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Comparison of placental transfer of local anesthetics in perfusates with different pH values in a human cotyledon model

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Abstract
Purpose. We aimed to investigate the placental transfer of local anesthetics in perfusates with different pH values, using a dual-perfused human cotyledon model.
Methods. The dual-perfused human cotyledon model was prepared from placentas obtained following cesarean delivery (n = 5). Protein-free solution was perfused through both maternal and fetal arteries. Four amide-type local anesthetics (mepivacaine [Mep]; lidocaine [Lid]; bupivacaine [Bup]; and ropivacaine [Rop]) were added to the maternal perfusate at 1 $\mu g \cdot ml^{-1}$. Three conditions were tested (stage 1, maternal pH 7.4, fetal pH 7.4; stage 2, maternal pH 7.4, fetal pH 6.9; and stage 3, maternal pH 6.9, fetal pH 6.9). Venous blood samples were collected from the fetal circuit after stabilization. The fetal vein/maternal artery concentration ratio (F/M ratio) of the local anesthetics was used as an index of placental transfer. The concentration of human chorionic gonadotropin (hCG) in the maternal vein was measured at the end of each stage.
Results. The F/M ratios in all stages were in the order of: Mep > Lid > Bup ≒ Rop. The F/M ratios of Mep were significantly higher than those of the other local anesthetics in all stages. The F/M ratios of Lid were higher than those of Rop in stages 2 and 3. The F/M ratios of Lid and Rop were higher in stage 2 than in stage 3. However, the differences between the F/M ratios in the three stages were not as large as expected from the basic uncharged ([B]) condition and pH gap. The concentration of hCG showed a time-dependent decrease with increasing stage (stage 1, 81.0 ± 58.9 $mIU \cdot ml^{-1}$; stage 2, 57.4 ± 31.8 $mIU \cdot ml^{-1}$; stage 3, 32.1 ± 19.7 $mIU \cdot ml^{-1}$).
Conclusion. Our data clearly show that it is the basic uncharged concentration that mainly determines the placental transfer of amide-type local anesthetics with protein-free perfusate. This finding suggests that Rop and Bup can be used more safely than Mep in terms of placental transfer.

Key words Placental transfer · Local anesthetics · Cotyledon · pH gap

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Introduction

Local anesthetics are commonly used for epidural anesthesia and analgesia during labor. The accidental intravascular injection of local anesthetics presents the dangers of maternal cardiac arrest and neonatal depression. The extent of local anesthetic transfer across the placenta is dependent on several factors: the molecular weight, protein binding, and lipid solubility of the anesthetic; the maternal serum drug concentration; and the maternal and fetal serum pH [1].

Maternal and fetal serum pH may play an important role in placental transfer by determining the quantity of basic uncharged ([B]) local anesthetic [2–5]. However, it is difficult to clearly identify the pH-dependent placental transfer of local anesthetics because placental transfer also depends on the drug's protein-binding ratio [6]. Additionally, the results of experiments on the placental transfer of local anesthetics may be subject to errors, given that a single local anesthetic was usually tested using a single placenta which may have been time-dependently damaged.

In this study, therefore, we compared the placental transfer of local anesthetics by simultaneously administering local anesthetics in protein-free perfusates with different pH values. The purpose of this study was to provide experimental evidence of the pH-dependent transfer of local anesthetics in a well-established dual-perfused human cotyledon model.

Patients, materials, and methods

The institutional review board approved this study (no. 184, 527), and written informed consent was obtained from each patient from whom a placenta was obtained after cesarean delivery. Four local anesthetics were tested: mepivacaine (Mep), lidocaine (Lid), bupivacaine (Bup), and ropivacaine (Rop). Mep, Lid, and Bup were

obtained from Sigma-Aldrich (St. Louis, MO, USA) and Rop was obtained from AstraZeneca (London, UK).

Placental perfusion

Five placentas from healthy parturients undergoing cesarean delivery (gestational age, 36–38 weeks) were collected and immediately transported to the laboratory. Cotyledons with easily recognizable maternal and fetal vessels were selected and perfused as previously reported [7–9]. Maternal and fetal circuits were perfused in a single-pass model at the rates of 15 and 2 ml·min^{-1}, respectively (Fig. 1). The perfusate was composed of tissue culture medium 199 modified with Earl's salts (ICN Biomedicals, Aurora, OH, USA) containing heparin (2500 U·l^{-1}), gentamycin (50 mg·l^{-1}), and glucose (1.0 g·l^{-1}). HCl or $NaHCO_3^-$ was used to adjust the pH of each solution. Initially, the maternal and fetal perfusates were adjusted to pH 7.4. The perfusates for the fetal and maternal circuits were equilibrated with 95% nitrogen/5% CO_2 and 95% oxygen/5% CO_2, respectively. The temperature of the container was maintained at 36°C using a water bath. After a stabilization period (30 min), the four local anesthetics were simultaneously added to the maternal perfusate, each to a final concentration of 1 µg·ml^{-1}.

The perfusate was tested in the following three stages: stage 1 (maternal pH 7.4, fetal pH 7.4); stage 2 (maternal pH 7.4, fetal pH 6.9); and stage 3 (maternal pH 6.9, fetal pH 6.9). The perfusion time for each stage was 30 min and the interval between stages was more than 20 min. A pilot single-pass perfusion experiment confirmed that local anesthetic concentrations in fetal venous samples reached a plateau within 10 min; therefore, venous samples from the fetal circuit were obtained at the start and 30 min after perfusion had been initiated. The concentration of human chorionic gonadotropin (hCG) in the maternal vein was measured at the end of each stage by chemiluminescent enzyme immunoassay to determine the viability of the placenta. Maternal and fetal perfusion pressures were measured using a pressure transducer (Yamasu, Tokyo, Japan). The perfusion pressures on the maternal and fetal side were less than 70 mm Hg.

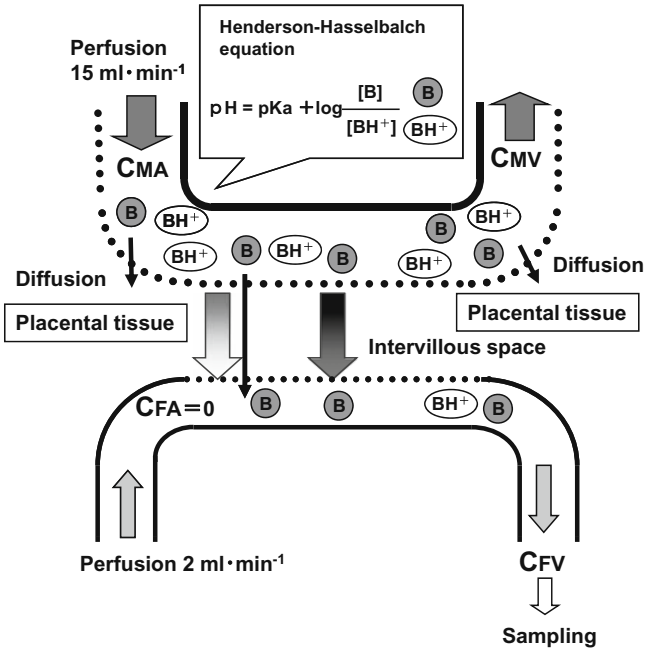

Fig. 1. Diagram of perfused cotyledon model. *MA*, maternal artery; *MV*, maternal vein; *FA*, fetal artery; *FV*, fetal vein; *C*, concentration of local anesthetics; *B*, basic uncharged form; BH^+ charged cationic form

The concentrations of the local anesthetics were measured simultaneously, using HPLC (model 540; CoulArray Medical System, ESA, Chelmsford, MA, USA), as previously reported [10]. This method is very sensitive and enables the simultaneous identification and quantification of multiple drugs. An MCM column (ODS [octadecyl silane] 4.6 × 250 mm; MC MEDICAL INC, Tokyo, Japan) was used for local anesthetic analysis with the above-mentioned detector (electrical potential 650–850 mV). The mobile phase included solution A, consisting of 0.035 mol·l^{-1} phosphate buffer (pH 6.2) and 19:1 (v/v) acetonitrile, and solution B, consisting of 0.12 mol·l^{-1} phosphate buffer (pH 6.2) and 50:50 (v/v) acetonitrile. The fetal vein/maternal artery concentration ratio of local anesthetic (F/M ratio) was calculated as an index of placental transfer. The concentration gradients of the [B] form of local anesthetics in the maternal artery and fetal vein were calculated using the Henderson-Hasselbalch equation. The pKa values of these local anesthetics are follows: Mep, 7.76; Lid, 7.91; Bup, 8.16; and Rop, 8.2 [11].

Statistical analysis

All data are expressed as means ± SD. The concentrations of each local anesthetic were analyzed with Student's *t*-test or one-way analysis of variance (ANOVA). Differences were considered statistically significant when $P < 0.05$.

Results

The F/M ratios of the local anesthetics in the three stages are shown in Fig. 2. The mean values of the F/M ratios of the local anesthetics in all stages were in the order of Mep > Lid > Bup ≒ Rop. The F/M ratios in stage 1 were 0.53 ± 0.34 for Mep, 0.31 ± 0.18 for Lid, 0.25 ± 0.20 for Bup, and 0.25 ± 0.18 for Rop. The F/M

ratios of Mep were significantly higher than those of the other local anesthetics in all stages. The F/M ratios of Lid were higher than those of Rop in stages 2 and 3. The F/M ratios of Rop and Bup were almost half that of Mep, and that of Lid was about two-thirds that of Mep. The differences in the F/M ratios of the local anesthetics between the stages were significant only for Lid and Rop between stage 2 and stage 3.

Figure 3 shows the calculated concentration gradients of the basic uncharged ([B] form) of the local anesthetics between the maternal artery and fetal vein. In the comparison between stage 1 and stage 2, only Mep appeared to have a large concentration gradient in stage 2 when compared with stage 1. The gradients of the [B] form in stage 3 were one-third of those in stage 2 for all the local anesthetics.

The concentration of hCG ($mIU \cdot ml^{-1}$) decreased time-dependently with increasing stages, with values of 81.0 ± 58.9 in stage 1, 57.4 ± 31.8 in stage 2 and 32.1 ± 19.7 in stage 3.

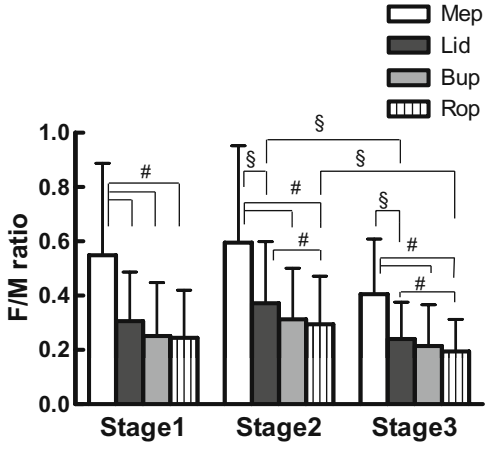

Fig. 2. The fetal vein /maternal artery concentration ratio (*F/M ratio*) of each local anesthetic in three different pH conditions. *Mep*, mepivacaine; *Lid*, lidocaine; *Bup*, bupivacaine; *Rop*, ropivacaine. [#]Significant difference ($P < 0.01$); [§]significant difference ($P < 0.05$)

Discussion

This study investigated the placental transfer of four local anesthetics in perfusates with different pH values. In all pH conditions tested, the F/M ratios of local anesthetics showing transfer across the placenta were, from greatest to least, Mep > Lid > Rop ≒ Bup. The F/M ratios of each anesthetic agent were comparable to those previously reported [12–17]. The umbilical vein-to-maternal vein concentration ratios of anesthetics in painless delivery with epidural anesthesia were reported to be: Mep, 0.64; Lid, 0.52; and Bup, 0.27 [12]; and Mep, 0.53; Lid, 0.41; and Bup, 0.25 [17]. Another study showed higher values for Bup and Rop, with values of 0.69 for Bup and 0.72 for Rop [18]. Given that the sequence of F/M ratios in our study was consistent with that of the calculated differences in the basic uncharged concentration ([B] form) of the local anesthetics, our data clearly demonstrate that the placental transfer of local anesthetics mainly depends on the basic uncharged concentration of local anesthetics. On this basis, the effect of competition on the transfer ratios for local anesthetics by simultaneous administration was considered to have been negligible.

However, the differences we found in the placental transfer of local anesthetics between the stages were not as large as expected from the [B] form concentration. For example, in stage 3, the gradient of the [B] form was one-third of that in stage 1, but the F/M ratios of local anesthetics did not show significant differences between stage 1 and stage 3. Additionally, the gradient of the [B] form in stage 2 was almost three times as great as that in stage 3. However, the F/M ratios of Lid and Rop in stage 2 were only 30% larger than those in stage 3. These discrepancies between the F/M ratios and the gradients of the [B] form may be reasonably explained in terms of placental cell viability, suggested by the time-dependent decrease of hCG with increasing stages. The loss of cell viability between the three stages may have been accelerated by acidosis. It is unlikely that the local anesthetics would have been mainly responsible

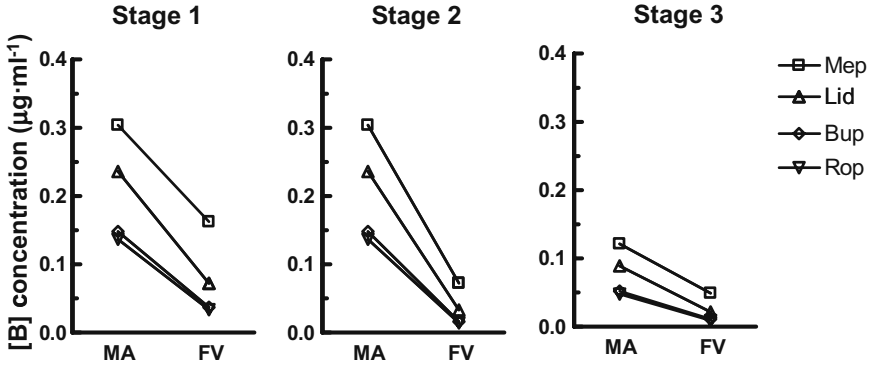

Fig. 3. Concentration gradients of the basic uncharged form (*[B] form*) of local anesthetics in three different pH conditions. The concentration gradients of the [B] form of local anesthetics between the maternal artery (MA) and fetal vein (FV) were calculated from the local anesthetic concentrations in the MA and FV using the Henderson-Hasselbalch equation. *Mep*, mepivacaine; *Lid*, lidocaine; *Bup*, bupivacaine; *Rop*, ropivacaine

for the decrease in hCG. A previous report [19] showed that cocaine suppressed hCG production, but this effect would not have been the case with our study, because the hCG concentration in that study increased time-dependently in their control. Placental cell damage resulting from acidosis may increase placental permeability [20,21] and thus increase the F/M ratios of local anesthetics. In our study, this time-dependent loss of placental cell viability would not have affected the comparison of placental transfer between local anesthetics in the same stage because the local anesthetics were administered simultaneously. Thus, our dual-perfused human cotyledon model has an advantage in that changes in placental cell viability are unlikely to change the result.

Our finding that the [B] form concentration of each local anesthetic was an important factor in determining placental transfer in all pH stages provides information regarding the optimal choice of local anesthetics for continuous epidural anesthesia for painless delivery. In regard to the [B] form concentrations of the local anesthetics, we found that these concentrations of Rop and Bup were similar regarding placental transfer in all stages. Rop may be recommended because Bup has the disadvantage of cardiotoxicity when used for continuous epidural anesthesia for painless delivery. It remains to be investigated how the decrease of protein binding in cases of maternal acidosis affects the placental transfer of local anesthetics.

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