The Use of a Selected Ion Monitoring Technique to Study the Disposition of Bupivacaine in Mother, Fetus, and Neonate Following Epidural Anesthesia for Cesarean Section

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It is well known that the concentration of bupivacaine in umbilical cord blood at birth is low compared with the concentration in maternal blood. It is not clear whether this low fetal/maternal ratio (F/M) is due to decreased placental transfer or increased uptake by fetal tissues. The purposes of this study were to develop an appropriate analytic method and to clarify this issue by studying the disposition of bupivacaine in mother, fetus and neonate following epidural anesthesia. The study population included 14 parturients who were delivered by Cesarean section, and their infants. Gas chromatography–mass spectrometry techniques were developed which could simultaneously determine bupivacaine and its metabolite 2,6-piperidinolxylidine (PPX) in maternal, fetal and neonatal body fluids to <4 ng/ml. The results indicate several points: First, that bupivacaine and PPX remain detectable in neonatal blood for at least three days. Second, that plasma levels of PPX decrease more slowly in mother and neonate than bupivacaine. Also, both mother and neonate excrete primarily PPX in urine, but a higher percentage of unchanged bupivacaine is excreted by the neonate. Finally, urinary excretion of PPX by the neonate remains relatively constant during the first 48 h of life. In contrast, the mother excretes the highest amount of PPX between 12–24 h postpartum. The persistence of bupivacaine and PPX in neonatal body fluids suggests that the low F/M ratio of bupivacaine at birth is due to considerable uptake of bupivacaine by fetal tissues and is not due to diminished placental transfer. (Key words: Anesthesia: obstetric. Anesthetic techniques: epidural, peridural, lumbar. Anesthetics, local: bupivacaine. Measurement techniques: gas chromatography, mass spectrometry, selected ion monitoring. Metabolism: metabolites, 2,6-piperidinolxylidine (PPX).)

Despite the increasing popularity of bupivacaine for obstetric anesthesia, study of its disposition and metabolism in peripartum patients has not been adequate. Of particular interest are the amount of bupivacaine actually reaching the fetus and its detoxification by metabolism to 2,6-piperidinolxylidine (PPX) (fig. 1). Most studies have been limited to reporting levels of bupivacaine in maternal and fetal blood at birth.1-8 These levels were then used to calculate ratios of the levels of drug in fetal cord vein blood to the levels of drug in maternal vein (F/M). Only one other study has previously monitored the metabolism of bupivacaine to PPX in these patients.9

Previous studies have all shown that the concentration of bupivacaine in umbilical cord blood at birth is much lower than that in maternal blood.1-9 However, it is not clear what this low F/M ratio actually represents. The difference in concentration is thought by some to result from the high binding of bupivacaine to maternal plasma proteins and subsequent low rate of passage across the placenta;10 while others have suggested that the low F/M ratio is not due to diminished placental transfer, but

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BUPIVACAINE

\[
\text{CH}_3 \quad \text{O} \quad \text{C}_4 \text{H}_9 \\
\text{CH}_3 \quad \text{dealkylation} \\
\text{CH}_3 \quad \text{N} \quad \text{CH} \quad \text{N}
\]

Desbutyl-bupivacaine
2,6 - pipexocolylxylidine

FIG. 1. The metabolism of bupivacaine to PPX.

due to increased uptake by fetal tissues.\(^{39}\) The latter is thought to be possible due to the essentially instantaneous dissociation of the drug from plasma proteins\(^{11}\) and to the high lipid solubility of bupivacaine.

Most reports of bupivacaine levels in obstetric patients and neonates have been limited by lack of sensitive analytical techniques for determining low levels of bupivacaine. In addition, the analysis of PPX has been hampered by the lack of efficient extraction procedures which allow simultaneous determination of bupivacaine and PPX in small sample volumes. Neither drug can be determined adequately in plasma without specific gas chromatography/mass spectrometry (GC/MS) techniques.

The purpose of this study was two-fold: first, to develop a sensitive GC/MS technique for simultaneous determination of bupivacaine and PPX and second, to investigate the disposition of bupivacaine and its metabolism to PPX in mother, fetus and neonate following epidural anesthesia for Cesarean section.

**Methods**

**SAMPLE ANALYSIS**

Reference crystals of bupivacaine HCl hydrate and PPX were provided by Sterling-Winthrop Research Institute, Rensselaer, NY. The internal standard (W-12174), a lidocaine derivative (N-ethyl-N-sebutylglycinexylidide), was provided by Astra Pharmaceutical Products, Inc., Worcester, Massachusetts. Stock solutions of both standards were made to the equivalent of 100 \(\mu\)g/ml free base in 0.01 N HCl. The internal standard stock solution was made up to 10 \(\mu\)g/ml in 0.01 N HCl.

A Hewlett Packard\(^ \circ \) 5995 A quadrupole table-top mass spectrometer equipped with a direct injection probe was used to obtain 70 eV electron impact mass spectra from crystals of the reference compounds. For selected ion monitoring the gas chromatogram was interfaced to the mass spectrometer with a jet separator. The chromatograph was fitted with a 1.0 m \(\times\) 2 mm (ID) AW DMCS treated glass coil packed with 3 per cent OV-17 coated on an 100-120 mesh Gas Chrom Q support (Applied Sciences, State College, PA). The instrument conditions were: carrier gas flow—20 ml/min; injection port temperature—250°C; oven temperature—programmed from 200°C (0 min) at 16/min to 244°C; and the entire run time was 3.5 min. The optics of the mass spectrometer were optimized by autotuning at m/z 169. The ion intensities at m/z 84 for PPX, m/z 114 for the internal standard, and m/z 140 for bupivacaine were monitored with a window width of 0.1 amu and dwell times of 100 ms for PPX, 50 ms for bupivacaine, and 25 ms for the internal standard. Standard curves were prepared using freshly obtained urine and plasma from volunteers. Blood bank plasma could be used if it was carefully checked for the presence of interfering contaminants. Patient samples were separated by centrifugation and the plasma was stored frozen until analyzed. Patient samples (0.2-1 ml) and spiked plasma or urine were extracted using a modification of the procedure described by Lesko et al.\(^ {12}\) The modifications were: use of W-12174 as the internal standard; saturation of the sodium carbonate solution with sodium chloride; use of 30 \(\mu\)l of benzene for the final solvent; and injection of 2 \(\mu\)l of the final solution.

Standard curves were prepared and the samples were quantitated using the Hewlett Packard software for automatic quantitation of SIM data by area normalization on m/z 114.\(^ {13}\) Standard curves ranged from 30 to 2,000 ng/ml for urine and from 4 to 1,000 ng/ml for plasma.

**PATIENTS AND SAMPLE COLLECTION**

The protocol was reviewed and approved by the Human Investigation Committee of Cleveland Metropolitan General Hospital. Samples from 14 pregnant patients were obtained at Cesarean section or postpartum after obtaining the appropriate informed consent. Ten deliveries were scheduled repeat Cesarean sections and four were primary Cesarean sections for fetal position (one patient), failed induction (two patients) or herpes infection (one patient). All patients were free of liver or kidney disease and history of drug-addiction. Patients with mild complications of pregnancy such as gestational diabetes, mild pre-eclampsia, premature rupture of membranes, or herpes infection were included if epidural anesthesia was indicated. In addition, two patients had insulin dependent diabetes mellitus. The characteristics of the study population were: age 28 ± 2 years (mean ± SE); race four black, ten white; parity three nulliparous, eleven multiparous. All the infants were healthy with
Apgar ratings of 8 or more by 5 min of life. The mean birthweight was 3,229 ± 156 g and mean gestational age based on modified Dubowitz exam was 38.5 ± 0.4 weeks. Two of the infants were preterm, one was born at 34 weeks gestation and the other at 37 weeks gestation.

Bupivacaine was administered as needed for pain relief. The epidural space was entered at the L3–L4 interspace with a 17-gauge Touhy needle and identified with the loss of resistance technique. After a negative aspiration for blood and cerebrospinal fluid and after obtaining a preinjection maternal blood sample, 0.75 or 0.5% bupivacaine without epinephrine was injected. The number of doses needed by individual patients was 1.5 ± 0.2, (mean ± SE) and the number of milligrams administered was 164 ± 15. The mean time intervals between the initial injection and delivery was 41 ± 3 min, the mean interval between the final injection and delivery was 19 ± 4 min.

Maternal blood samples (3 ml) were collected from seven mothers through an indwelling cannula in a superficial vein on the dorsum of the hand at exact 5, 10, 15, 20, 30, 45, and 60 min intervals until delivery. The collection sequence was restarted after a repeat dose. A maternal sample was obtained at delivery-coincident with clamping of the umbilical cord, from eight mothers. All samples were drawn into heparin anticoagulant tubes and immediately placed on ice. The plasma was removed following centrifugation and frozen until assayed by GC-MS. After delivery, a 24-h blood sample was collected from seven of the mothers, and six consecutive 12-h urine samples were collected in standard containers from 12 of the mothers. Complete sets of blood and/or urine were not always obtained.

A total of 11 umbilical vein and seven umbilical artery blood samples (1–10 ml) were collected from 11 infants at birth from doubly clamped sections of umbilical cord. Data from 13 neonatal blood samples collected consecutively for up to 72 h were obtained from five of the infants. Urine samples were collected from 11 of the infants but complete sequential collections could not always be obtained. All of the neonatal urines were collected in plastic newborn urine collection bags and frozen until assayed by GC. Because of the inherent difficulty in collecting complete 12-h samples from newborns, data from all urine collections are expressed as micrograms of drug excreted per milligram of creatinine; urine creatinine was determined by the picric acid method.

**Results**

**Methodology**

The mass spectra of the bupivacaine, PPX and W-12174 standards are shown in figure 2. In each case, the intensity of the base peak was considerably greater than the intensity of the other fragment ions and the base peak was chosen for selected ion monitoring.

The selected ion current profile of a urine extract containing 3.74 μg/ml PPX, 0.39 μg/ml bupivacaine, and 0.25 μg/ml internal standard is shown in figure 3. The retention times of the internal standard, PPX, and bupivacaine are approximately 1.4, 2.1, and 2.7 min, respectively. Frequently a second contaminant peak at m/z 140 can be seen after the bupivacaine peak; a spectrum obtained from a total ion current profile matched a library spectrum of tri-2-butoxyethyl phosphate, a plasticizer contaminant from the Becton-Dickenson Vacutainers®. While this peak might be expected
SELECTED ION CURRENT PROFILE OF A URINE EXTRACT

ION = m/z 140
Full scale = 45

Unknown contaminant
Bupivacaine
BD Contaminant

ION = m/z 114
Full scale = 587
W-12174

ION = m/z 84
Full scale = 1198

MINTUES

FIG. 3. Selected ion current profile of a urine extract showing the lack of interference from urine contaminants and the presence of the plasticizer contaminant from the BD vacutainer stoppers.

in plasma but not urine, this particular sample had been stored in a reused green top vacutainer.

Calibration curves obtained by normalizing the peak area to m/z 114 were linear over the ranges studied. The least squares linear regression line which describes these curves in plasma are $y = 0.024x + 0.002$ for bupivacaine and $y = 0.003x + 0.0001$ for PPX ($r = 1$ for both curves). Using these curves, samples containing less than 4 ng/ml PPX or bupivacaine could be quantitated.

The precision of the method was determined by repeated analysis of spiked samples containing low (15 ng/ml) and high (500 ng/ml) concentrations of bupivacaine and PPX. The relative standard deviation (coefficient of variation) for bupivacaine was 6.2 and 2.4 per cent for the low and high samples, respectively; for PPX these values were 10.6 and 5.4 per cent.

MATERNAL PLASMA

Figure 4 is a typical maternal plasma profile of bupivacaine and PPX from one patient following epidural anesthesia with bupivacaine for Cesarean section. PPX was detectable in the 5 min sample and after 15 min increased steadily until delivery at 45 min. The early fluctuations in PPX levels between 5 and 15 min, which paralleled the fluctuations in bupivacaine, are thought to be due to the small amount of PPX measurable in the commercial bupivacaine preparations. (Three different lot numbers of commercial bupivacaine were tested and were found to contain an average of 4.25 µg PPX/ml bupivacaine solution.) Twenty-four hours after delivery, bupivacaine had decreased greatly while the PPX levels were only slightly lower than the previous day. The concentrations of bupivacaine and PPX in eight maternal delivery samples were 1178 ± 214 and 101 ± 38 ng/ml (mean ± SE), respectively (table 1). Twenty-four hours later the mean concentrations were 85 ± 18 and 102 ± 11 ng/ml, respectively.

MATERNAL URINE

The pattern of urinary excretion and metabolism of bupivacaine is shown in figure 5. Most of the bupivacaine was excreted as PPX which reached its highest levels in

FIG. 4. The appearance of bupivacaine and PPX in maternal venous plasma from a typical patient following 188 mg bupivacaine administered for epidural anesthesia for Cesarean section.
the 12–24 h postpartum sample. In contrast, unchanged bupivacaine was present only in low levels which decreased steadily over the 72-h period of collection.

**Fetal Plasma**

The levels of bupivacaine and PPX in cord vein and artery plasma are shown in Table 1. The ratio of the bupivacaine concentration in umbilical cord vein compared to that in maternal venous plasma (F/M) ratio was 0.31 and that for PPX was 0.77. Two of the infant samples had higher plasma levels of PPX than the corresponding maternal sample. In two cases, cord artery levels of PPX were higher than the corresponding cord vein levels.

**Neonatal Plasma**

Bupivacaine and PPX levels were obtained in samples from five infants. In most cases the bupivacaine concentration decreased greatly during the first 24 h and then decreased more slowly during the next 48 h. In contrast, the PPX levels first increased and then decreased more slowly than the bupivacaine. Four of five babies had detectable plasma levels of both bupivacaine and PPX for 72 h.

**Neonatal Urine**

Figure 5 also illustrates the neonatal urinary excretion and metabolism of bupivacaine. It can be seen that the neonate excretes a higher percentage of unchanged bupivacaine than the mother. In addition, it can be seen that the urinary excretion of PPX remains relatively constant for the first 48 h of life. For all three days more PPX than parent compound was excreted and both compounds were detectable.

**Discussion**

The purpose of this investigation was twofold: first, to develop an appropriately sensitive analytic technique, and, second, to investigate the disposition of bupivacaine and its metabolism in mother, fetus, and neonate. The data from this study regarding both bupivacaine and PPX suggest that with an appropriate analytic method, considerable uptake of bupivacaine by both fetal and maternal tissue can be demonstrated.

**Methodology**

The increasing popularity of bupivacaine has led to a demand for a rapid and specific analytic method for measuring bupivacaine and PPX in body fluids. Gas chromatographic methods are available and these have recently been summarized. Most use a relatively insensitive flame ionization detector and are not suitable for the analysis of PPX. Recently, Lesko et al., using a more sensitive nitrogen phosphorus detector, reported simultaneous determination of bupivacaine and PPX; with their method, however, the bupivacaine retention time is 15 min and the limit of detection is 100 ng/ml. In comparison, we report retention times of less than 3 min and a sensitivity of <4 ng/ml. Methods such as this using very sensitive mass spectrometry techniques have not previously been published.

One reason for the sensitivity of this assay is the use...
of selected ion monitoring. The intense base peak produced by electron impact for each of the three compounds allows the procedure to be maximized for sensitivity. The intense base peaks are thought to result from cleavage of the carbon–carbon bond at the carbonyl group which is characteristic of substituted amines.17

Other compounds could be analyzed or used for internal standards with this procedure. For example, lidocaine (base peak m/z 86) or similar anilide-type local anesthetics may be analyzed by monitoring the appropriate ion fragments. However, it should be noted that the use of mepivacaine, (base peak m/z 98) is not suitable as an internal standard for PPX analysis because its structure is different from PPX by only one methyl group. Consequently, mepivacaine and PPX are hard to separate chromatographically, and the two compounds have many ion fragments, including m/z 98, in common. Similarly, without derivatization, it would be difficult to determine PPX, which is also a metabolite of mepivacaine in patients who received mepivacaine for local anesthesia.

MATERNAL PLASMA

The levels of bupivacaine at delivery and the shape of the plasma profile that we report are similar to those reported previously following Cesarean section.3,5,7 Levels of PPX following bupivacaine for Cesarean section have not previously been reported in maternal plasma. However, Reynolds and Taylor reported detectable PPX levels in plasma of laboring patients following multiple doses of bupivacaine.9 These were primarily patients who labored 4 h or longer following induction of epidural anesthesia. The levels reported in the present series following fewer doses or shorter drug to delivery intervals are understandably lower than those reported previously. A study of the peripartum metabolism of a similar drug also exists: Meffin et al.18 reported measurable levels of PPX in maternal blood at the time of vaginal delivery following epidural anesthesia with mepivacaine.

The shape of the maternal PPX appearance curve deserves some comment. During the first few minutes, the shape mirrors the bupivacaine curve and suggests that some PPX was present in the commercial bupivacaine solutions. PPX was found in the solutions but this was not unexpected since metabolites have been previously found in other commercial drug preparations.19 The amount of PPX received in this manner is probably insignificant in view of the large amount subsequently produced by metabolism. In addition, this metabolite is not pharmacologically active.

MATERNAL URINE

The urine data show that the pregnant woman can rapidly metabolize bupivacaine; very little unchanged drug was excreted in the urine of patients even in the first 12-h collection period. More PPX was excreted in the second 12-h collection period than in the first period. In contrast, the bupivacaine levels decreased by approximately 25 per cent over the same time period. This supports the concept of extensive tissue uptake of bupivacaine by maternal tissues; i.e., even though plasma and urine levels are low, considerable substrate must be available to produce such high amounts of PPX. Considerably different excretion patterns are seen in urine from similar peripartum patients who received less highly bound drugs such as lidocaine20 and meperidine.21

FETAL PLASMA

The low F/M ratio (0.31) observed for bupivacaine compares well with what has been reported previously.1,9 The higher (0.77) F/M ratio for PPX is similar to that reported by Reynolds and Taylor (0.85) for laboring patients.9 The difference is probably due to the longer length of time between initial administration of the drug and more frequent doses in the previous study. Meffin et al.18 studied mepivacaine, a similar compound with the same metabolite, and also reported the F/M ratio for PPX (2.05), to be much higher than the F/M ratio for the parent compounds (0.41). Similarly, higher F/M ratios have also recently been reported for MEGX than for lidocaine, the parent compound.19 That the F/M ratio for the metabolite is consistently higher than for the parent compound, and that a few infants had higher plasma levels of PPX than their mothers suggest that some of the PPX measured in fetal plasma resulted from in utero metabolism of bupivacaine.

NEONATAL PLASMA

Finding bupivacaine in neonatal plasma for as long as 72 hours suggests that considerable placental transfer occurs and that the half-life of bupivacaine in neonates might be considerably longer than that reported previously.4,6 However, accurate plasma half-lives could not be calculated from the data points obtained in this study from so few neonates. Similar levels of bupivacaine have been detected previously in neonatal plasma for as long as the first 25 h of life following Cesarean section.4,5 Other studies reporting nondetectable levels at 4 or 8 h of age were probably hampered by the analytic techniques involved.2

In contrast to the rapid decline in bupivacaine levels, PPX levels decreased more slowly in neonatal plasma. This, again, seems to support the idea of increased uptake of bupivacaine by fetal tissues. In other words, the bupivacaine in the tissues was now being metabolized and contributing to the plasma levels of PPX. It is not likely that the excretion of PPX by the neonatal kidney cannot keep up with the rapid production of PPX from bupi-
Bupivacaine disposition

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vaccine. PPX is more polar than the parent compound and would be expected to be excreted more readily. Never- therelhese, other interpretations of these data might be that the enzymes responsible for the dealkylation of bupivacaine are quickly saturated, or subsequent pathways for the elimination of PPX are inefficient. These questions cannot be resolved from this study.

Neonatal Urine

The urinary excretion pattern for neonates observed in this study is quite different than the adult pattern for several reasons: First, a greater proportion is excreted as unchanged drug. This illustrates the neonate's more limited capacity to produce PPX. This was also noticed by Meffin et al. for mepivacaine.18 Second, although more PPX than unchanged drug is excreted during each period, the PPX does not increase very much between 12 and 24 h, nor does it decrease from 24–48 h as was seen in maternal urine. This suggests several points: First, that the PPX is coming primarily from neonatal metabolism. If most of it had arisen from maternal metabolism and placental transfer, there probably would have been a steady decrease each collection period. Second, it again suggests that there is considerable tissue uptake of bupivacaine, whose elimination from the tissues maintains a level of substrate for PPX formation. And finally, the steady urine levels of PPX over time suggest the possibility of saturation of an enzyme system or the inability to further metabolize PPX. The excretion patterns obtained suggest that the infants ability to metabolize bupivacaine is similar to its ability to dealkylate lidocaine;20 but much more efficient than its ability to demethylate meperidine.21

In summary, the purpose of this study was to investigate the disposition of bupivacaine and its metabolism to PPX by mother, fetus and neonate using sensitive GC/MS techniques. The data suggest that a considerable amount of bupivacaine is transferred across the placenta following epidural anesthesia. Detectable levels of bupivacaine and sustained levels of PPX in both plasma and urine for several days after birth support this. It appears that the low levels of bupivacaine in umbilical cord blood might be due more to rapid uptake of bupivacaine by fetal tissues than to decreased placental transfer. This seems reasonable in spite of the high percentage of drug bound to maternal plasma proteins for two reasons: First, dissociation of drug from protein can occur essentially instantaneously;9 and second, because the high lipid solubility of bupivacaine would tend to facilitate, not impede, placental transfer.9

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