**ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine with opioids for Caesarean delivery**

L. Bouvet1*, X. Da-Col2, D. Chassard2, F. Dalèry2, L. Ruynat2, B. Allaouchiche1, E. Dantony3,4 and E. Boselli1

1 Department of Anaesthesia and Intensive Care, Édouard Herriot Hospital, Hospices Civils de Lyon, 2 Department of Anaesthesia and Intensive Care, Femme Mère Enfant Hospital, Hospices Civils de Lyon, 3 Department of Biostatistics, Hospices Civils de Lyon and 4 CNRS UMR 5558, Pierre-Bénite, University of Lyon, Claude Bernard Lyon 1, Lyon, France

* Corresponding author: Service d’Anesthésie Réanimation, Hôpital Édouard Herriot, 5 Place d’Arsonval, Lyon 69003, France. E-mail: lionel.bouvet@chu-lyon.fr

**Editor’s key points**

- This study was conducted to determine the ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine, combined with intrathecal sufentanil and morphine, for Caesarean delivery anaesthesia.
- The ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine combined with intrathecal morphine and sufentanil were 6.2 mg and 12.9 mg, respectively.
- A combined spinal–epidural technique should be used when doses of levobupivacaine less than the ED$_{95}$ are administered.

**Background.** This prospective randomized double-blind dose–response study aimed to determine the ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine combined with morphine and sufentanil for elective Caesarean delivery.

**Methods.** Parturients undergoing elective Caesarean delivery were included and allocated to five levobupivacaine dose groups (6, 8, 10, 12, or 14 mg). Combined spinal–epidural (CSE) anaesthesia was performed, allowing intrathecal administration of the allocated dose of levobupivacaine with intrathecal morphine 100 µg and intrathecal sufentanil 2.5 µg, and insertion of epidural catheter for completing anaesthesia in the case of failure. The dose was considered as successful if a bilateral T6 sensory block to pinprick occurred in 15 min and if no epidural supplement was required during surgery. A probit regression analysis was performed to calculate the ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine for Caesarean delivery.

**Results.** Eighty-five parturients were included. A block to T6 sensory level was reached in 15 min for most of the patients. The ED$_{50}$ and ED$_{95}$ of levobupivacaine were 6.2 mg (95% CI: 2.6–7.6) and 12.9 mg (11.1–17.9), respectively. Haemodynamic stability and the rate of nausea and vomiting were similar among groups. Greater doses of levobupivacaine were associated with increased motor block duration.

**Conclusions.** When combined with intrathecal sufentanil 2.5 µg and intrathecal morphine 100 µg, the ED$_{95}$ of intrathecal levobupivacaine is 12.9 mg for Caesarean delivery. If doses of levobupivacaine less than the ED$_{95}$, particularly near the ED$_{50}$, are used, these doses should be administered under a CSE technique.

**Keywords:** Caesarean; effective dose; levobupivacaine; spinal anaesthesia

Accepted for publication: 13 August 2010

Levobupivacaine, the pure $S$(-) enantiomer of racemic bupivacaine, was introduced for obstetric spinal and epidural anaesthesia during the last decade.1 It was assessed for intrathecal and epidural labour analgesia and was reported to be less potent than bupivacaine, but more potent than ropivacaine.2 3 However, other authors reported that intrathecal levobupivacaine had a similar clinical profile to racemic bupivacaine for intrathecal labour analgesia, but caused less motor block at similar doses.4

The efficacy of neuraxial local anaesthetic is enhanced by the addition of intrathecal opioids.5 6 The effective doses providing 50% (ED$_{50}$) and 95% (ED$_{95}$) success of ropivacaine, isobaric and hyperbaric bupivacaine in combination with opioids, for Caesarean delivery, have been investigated previously.7-9 When administered for Caesarean delivery, intrathecal levobupivacaine seems to have a lower potency for motor block than bupivacaine,10-12 but the ideal dose of intrathecal levobupivacaine for Caesarean delivery remains uncertain.

One previous study calculated the minimum local anaesthetic dose (MLAD), corresponding to the median ED$_{50}$, of intrathecal plain levobupivacaine,13 and more recently, another study determined the ED$_{50}$ of intrathecal levobupivacaine combined with sufentanil.14 To date, the more clinically relevant ED$_{95}$ of intrathecal levobupivacaine co-administered with opioids for Caesarean delivery is unknown.

The aims of this randomized, double-blind, prospective dose–response study were to determine the ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine, when co-administered with intrathecal sufentanil 2.5 µg and intrathecal morphine 100 µg, for Caesarean delivery anaesthesia.

© The Author [2010]. Published by Oxford University Press on behalf of the British Journal of Anaesthesia. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
Methods

Design

We designed a prospective, randomized, double-blind predetermined multiple dose study to determine the ED50 and ED95 of intrathecal levobupivacaine for Caesarean delivery.

Subjects and setting

Healthy, term parturients presenting for elective Caesarean delivery were enrolled in this study. The study was conducted at the Femme Mère Enfant Hospital (Lyon, France). Parturients were enrolled over an 8 month period. Institutional review board approval given for the study and signed informed consent was obtained from all subjects.

Inclusion criteria were ASA physical status class I or II, age between 18 and 45 yr, weight <110 kg, height >150 cm, singleton pregnancy, gestational age of more than 37 completed weeks, and scheduled elective Caesarean delivery. Exclusion criteria were active labour, ruptured membranes, diabetes or gestational diabetes, three or more previous Caesarean deliveries, pregnancy-induced hypertension or pre-eclampsia, intrauterine growth retardation, placenta praevia, and congenital anomaly.

Study protocol

All Caesarean sections were performed in the morning, between 8 a.m. and 1 p.m. All parturients were premedicated with hydroxyzine 50 mg and cimetidine 200 mg, orally given 30 min before the combined spinal–epidural (CSE) anaesthesia. An i.v. catheter was inserted in a peripheral arm vein, and a 1000 ml lactated Ringer’s solution was slowly infused after the arrival of the patient in the operating theatre. Patients were randomized by a computer-generated list to receive one of five doses of levobupivacaine (6, 8, 10, 12, or 14 mg intrathecal levobupivacaine). Allocation concealment was ensured by the use of coded, sealed opaque envelopes. Throughout the study, the same anaesthetist opened the envelope, added 2.5 μg sufentanil (0.5 ml) and 100 μg morphine (0.1 ml) to the allocated dose of levobupivacaine and performed the CSE anaesthesia. This physician was not involved in the subsequent management of the patient. CSE anaesthesia was performed at the L3–L4 or L4–L5 interspace using a loss-of-resistance-to-saline technique, with the patient in the sitting position. The spinal component was performed with a needle-through-needle technique using a 26 G needle (Espocan®, B. Braun, Germany). The intrathecal dose was injected over 100–120 s. The spinal needle was withdrawn, and a 20 G multiple-orifice epidural catheter was thereafter inserted 4 cm into the epidural space. The catheter was gently aspirated and checked for the presence of blood or cerebrospinal fluid, but no test dose was administered. The catheter was taped into place, and the patient was immediately laid in the supine position with a 10° left lateral tilt. Once intrathecal anaesthesia was completed, an anaesthetist, blinded to the levobupivacaine dosage, was allowed to enter into the operating theatre to manage the patient. This anaesthetist recorded the data for the present study.

The success or failure of the intrathecal block was the primary outcome of this study. A successful block was defined as a bilateral sensory level to pinprick to T6 within 15 min with no additional epidural anaesthetic required during surgery. A failure was recorded when a T6 sensory level was not obtained within 15 min after intrathecal drug administration, or when supplemental epidural analgesia was required to complete surgery because of either patient’s request for additional analgesia or a visual analogue pain scale (VAPS) score ≥40 mm. In the case of failure, 5 ml bolus injections of 5 mg ml⁻¹ levobupivacaine were administered through the epidural catheter.

Once intrathecal anaesthesia was completed, 500 ml lactated Ringer’s solution in which epidrane 30 mg and phenylephrine 100 μg were diluted was rapidly infused during the first 5 min after induction of anaesthesia, and the flow was afterward slowed and adjusted in order to prevent hypotension during the surgery. Hypotension was defined as a decrease in systolic arterial pressure (SAP) of more than 20% in comparison with the baseline value or an SAP of <90 mm Hg. When hypotension occurred, supplemental boluses of a mixture combining ephedrine 3 mg ml⁻¹ with phenylephrine 25 μg ml⁻¹ were administered in order to reach at least 80% of the baseline value of the SAP.

Measurements

Patient characteristic data recorded included age, height, weight, parity, number of previous Caesarean deliveries, and gestational age. Neonatal weight and Apgar scores were recorded after delivery. The SAP was determined by non-invasive arterial pressure measurements made at baseline, at 2 min intervals after drug injection for the first 15 min, at 5 min intervals until the end of the surgery, and at 10 min intervals in the post-anaesthesia care unit.

The sensory level was determined bilaterally by pinprick at 2 min intervals after drug injection for the first 15 min and then was assessed and recorded at 20, 25 min, and thereafter at 15 min intervals until the complete recovery from sensory block. The time for bilateral T6 sensory level was recorded.

Pain was assessed using a VAPS, from 0 (no pain) to 100 mm (worse pain imaginable), at 10 min intervals during the surgery. The time between intrathecal anaesthesia and of the need for epidural administration of 5 mg ml⁻¹ levobupivacaine due to patient’s request for additional analgesia or a VAPS score ≥40 mm was recorded. Pruritus and nausea were recorded using a visual analogue scale (from 0 to 100 mm).

Motor block was assessed using the modified Bromage scale: 0, able to lift extended leg at hip; 1, able to flex knee but unable to lift extended leg; 2, able to move foot only; and 3, unable to move even foot. In the case of success, for each patient, motor block was recorded at the entrance...
of the patient in the post-anaesthesia care unit, and the time between intrathecal injection and recovery, as defined by the modified Bromage scale = 0, was registered. The patient was authorized to leave the post-anaesthesia care unit when she had totally recovered from both sensory and motor blocks.

**Statistical analysis**

Using a Cochran–Armitage test for trend in proportions, a sample size of 17 patients per group was obtained based on five groups with levobupivacaine dosage values of 6, 8, 10, 12, and 14 mg and proportions of success equal to 0.5, 0.6, 0.7, 0.8, and 0.9, respectively. Our calculation indicated that a total sample size of 85 subjects would give 81% power to detect a linear trend using a two-sided Z-test with continuity correction and a significance level of 0.05 (PASS® 8.0.05; NCSS, LCC, Kaysville, UT, USA). The STATISTICA® version 6.0 computer software package (Statsoft, Tulsa, OK, USA) was used for the analysis of the haemodynamic data. Mean doses of ephedrine were analysed using one-way analysis of variance after assessment of the normality of the distribution of the data using a Shapiro–Wilk’s W-test. Repeated measures of haemodynamic values were analysed by a two-way analysis of variance. Incidence data were analysed by $\chi^2$ test for trend. The level of significance was set at $P<0.05$.

The mean times to T6 sensory block and for achieving complete recovery from motor block were analysed according to the assumption that these times would be, respectively, decreased or increased when increasing the dose of levobupivacaine. The analysis was performed using a test for trend for continuous variables as previously described by Cuzick. The statistical software R was used for these analyses.

Probit regression based on ‘success’ or ‘failure’ for each patient was performed using SPSS version 12.0 (SPSS®, Chicago, IL, USA). The ED$_{50}$ and ED$_{95}$ of levobupivacaine added with opioids were calculated.

**Results**

One hundred and eighteen patients were screened for this study. Seven declined to participate, 26 did not meet the inclusion criteria. Eighty-five patients ($n=17$ patients per group) were enrolled and randomly assigned to a dose group, from June 2008 to February 2009. They all completed the study according to the protocol and were included in the analysis.

Patient and baseline obstetric characteristics were similar across the five groups (Table 1). The mean duration of surgery was similar among groups (Table 1).

There was no significant difference among groups as regards the rates of success for a bilateral T6 sensory level reached in <15 min, and the mean time to T6 sensory block onset was not different between groups (Table 2). However, anaesthetic failure during surgery occurred significantly more frequently with smaller levobupivacaine doses (Table 2). A VAPS score $\geq 40$ mm was taken to indicate failure in nine patients. In four patients, failure was due to discomfort caused by the sensation of abdominal stretching during the Caesarean section. All of these patients had a VAPS score of $\geq 40$ mm: it was 20 in one patient and 30 for three patients. The mean time to failure during surgery was not statistically different among groups (Table 2).

Using probit analysis, the ED$_{50}$ of levobupivacaine was 6.2 mg (95% CI: 2.6–7.6) and the ED$_{95}$ was 12.9 mg (95% CI: 11.1–17.9) (Fig. 1).

Two-way analysis of variance of SAP did not show any statistically significant difference among groups, nor significant changes of SAP before and after spinal anaesthesia (Table 3). The greatest decrease in SAP ranged from 9% to 14.9% across the five groups (Table 3). No significant difference was found between groups with respect to supplemental vasopressor requirements (Table 3). No significant difference was found in the incidences of pruritus, nausea, and vomiting. The number of ‘success’ patients with the modified Bromage score $\geq 2$ at the end of surgery was significantly increased with increasing levobupivacaine dosage, and the mean time to complete recovery from motor block significantly increased when increasing the dose of levobupivacaine (Table 3). Oxytocin requirements and Apgar scores were similar across the groups.

**Discussion**

In this study, we were able to determine the ED$_{50}$ and ED$_{95}$ of intrathecal levobupivacaine, combined with intrathecal morphine and sufentanil, for elective Caesarean delivery. Previous dose–response studies assessing the potency of intrathecal local anaesthetics for Caesarean section used varied definitions of a successful block, leading to some difficulties for the comparison of the results among these trials. In our study, success was defined as the combination of a successful bilateral T6 sensory block to pinprick reached in <15 min after the intrathecal drug administration with no additional epidural anaesthetic required during surgery. Using this definition of successful block, failure occurred mainly during surgery, as T6 sensory level to pinprick was reached in 15 min for most of the patients. One can assume that the local concentration of levobupivacaine was probably low at the distant effect sites in the lower levobupivacaine dose groups, leading to inadequate potency for providing surgical anaesthesia in these groups of patients. Hence, as previously reported, the initial T6 block to pinprick did not reliably predict overall success, and it would have been probably more appropriate in the present study to test the block to touch rather than to pinprick to minimize the risk of inadequate anaesthesia.

Previous studies have used varied definitions of failure during surgery. In the studies by Ginosar and colleagues and Carvalho and colleagues, failure was defined by either a VAPS score $\geq 20$ mm or patient’s request for additional analgesia, while it was defined in the present study by either a VAPS score $\geq 40$ mm or patient’s request for additional analgesia. This cut-off value of 40 mm for the VAPS score was chosen as it has been reported that...
this value allows the identification of a significant level of pain during the perioperative period. However, this cut-off value was probably not optimal, since supplemental analgesia was requested for four patients with VAP score of 40 mm. It may be more appropriate to use a level for VAP score of 10 mm for defining failure during surgery. The MLAD, corresponding to the ED50, of intrathecal levobupivacaine for Caesarean section was assessed in two previous studies. The MLAD of intrathecal plain levobupivacaine was larger than the ED50 value calculated in our study (10.6 vs 6.2 mg), since the dose-sparing effect of sufentanil added to levobupivacaine in our study probably contributed to decrease the value of ED50 of levobupivacaine. A more recent study assessing the MLAD of levobupivacaine combined with 3 μg sufentanil for Caesarean section reported a similar value for ED50 to that calculated in our study (4.73 vs 6.2 mg). Although the modified Dixon’s up-and-down method followed by the probit analysis method allows accurate calculation of ED50 as performed in the studies by Parpaglioni and colleagues, our dose-response study design was more appropriate for accurate calculation of ED95, which is more relevant for clinical practice than the ED50.

The ED50 and ED95 of intrathecal levobupivacaine do not differ from the ED50 (7.25 mg) and ED95 (13.0 mg) of intrathecal isobaric bupivacaine combined with fentanyl and morphine previously reported by Carvalho and colleagues, although some previous studies have suggested that levobupivacaine is less potent than bupivacaine for Caesarean section. Thus, the values for ED50 and ED95 of levobupivacaine are less than might have been expected. This may be due to two reasons: first, as mentioned above, the definition of success or failure differed between our study and the study by Carvalho and colleagues. Secondly, it has been reported that 2.5 μg intrathecal sufentanil is more potent than 10 μg intrathecal fentanyl used in the study by Carvalho and colleagues, through significantly increasing duration of effective analgesia in the early

Table 1 Patient characteristic and obstetric data; values are presented as mean (SD), except age range, which is presented as number of patients, and parity, which is presented as median

<table>
<thead>
<tr>
<th>Dose of levobupivacaine</th>
<th>6 mg (n=17)</th>
<th>8 mg (n=17)</th>
<th>10 mg (n=17)</th>
<th>12 mg (n=17)</th>
<th>14 mg (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33</td>
<td>32</td>
<td>33</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 (6)</td>
<td>163 (6)</td>
<td>165 (6)</td>
<td>163 (7)</td>
<td>162 (5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69 (14)</td>
<td>64 (14)</td>
<td>67 (10)</td>
<td>66 (11)</td>
<td>60 (9)</td>
</tr>
<tr>
<td>Parity</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39 (1)</td>
<td>38 (0)</td>
<td>39 (0)</td>
<td>38 (1)</td>
<td>39 (0)</td>
</tr>
<tr>
<td>Neonatal weight (kg)</td>
<td>3.3 (0.3)</td>
<td>3.2 (0.5)</td>
<td>3.4 (0.4)</td>
<td>3.3 (0.4)</td>
<td>3.2 (0.3)</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>54 (17)</td>
<td>58 (14)</td>
<td>51 (14)</td>
<td>59 (15)</td>
<td>50 (12)</td>
</tr>
</tbody>
</table>

Table 2 Success and failures among groups; values are presented as number of patients, mean (SD), median (inter-quartile range), and observed time for failure during surgery for each patient in groups 8, 10, and 12 mg. *P=0.0003 and †P=0.0001 between groups when increasing levobupivacaine dose (χ2 test for trend)

<table>
<thead>
<tr>
<th>Dose of levobupivacaine</th>
<th>6 mg (n=17)</th>
<th>8 mg (n=17)</th>
<th>10 mg (n=17)</th>
<th>12 mg (n=17)</th>
<th>14 mg (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6 sensory block in ≤15 min (n)</td>
<td>16</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Time to T6 sensory block (min)</td>
<td>11.2 (5.6)</td>
<td>10.1 (5.1)</td>
<td>9.4 (7.3)</td>
<td>7.9 (6.4)</td>
<td>6.9 (3.1)</td>
</tr>
<tr>
<td>Failure during surgery (n)*</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Time to failure during surgery (min)</td>
<td>25 (22–36)</td>
<td>34 (40)</td>
<td>42 (51)</td>
<td>58 (——)</td>
<td>—</td>
</tr>
<tr>
<td>Overall success [n (%)]†</td>
<td>8 (47)</td>
<td>12 (71)</td>
<td>14 (82)</td>
<td>15 (88)</td>
<td>17 (100)</td>
</tr>
</tbody>
</table>

Fig 1 Probit regression of the probability of success of spinal anaesthesia as a function of the dose of intrathecal levobupivacaine. Horizontal bars denote 95% CI for ED50 and ED95.
postoperative period when combined with bupivacaine for Caesarean section. Capogna and colleagues reported a six-fold potency ratio for sufentanil and fentanyl for epidural analgesia. Therefore, in patients having received low doses of levobupivacaine, one can assume that sufentanil increased the duration of analgesia to a greater extent than fentanyl and contributed to success of low doses of levobupivacaine during surgery explaining in part the lower ED50 and ED95 of intrathecal levobupivacaine in comparison with the expected values.

All Caesarean sections were performed in the morning, in order to avoid any significant statistical bias due to the effects of chronobiological conditions in intrathecally administered local anaesthetic and opioids, as, for example, sufentanil, for which duration of intrathecal analgesia was reported to exhibit a temporal pattern with 30% variation through the day.

Results of this study are valid for elective Caesarean delivery with time to wait 15 min for the bilateral T6 sensory level after spinal dose injection. Hence, our results probably do not apply to urgent Caesarean delivery requiring a rapid spread of spinal anaesthesia. Further studies are required for determining the optimal dose of spinal levobupivacaine, and assessing its clinical tolerance, for urgent Caesarean section. Our results demonstrate that a sufficient dose (more than 12 mg, i.e. larger than the doses previously assessed in the literature) of intrathecal levobupivacaine added with sufentanil and morphine is required for providing effective anaesthesia for elective Caesarean delivery. If doses of levobupivacaine less than the ED95, particularly near the ED50, are used, they should be administered under a CSE technique, in order to be able to complete insufficient analgesia during surgery.

In conclusion, the ED95 of intrathecal levobupivacaine combined with intrathecal morphine 100 μg and intrathecal sufentanil 2.5 μg for elective Caesarean delivery anaesthesia was 12.9 mg. Haemodynamic and overall tolerance as well as motor block duration of such a protocol of spinal anaesthesia seem acceptable for elective Caesarean sections, provided that an appropriate strategy for preventing hypotension due to spinal anaesthesia is used.

### Table 3: Adverse effects of the various intrathecal levobupivacaine doses; data are presented as mean (so), median (inter-quartile range), or number of patients (n). *Administered volume of the mixture combining ephedrine 3 mg ml⁻¹ with phenylephrine 25 μg ml⁻¹. †P<0.0001 between groups when increasing levobupivacaine dosage (χ² test for trend). ‡P=0.00005 between groups when increasing levobupivacaine dosage (Cuzick’s test for trend).

<table>
<thead>
<tr>
<th>Dose of levobupivacaine</th>
<th>6 mg (n=17)</th>
<th>8 mg (n=17)</th>
<th>10 mg (n=17)</th>
<th>12 mg (n=17)</th>
<th>14 mg (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest SAP (mm Hg)</td>
<td>114 (24)</td>
<td>111 (17)</td>
<td>111 (16)</td>
<td>110 (14)</td>
<td>109 (17)</td>
</tr>
<tr>
<td>Maximal SAP reduction (%)</td>
<td>14.9 (6)</td>
<td>14.5 (5)</td>
<td>11 (4)</td>
<td>9 (3)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>Supplemental vasopressor requirement (n)</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Volume of vasopressor (ml)*</td>
<td>4 (2–6)</td>
<td>3 (2–3)</td>
<td>3 (3–3)</td>
<td>2 (1–4)</td>
<td>5 (3–6)</td>
</tr>
<tr>
<td>Nausea/vomiting (n)</td>
<td>1/0</td>
<td>2/1</td>
<td>1/0</td>
<td>1/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Modified Bromage’s scale ≥ 2 at the end of surgery (n)†</td>
<td>5</td>
<td>6</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Time for modified Bromage’s scale=0 (min)‡</td>
<td>106 (79)</td>
<td>110 (42)</td>
<td>149 (79)</td>
<td>164 (58)</td>
<td>183 (67)</td>
</tr>
</tbody>
</table>

### Conflict of interest
None declared.

### Funding
Support was provided solely by institutional sources.

### References
1. Bader AM, Tsen LC, Camann WR, Nephew E, Datta S. Clinical effects and maternal and fetal plasma concentrations of 0.5% epidural levobupivacaine versus bupivacaine for cesarean delivery. *Anesthesiology* 1999; 90: 1596–601.
24 Pace NL, Stylianou MP. Advances in and limitations of up-and-down methodology: a precis of clinical use, study design, and dose estimation in anesthesia research. Anesthesiology 2007; 107: 144–52
27 Chassard D, Bruguerolle B. Chronobiology and anesthesia. Anesthesiology 2004; 100: 413–27