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Systemic Toxicity of Levobupivacaine, Bupivacaine, and Ropivacaine during Continuous Intravenous Infusion to Nonpregnant and Pregnant Ewes

Alan C. Santos, M.D., M.P.H., Pedro I. DeArmas, M.D.†*

Background: **Levobupivacaine, the single levorotatory isomer of bupivacaine, is now available for clinical use. This study was undertaken to determine whether pregnancy affects the systemic toxicity of levobupivacaine and to compare the systemic toxicity of levobupivacaine with that of bupivacaine and ropivacaine.**

Methods: **Chronically prepared nonpregnant and pregnant sheep were randomized to receive an intravenous infusion of 0.52% levobupivacaine, 0.52% bupivacaine, or 0.50% ropivacaine at a constant rate of 0.1 ml · kg^{-1} · min^{-1} until circulatory collapse. The investigators were blinded to the identity of the local anesthetic. Physiologic parameters, including cardiac rhythm, were monitored throughout the study. Arterial blood samples were obtained before infusion and at the onset of toxic manifestations. These were analyzed for total and free serum drug concentrations as well as arterial blood *p*H and gas tensions.**

Results: **The doses of all three drugs required to produce convulsions were lower in pregnant than nonpregnant animals. However, as the infusion continued, there were no significant differences between pregnant and nonpregnant ewes in the dose of drug required to produce more advanced manifestations of toxicity: hypotension, apnea, and circulatory collapse. The mean cumulative dose and serum concentration at each toxic manifestation was lowest for bupivacaine, intermediate for levobupivacaine, and highest for ropivacaine in both pregnant and nonpregnant animals. For all three local anesthetics, there were no significant differences between pregnant and nonpregnant ewes in total and free serum drug concentrations, except that at circulatory collapse, these were higher in pregnant animals.**

Conclusions: **Pregnancy increases the risk of convulsions but not of more advanced manifestations of local anesthetic toxicity. The risk of toxicity is greatest with bupivacaine and least with ropivacaine. However, in actual clinical practice, the risk of systemic toxicity may also be affected by the relative potency and effectiveness of these drugs.**

BUPIVACAINE is probably the most commonly used drug for epidural analgesia in obstetrics, although its margin of safety is narrower than that of less potent agents such as lidocaine and mepivacaine.[1–3] Indeed, unintended intravascular injection of bupivacaine during attempted epidural anesthesia for labor or cesarean delivery has resulted in almost simultaneous convulsions and cardiovascular collapse, often refractory to resuscitation.[4,5] As a result, there has been a search for alternative drugs with the desirable blocking properties of bupivacaine but having a greater margin of safety.

The development of new long-acting amides has taken advantage of the fact that most amide local anesthetics have a chiral center, a carbon atom bonded to four different molecules, and thus can exist as dextro (*R*+) and levorotatory (*S*−) stereoisomers. This is important because the levorotatory isomer of most long-acting amide local anesthetics generally has lower potential for systemic toxicity than the dextro form of the drug.[6] Until recently, formulations of local anesthetic for clinical use have contained a racemic mixture of both the levorotatory and dextrorotatory isomers.

Ropivacaine, the first single levorotatory isomer formulation of local anesthetic for clinical use, became available in the early 1990s as a potential alternative to bupivacaine. Although it is less potent,[7] ropivacaine has many of the beneficial blocking properties of bupivacaine[8] but a somewhat greater margin of safety.[9] The systemic toxicity of ropivacaine is not enhanced by pregnancy.[9]

The other drug recently approved for clinical use is levobupivacaine, the single levorotatory isomer of bupivacaine. Unlike ropivacaine, it is equipotent to bupivacaine[10] and may have a greater margin of safety than bupivacaine.[11–14] The potential effects of pregnancy on the systemic toxicity of levobupivacaine are unknown.

The purpose of the current study involving nonpregnant and pregnant sheep was twofold: (1) to determine whether pregnancy affects the systemic toxicity of levobupivacaine; and (2) to compare the *in vivo* systemic toxicity of levobupivacaine with that of racemic bupivacaine, currently the most frequently used amide local anesthetic in obstetrics, and ropivacaine, the only other single levorotatory isomer of local anesthetic in clinical use.

Materials and Methods

Thirty-eight pregnant ewes near term of gestation and 37 nonpregnant ewes were studied in a protocol approved by the Institutional Animal Care and Use Committee of the State University of New York–Stony Brook and Montefiore Medical Center (Bronx, NY). Only water

* Professor, Department of Anesthesiology, † Instructor, Department of Medicine.

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Address correspondence to Dr. Santos: Department of Anesthesiology, St. Luke's–Roosevelt Hospital Center, 1111 Amsterdam Avenue, New York, New York 10025. Address electronic mail to: obanesdoc@aol.com. Reprints will not be available from the authors. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

was provided to the animals the night before surgery. A neck cutdown was performed during general endotracheal anesthesia with 2–3% halothane in nitrous oxide and oxygen, and two polyethylene catheters were inserted, side by side, into the common carotid artery for simultaneous blood sampling and monitoring. An additional single catheter was placed in the jugular vein. Ewes were then allowed to recover for a least 5 days. Antibiotics and an analgesic (flunixin meglumine) were administered for 2 days postoperatively.

On the day of study, the ewe was weighed and contained in a cart with freedom to stand or lie down. Arterial blood pressure and heart rate (determined by cardiotachometer) were recorded continuously on a polygraph. Cardiac rhythm was also monitored using a transvenous intracardiac electrode placed percutaneously *via* the contralateral jugular vein on the day of study. At the conclusion of a control period of at least 30 min, ewes were randomized to receive an intravenous infusion of levobupivacaine (0.52%), bupivacaine (0.52%), or ropivacaine (0.50%) at a constant rate of 0.1 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}. These drug concentrations were chosen to administer equimolar amounts of the three local anesthetics. Accordingly, there were six groups of animals: levobupivacaine pregnant (n = 14), levobupivacaine nonpregnant (n = 12), bupivacaine pregnant (n = 12), bupivacaine nonpregnant (n = 12), ropivacaine pregnant (n = 12), and ropivacaine nonpregnant (n = 13). Test substances were supplied by the sponsor (Chiroscience R & D, Ltd., Cambridge, United Kingdom) in ampules designated by experiment number only; thus, all investigators were blinded to the local anesthetic being infused.

Arterial blood samples were obtained in duplicate (anticoagulated and nonanticoagulated) before drug infusion and at the onset of each toxic manifestation, which usually appeared in sequence: convulsions, hypotension, apnea, and circulatory collapse. Convulsions were considered to begin with the onset of tonic–clonic movements. Hypotension was defined *a priori* by a decrease in blood pressure of at least 40%, usually precipitously, from levels that had been recorded during convulsions. Apnea was diagnosed after the absence of respirations for 15 s. Finally, the disappearance of pulsatile blood pressure signaled circulatory collapse. Blood *p*H and gas tensions were determined from arterial samples anticoagulated with heparin. Nonanticoagulated blood samples were allowed to clot, and serum was separated after centrifugation. Contact with polystyrene plastic or stoppers containing tributoxyethyl phosphate ester plasticizer was avoided. Serum rather than plasma was studied to avoid the artifactual effects of *in vitro* lipolysis, which are particularly significant in the plasma of pregnant animals.[15,16] After an aliquot of serum had been removed for drug determination, the pH of the remaining serum was adjusted with microliter quantities of 0.1 N hydrochloric acid or sodium hydroxide to be equal ($\pm$ 0.02) to the blood *p*H determined at the time of sampling corresponding to the toxic event. Using an ultrafiltration system (Centrifree®; Amicon, Worchester, MA), serum water was obtained from 1-ml aliquots of serum after centrifugation for 45 min at 2,000*g*. All serum and serum water samples were kept frozen until drug analysis using chiral normal phase high-performance liquid chromatography with ultraviolet spectrophotometric detection. Electrocardiograms were analyzed for the presence of malignant ventricular arrhythmias at the time of death by a cardiologist blinded to the animal's group assignment.

All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC) by an independent contractor (Parexel International Ltd., Uxbridge, United Kingdom) blinded to drug treatment. Cox proportional hazards model, analyses of variance, Wald chi-square, Kruskal-Wallis and Mantel-Haenszel chi-square tests were used where appropriate (see Appendix for details of statistical analyses). It was estimated that 12 ewes would be required in each group to detect a difference of 30% in the dose of drug needed to produce circulatory collapse between pregnant and nonpregnant ewes (α = 0.05, β = 0.8). All results are expressed as the mean $\pm$ SD.

Results

The mean weight of animals in each group was as follows: levobupivacaine pregnant, 54 $\pm$ 7 kg; levobupivacaine nonpregnant, 55 $\pm$ 11 kg; bupivacaine pregnant, 54 $\pm$ 7 kg; bupivacaine nonpregnant, 56 $\pm$ 9 kg; ropivacaine pregnant, 52 $\pm$ 5 kg; and ropivacaine nonpregnant, 55 $\pm$ 10 kg. There were no significant differences among the three groups of pregnant animals in gestational age. All ewes were in good condition at the start of the study, and there were no significant differences among the groups in mean heart rate, mean arterial blood pressure, *p*H, and gas tensions at control (tables 1–3).

The manifestations of systemic toxicity occurred in the majority of animals as anticipated: convulsions followed by hypotension, apnea, and, finally, circulatory collapse (figs. 1–3). However, in some ewes, convulsions were directly followed by circulatory collapse. This occurred in five animals in the levobupivacaine-nonpregnant group, two in the bupivacaine-pregnant group, three in the bupivacaine-nonpregnant group, two in the ropivacaine-pregnant group, and two in the ropivacaine-nonpregnant group. In some other animals, hypotension and apnea occurred almost contemporaneously: levobupivacaine pregnant = 2, bupivacaine nonpregnant = 2, and ropivacaine nonpregnant = 1 (figs. 1–3). The dose of drug required to produce convulsions was lower for pregnant as compared with nonpregnant animals: 0.015 $\pm$ 0.003 mmol/kg levobupivacaine pregnant *ver-*

Table 1. Physiologic Variables for Levobupivacaine Groups

	Control	Convulsion	Hypotension	Apnea	Circulatory Collapse
Nonpregnant					
HR	86 ± 11 (n = 12)	222 ± 32 (n = 12)	167 ± 48 (n = 7)	124 ± 27 (n = 7)	—
MABP	113 ± 14 (n = 12)	198 ± 33 (n = 12)	72 ± 18 (n = 7)	49 ± 11 (n = 7)	—
*p*H	7.48 ± 0.03 (n = 12)	7.48 ± 0.06 (n = 12)	7.25 ± 0.1 (n = 7)	7.25 ± 0.1 (n = 7)	7.31 ± 0.2 (n = 12)
P_{CO_2}	31 ± 3 (n = 12)	28 ± 5 (n = 12)	52 ± 14 (n = 10)	53 ± 17 (n = 7)	48 ± 18 (n = 12)
P_{O_2}	94 ± 5 (n = 12)	100 ± 9 (n = 12)	37 ± 31 (n = 10)	32 ± 35 (n = 7)	50 ± 40 (n = 12)
Pregnant					
HR	98 ± 13* (n = 14)	204 ± 42† (n = 14)	121 ± 16 (n = 14)	100 ± 15 (n = 12)	—
MABP	98 ± 14‡ (n = 14)	190 ± 19† (n = 14)	72 ± 8 (n = 14)	50 ± 11 (n = 12)	—
*p*H	7.51 ± 0.03 (n = 14)	7.48 ± 0.04 (n = 14)	7.22 ± 0.05 (n = 14)	7.21 ± 0.05 (n = 12)	7.20 ± 0.05 (n = 14)
P_{CO_2}	31 ± 2 (n = 14)	29 ± 3 (n = 14)	58 ± 4 (n = 14)	59 ± 5 (n = 12)	61 ± 5 (n = 14)
P_{O_2}	92 ± 10 (n = 14)	103 ± 12 (n = 14)	21 ± 8 (n = 14)	17 ± 5 (n = 12)	15 ± 4 (n = 14)

Heart rate (HR, beats/min), mean arterial blood pressure (MABP, mmHg), arterial blood *p*H, partial pressure of carbon dioxide (P_{CO_2}, mmHg), and partial pressure of oxygen (P_{O_2}, mmHg) during the control period and at the onset of each toxic manifestation during levobupivacaine infusion.

* Significantly greater than nonpregnant at control. † Significantly lower than nonpregnant at convulsions. ‡ Significantly lower than nonpregnant at control.

sus 0.018 ± 0.006 mmol/kg levobupivacaine nonpregnant ($P < 0.001$), 0.013 ± 0.002 mmol/kg bupivacaine pregnant *versus* 0.014 ± 0.003 mmol/kg bupivacaine nonpregnant ($P < 0.001$), and 0.019 ± 0.005 mmol/kg ropivacaine pregnant *versus* 0.021 ± 0.003 mmol/kg ropivacaine nonpregnant ($P < 0.001$). However, as infusion continued, there was no significant difference between pregnant and nonpregnant animals in the cumulative dose of levobupivacaine, bupivacaine, or ropivacaine required to produce more advanced manifestations of toxicity, such as hypotension, apnea, and circulatory collapse (figs. 1–3).

The total and free serum concentrations of drug were not significantly different in pregnant as compared with nonpregnant animals at the onset of convulsions, hypotension, and apnea (table 4). However, by the time

Table 2. Physiologic Variables for Bupivacaine Groups

	Control	Convulsion	Hypotension	Apnea	Circulatory Collapse
Nonpregnant					
HR	85 ± 12 (n = 12)	195 ± 50 (n = 12)	122 ± 40 (n = 9)	101 ± 42 (n = 5)	—
MABP	115 ± 11 (n = 12)	190 ± 10 (n = 12)	78 ± 10 (n = 9)	59 ± 13 (n = 7)	—
*p*H	7.48 ± 0.04 (n = 11)	7.48 ± 0.07 (n = 11)	7.21 ± 0.06 (n = 6)	7.29 ± 0.05 (n = 6)	7.25 ± 0.15 (n = 11)
P_{CO_2}	32 ± 2 (n = 11)	30 ± 7 (n = 11)	61 ± 12 (n = 6)	65 ± 10 (n = 6)	58 ± 18 (n = 11)
P_{O_2}	90 ± 6 (n = 11)	95 ± 13 (n = 11)	25 ± 2 (n = 6)	21 ± 2 (n = 6)	35 ± 32 (n = 11)
Pregnant					
HR	95 ± 18* (n = 12)	188 ± 35† (n = 11)	109 ± 22 (n = 10)	81 ± 29 (n = 10)	—
MABP	96 ± 12‡ (n = 12)	179 ± 18† (n = 11)	70 ± 10 (n = 10)	49 ± 12 (n = 10)	—
*p*H	7.51 ± 0.03 (n = 12)	7.48 ± 0.04 (n = 11)	7.21 ± 0.05 (n = 10)	7.19 ± 0.05 (n = 10)	7.24 ± 0.14 (n = 12)
P_{CO_2}	30 ± 3 (n = 12)	27 ± 2 (n = 11)	54 ± 9 (n = 10)	57 ± 7 (n = 10)	51 ± 14 (n = 12)
P_{O_2}	93 ± 9 (n = 12)	105 ± 6 (n = 11)	32 ± 19 (n = 10)	23 ± 11 (n = 10)	32 ± 31 (n = 12)

Heart rate (HR, beats/min), mean arterial blood pressure (MABP, mmHg), arterial blood *p*H, partial pressure of carbon dioxide (P_{CO_2}, mmHg), and partial pressure of oxygen (P_{O_2}, mmHg) during the control period and at the onset of each toxic manifestation during bupivacaine infusion.

* Significantly greater than nonpregnant at control. † Significantly lower than nonpregnant at convulsions. ‡ Significantly lower than nonpregnant at control.

Table 3. Physiologic Variables for Ropivacaine Groups

	Control	Convulsion	Hypotension	Apnea	Circulatory Collapse
Nonpregnant					
HR	91 ± 12 (n = 13)	236 ± 37 (n = 12)	156 ± 55 (n = 9)	107 ± 37 (n = 5)	—
MABP	109 ± 7 (n = 12)	211 ± 48 (n = 12)	78 ± 12 (n = 9)	58 ± 14 (n = 10)	—
*p*H	7.48 ± 0.04 (n = 13)	7.45 ± 0.06 (n = 13)	7.27 ± 0.16 (n = 10)	7.26 ± 0.17 (n = 9)	7.26 ± 0.16 (n = 13)
P_{CO_2}	31 ± 2 (n = 13)	33 ± 4 (n = 13)	50 ± 16 (n = 10)	53 ± 18 (n = 9)	53 ± 18 (n = 13)
P_{O_2}	94 ± 10 (n = 13)	94 ± 10 (n = 13)	42 ± 37 (n = 10)	37 ± 38 (n = 9)	39 ± 37 (n = 13)
Pregnant					
HR	100 ± 14* (n = 12)	201 ± 54† (n = 12)	123 ± 37 (n = 10)	98 ± 31 (n = 10)	—
MABP	96 ± 16‡ (n = 12)	183 ± 21† (n = 12)	68 ± 10 (n = 10)	47 ± 11 (n = 10)	—
*p*H	7.48 ± 0.05 (n = 12)	7.42 ± 0.09 (n = 12)	7.19 ± 0.12 (n = 10)	7.18 ± 0.14 (n = 10)	7.24 ± 0.14 (n = 12)
P_{CO_2}	32 ± 2 (n = 12)	36 ± 13 (n = 12)	54 ± 12 (n = 10)	56 ± 14 (n = 10)	55 ± 14 (n = 12)
P_{O_2}	94 ± 8 (n = 12)	92 ± 13 (n = 12)	31 ± 18 (n = 10)	25 ± 20 (n = 10)	30 ± 27 (n = 12)

Heart rate (HR, beats/min), mean arterial blood pressure (MABP, mmHg), arterial blood *p*H, partial pressure of carbon dioxide (P_{CO_2}, mmHg), and partial pressure of oxygen (P_{O_2}, mmHg) during the control period and at the onset of each toxic manifestation during ropivacaine infusion.

* Significantly greater than nonpregnant at control. † Significantly lower than nonpregnant at convulsion. ‡ Significantly lower than nonpregnant at control.

circulatory collapse occurred, pregnant ewes had higher total and free serum concentrations of all three drugs than nonpregnant ewes ($P = 0.022$ and 0.013, respectively; table 4).

Before the start of infusion, pregnant ewes had a higher mean heart rate and lower mean arterial blood pressure than nonpregnant ewes ($P < 0.023$ and $P < 0.001$, respectively; tables 1–3). In contrast, by the onset of convulsions, hypotension, and apnea, pregnant animals had a lower heart rate and mean arterial blood pressure than nonpregnant animals ($P < 0.05$; tables 1–3). Heart rate and mean arterial blood pressure at the onset of each toxic manifestation were not affected by the individual drug administered.

There were no significant differences in acid-base state between pregnant and nonpregnant ewes at control. Animals receiving levobupivacaine demonstrated a lower mean arterial blood oxygen tension at the onset of hypotension than those given bupivacaine or ropivacaine ($P = 0.04$; tables 1–3). A lower mean arterial blood carbon dioxide tension was found at convulsions in the bupivacaine as compared with the levobupivacaine and ropivacaine groups ($P = 0.003$), whereas ropivacaine-treated ewes had a lower mean arterial *p*H ($P = 0.049$)

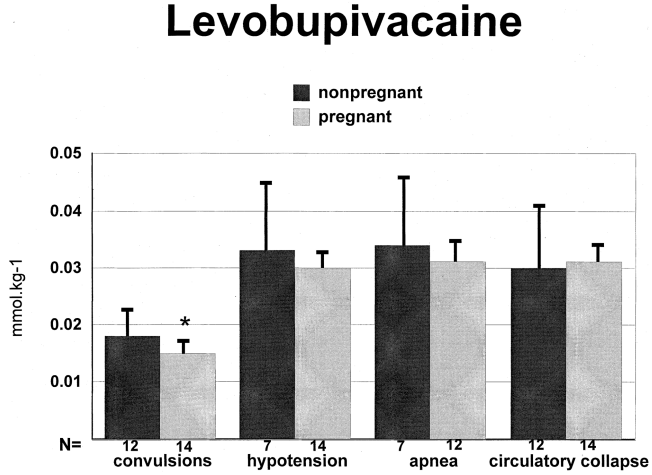

Fig. 1. The cumulative dose of levobupivacaine at the onset of each toxic manifestation. N = number of animals. *Significantly lower than nonpregnant.

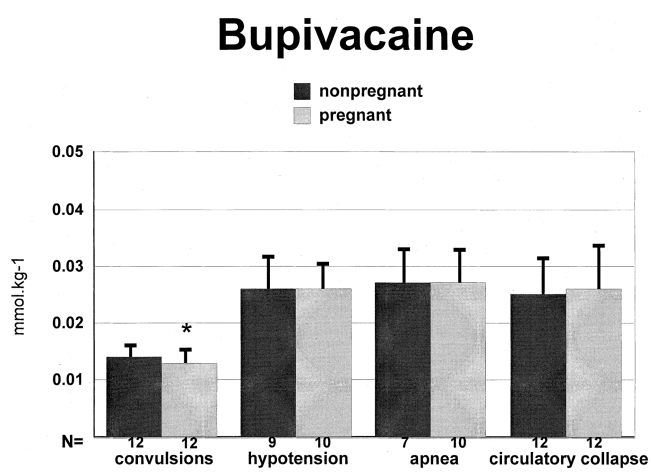

Fig. 2. The cumulative dose of bupivacaine at the onset of each toxic manifestation. N = number of animals. *Significantly lower compared with nonpregnant animals.

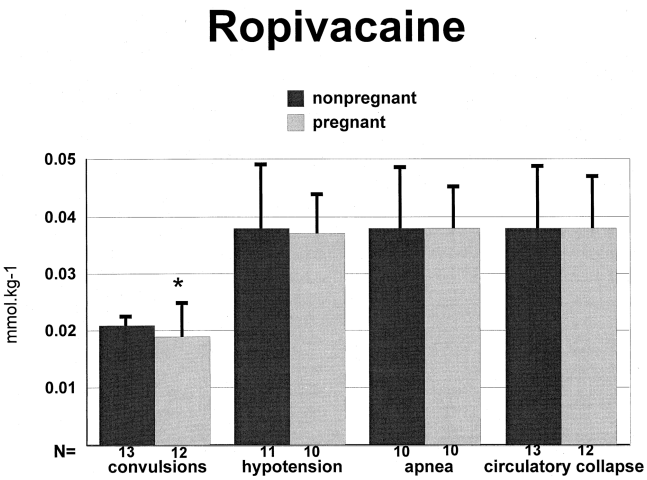

Fig. 3. The cumulative dose of ropivacaine at the onset of each toxic manifestation. N = number of animals. *Significantly lower compared with nonpregnant animals.

and lower mean arterial blood oxygen tension at convulsions than the other two drug groups (tables 1–3).

There were also differences in the dosages and serum levels among the three local anesthetics studied. The mean cumulative dose at most toxic manifestations was lowest for bupivacaine, intermediate for levobupivacaine, and highest for ropivacaine in both pregnant and nonpregnant ewes (figs. 4 and 5).

The mean total and free serum concentrations of drug at each toxic manifestation were lowest for bupivacaine in both pregnant and nonpregnant sheep ($P = 0.001$; table 4). Although there were no significant differences in the total serum concentrations of ropivacaine and levobupivacaine at each toxic manifestation, free drug concentrations were greater for ropivacaine ($P = 0.001$).

There were no significant differences among the three drugs in the incidence of ventricular arrhythmias as the terminal event (fig. 6). A lower proportion of pregnant animals (13%) had ventricular tachycardia or fibrillation as the terminal event compared with nonpregnant animals (35%; $P < 0.028$).

The ratio of the cumulative dose required to produce circulatory collapse compared with the dose resulting in convulsions (CC/CNS ratio) was higher for each drug in

Table 4. Drug Determinations

	Convulsion	Hypotension	Apnea	Circulatory Collapse
Nonpregnant				
Levobupivacaine				
Total	5.59 ± 1.80 (n = 12)	7.36 ± 2.51 (n = 7)	7.30 ± 2.78 (n = 6)	6.82 ± 2.43* (n = 12)
Free	2.28 ± 0.56 (n = 12)	3.74 ± 0.98 (n = 7)	3.93 ± 1.05 (n = 7)	3.47 ± 1.22* (n = 12)
Ropivacaine				
Total	4.70 ± 0.99 (n = 13)	6.57 ± 2.11 (n = 10)	7.45 ± 1.67 (n = 8)	7.44 ± 1.64* (n = 13)
Free	2.45 ± 0.39† (n = 11)	5.01 ± 1.24† (n = 10)	5.27 ± 1.52† (n = 8)	5.14 ± 1.55*† (n = 12)
Bupivacaine				
Total	2.49 ± 0.76‡ (n = 11)	3.03 ± 0.67‡ (n = 6)	3.49 ± 1.00‡ (n = 6)	3.44 ± 1.77*‡ (n = 11)
Free	0.94 ± 0.44‡ (n = 11)	1.73 ± 0.37‡ (n = 6)	1.88 ± 0.49‡ (n = 6)	1.59 ± 0.68*‡ (n = 11)
Pregnant				
Levobupivacaine				
Total	4.86 ± 1.47 (n = 14)	7.40 ± 1.19 (n = 14)	7.58 ± 1.30 (n = 12)	7.64 ± 1.22 (n = 13)
Free	1.93 ± 0.21 (n = 13)	4.09 ± 0.80 (n = 14)	4.22 ± 0.87 (n = 11)	4.40 ± 0.91† (n = 13)
Ropivacaine				
Total	5.61 ± 2.85 (n = 12)	9.11 ± 3.40 (n = 10)	9.45 ± 3.61 (n = 10)	9.61 ± 3.70 (n = 12)
Free	2.74 ± 1.10† (n = 11)	5.57 ± 1.46† (n = 9)	5.93 ± 1.57† (n = 9)	5.99 ± 1.92† (n = 10)
Bupivacaine				
Total	2.93 ± 0.59‡ (n = 11)	3.95 ± 1.04‡ (n = 10)	3.92 ± 1.08‡ (n = 10)	4.02 ± 1.49‡ (n = 12)
Free	0.86 ± 0.23‡ (n = 11)	1.98 ± 0.49‡ (n = 10)	2.17 ± 0.58‡ (n = 9)	2.95 ± 0.87‡ (n = 12)

Serum concentrations (μg/ml) at each toxic manifestation.

Mean ± SD.

* Significantly lower than corresponding pregnant (total $P = 0.022$; free $P = 0.013$). † Significantly greater than bupivacaine and levobupivacaine. ‡Significantly lower than other drugs (< 0.001).

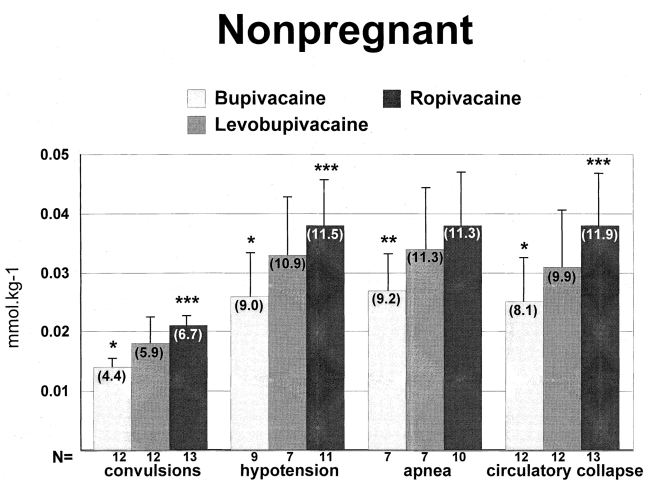

Fig. 4. The cumulative dose of bupivacaine, levobupivacaine, and ropivacaine at the onset of each toxic manifestation in nonpregnant ewes. N = number of animals. Numbers in parentheses indicate approximate amount of local anesthetic (milligram per kilogram) calculated by multiplying the mean amount (milliliters per kilogram) administered by the drug concentration. *Significantly lower compared with levobupivacaine and ropivacaine. **Significantly lower compared with ropivacaine only. ***Significantly greater compared with bupivacaine and levobupivacaine.

pregnant than nonpregnant animals (table 5). There were no significant differences among the three drugs studied in the CC/CNS ratio of doses or serum concentrations.

Discussion

In clinical practice, systemic toxicity would be most likely to occur during epidural anesthesia for cesarean section rather than for labor analgesia because of the higher drug concentrations and volumes administered. For this reason, in the current study, we administered equimolar solutions of local anesthetic at a concentration of approximately 0.5%, which is, according to some reports, the concentration that is effective for epidural anesthesia during cesarean delivery.[8,17] However, the appropriate drug doses and concentrations still need to be determined in a single randomized, controlled, clinical trial comparing levobupivacaine, ropivacaine, and bupivacaine. Our data suggest that the order of toxicity was bupivacaine > levobupivacaine > ropivacaine in both pregnant and nonpregnant animals. This is consistent with the preliminary results of a study performed in dogs that suggested that resuscitation from a local anesthetic–induced cardiac arrest was most difficult with bupivacaine and least difficult with ropivacaine, with levobupivacaine being intermediate.[18] Although the *in vitro* potency of bupivacaine and levobupivacaine are similar, but that of ropivacaine is approximately 30% less,[6] a recently published study demonstrated that ropivacaine, even at equipotent doses to bupivacaine, has lower systemic toxicity in rats.[19] However, our findings may not be applicable to epidural analgesia during labor, where the median local analgesic concentration of ropivacaine is approximately 67% greater than for bupivacaine,[7] whereas bupivacaine and levobupivacaine are comparable.[10] Unfortunately, there is no single study comparing the median local analgesic concentration of the three drugs for epidural analgesia during labor.

Our data also suggest that, with the exception of convulsions, the systemic toxicity of levobupivacaine is not affected by pregnancy in sheep, but neither is that of bupivacaine or ropivacaine. Convulsions occurred in pregnant ewes at lower doses of all three drugs than in nonpregnant ewes. Generally speaking, the difference was small, in the order of 10–15%. This is in contrast to earlier studies by our group and other investigators, demonstrating that pregnancy does not reduce the convulsive dose threshold for lidocaine,[20,21] mepivacaine,[22] bupivacaine,[3,9] or ropivacaine.[9] The reason for the apparent difference between this and previous studies is unclear but may be related to the use of survival analysis, which, in contrast to the parametric tests applied in our previous studies, better accounts for absent data points by modeling them as censored rather than missing and also does not rely on the assumption of normality. Nonetheless, more advanced manifestations of systemic toxicity, such as hypotension, apnea, and circulatory collapse, developed at similar doses of all three drugs in pregnant and nonpregnant sheep. This is particularly important because there has been controversy as to whether pregnancy-enhanced sensitivity to bupivacaine was responsible for the epidemic of cardiac arrests

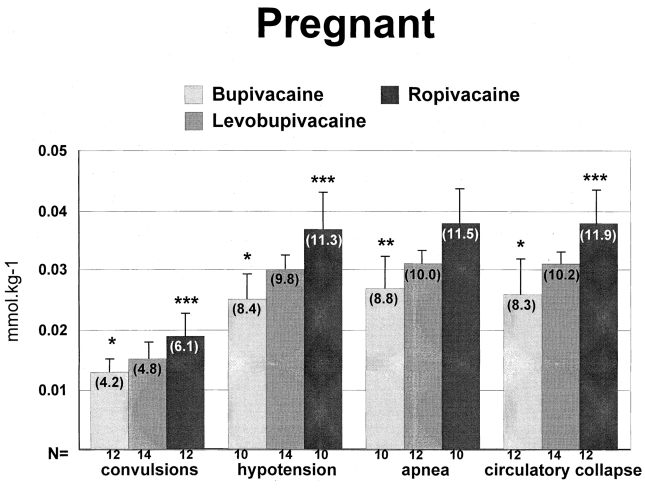

Fig. 5. The cumulative dose of bupivacaine, levobupivacaine, and ropivacaine at the onset of each toxic manifestation in pregnant ewes. N = number of animals. Numbers in parentheses indicate approximate amount (milligrams per kilogram) of local anesthetic calculated by multiplying the mean amount (milliliters per kilogram) administered by the drug concentration. *Significantly lower compared with levobupivacaine and ropivacaine. **Significantly lower compared with ropivacaine only. ***Significantly greater compared with bupivacaine and levobupivacaine.

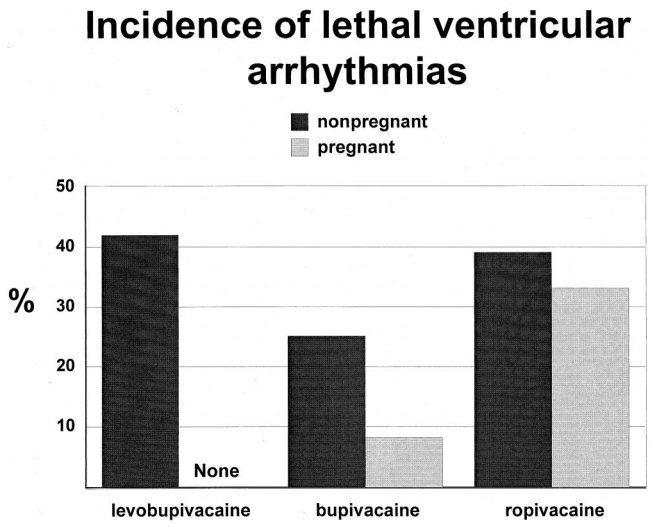

Fig. 6. The incidence (%) of ventricular tachycardia–fibrillation as the terminal event.

among parturients when clinical doses of the drug were unintentionally injected into an epidural vein.[5] In contrast to lidocaine[20] and mepivacaine,[22] an early *in vivo* study, without the use of blinding and randomization, suggested that pregnant sheep were indeed more vulnerable to the toxic effects of bupivacaine than nonpregnant sheep.[3] In addition, electrophysiologic studies of rabbit myocardium have demonstrated that progesterone, the major hormone of late pregnancy, enhances the arrhythmogenicity of bupivacaine, but not of other amide local anesthetics.[23,24] However, this is now the second *in vivo* study involving a large number of animals, and the use of blinding and randomization, to demonstrate that pregnancy does not enhance the cardiotoxicity of bupivacaine.[9]

Although convulsions occurred in pregnant ewes at lower doses of all three drugs, there was no significant difference between pregnant and nonpregnant animals in the total and free serum concentrations of drug at the onset of convulsions. For bupivacaine and ropivacaine, this could be explained by the fact that the volumes of the central compartment and distribution are lower in pregnant as compared with nonpregnant sheep.[25] Conversely, although the doses required to produce cardiac arrest were similar in pregnant and nonpregnant ewes, the total and free serum concentrations of all three drugs were greater in pregnant animals, possibly because of a smaller volume of distribution.[25] Unfortunately, there are no studies of the effects on pregnancy on the pharmacokinetics of levobupivacaine.

The difference in total and free drug concentrations between pregnant and nonpregnant animals at hypotension and apnea did not achieve statistical significance, probably because of low power of statistical analysis, as some animals died before progression through all toxic end points. However, by design, all animals reached circulatory collapse, and the total and free concentrations of all three local anesthetics were greater in pregnant as compared with nonpregnant sheep. It is not surprising that the free serum concentration of all drugs at circulatory collapse was greater in pregnant sheep, probably because of pregnancy-induced reductions in serum albumin and α_1 acid glycoprotein concentrations.[26] This is in contrast to our previous study, in which there were no significant differences in total and free concentrations of bupivacaine and ropivacaine at circulatory collapse between pregnant and nonpregnant ewes.[9] The apparent difference between the two studies may be related to the mean arterial *p*H of pregnant animals at circulatory collapse. In the current study, it was 7.24 for bupivacaine and ropivacaine, whereas in our previous study the corresponding values were 7.33 and 7.27, respectively.[9] It has been shown that the free fraction of local anesthetic increases as the *p*H decreases.[27]

Table 5. Margin of Safety Ratios

CC/CNS	Dose	Total
Levobupivacaine		
NP	1.67 ± 0.45	1.22 ± 0.23
PR	2.15 ± 0.36*	1.73 ± 0.66
Bupivacaine		
NP	1.86 ± 0.69	1.55 ± 1.29
PR	2.08 ± 0.45*	1.41 ± 0.27
Ropivacaine		
NP	1.81 ± 0.53	1.64 ± 0.47
PR	2.04 ± 0.48*	1.83 ± 0.53

Ratio of drug doses and serum drug concentrations required to produce circulatory collapse compared with convulsions (CC/CNS).

* Significantly greater than corresponding values for nonpregnant.

NP = nonpregnant; PR = pregnant.

All ewes were in good condition before the start of the study. As in our previous study,[9] the lower heart rate and blood pressure recorded in pregnant ewes at the onset of toxic manifestations, regardless of drug allocation, may be a result of attenuation of cardiovascular responses to adrenergic stimulation during pregnancy.[27]

The incidence of lethal ventricular arrhythmias at the time of death was lower in pregnant than nonpregnant ewes, suggesting that pregnant animals may be less vulnerable to this complication. The incidence of ventricular tachycardia–fibrillation was similar with the three drugs. This finding is consistent with our previous study of bupivacaine and ropivacaine.[9]

For each drug, the ratio of the dose required to produce circulatory collapse to that leading to convulsions (CC/CNS) was greater in pregnant animals because the convulsive dose was lower in these animals, whereas circulatory collapse occurred at similar drug doses in both groups of sheep. As with our earlier study,[9] relying on CC/CNS ratios alone to evaluate drug safety can be misleading because, although two drugs may have a similar ratio, they may be vastly different in the absolute

drug doses required to produce manifestations of systemic toxicity.[9]

Finally, our data may not pertain to the clinical setting of an unintended intravascular injection. Whereas in the current study, toxic manifestations were clearly delineated in most animals and circulatory collapse occurred after approximately 15–25 min of infusion, it was a bolus injection of bupivacaine that resulted in immediate cardiac arrest in the reported clinical cases.[4,5] Yet our data are consistent with the results of another study showing no difference in the doses or serum concentrations of the three drugs required to produce toxic manifestations in pregnant and nonpregnant sheep given incremental bolus doses of levobupivacaine, bupivacaine, and ropivacaine at 1-min intervals until circulatory collapse.[28]

In conclusion, pregnancy increased the risk of convulsions but not of other serious manifestations of local anesthetic toxicity, such as hypotension, apnea, and circulatory collapse. During the conditions of this study, the risk of systemic toxicity was greatest with bupivacaine and least with ropivacaine. However, the potential systemic toxicity of these drugs in clinical practice may also be affected by their relative potency and effectiveness when used for regional anesthesia.

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Appendix: Statistical Analyses

For pregnant ewes, possible imbalances between treatment groups at control with respect to gestational age were determined using an analysis of variance model. Overall difference between treatment groups was assessed using the type I sums of squares. The Scheffé multiple comparison procedure was then used to assess pairwise comparisons in the event of a statistically significant overall difference.

Differences between treatment groups and pregnancy status with respect to weight at entry were assessed using an analysis of variance model. The interaction between treatment and pregnancy status was assessed and, provided no interaction effect was observed ($P < 0.05$), main effects were then assessed *via* a main effects model using the type I sums of squares. The Scheffé multiple comparison procedure was then used to assess pairwise comparisons.

Accumulated dose at the onset of each toxic manifestation was compared using Cox proportional hazards model for survival data using SAS procedure PHREG (SAS Institute). A survival analysis approach was felt to be appropriate as it does not rely on the parametric assumption of normality and better accounts for missing data values by modeling them as censored data.

The treatment-by-pregnancy status interaction effect was assessed in each case by comparing the "−2 LOG L" statistic obtained *via* a model of main effects (treatment and pregnancy status) and interaction with a model of main effects only. In cases of statistical significance at the 5% level, the interaction effect was further investigated. Where the interaction was not deemed statistically significant, the treatment was assessed by comparing the "2-LOG L" statistic obtained *via* a model of main effects (treatment and pregnancy status) with a model of pregnancy status only. The effect of pregnancy status was determined directly *via* the associated Wald chi-square statistic from the main

effects of model. Where the treatment effect was deemed statistically significant, pairwise comparisons of treatments were assessed *via* the associated Wald chi-square statistics obtained from the main effects model.

The ratio of dose to circulatory collapse/dose to convulsion was analyzed using an analysis of variance model. Interaction effects between treatment and pregnancy status were assessed using a factorial analysis of variance model. Where no interaction effect was observed ($P < 0.05$), main effects were then assessed *via* a main effects model using the type I sums of squares. The Scheffé multiple comparison procedure was then used to assess pairwise comparisons.

The serum drug concentration parameters of interest were total serum concentration and serum concentration of free drug. With the exception of the serum ratio values of circulatory collapse/convulsion, an analysis of variance approach was deemed appropriate. The interaction effect between treatment and pregnancy status was assessed using a factorial analysis of variance model at each toxic manifestation. Where no interaction effect was observed ($P < 0.05$), main effects were then assessed *via* a main effects model using the type I sums of squares. The Scheffé multiple comparison procedure was then used to assess pairwise comparisons. For the ratio of serum concentrations at circulatory collapse/serum concentrations at convulsion, the analysis of variance approach was not used because of highly influential outliers in the data. Instead, nonpregnant and pregnant ewes were analyzed separately using the nonparametric Kruskal-Wallis test.

The proportion of animals having malignant ventricular arrhythmias at the time of death was analyzed using the stratum-adjusted Mantel-Haenszel chi-square test. The tests were performed using SAS procedure FREQ, with rank scores and comparisons evaluated *via* the statistics presented for "row mean scores differ." The effect of pregnancy status was investigated by testing for association between pregnancy status and arrhythmias, stratifying by treatment. Similarly, the treatment effects were investigated by testing for association between treatment and arrhythmias, stratifying by pregnancy status.

For the physiologic parameters, a repeated-measures approach was not used as it was deemed inappropriate in light of missing data for hypotension and apnea. Because normality of residual plots following an analysis of variance approach was not shown by all variables for all manifestations, two separate approaches were used. For heart rate and mean arterial blood pressure, because the residuals were reasonably normally distributed, the analysis of variance approach was deemed appropriate. However, for mean arterial *p*H, arterial blood carbon dioxide tension, and arterial blood oxygen tension, a parametric analysis of variance was inappropriate; therefore, data from nonpregnant and pregnant animals were analyzed separately using the nonparametric Kruskal-Wallis test. Where the analysis of variance approach was used, interaction effects between treatment and pregnancy status were investigated. Where no interaction effect was observed ($P < 0.05$), main effects were then assessed *via* a main effects model using the type I sums of squares. The Scheffé multiple comparison procedure was then used to assess pairwise comparisons. Where the nonparametric Kruskal-Wallis test was used and there was evidence of a treatment difference ($P < 0.05$), pairwise comparisons were made again using the Kruskal-Wallis.

The assumption of normality in the residuals for the analysis of variance approach for demographics, ratio parameters, serum parameters, and physiologic parameters was checked using the UNIVARIATE procedure in SAS. No normality checks were necessary for accumulated dose because a nonparametric Cox proportional hazards model was used.

Because the residuals from the analysis of variance models fitted for the demographics showed no strong signs of non-normality, the parametric analysis of variance model was deemed suitable. Because the residuals from the analysis of variance models for the three dose ratio parameters showed no strong skewness, an analysis on log transformed data was not deemed necessary.

For the serum drug concentration parameters, the residuals from an analysis of variance model displayed reasonable normality with occasional outliers. These outliers were investigated and, in most cases, were found not to have a great influence so as to affect the conclusion of the analysis. However, for the serum ratio parameters, the outliers were found to be highly influential. As a result, the ratio parameters were analyzed *via* nonparametric methods.

For the physiologic variables, the residuals for heart rate and mean arterial blood pressure showed reasonable normality and were thus analyzed using analysis of variance. However, residuals from the analysis of variance models for mean arterial blood *p*H, carbon dioxide tension, and oxygen tension showed strong non-normality. Because no suitable transformations could be found for these three parameters, the nonparametric approach was adopted.