses. The duration of antinociceptive effect was longer with epidural administration of 0.5 and 0.75 mg kg⁻¹ doses.

Conclusions. Epidural administration of methadone in rats resulted in systemic exposure similar to that after i.v. administration, but improved thermal antinociceptive efficacy, and reduced supraspinal undesired effects. The findings suggest the presence of local effect at the spinal cord level, in addition to the systemic effect produced by epidural methadone.

Keywords: analgesia, epidural; methadone; models, animal; pharmacokinetics

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Acute postoperative pain is a seriously undertreated condition that can be associated with increased morbidity and even mortality. Opioids are the most commonly administered analgesics in this setting. Their epidural administration has shown advantages over the i.v. route in managing acute pain after several types of surgery. The proposed mechanism of superior pain relief and favourable adverse effect profile is by direct action at spinal opioid receptors.

Methadone is a synthetic opioid, mainly used orally to manage heroin dependence and chronic pain. Its long half-life, negative association with drug abuse, and potential for accumulation after repetitive systemic use frequently hinder its clinical utility. Despite its lack of widespread popularity, it is frequently administered epidurally at Hadassah-Hebrew University Medical Center for postoperative pain relief.

Methadone is an intermediately lipophilic opioid (log P 3.9–4.2) but has the highest dissociation constant among opioids (pKa 8.2 – 9.2) and is more than 90% ionized at physiological pH. These properties, supported by some animal data, hypothetically provide an advantage for epidural administration of methadone in terms of low potential for cephalad migration in the cerebrospinal fluid (CSF) and increased residence time in the epidural space.

The clinical efficacy of epidural methadone in acute postoperative pain has been demonstrated. However, results are contradictory regarding its systemic contribution...
Comparison of epidural and i.v. methadone

Methods

Animals and surgery

All surgical and experimental procedures were approved by the Animal Experimentation Ethics Committee of The Hebrew University Hadassah Medical School, Jerusalem. Male Sprague–Dawley rats (Harlan, Israel) were used. Before surgery, rats were kept at 12-h light/dark cycle with ad libitum access to food and water. On each of 2 days before surgery, the rats were habituated for handling and thermal nociception testing. Surgical anesthesia consisted of 1 ml kg$^{-1}$ of ketamine 20 mg ml$^{-1}$ (Fort Dodge, IA, USA): xylazine 100 mg ml$^{-1}$ (90:10 v/v) by intra-peritoneal injection, maintained by pure ketamine as needed.

A catheter of polyethylene tubing (PE50, Intramedic®, Becton Dickinson, MD, USA) was placed in the right jugular vein of all rats to allow drug administration and blood sampling. An epidural catheter was placed in half of the animals in the same anaesthesia session. Briefly, the dorsal thoracolumbar spinal region was clipped and sterilized with isopropanol. A 1–2 cm midline skin incision was made caudal to the T13 spinous process. Muscles were bluntly dissected from the vertebrae and retracted to expose the L1–L2 intervertebral space. The intervertebral ligament was carefully cut and an epidural catheter (a 14 cm polyethylene tube (PE-10; Braintree Scientific, Braintree, MA, USA)) was inserted into the epidural space 1.5 cm caudally. To reduce the possibility of catheter migration, two knots were tied on the catheter with 1 cm of PE-50 tubing, and on top of it, with 3 mm wide Silastic® tubing (Dow Corning, Midland, MI, USA). The first knot was made 2.0 cm from the caudal catheter tip, and was sutured to the paravertebral muscles between the L1 and L2 vertebrae. The second knot was made 9.0 cm distal to the first knot and was sutured at the dorsal neck catheter exteriorization site. The dead space in the catheter was 8 ± 1 μl. No hole or groove in the bone was made and no bone was dissected. The catheter tip was located at the L3–L4 level. Fluid-free aspiration ensured that the catheter placement was not intrathecal or intravascular. The catheter was flushed with 20 μl of sterile saline and plugged with a 28G steel wire. The muscles and fascia were sutured, and then the skin was closed using stainless steel wound clips. Both i.v. and epidural catheters were tunnelled subcutaneously and were exteriorized at the dorsal. After the surgery, animals were transferred to individual cages with ad libitum access to food and water to recover for 24 h. Eight and 24 h after epidural catheter placement surgery, animals were evaluated for sensory and motor function of the hind limbs, and thermal latencies of the tail. Only rats with no motor or sensory disturbance on the first postoperative day (compared with preoperative values) were used.

Experimental procedure

Experiments were initiated 26–28 h after catheter placement to prevent fibrotic tissue formation around the catheter tip. Racemic methadone hydrochloride (Rafa Laboratories, Jerusalem, Israel) was dissolved in normal saline for epidural or i.v. administration. Each animal received a single dose of methadone by either i.v. or epidural routes at a dose of 0.1, 0.25, 0.5, or 0.75 mg kg$^{-1}$. For i.v. administration, methadone was diluted to a final volume of 100–200 μl. For epidural administration, methadone was diluted to a final volume of 25–50 μl. In the i.v. control group, 200 μl normal saline was administered via the i.v. catheter, and in the epidural control group, 50 μl of normal saline was administered via the epidural catheter.

Serial blood samples (220 μl), with immediate 100 μl i.v. fluid replacement with normal saline, were collected into heparinized tubes (15 U ml$^{-1}$) at: 0, 2, 8, 15, 30, 60, 90, 120, 150, and 180 min after i.v. administration, and 0, 2, 8, 15, 30, 60, 90, 120, 180, and 240 min after epidural administration of methadone. Plasma was separated by centrifugation (4000g for 5 min at 4°C) and stored at −20°C for analysis.

To verify the location of the epidural catheter, at the end of the experimental procedures 150 μl of lidocaine 2% solution (Rafa Laboratories) was injected to the epidural catheter. Three types of responses were obtained after lidocaine injection: (i) lack of sensory or motor deficit indicated subcutaneous location of the catheter. Post-mortem examination of these animals confirmed epidural catheter migration to the subcutaneous tissue. (ii) Sensory and motor deficit starting with hind limbs and progressing to forelimbs, with substantial systemic response (e.g. generalized seizures or cardiac arrest) indicated intrathecal location of the catheter. (iii) Transient sensory and motor deficit of hind limbs, with normal motor function of forelimbs and with return to full sensory and motor function within 30 min indicated epidural location of the catheter. Only data obtained from the latter group were included.

At the end of the study, all animals were killed with an overdose of i.v. pentobarbital (100 mg kg$^{-1}$) (pentobarbital sodium 200 mg ml$^{-1}$, CTS, Israel) and the position of the epidural catheter tip was verified visually.

Assessment of nociceptive response

The effect of methadone after administration by each route was investigated using two thermal nociception methods: hot water tail immersion test (by immersing the distal 2 cm of the tail in 49°C water) and by tail-flick test (Tail Flick Analgesia Monitor, Accuscan Instruments, OH, USA). With both methods, the time to tail-withdrawal (in seconds) was assessed as a measure of nociception. In order to control for inter-animal differences in thermal nociception models, antinociception was expressed as the per cent of maximal possible effect (%MPE) over time, calculated for each dose and time point.

%MPE = \frac{\text{measured latency} - \text{baseline latency}}{\text{cutoff latency} - \text{baseline latency}} \times 100\% \quad (1)
Latency was determined from an average of three measurements. To minimize the possibility of thermal injury, the cut-off latency for hot water immersion was 20 s and the cut-off latency for tail-flick was 15 s.

Supraspinal effects

Pinna reflex (by alternatively touching the auditory meatus to elicit a head shake) and corneal reflex (by light alternative touching of both corneas with a blunt paper instrument to elicit an eye blink) were assessed at 3, 6, and 9 min after methadone administration. The absence of the reflexes was indicative of undesired opioid supraspinal effects.21–23

Methadone analysis

Determination of methadone plasma concentration was performed using a Waters Millennium HPLC-MS equipped with Micromass QZ detector, Waters 600 Controller gradient pump, Waters 717 auto-sampler, and Xterra® MS C8 column (3.5 μm, 2.1 × 100 mm, Waters, Ireland). The mobile phase consisted of methanol–water (35:65) containing 0.1% (v/v) formic acid, and the flow was 0.3 ml min⁻¹. Solvents were purchased from J.T. Baker, USA. Nitrogen flow was 500 litre h⁻¹, source temperature was 400°C, the cone voltage was 12 V. Retention times for methadone and internal standard midazolam (Sigma, St Louis, MO, USA) were 7.2 and 3.1 min. The quantification range for methadone was 5–500 ng ml⁻¹.

Pharmacokinetic and pharmacodynamic data analysis

The concentration–time data for methadone after i.v. and epidural administration were analysed by a non-compartmental approach. Terminal half-life, area under the concentration–time curve from zero to infinity (AUC), volume of distribution at steady state (Vss), clearance (Cl), and mean residence time (MRT) were calculated for individual concentration–time profiles. To obtain a measure for cumulative antinociceptive effect over time, the area under the concentration–time curve (AUEC) for each animal was calculated using the trapezoidal rule (without extrapolation to infinite time).24 To further investigate pharmacokinetic–pharmacodynamic (PK–PD) relationships, a model-based approach was utilized. Initially, the PK model describing the disposition and elimination of methadone after i.v. administration was established; then, the model was extended to describe the tail-flick antinociceptive effect for i.v. administration. As the PK profiles after epidural administration were not significantly different from those obtained after i.v. injection (see Results), a separate PK model for epidural data was not developed. The PD model for epidural methadone included two sites of action. Finally, i.v. concentration–time data (three dose levels) were co-modelled with tail-flick data after both i.v. and epidural routes of administration (four dose levels), and all model parameters were estimated simultaneously. The following equations were used to describe the model:

\[
\frac{dC_p}{dt} = -k_{pt} \cdot C_p + k_{tp} \cdot \frac{A_t}{V_c} \cdot \frac{V_{max} \cdot C_p}{K_m + C_p} \tag{2}
\]

\[
\frac{dA_t}{dt} = k_{pt} \cdot C \cdot \frac{V_{max} \cdot C_p}{K_m + C_p} - k_{tp} \cdot A_t \tag{3}
\]

\[
E_{IV} = e \cdot C_p \tag{4}
\]

\[
E_{total}^{epi} = e \cdot C_p + \frac{E_{max} \cdot C_p}{EC_{50} + C_p} \tag{5}
\]

where \(C_p\) represents methadone concentration at the central distribution compartment, \(A_t\) is the amount of the drug at the peripheral compartment, and \(k_{pt}\) and \(k_{tp}\) are the corresponding first-order distribution rate constants. The effect after i.v. administration \((E_{IV})\) is proportional to concentration in the central compartment. Total drug effect after epidural administration \((E_{total}^{epi})\) is composed of the effect achieved due to systemic uptake \((e)\) and the effect achieved at the spinal level \((E_{max} \text{ and } EC_{50})\). Model fitting and parameter estimation were performed using MATLAB R2009a (The MathWorks, Natick, MA, USA).

Statistics

Comparisons between cumulative antinociceptive effects and areas under concentration–time curve (AUCs) of i.v. vs epidural methadone in same dose groups were carried out using Student’s t-test. Intergroup PD differences among different doses of same route of methadone administration were tested using one-way analysis of variance (ANOVA), with pairwise multiple comparison procedures performed using the Holm–Sidak method. The comparison between binary outcome of maximum antinociceptive effect at 1 between epidural and i.v. methadone administration was performed using Fisher’s exact test. Plasma concentrations of epidural vs i.v. methadone at each time point (per dose) were compared by Student’s test with Bonferroni correction for multiple comparisons. Statistical analysis was performed using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA), with differences being considered significant at \(P \leq 0.05\).

Results

Animals

The mean (SD) weight of the animals before surgery was 309 (37) g, and 24 h after surgery was 290 (35) g (\(n = 56\)). Three animals did not return to baseline sensory and motor function of the hind limbs 8 h after surgery. The epidural catheter was found in the subcutaneous tissue in three animals and in the intrathecal space in four animals. Data from 46 animals were included in the analysis. Baseline tail flick and hot water tail immersion latencies were not different before and 24 h after catheter placement.
Methadone pharmacokinetics

Mean serum concentration–time profiles of methadone after i.v. (0.25, 0.5, and 0.75 mg kg\(^{-1}\)) and epidural (0.1, 0.25, 0.5, and 0.75 mg kg\(^{-1}\)) administration are presented in Figure 1. The AUCs were not significantly different between the two routes of administration at equivalent doses. Plasma concentrations at each time point were not different between epidural and i.v. administration, with the exception of one time point (2 min 0.5 mg kg\(^{-1}\) dose). The corresponding PK parameters obtained by non-compartmental analysis are listed in Table 1. Following i.v. administration, profiles demonstrated a polyexponential decline. Highest plasma concentrations were observed at 2 min with both administration routes. The dose-normalized curves were not superimposable; therefore, a model with linear elimination rate could not capture methadone pharmacokinetics. Alternatively, a two-compartment model with a saturable Michaelis–Menten type of elimination provided a good fit of the experimental data (Fig. 1, solid line and Table 2).

**Table 1** Mean (so) pharmacokinetic parameters of methadone after i.v. and epidural administration to rats calculated by non-compartmental analysis. AUC, area under the plasma concentration–time curve; CL, clearance; \(T_{1/2}\), terminal half-life; MRT, mean residence time; \(V_{SS}\), steady-state volume of distribution; NC, not calculated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>I.V. dose</th>
<th>Epidural dose</th>
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<tr>
<td></td>
<td></td>
<td>0.25 mg kg(^{-1})</td>
<td>0.5 mg kg(^{-1})</td>
</tr>
<tr>
<td>AUC</td>
<td>ng min ml(^{-1})</td>
<td>2116 (886)</td>
<td>5735 (1051)</td>
</tr>
<tr>
<td>CL</td>
<td>ml min(^{-1}) kg(^{-1})</td>
<td>131 (41)</td>
<td>89 (15)</td>
</tr>
<tr>
<td>(V_{SS})</td>
<td>ml kg(^{-1})</td>
<td>8711 (3596)</td>
<td>9643 (3731)</td>
</tr>
<tr>
<td>(T_{1/2})</td>
<td>min</td>
<td>53 (14)</td>
<td>85 (35)</td>
</tr>
<tr>
<td>MRT</td>
<td>min</td>
<td>66 (13)</td>
<td>109 (38)</td>
</tr>
</tbody>
</table>

Fig 1 Time course of plasma methadone concentrations after i.v. (open circle) and epidural (filled circle) administration to rats (mean and SEM). Solid lines indicate model fitted profiles.
Mean (SD) baseline tail-flick and hot water tail immersion test latencies were 7.1 (1.0) and 9.2 (2.2) s, immediately before administration of the drug. In control groups, administration of normal saline via the epidural (n = 4) or i.v. (n = 4) catheter did not change baseline latency values for either test over the 180 min period.

Methadone pharmacodynamics showed high intragroup variability. In both epidural and i.v. groups, the onset of antinociception was fast and the maximal effect was observed at the first measurement (in a majority of animals). The antinociceptive effect after epidural administration was stronger than with i.v. administration, except for the lowest dose of 0.1 mg kg\(^{-1}\), at which the effect was very mild. Mean %MPE data vs time for the tail-flick test for both routes of administration and four doses is shown in Figure 2. Experimental data were reasonably captured by the proposed model structure (Fig. 2) and parameters were estimated with good precision.

### Table 2: Final pharmacokinetic and pharmacodynamic model-estimated parameters. %CV, coefficient of variation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Estimate</th>
<th>%CV</th>
</tr>
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<tbody>
<tr>
<td>( V_c ) (volume of the central compartment)</td>
<td>litre kg(^{-1})</td>
<td>3.53</td>
<td>11</td>
</tr>
<tr>
<td>( k_{pl} ) (first-order distribution rate constant, central to peripheral compartment)</td>
<td>min(^{-1})</td>
<td>0.0697</td>
<td>29</td>
</tr>
<tr>
<td>( k_{tp} ) (first-order distribution rate constant, peripheral to central compartment)</td>
<td>min(^{-1})</td>
<td>0.0488</td>
<td>20</td>
</tr>
<tr>
<td>( V_{max} ) (maximum elimination rate)</td>
<td>( \mu ) litre(^{-1}) min(^{-1})</td>
<td>1.87</td>
<td>26</td>
</tr>
<tr>
<td>( K_m ) (Michaelis–Menten constant)</td>
<td>( \mu ) litre(^{-1})</td>
<td>47.7</td>
<td>35</td>
</tr>
<tr>
<td>( \varepsilon ) (systemic drug effect constant)</td>
<td>% litre ( \mu )g(^{-1})</td>
<td>0.849</td>
<td>9</td>
</tr>
<tr>
<td>( E_{max} ) (maximum effect at the spinal level)</td>
<td>%</td>
<td>38.8</td>
<td>30</td>
</tr>
<tr>
<td>( EC_{50} ) (concentration to achieve 50% of effect at the spinal level)</td>
<td>( \mu ) litre(^{-1})</td>
<td>8.70</td>
<td>57</td>
</tr>
</tbody>
</table>

![Fig 2](image_url)  
Time course of antinociceptive effect (tail-flick test) after i.v. (open circle and solid lines) and epidural (filled circle and dashed lines) administration to rats. Symbols represent mean (SEM) observed data, and lines are model fitted profiles.
The duration of antinociceptive effect was longer with epidural administration of methadone. Sixty minutes after methadone administration, the percentage of rats having maximum antinociceptive effect (100% MPE) was higher after epidural administration of 0.5 mg kg$^{-1}$ (50% vs 0% of animals, $P=0.05$), and 0.75 mg kg$^{-1}$ (83% vs 0% of animals, $P=0.015$).

**Methadone supraspinal effects**

Corneal reflex was temporarily absent only at 0.75 mg kg$^{-1}$ i.v. dose. It was present at all time-points with all epidural doses. The absence of pinna reflex was observed in the majority of animals that received i.v. methadone at 0.25 mg kg$^{-1}$ or higher, indicating a certain degree of supraspinal methadone effect. With epidural administration, with the exception of one animal in the 0.5 mg kg$^{-1}$ group, pinna reflex was present at all time-points in all dose groups (Table 3).

**Discussion**

The results demonstrate an advantage of epidural compared with i.v. administration of methadone in terms of improved antinociceptive efficacy and reduced undesired supraspinal opioid effects in this model.

The plasma concentration profiles of methadone obtained after epidural administration were not different from those of i.v. administration. Similar findings have previously been reported for other lipophilic opioids. The epidural space is rich in blood vessels, and methadone might undergo rapid absorption and distribution to the systemic circulation. The dose–response curves obtained with each of the thermal nociception models show separation of the PD effect at doses ≥ 0.25 mg kg$^{-1}$, suggesting that antinociception after epidural administration of methadone is beyond that contributed by its systemic effect.

The magnitude and duration of the analgesic and toxic effects of epidural opioids are difficult to predict based solely on physicochemical properties. While lipophilicity and
ionization usually predict a molecule’s ability to cross biological membranes, these parameters, together with molecular mass, volume, and surface area, are not linearly correlated to meningeal permeability. The spinal effect of epidural opioids is mediated by local administration at the proximity to the spinal cord, and by the supraspinal effects achieved by distribution to the brainstem and brain by cephalad CSF spread and systemic circulation. Many opioids including morphine, hydromorphone, fentanyl and alfentanil appear in the cerebello-medullary system after lumbar epidural or intrathecal administration. With epidural methadone, on the contrary, cephalad spread is unlikely, as methadone is not detectable in the CSF at cervical or intracerebroventricular levels after lumbar intrathecal or epidural administration.

Systemic administration of opioids result mainly in supraspinal sites of action, but local action at the spinal site after neuraxial administration is sufficient to provide complete antinociception. Independent spinal and supraspinal administration of opioids provide synergistic antinociceptive effect, considering negligible rostral spread of methadone after lumbar epidural administration, the supraspinal effect achieved from systemic methadone distribution after epidural injection could potentiate the local spinal effect and result in the enhanced antinociception we observed with the epidural administration.

Despite similar PK profile, we observed reduced supraspinal effects with epidural methadone which provides further indirect evidence for the lack of cephalad spread. This finding was consistently observed at doses above 0.1 mg kg⁻¹. Similar findings of differences in corneal and pinna reflexes were reported for i.v. and epidural sufentanil. We suggest that i.v. bolus administration of lipophilic methadone, especially into the jugular vein, produces a peak brain concentration, higher than after epidural administration despite similar plasma concentration measured 2 min after injection. This is supported by ~50% higher plasma concentration of methadone measured at 1 min compared with 2 min after i.v. injection in rats. An absorption phase from the epidural space to the systemic circulation might have caused lower initial plasma and, therefore, brain concentrations of methadone during the first 2 min after the injection, leading to less supraspinal effect. The absorption phase might also explain the finding of higher plasma concentration of methadone 2 min after the epidural 0.5 mg kg⁻¹ dose, which might have coincided with the peak plasma concentration. However, this hypothesis will require further investigation with more frequent initial blood sampling.

A PK–PD modelling approach was used to further evaluate the experimental findings. Initially, the PK model included a separate epidural compartment, but due to a very fast absorption and lack of the absorption phase in the PK profiles, the absorption rate constant could not be estimated. To describe the

| Table 3 | Presence of pinna and corneal reflexes after methadone administration. Epi, epidural; I.V., intravenous |
|---|---|---|---|---|---|---|---|
| Methadone dose | 0.1 mg kg⁻¹ | 0.25 mg kg⁻¹ | 0.5 mg kg⁻¹ | 0.75 mg kg⁻¹ |
| Time (min) | I.V. Epi | I.V. Epi | I.V. Epi | I.V. Epi | I.V. Epi | I.V. Epi | I.V. Epi |
| 3 | 0 0 0 0 | 75 0 0 0 | 66 10 0 0 | 83 0 66 0 |
| 6 | 0 0 0 0 | 50 0 0 0 | 100 10 0 0 | 83 0 66 0 |
| 9 | 0 0 0 0 | 0 0 0 0 | 100 10 0 0 | 17 0 0 0 |
effect at the spinal level, a biophase model was evaluated; however, it provided a fit that was similar to the fit shown in Results. For simplicity, in the final PD model, the effect from ‘central’ and ‘spinal’ sites of action was driven by the plasma concentration. This model provided a reasonable description of the experimental data after i.v. and epidural administration. A more mechanistic model might be proposed to further distinguish the relative contribution from these two sites, but it would require measurement of drug concentration in the epidural space. The CSF clearance of methadone in humans is 10–20 times more rapid than its clearance from plasma, but however, single epidural and i.v. administration result in comparable duration of analgesia of 4–6 h. A more detailed preclinical investigation of epidural and CSF clearance to evaluate whether this ratio between clearances is preserved across species will allow better translation between the pre-clinical and clinical models.

We used racemic (DL) methadone in this study. L-Methadone is an opioid agonist, whereas D-methadone has lower affinity for opioid receptors. Both isomers exhibit N-methyl-D-aspartate (NMDA)-type glutamate receptor binding. The debate over the role of NMDA antagonism as a clinically relevant antinociceptive mechanism of methadone is ongoing, but our model of thermal nociception would capture only opioid-receptor-mediated effects of methadone. Future research with antinociceptive models of nerve injury or formalin injection might reveal additional NMDA-receptor-mediated antinociceptive effects of methadone.

Potential limitations of our study include our inability to perform a priori sample size calculation to determine the optimal group size to detect differences in plasma concentrations between the two routes of administration, since no pharmacokinetic data on epidural methadone were available in rats. In addition, our blood sampling schedule did not capture the PK profile of methadone in the first 2 min after administration, which potentially could be different between the two administration routes due to a rapid redistribution phase after i.v. and absorption phase after epidural administration. Few studies have investigated epidural methadone clinically. Prieto-Alvarez and colleagues reported effective postoperative analgesia with epidural methadone, without accumulation in plasma for 72 h after operation in abdominal and lower-limb surgery. Shir and colleagues also demonstrated good postoperative pain relief, but reported increasing plasma concentrations after continuous epidural methadone administration, reaching those obtained with systemic dosing. Epidural methadone was as effective as epidural fentanyl after abdominal surgery and superior to epidural morphine for Caesarean section in terms of analgesia or urinary retention and need for bladder catheterization. Based on currently available clinical trials, it is difficult to draw conclusions on the clinical utility of epidural methadone. It is especially challenging to compare its efficacy and safety to other opioids, because studies have utilized different dose regimens of epidural methadone, and some of the differences might be accounted for by the use of non-equianalgesic dose ratios. Methadone by the epidural route is not approved by most regulatory agencies, including the US FDA. Its clinical use is limited, and despite a few studies reporting long-term use of neuraxial methadone, initial data in dogs suggest that methadone, like other NMDA antagonists, can cause spinal cord pathology after long-term intrathecal infusion. More formal safety data on epidural methadone might be required before further clinical studies are performed.

The findings of comparable plasma concentrations and the advantage in antinociceptive and supraspinal effect profiles of epidural methadone in this study, combined with its physicochemical properties and evidence supporting the lack of cephalad spread in the CSF after epidural administration, make methadone a particularly interesting molecule warranting further preclinical and clinical investigation in the setting of postoperative epidural analgesia.

Authors’ contributions
S.H., I.Y.-D., E.D., A.H. designed the study, S.H., I.Y.-D., Y.R. conducted the study, S.H., L.K., Y.I. analysed the results, and S.H., L.K., E.D., Y.R., A.H. wrote the manuscript. All authors have seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Declaration of interest
None declared.

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References
18 Durant PA, Yaksh TL. Epidural injections of bupivacaine, morphine, and suxamethonium into the intrathecal gray matter: Advances in Pain Research and Therapy 1986; 19: 832–6


20 Hammond DL. Intrathecal administration of analgesics. Anesthesiology 1986; 64: 43–53


27 Bernards CM, Hill HF. Morphine and alfentanil permeability through the spinal dura, arachnoid, and pia mater of dogs and monkeys. Anesthesiology 1990; 73: 1214–9

28 Bernards CM, Hill HF. Physical and chemical properties of drug molecules governing their diffusion through the spinal meninges. Anesthesiology 1992; 77: 750–6


31 Yeung JC, Rudy TA. Multiplicative interaction between narcotic antagonists expressed at spinal and supraspinal sites of antinociceptive action as revealed by concurrent intrathecal and intracerebroventricular injections of morphine. J Pharmacol Exp Ther 1980; 215: 626–32


37 Chaplan SR, McLainberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allosmia in the rat. J Pharmacol Exp Ther 1997; 280: 829–38


43 Eisenach JC, Schafer SL, Yaksh T. The need for a journal policy on intrathecal, epidural, and perineural administration of non-approved drugs. Pain 2010; 149: 417–9

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