Pharmacokinetics of Bupivacaine after Continuous Epidural Infusion in Infants with and without Biliary Atresia

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Background: Continuous epidural infusion of bupivacaine is widely practiced for postoperative pain relief in pediatric patients. However, bupivacaine may induce adverse effects in infants (convulsions or cardiac arrhythmias), likely because of decreased hepatic clearance and serum protein binding capacity. The authors wanted to examine the complex relations between age, α-1 acid glycoprotein (AAG) concentration, and unbound and total bupivacaine serum concentrations in infants receiving bupivacaine epidurally for 2 days.

Methods: Twenty-two infants aged 1–7 months (12 with biliary atresia and 10 with another disease) received a continuous epidural infusion of 0.375 mg · kg⁻¹ · h⁻¹ bupivacaine during 2 days (during and after surgery). Unbound and total bupivacaine concentration in serum was measured 0.5, 4, 24, and 48 h after infusion initiation. AAG concentration was measured in serum before and 2 days after surgery. In eight additional infants, the blood/plasma concentration ratio was measured in vitro at whole blood concentrations of 2 and 20 μg/ml. Bupivacaine concentration was fitted to a one-compartment model to calculate basic pharmacokinetic parameters.

Results: No adverse effects were observed. AAG increased markedly after surgery, and the increase was correlated to both age and preoperative AAG concentration. Two infants aged 1.8 months had unbound concentrations greater than 0.2 μg/ml. Clearance of unbound drug significantly increased with age. Because of increased drug binding, clearance of bupivacaine decreased both with time (from 0.5 to 48 h) and with age. Blood/plasma ratio was 0.77 ± 0.08 and 0.85 ± 0.24 at 2 and 20 μg/ml, respectively.

Conclusions: Because of a low AAG concentration and a low intrinsic clearance, unbound bupivacaine increased to concentrations greater than 0.2 μg/ml in two infants younger than 2 months, after 2 days of infusion at a rate of 0.375 mg · kg⁻¹ · h⁻¹. The increase in AAG observed after surgery did not fully buffer this unbound fraction. Similarly, the buffer capacity of erythrocytes did not sufficiently increase at high concentration to compensate the saturation of the AAG system. Thus, we propose the use of a maximum dose of 0.25 mg · kg⁻¹ · h⁻¹ in infants younger than 4 months and a maximum of 0.3 mg · kg⁻¹ · h⁻¹ in infants older than 4 months.

CONTINUOUS epidural infusion of local anesthetics with or without opioids is widely practiced for postoperative pain relief in pediatric patients. However, the use of long-acting local anesthetics such as bupivacaine may induce adverse effects, either convulsions or cardiac arrhythmias. Despite an expected lower intrinsic susceptibility to toxic effects of local anesthetics, infants are considered at greater risk of toxicity than older children and adults, likely because of pharmacokinetic considerations. The main reasons advanced for this susceptibility are a lower clearance of bupivacaine and a lower serum protein binding in infants as compared with children and adults. Like all other amide local anesthetics, bupivacaine is highly bound to α-1 acid glycoprotein (AAG) and to serum albumin (HSA). AAG serum concentration is lower in infants than in children and adults. These facts can explain why toxic reactions may occur in infants receiving continuous perineural infusion of bupivacaine for pain relief. There is particular risk for these toxic reactions because cardiac manifestation may occur without any neurologic prodrome or accompanying symptoms.

To date, some studies have investigated the pharmacokinetics of bupivacaine after single injection or during the first 1–2 days after surgery in infants aged less than 6 months. However, the complex relations between age, AAG, and HSA serum concentrations and unbound and total bupivacaine concentrations have not been fully studied in infants receiving bupivacaine for more than 1 day. In our institution, epidural infusion of bupivacaine with or without opioids is routinely used for postoperative pain relief during 1–4 days in children and infants. Therefore, we wanted to measure the unbound and total bupivacaine concentration during the first 2 days after surgery in this population. An important number of these patients are scheduled for a Kasai procedure for biliary atresia. These infants have usually only mild hepatic dysfunction with normal prothrombin time but an important cholestasis. Although hyperbilirubinemia alters primarily serum binding of acidic drugs, we wanted to know if this association of cholestasis and mild hepatic dysfunction might modify the kinetics of bupivacaine in these infants.

Materials and Methods

After obtaining approval from the local ethical committee (Comité Consultatif pour les Personnes se Livrant à la Recherche Biomédicale, Hôpital Cochin, Paris, France), 20 infants (12 with biliary atresia and 8 with another disease) received a continuous epidural infusion of bupivacaine for pain relief. There is particular risk for these reactions because cardiac manifestation may occur without any neurologic prodrome or accompanying symptoms.


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France) and informed parental consent, 12 infants with biliary atresia scheduled for Kasai procedure and 10 infants without cholestasis scheduled for urologic or digestive procedure were studied. None of them had an American Society of Anesthesiologists physical status greater than II. No premedication was given. Anesthesia was induced with either sevoflurane or halothane and was maintained with isoflurane. After insertion of a peripheral intravenous line and tracheal intubation, a central venous catheter was inserted. A 24-gauge epidural catheter was inserted at the L3-L4 or L4-L5 interspace in the left lateral position using a 20-gauge Tuohy needle. After injection of a test dose (1 ml bupivacaine 0.25% with 1/200,000 epinephrine) the injection was completed to a total dose of 1.25 mg/kg bupivacaine using the same solution. This initial bolus injection was immediately followed by a continuous epidural infusion of 0.375 mg · kg⁻¹ · h⁻¹ plain bupivacaine 0.125%. Epidural infusion was not stopped before the 48th h of administration. During the 48-h study period, only crystalloid or hydroxyethyl starch were infused, according to blood loss, and no serum albumin was infused. Heart rate, noninvasive blood pressure, oxygen saturation, end-tidal carbon dioxide tension, volatile agent tension, and body temperature were continuously monitored during the procedure. All infants were extubated after surgery. During the postoperative period, pain intensity was assessed using a pain score derived from the score of Attia et al.²⁰

**Sampling and Assay**

In all infants, AAG and HSA were measured in serum before surgery and at day 2 using an automated nephelometric technique using control serum from neonates and infants.²¹ Venous blood (2.5 ml) was sampled in additive free tubes (Vacutainer, Le Pont de Claix, France) 0.5, 4, 24, and 48 h after bupivacaine injection. After centrifugation, the serum (1.2–1.4 ml) was removed and kept frozen at −20°C until assayed. After pH adjustment by equilibration during 2 h in an agitated water bath with 95% N₂ and 5% CO₂ at 37°C, protein binding was determined in 800 μl to 1 ml serum using ultrafiltration at 35°C using YMT membranes (Amicon, Grace SA, Epernon, France). Total and free bupivacaine were then assayed by gas chromatography.²² The limit of detection was 0.01 μg/ml and the within- and between-day coefficients of variation were 8 and 12% at 0.02 μg/ml and 5 and 7% at 200 μg/ml, respectively.

**In Vitro Erythrocyte Binding**

In eight additional infants aged 0.6–7 months with an hematocrit of 0.33 ± 0.04, venous blood (2.2 ml) was sampled on EDTA and divided in two 1-ml aliquots to which bupivacaine was added in vitro to obtain approximate concentrations in whole blood of 2 and 20 μg/ml, respectively. After gentle agitation during 15 min at 35°C, bupivacaine was assayed in whole blood and in plasma.

**Statistics**

The two groups were compared for age, weight, preoperative and postoperative hemoglobin concentration in blood, and preoperative AAG and HSA serum content using the Student t test. Blood/plasma concentration ratio measured in vitro at 2 and 20 μg/ml, respectively, was compared using the Student t test for paired data. Because AAG and HSA concentrations in serum were not available at all four sampling times, we associated AAG and HSA content in serum measured before surgery to 0.5 and 4 h and AAG and HSA content in serum measured on the second postoperative day to 24 and 48 h. We used a stepwise multiple linear regression to test the correlation between preoperative AAG concentration in serum with age and sex, and postoperative AAG concentration in serum with preoperative AAG, age, and sex.

Pharmacokinetic analysis was performed using a four-point sampling design. The whole data set (unbound and bound concentration–time data) was simultaneously fitted using mixed-effect modeling. Because of the relatively small number of data points, great care was paid not to overparameterize the model. Therefore, we used a simple one-compartment model of unbound concentration (C_u) Total concentration in serum (C_T) was related to C_u by a simple linear link proportional to AAG concentration. The following four parameters were estimated: (1) k_p, the absorption rate constant from epidural space; (2) CL_u, the intrinsic clearance of unbound drug; (3) V_u, the volume of distribution of unbound drug; and (4) K_u, the parameter relating bound to unbound concentration: C_b = K_b · C_u. An intersubject variability parameter (η) with mean zero and variance ω² was associated to each of these structural parameters, namely, η_CL, η_V, and η_K, respectively. No intersubject variability parameter was associated to k_p. It is important to note that, because clearance and volume of unbound drug are the basic structural parameters, clearance and volume of total drug (CL_T and V_T) may vary with time within the same individual. Because the first concentration–time point was sampled 30 min after epidural injection, the relevance of the model with absorption from epidural space was tested against a simple model of intravenous administration using the Akaike criterion.²³ In a second step, the effects of covariates (AAG and HSA concentration, weight, age, sex, and disease status) have been tested to build a full model including the vector of covariates (Appendix). We used the results of the regression analysis described previously to make a choice between the potential covariates. The relevant effect of these covariates was assessed as usual using the log likelihood ratio test.²⁴ A difference in the objective function of 6.65 was considered significant (P < 0.01). We estimated bias and error by calculating the median.
weighted residual and median absolute weighted residual for unbound and total concentration–time data separately (Appendix).25

To better illustrate the influence of clearance and AAG concentration on bupivacaine unbound and total concentrations, we performed a simulation step for infants aged 1 and 6 months using parameters obtained with the best reduced model. We calculated the average population CL\textsubscript{u} value after 3 and 48 h of infusion for infants aged 1–7 months with mean preoperative and postoperative AAG concentrations for the age and simulated values of CL\textsubscript{u} for infants aged 1 and 6 months to calculate a confidence interval of this parameter. The 95% confidence interval of CL\textsubscript{u} was obtained with 1,000 replicates (Appendix).

In addition, although the postoperative period cannot be considered as a steady state, we compared the individual clearances (unbound and total) calculated by post hoc procedure to the individual clearances calculated as CL = Dose/C\textsubscript{ss}. Here, Dose is the infusion rate (milligrams per hour per kilogram) and C\textsubscript{ss} is the pseudo-steady state concentration measured at 48 h. For comparing parametric and nonparametric methods used for estimating clearances, median weighted residual and median absolute weighted residual were normalized (weighted) by the mean value.26

Raw data are reported as the mean ± SD. Although bioavailability (f) of amide local anesthetics is usually considered complete by the epidural route, CL and V are presented corrected for bioavailability (CL/f and V/f).

Results

Twelve infants with biliary atresia were studied. Their mean age was 1.9 ± 0.6 months (range, 1–3.5 months), and their mean weight was 4.4 ± 1.0 kg. Their biologic status was as follows: total serum bilirubin, 163 ± 39 µM; alkaline phosphatase, 531 ± 177 U/l; γ-glutamyltransferase, 861 ± 435 U/l; prothrombin time, 100%. The preoperative and postoperative hemoglobin concentration was 10.8 ± 1.1 and 8.9 ± 0.7 g/dl, respectively. Ten infants with digestive or urologic diseases were studied. Their mean age was 4.6 ± 1.6 months (range, 2.5–7 months), and their mean weight was 6.3 ± 1.5 kg. Their preoperative and postoperative hemoglobin concentration was 10.7 ± 1.4 and 8.6 ± 1.2 g/dl, respectively. Age and weight were significantly lower in patients with biliary atresia than in the other patients. No difference in preoperative and postoperative hemoglobin concentration was found between the two groups. There was no clinical evidence of bupivacaine toxicity in any infant during the infusion period. Postoperative analgesia was excellent. AAG concentration was significantly lower in the biliary atresia group than in the other group either before surgery (0.46 ± 0.15 vs. 0.66 ± 0.11 g/l, respectively, at 0 h, biliary atresia group vs. the other group) than after surgery (0.78 ± 0.19 vs. 1.30 ± 0.39 g/l at 48 h, biliary atresia group vs. the other group). Preoperative AAG was significantly correlated with age (P < 0.0001; fig. 1). Postoperative AAG concentration was significantly correlated with age and preoperative AAG concentration (P < 0.0001), but not with sex or with disease status: AAG at 48 h = 0.139 + 0.123 mg/l × age (months) + 0.904 × AAG at 0 h, r² = 0.625, i.e., 62% of the variance is explained by the regression. HSA was not significantly different between the two groups either before (36 ± 5 vs. 39 ± 5 g/l, biliary atresia group vs. the other group) or after surgery (31 ± 5 vs. 31 ± 4 g/l, biliary atresia group vs. the other group). Preoperative but not postoperative HSA was significantly correlated with age (P < 0.05).

Because of ethical considerations, we did not draw blood in infants when samples for clinical purpose were not mandatory or when the amount needed for clinical purpose was higher than a specified value. When a limited blood volume was available, only total concentration was measured. Therefore, 17 data points were missed because of sampling limitations (7 corresponding to total concentration, and 10 corresponding to free concentration). Maximum total and unbound bupivacaine were observed at 48 h (3.58 and 0.33 µg/ml, respectively; table 1 and fig. 2). The free fraction decreased with increased AAG concentration and with age (fig. 3).

In Vitro Erythrocyte Binding

The eight infants in whom binding was measured were aged 0.6–7 months, and their mean hematocrit was 0.35 ± 0.03. Total blood concentration measured after in vitro addition of bupivacaine was 2.0 ± 0.1 and 20.1 ± 0.3 µg/ml with a blood/plasma concentration ratio of 0.77 ± 0.08 and 0.85 ± 0.10, respectively (P < 0.05). Thus, the ratio of drug amount between erythrocytes and plasma increased from 0.36 ± 0.19 to 0.56 ±
0.27 when the amount added to whole blood increased from 2 to 20 µg/ml \((P < 0.05)\). However, the proportion of molecules between erythrocytes and whole blood increases only from 0.15 ± 0.07 to 0.22 ± 0.09, showing that the proportion of molecules trapped by erythrocytes is not major, even at the highest concentrations.

**Pharmacokinetics**

The best model was the model with first-order absorption considering bound concentration related to unbound concentration by a linear relation without inter-subject variability for \(K_b\) and with \(V_u\) independent of age (table 2). The use of disease status, sex, or HSA concentration as covariates did not improve the quality of fitting (table 2). Estimates of pharmacokinetic parameters are reported in table 3. Bias (median weighted residual) was 4 and 6%, and error (median absolute weighted residual) was 14 and 19% for unbound and bound concentration–time data, respectively. The relation between predicted and measured values is displayed in figure 4. Table 4 shows the evolution of average clearances (\(CL_u\) and \(CL_T\)) and concentrations (\(C_u\) and \(C_T\)) for infants aged 1–7 months. These values were calculated numerically using the average AAG concentration for the age. Although \(CL_T\) increased with increasing age at the time of first injection, \(CL_T\) decreased with increasing age 48 h later. This phenomenon has been numerically simulated, and the results are displayed in figure 5. \(CL_u\) and \(CL_T\) calculated by the two techniques (parametric and non-parametric) were in excellent agreement: \(CL_u\) and \(CL_T\) were calculated at 48 h by the parametric Bayesian procedure (NONMEM) and by the nonparametric method. The ratio of the values given by the two methods was 0.96 for \(CL_u\) and 1.04 for \(CL_T\), but with a coefficient of variation of 26 and 30%, respectively. Bias (median weighted residual) and error (median absolute weighted residual) were 1.6 and 21% for \(CL_u\) and 3.9 and 21% for \(CL_T\), respectively.

**Discussion**

**Pharmacokinetic Model**

We measured total and unbound serum bupivacaine in infants aged 1–7 months receiving continuous epidural bupivacaine for postoperative pain relief. We used a four-point sampling design with 22 replicates at each sampling time (with 17 missing values). This design was chosen rather than a sampling scheme with unequal time points because misspecification of the model was not of major importance since steady state was rapidly reached for unbound concentration, and because the patients were sampled at the time of sampling for clinical purpose. A very simple model with intravenous administration failed to correctly fit the data. Therefore, we used a one-compartment model with first-order absorption, and at a second time, concurrently built two different models. The first model, based on intrinsic hepatic clearance concept,\(^{27}\) showed better adequacy of fitting.
than the model, which considered unbound and total concentrations separately. Fitting was adequate as assessed by the low bias and moderate error (fig. 4). Bupivacaine is considered to be restrictively cleared, i.e., rate limited in its metabolism. Therefore, \( \text{CL}_{T} \) mainly depends on \( \text{CL}_{u} \), the intrinsic clearance that is directly linked to hepatic microsomal activity, and protein binding. Although one may expect some departure from this theoretical model (mainly because bupivacaine hepatic extraction ratio is not extremely low), this model proved to adequately fit the data. \( \text{CL}_{T} \) and \( \text{V}_{T} \) are derived parameters, nonlinearly depending on \( \text{CL}_{u} \) and \( \text{V}_{u} \) and on AAG concentration. Therefore, \( \text{CL}_{T} \) and \( \text{V}_{T} \) vary with time because AAG concentration increases during the postoperative period.

**Covariates**

Clusters corresponding to subgroups of patients were searched by the mean of mixed-effect modeling. Only two covariates entered successfully in the model. \( \text{CL}_{u} \) was related to age, and the ratio linking total and unbound concentration was proportional to AAG concentration in serum. These two covariates are direct factors of our model. Because bupivacaine is mainly metabolized by the cytochrome P450 isoform CYP3A4, which reaches only 50% of adult concentrations between 6 and 12 months of age, age may be considered as the main determinant of intrinsic hepatic clearance. Similarly, AAG concentration is the main factor of protein binding and is therefore a direct factor of the physiologically based model. Patients with biliary atresia were significantly younger, and they had a lower AAG concentration in serum than the other patients. This lower AAG concentration appeared to be related only to the difference in age between the two groups. Two days after surgery, AAG concentration was higher than before surgery in all infants. Moreover, this increase was significantly related to age and basal AAG concentration (fig. 1). AAG increased on average by a factor of 1.5 at the age of

**Table 2. Model Building and Statistical Significance**

<table>
<thead>
<tr>
<th>Reduced models</th>
<th>OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No difference between the two groups for ( V_u )</td>
<td>( \text{OF2} = -476.308 )</td>
</tr>
<tr>
<td>( K_b ) without random effect parameter</td>
<td>( \text{OF3} = -476.282 )</td>
</tr>
<tr>
<td>( V_u ) only, dependent of ( \text{BW} ) (one parameter)</td>
<td>( \text{OF4} = -474.985^{*} )</td>
</tr>
<tr>
<td>( \text{CL}_{u} ) only, dependent of ( \text{BW} ) (one parameter)</td>
<td>( \text{OF5} = -466.245 )</td>
</tr>
<tr>
<td>( \text{CL}_{u} ) and ( V_u ) only, dependent of ( \text{BW} ) ( K_b ) independent of AAG concentration</td>
<td>( \text{OF6} = -463.780 )</td>
</tr>
<tr>
<td>( K_b ) independent of AAG concentration</td>
<td>( \text{OF7} = -428.291 )</td>
</tr>
</tbody>
</table>

The full model considered the intrinsic clearance of unbound drug (\( \text{CL}_{u} \)) dependent of body weight, disease status, and age and considered \( V_u \) dependent of body weight and age. It was not possible to consider the disease status for volume because of overparameterization. The successive reduced models are nested, and each one follows from the preceding until model 4, which was the best reduced model with \( \text{CL}_{u} \) dependent of body weight and age, and \( V_u \) independent of age and without intersubject variability for \( K_b \). Models 5–7 are reduced from model 4. Goodness of fit is represented by the objective function (OF). A difference in OF of 6.63 (with one degree of freedom, \( P < 0.01 \)) was considered significant.

* Best reduced model.

\( \text{BW} \) = body weight; AAG = \( \alpha-1 \)-acid glycoprotein.
1 month and by a factor of 2.5 at the age of 7 months. Other investigators have found a quantitatively lower increase in AAG concentration after surgery, without this proportionality between increase and age. However, an immediate consequence of this greater increased AAG concentration in the older infants is the much greater decrease in total clearance observed in older infants than in the younger infants (table 4 and fig. 3). The relation between HSA and age was weak, whereas AAG concentration is serum was significantly related to age. This is in perfect agreement with our previous findings. HSA concentration in serum did not enter successfully in the model. This is not surprising because it is well established that binding of basic drugs to HSA is nonspecific and not related to the HAS-specific site for bilirubin and acidic drug binding. Therefore, competitive displacement between bilirubin and bupivacaine is not expected to occur. Twelve infants had

Table 3. Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLu/f*</td>
<td>(ml · min⁻¹ · kg⁻¹)</td>
<td>30.2 (24%)</td>
<td>6.13 (30%)</td>
</tr>
<tr>
<td>θ1</td>
<td>(ml · min⁻¹ · kg⁻¹ · mo⁻¹)</td>
<td>0.0893</td>
<td></td>
</tr>
<tr>
<td>θ2</td>
<td>(ml · min⁻¹ · kg⁻¹ · mo⁻¹)</td>
<td>6.13 (30%)</td>
<td></td>
</tr>
<tr>
<td>Vc/f</td>
<td>(l/kg)</td>
<td>16.1 (17%)</td>
<td></td>
</tr>
<tr>
<td>k&lt;sub&gt;a&lt;/sub&gt;</td>
<td>(min⁻¹)</td>
<td>0.067 (47%)</td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;b&lt;/sub&gt;</td>
<td>(ml · min⁻¹ · kg⁻¹)</td>
<td>12.2 (7%)</td>
<td></td>
</tr>
</tbody>
</table>

* CLu/f (ml · min⁻¹ · kg⁻¹) = θ1 + θ2 · age (months).

Data are typical value (% coefficient of variation of estimate). \( \omega^2 \) is the variance of the interindividual variability parameter (\( \eta \)). Data are given with three significant digits. Clearance terms are presented corrected for bioavailability (f).

CLu = intrinsic clearance of unbound drug; Vc = volume of distribution of unbound drug; k<sub>a</sub> = absorption rate constant from epidural space; K<sub>b</sub> = parameter relating bound to unbound concentration.

Fig. 4. Goodness of fit. Measured versus predicted unbound (top) and total (bottom) bupivacaine concentration. Data are represented on a Cartesian plot (left) and on a log–log plot (right). A small bias is obvious on the upper part, likely because of the use of a single proportional intraindividual error and to the simultaneous fitting of unbound and bound concentrations.
biliary atresia with cholestasis, but with normal liver function and normal nutritional status. The use of the disease status (biliary atresia or not) as covariate did not improve the quality of fitting.

**Binding to Erythrocytes**

After AAG and HSA, erythrocytes represent the third buffer system. In infancy, physiologic anemia diminishes the importance of the erythrocyte system. When total blood concentrations increased from 2 to 20 mg/ml, the proportion of bupivacaine molecules buffered by the erythrocytes increased from 15 to 22%. Clearly, erythrocytes are unable to compensate when the usual buffer system, represented mainly by AAG, is saturated.

**Total and Unbound Concentration**

At the end of the 48-h infusion period, the unbound concentration remained stable, whereas total concentration showed a trend to increase (fig. 2). This lack of steady state clearly shows that the inflammatory process leading to increase in AAG concentration was obviously active 48 h after surgery. Because bupivacaine is restrictively cleared by the liver, CLT mainly depends on intrinsic hepatic clearance, i.e., microsomal activity, and on the fraction unbound in plasma. The unbound bupivacaine concentration increased until pseudo–steady state at 24 h, but the total bupivacaine concentration continued to increase between 24 and 48 h (fig. 2). As a result of this continuous increase in protein binding caused by postoperative inflammation, the free fraction markedly decreased between 0.5 and 48 h (fig. 3). The resulting decrease in CLT was 29% in infants aged 1 month and 54% in infants aged 7 months (table 4). It is important to recognize that, despite a decrease in free fraction from 0.5 to 48 h, the unbound concentration (which is considered the toxic moiety) increases during the whole study period (fig. 3).

After 2 days of continuous epidural infusion of bupivacaine (0.375 μg · kg⁻¹ · h⁻¹), the unbound concentration was higher than 0.2 μg/ml in two infants aged 1.8 months (0.29 and 0.33 μg/ml, respectively). These concentrations, which may be considered above the toxic threshold (see below), were associated with AAG concentrations in serum of 0.64 and 0.89 mg/l, i.e., close to the average 0.81 mg/l for the age, 48 h after surgery. The unbound bupivacaine concentration threshold for toxicity during continuous administration is still un-

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**Table 4. Average Population Values of CLₜ for Infants Aged 1–7 Months**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>CLₜ/f (ml · min⁻¹ · kg⁻¹)</th>
<th>AAG (mg/ml)</th>
<th>CLₜ/f (ml · min⁻¹ · kg⁻¹)</th>
<th>Cₜ (μg/ml)</th>
<th>Cₜ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.3</td>
<td>0.46</td>
<td>5.54</td>
<td>0.11</td>
<td>0.70</td>
</tr>
<tr>
<td>2</td>
<td>42.5</td>
<td>0.50</td>
<td>5.99</td>
<td>0.10</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>48.6</td>
<td>0.54</td>
<td>6.38</td>
<td>0.10</td>
<td>0.74</td>
</tr>
<tr>
<td>4</td>
<td>54.7</td>
<td>0.59</td>
<td>6.72</td>
<td>0.09</td>
<td>0.76</td>
</tr>
<tr>
<td>5</td>
<td>60.9</td>
<td>0.63</td>
<td>7.02</td>
<td>0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>6</td>
<td>67.0</td>
<td>0.67</td>
<td>7.29</td>
<td>0.09</td>
<td>0.78</td>
</tr>
<tr>
<td>7</td>
<td>73.1</td>
<td>0.71</td>
<td>7.53</td>
<td>0.08</td>
<td>0.79</td>
</tr>
</tbody>
</table>

The corresponding theoretical α-1 acid glycoprotein (AAG) concentration, average population values of CLₜ and average unbound (Cₜ) and total bupivacaine concentration (Cₜ) have been calculated numerically at the 3rd and 48th h after bolus injection and immediate infusion initiation. Because the increase in AAG concentration in the postoperative period is greater in older infants than in the younger ones, CLₜ not only decreases with time, but also decreases with age 2 days after bupivacaine infusion initiation. At any time, Cₜ is higher in the older infants than in the younger ones because AAG concentration is higher in the older infants as compared with younger ones. However, the true “toxic” concentration, Cₜ, is always lower in older infants because the intrinsic clearance (CLₜ) increases with age. Clearance terms are presented corrected for bioavailability (f).

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**Fig. 5. Simulation of unbound bupivacaine concentration during 48-h infusion in infants aged 1 month (top) and 6 months (bottom).** The curves were drawn with typical population values and 95% confidence intervals of unbound clearance. The volume of distribution has been set to its typical population value for all calculations.

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known in humans. Convincing arguments lead to consider that 0.2 μg/ml is the frontier of toxicity.\textsuperscript{16,31,32} These arguments are mainly based on calculations made using the free fraction and the total concentration observed. In addition, Knudsen et al.\textsuperscript{32} showed, in volunteers, that neurologic signs of toxicity occurred with bupivacaine free concentration reaching 0.3 μg/ml. We did not observe any adverse reaction in the infants studied. Luz et al.\textsuperscript{16} used a similar therapeutic scheme described the occurrence of irritability and jitteriness in some infants, and they attributed these signs to bupivacaine accumulation. Other investigators did not found any sign of toxicity, although the bupivacaine concentrations reported were similar in all of these studies.\textsuperscript{13,15}

To better understand the evolution of clearances and concentrations, both unbound and total, in various circumstances, we performed several simulations. We calculated the average population CL\textsubscript{g} value after 3 and 48 h of infusion for infants aged 1–7 months with mean preoperative and postoperative AAG concentrations for the age (table 4), and we simulated values of CL\textsubscript{u} for infants aged 1 and 6 months to calculate a confidence interval of preoperative and postoperative AAG concentrations for infants aged 1 and 6 months (fig. 5). Although these simulations must be viewed as rough computer estimates, they clearly show that total bupivacaine concentration may not represent the best warning of toxicity.

Most reported serious adverse effects were observed after several hours of very high dosage (2.5 mg · kg\textsuperscript{-1} · h\textsuperscript{-1}).\textsuperscript{8,9} At present, most investigators recommend lower dosages.\textsuperscript{3,13–16} However, these recommendations vary between 0.2\textsuperscript{13,14} and 0.375 mg · kg\textsuperscript{-1} · h\textsuperscript{-1}.\textsuperscript{14} Using this maximum recommended dose (0.375 mg · kg\textsuperscript{-1} · h\textsuperscript{-1}), we observed an unbound bupivacaine concentration higher than 0.2 μg/ml in two infants aged 1.8 months. As in our preceding study,\textsuperscript{12} we found a marked difference in C\textsubscript{u} between infants younger than 2–3 months and those older than 3–4 months (fig. 3). We may propose the use of a maximum dose of 0.25–0.3 mg · kg\textsuperscript{-1} · h\textsuperscript{-1} in infants younger than 4 months and a maximum of 0.3–0.375 mg · kg\textsuperscript{-1} · h\textsuperscript{-1} in infants older than 4 months.

\[ CL_u = TVCL_u \cdot \exp(\eta_u) \]  \hspace{1cm} (1)
\[ V_u = TVV_u \cdot \exp(\eta_v) \]  \hspace{1cm} (2)
\[ K_0 = TVK_0 \cdot \exp(\eta_{k0}) \]  \hspace{1cm} (3)

where CL\textsubscript{u} is the intrinsic hepatic clearance of unbound drug (related to body weight), V\textsubscript{u} is the volume of distribution of the unbound drug (related to body weight), and K\textsubscript{0} is a parameter linearly relating bound drug concentration (C\textsubscript{b}) to unbound drug concentration (C\textsubscript{u}):

\[ C_u = K_0 \cdot C_b \]  \hspace{1cm} (4)

TVCL\textsubscript{u} and TVV\textsubscript{u} are population parameters (fixed-effect parameters), respectively, for intrinsic hepatic clearance and volume of distribution of unbound drug, and TVK\textsubscript{0} is the population parameter relating bound to unbound concentrations. \( \eta \) is an intersubject variability parameter (random-effect parameter) with mean zero and variance \( \sigma^2 \). Because clearance and volume of unbound drug are the basic parameters, clearance and volume of total drug (CL\textsubscript{T} and V\textsubscript{T}) may vary with time within the same individual.

The effect of potential covariates (AAG and HSA concentration, age, sex, and disease status) have been added to this model to build a full model that includes the vector of covariates to the typical value of parameter k as follows:

\[ TV_{\text{para}} = \theta_1 + \xi \cdot \theta_2 \]  \hspace{1cm} (5)

The full model was first built with only age and body weight as covariates for CL. The addition of these covariates to the volume term as well as the addition of other potential covariates (disease status, sex, HSA concentration) to the clearance and volume terms were performed each at a time in a stepwise manner. Once the full model was

\textbf{NONMEM Control Stream for the Full Model}

```
SOLUTION SUFFI FULL
$INPUT 1D TIME AMT RATE CVMT FLAG D V AAG HSA AVB HB AGS EW SEX $SUBROUTINES ADVAN2 TRANS2 LIBRARY $PK
TVCL1 = THETA(1) + AGE*THETA(2)
TVCL2 = THETA(3) + AGE*THETA(4)
TVCL = AVB*TVCL1 + (1-AVB)*TVCL2
CL  = TVCL*EXP(ETA(1))
TV  = TVCL*TVCL2*EXP(ETA(2))
KA = THETA(6)
KB = AAG*THETA(7)*EXP(ETA(3))
S2 = V
$ERROR
U = F
Y = F*(1+KB)
C = (1-FLAG)*F + FLAG*Y
Y = C*(1+ERR(1))
$THETA NOABORT
(0,3,100)
(0,3,10)
(0,3,10)
(0,3,1000)
(0,4,200)
(0,15,100)
$SIGMA
.5 .5 .5
$SIGMA
.5
$ESTIMATION SIGDIGIT=4 MAXEOVAL=6000 PRINT=10 POSTHOC $COVER
$SCATTER ...
$TABLE ...
```

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\textbf{Appendix}

Fitting was performed with NONMEM (version V level I)\textsuperscript{15} using the subroutines ADVAN2 and TRANS2, using the first order method. We fitted the data to the following one-compartment model of unbound drug concentration (C\textsubscript{u}). Total concentration in serum (C\textsubscript{b}) was related to C\textsubscript{u} by a simple linear link proportional to AAG concentration. The following equations for individual parameters were used

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obtained, covariates were successively deleted as usual (table 2). Two criteria were used to discriminate between the full model and reduced models, the log likelihood ratio test and the estimated coefficient of variation of estimates calculated as the ratio of the square root of the diagonal elements of the covariance matrix over the parameter estimates. A difference in the objective function of 6.63 was considered significant ($P < 0.01$). To avoid overparametrization of the model, we only considered parameters with an estimated coefficient of variation less than 50%. Fitting was performed considering a proportional intra-individual error (constant coefficient of variation). The 95% confidence interval of $Cl_n$ was obtained by sampling 1,000 replicates of the interindividual variability parameter $\eta$ of $\text{CL}$ from a normal distribution with mean zero and variance $\sigma^2$ using the NONMEM simulation step. For these simulations, $\sqrt{V_n}$ was set to it typical population value.

References

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