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Central Nervous System and Cardiac Effects From Long-Acting Amide Local Anesthetic Toxicity in the Intact Animal Model

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With the development of the newer long-acting amide local anesthetics,ropivacaine and levobupivacaine, numerous animal studies of LA systemic toxicity have emerged. Because of the complex nature of the human response to LA intoxication, the task of designing and interpreting these animal studies of LA toxicity can be difficult. Accordingly, this report will review the selection of an animal model for the study of LA toxicity; examine the pertinent in vivo animal studies that compare the central nervous system toxicity, cardiovascular toxicity, and the ease of resuscitation of the single enantiomer local anesthetics to racemic bupivacaine; and extrapolate these findings to the clinical setting. *Reg Anesth Pain Med 2003;28:3-11.*

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Since Albright's alarming editorial[1] on the anecdotal report of cardiac arrest associated with bupivacaine, there has been a decline in the frequency of reports of local anesthetic-induced cardiotoxicity. This is attributed to the improved clinical techniques that have assisted in the detection of potential local anesthetic (LA) toxicity and the restrictions placed on 0.75% bupivacaine for its use in intravenous regional and obstetrics. Another possible reason for the decline in LA toxicity is the increased awareness of the relationship between stereoselectivity and toxicity. As a consequence of preclinical laboratory investigations[2-4] demonstrating reduced toxic side effects with the single S(-) enantiomer bupivacaine as compared with its racemic formulation, ropivacaine and levobupivacaine were developed as long-acting local anesthetic alternatives to racemic bupivacaine with potentially greater margins of safety.

To better understand these preclinical investigations, this report will review the selection of an animal model for investigation of systemic LA toxicity, examine the pertinent in vivo animal studies that compare the central nervous system (CNS) toxicity, cardiovascular (CV) toxicity, and the ease of resuscitation of the single enantiomer local anesthetics to racemic bupivacaine, and extrapolate these experimental findings to the clinical setting. Equal anesthetic potency among the long acting agents, racemic bupivacaine, ropivacaine, and levobupivacaine, has been assumed despite the concern that ropivacaine may possess 60% to 75% the potency of racemic bupivacaine during labor epidural analgesia.[5,6] There are several reasons for this assumption. First, it has been debated whether the methodology used in these epidural analgesia studies, that is, up-down median effective estimates, can be applied to the overall dose-response curves.[7] Second, other labor epidural analgesia studies found no difference in hourly amounts of self-administered ropivacaine and bupivacaine.[8,9] Finally, intravenous ropivacaine at equivalent (3.0 mg/kg/min) and equipotent (4.5 mg/kg/min) nerve blocking doses were less cardiotoxic than racemic bupivacaine (3.0 mg/kg/min) in anesthetized rats.[10] Even though this report focuses on systemic toxicity from the long-acting amide local anesthetics, the

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shorter-acting agents, such as lidocaine and mepivacaine, should not be presumed innocuous.[11,12]

The task of designing and interpreting experimental animal models of LA toxicity can present a challenge to the researcher and clinician owing to the complex nature of the human response to LA intoxication. An animal model is defined as an animated object of imitation, or an "image of Man," used to investigate a physiologic or pathologic circumstance in question. There are no universal rules for the choice of the best animal model, or paradigm, due to the differing objectives of each experiment. In the case of LA toxicity, for example, studies are performed to (1) rationalize dose response relationships for particular toxic effects, including the establishment of threshold doses (i.e., convulsive doses, lethal doses, etc.); (2) characterize the likely principal factors influencing the severity of toxic effects (i.e., hypercarbia, acidosis, comedications, etc.); (3) provide a basis for structure-activity predictions; (4) identify target cell types or organs of toxicity; (5) determine the most susceptible species; and (6) increase the basic understanding of the pathophysiologic process. Indeed, underlying all of these objectives is the quest to extrapolate experimental findings to the human response, as it is clearly unethical to perform such studies in human volunteers.

Choice of Species

The validity of extrapolation to the human is, in part, a function of the appropriateness of the species used for the problem. In the context of LA toxicity, the rodent, canine, swine, and ewe are the most frequently used species. There are several advantages and disadvantages to each of these species. The rodent differs substantially from the human; however, its small size and uniformity (inbred) make it an economical (i.e., housing rodents *v* "higher" animals) and highly reproducible in vivo model. Another potential advantage is the wide availability of genetic variants. In contrast, the relatively larger-sized canine, while more costly, allows for intricate instrumentation and elaborate data collection (i.e, electroencephalogram [EEG] activity, arrhythmia detection, myocardial function, resuscitation [advanced cardial life support]). Even so, the canine model should be used with caution in CV studies as their abundance of coronary collaterals may provide added protection from ischemia. The swine, on the other hand, has coronary vasculature similar to the human, predisposing the myocardium to a greater number of arrhythmogenic events during ischemia. Additionally, the presence of a placenta and the high incidence of singleton progeny make the ewe a close model for the study of LA toxicity in relation to human pregnancy. Lastly, the ewe is more sensitive to LA toxicity than the canine, suggesting that it may be a more reliable "safeguard" when extrapolating to the human response. There is no doubt that this "plurispecies" approach to the issue of LA toxicity can be confusing if comparisons between studies are to be made. On the other hand, if all species respond similarly to the test stimulus, then the overall validity of the experimental findings becomes enhanced as does the success of extrapolation to the human response.

Choice of Paradigm

A diversity of experimental paradigms adds to the difficulty of interpretation among the different species. These paradigms can be broadly characterized by dosing regimen: acute (bolus) dosing versus chronic (continuous); site of LA administration: intravenous versus intracoronary versus intracerebral (or intracarotid); and the animal's state of being: conscious with or without sedation versus anesthetized, and spontaneous versus mechanically ventilated. Indeed, the study design that best simulates the unintentional intravenous injection of LA during peripheral nerve block placement is that which uses intravenous, bolus administration of LA into a conscious animal. However, if the objective of the experiment is to focus on the CV response, then early CNS excitation may negate any direct LA-mediated myocardial depressant effect. Accordingly, several investigators have used site-directed LA administration, or intracoronary delivery of LA in conscious, large animal preparations. The doses used produce similar plasma concentrations in cardiac circulation as during intravenous administration, and the lack of recirculation prevents neural and humoral factors from confounding the myocardial effects. Also, CNS site-directed carotid arterial infusions of LA (with minimal systemic circulation) into conscious animals have been used to evaluate direct CNS toxicity and the indirect cardiac sequelae from sympathetic nervous system activation. The other study design routinely used to examine cardiotoxic effects is the anesthetized, mechanically ventilated animal with continuous LA infusion. Although it far from emulates the clinical scenario of LA intoxication during the establishment of peripheral nerve block for surgical anesthesia, it too has several advantages. First, the design attenuates the confounding effects of seizures and their metabolic consequences, including hypoxia, hypercarbia, and acidosis.[13-15] Second, it allows for intricate invasive monitoring, regardless of the animal's size.[16] Third, it lends clinical relevance to the combined regional-

general anesthetic technique that is more frequently being used during major general and vascular procedures. Also, the continuous LA infusion paradigm allows ready observation of the progressive signs of toxicity. Selective hemodynamic and electrophysiologic measures, for instance, are often unobtainable in experimental models using bolus LA administration because of the minimal separation between blood concentrations of LA leading to CNS and CV toxicity. Finally, incremental elevations in LA blood concentrations imitate gradual overdosage or accumulation of LA that may occur with continuous infusions for postoperative pain management. While a diversity of paradigms exists, each paradigm offers unique information that is useful in the overall understanding of LA systemic toxicity.

CNS Toxicity

Systemic local anesthetic toxicity is a rare event, however, reactions can occur after an accidental intravascular injection or overdosage (i.e., enhanced absorption) at the intended site of action. The CNS and CV systems are the major sites of toxicity. The CNS is more sensitive to local anesthetic toxicity than the CV system.[17] That is, CNS intoxication usually manifests before signs of cardiovascular compromise (except in some cases of bupivacaine intoxication).[18,19] CNS intoxication is characterized by a 2-stage pathophysiologic process. Shivering, muscle twitching, and tremors precede tonic-clonic seizure activity as increased plasma levels of LA preferentially block inhibitory central pathways, leaving excitatory neurons unopposed.[20-22] With increasing LA concentrations, block of both inhibitory and excitatory pathways leads to generalized CNS depression resulting in hypoventilation and respiratory arrest.

Animal Studies

In the majority of conscious animal studies, CNS toxicity is evaluated qualitatively by the presence or absence of convulsions. The convulsive threshold dose is a measure of CNS toxicity. Recent work by Ladd and Mather[23] also describes a quantitative method of evaluating subconvulsive doses of LA in conscious sheep. In brief, before and after intravenous administration of LA in graded doses, observed behaviors representing the prodrome to the onset of convulsions (such as licking, swallowing, extension of the neck, head bobbing, splaying of legs, etc.) are ranked in severity. Ranked scores from 0 (no apparent affect) to 100 (death) are modeled according to a logistic population growth equation, and a Central Effects Index (CEI) is determined. Using standard repeated measures analyses, peak CEIs (or the area under the curve for the CEIs) are compared among LAs in question. For instance, in sheep, subconvulsive doses (75- and 100-mg doses) of levobupivacaine produce smaller peak CEI values than equivalent doses of racemic bupivacaine, suggesting that the CNS stimulatory potency of levobupivacaine is less than racemic bupivacaine at subconvulsive doses.[23] In anesthetized, ventilated animals, EEG activity is used to determine the threshold LA convulsive dose. Table 1 compares the convulsive LA doses of racemic bupivacaine, levobupivacaine, ropivacaine, and lidocaine among species and paradigms.

Not unexpectedly, a general relationship exists between the relative anesthetic potency and the dosage required to produce CNS toxicity in that the long-acting LAs are up to 4 times more toxic than lidocaine.[24] Within the N-n-alkyl piperidine xylidide family, the propensity for seizure activity after LA intoxication with the single S(-) isomers, levobupivacaine and ropivacaine, appears to be 1.5

Table 1. Convulsive Doses of Racemic Bupivacaine, Levobupivacaine, Ropivacaine, and Lidocaine in Various Animal Species and Experimental Paradigms

Species	Reference	Dosing Regimen	Injection Site	State of Being	Racemic Bupivacaine	LBupivacaine	Ropivacaine	Lidocaine
Mouse	(51)	Bolus	IP	Awake	58 mg/kg			111 mg/kg
Rat	(10)	Continuous	IV: equiv equipotent	Awake	2.8 mg/kg		4.5 mg/kg 2.9 mg/kg	
	(25)	Continuous	IV	Anesth/Ventilated	9.3 mg/kg	12.8 mg/kg	13.2 mg/kg	
Dog	(44)	Serial bolus	IV	Awake	5 mg/kg			22 mg/kg
	(47)	Infusion	IV	Awake	4.3 mg/kg		4.9 mg/kg	20 mg/kg
Sheep								
	(52)	Continuous	IV	Awake	.014 mmol/kg 2.49 μg/mL	.018 mmol/kg 5.59 μg/mL	.021 mmol/kg 4.7 μg/mL	
	(46)	Bolus/3min	IV	Awake	1.6 mg/kg (69 mg) 10 μg/mL		3.5 mg/kg (155 mg) 17 μg/mL	6.8 mg/kg (320 mg) 54 μg/mL
	(26,27)	Bolus/3min	IV	Awake	69-85 mg	103-127 mg		

Abbreviations: Lbupivacaine, levobupivacaine; IP, intraperitoneal; IV, intravenous; anesth, anesthetized.

to 2.5 times less than LA intoxication with the R(+) isomer or racemic bupivacaine (Table 1). Comparative CNS toxicity between levobupivacaine and ropivacaine reveals the differences are, in part, species dependent. In the anesthetized, ventilated rat, the cumulative convulsive doses were similar between levobupivacaine (12.8 mg/kg) and ropivacaine (13.2 mg/kg).[25] In the conscious sheep, convulsant doses were slightly greater with ropivacaine as compared with levobupivacaine (mean [95% confidence limits] = 156 mg [128 to 184 mg] *v* 101 mg [87 to 116 mg]).[26,27] Of course, the relative doses required to produce convulsions with all LAs are influenced by the route and rate of injection (intraperitoneal *v* intravenous; bolus *v* continuous infusion), the rapidity with which a particular blood level is achieved, and whether the animal is awake or anesthetized (or acid-base status). In the end, these differing paradigms often make comparisons among CNS toxicity studies difficult to interpret.

CV Toxicity

The pathophysiology of the CV system, the other target of systemic LA intoxication, can also be considered in 2 stages. During the CNS excitatory phase, activation of the sympathetic nervous system can lead to tachycardia and hypertension masking direct LA-mediated myocardial effects. However, as blood concentrations increase, arrhythmias and contractile dysfunction supersede sympathetic-mediated action. In addition to these direct effects, a central component of LA-induced cardiotoxicity has been suggested. Direct injection of LAs into the medullary vasomotor centers of various animal species[28-30] can produce bradycardia, hypotension, and ventricular arrhythmias similar to accidental intravascular administration.

Animal Studies

Because arrhythmias, myocardial depression, and/or cardiovascular collapse are manifestations of LA-induced cardiotoxicity, investigators have used measures of arrhythmogenicity, mechanical activity, and survivability as cardiovascular endpoints of LA intoxication.

Arrhythmogenicity

In common with in vitro studies, electrophysiologic studies in the intact animal show a dose-dependent prolongation of cardiac conduction with increasing concentrations of the long-acting LAs as reflected by increases in the PR interval and QRS duration of the electrocardiogram.[31,32] Depression of SA and AV nodal activity is further manifested by bradycardia and partial or complete atrioventricular block. With high blood levels of racemic bupivacaine, the heart is predisposed to re-entrant arrhythmias (prolongation of QT interval) as the incidence of ventricular tachycardia and ventricular fibrillation increase.

Comparison of arrhythmogenic potential of racemic bupivacaine and lidocaine has been consistent regardless of the animal species or paradigm. Doses required to induce nodal and ventricular arrhythmias were in the subconvulsant range for racemic bupivacaine, whereas even convulsant doses of lidocaine did not induce such arrhythmias.[33] Equipotent convulsant doses of racemic bupivacaine and lidocaine in conscious sheep produced severe arrhythmias with racemic bupivacaine, whereas only transient ST-segment depression or sinus tachycardia was seen with lidocaine.[34] Using intracoronary injection of LA in anesthetized pigs, Nath et al.[35] found comparable prolongation of the QRS interval with racemic bupivacaine and lidocaine at a dose ratio of 1:16. Moreover, 7 of 15 animals given 4 mg intracoronary bupivacaine died of ventricular fibrillation preceded by progressive QRS prolongation, whereas lidocaine-induced ventricular fibrillation occurred at 64 mg. These data suggest that the arrhythmogenic potential of racemic bupivacaine compared with lidocaine may be greater than their ratio of anesthetic potency (bupivacaine: lidocaine = 4:1[24]). Furthermore, the findings from intracarotid administration of LA suggest that bupivacaine-induced ventricular arrhythmias may not be related to CNS excitation.[36]

Studies comparing the electrophysiologic and arrhythmogenic potential of the long-acting LAs have shown, in general, that levobupivacaine has intermediate risk between ropivacaine and racemic bupivacaine. In a study in conscious rats that assumed equivalent nerve blocking potency between levobupivacaine and ropivacaine, the same bolus dose of levobupivacaine prolonged QRS duration more than ropivacaine.[37] At greater concentrations, ventricular tachycardia occurred in 7 of 8 levobupivacaine-treated rats compared with only 1 of 8 ropivacaine-treated rats. Similarly, in anesthetized rats, the cumulative intravenous dose and plasma concentrations of LA at the onset of the first arrhythmia were greater for ropivacaine as compared with levobupivacaine, and both were significantly greater than racemic bupivacaine.[25] In anesthetized swine, using an escalating intracoronary LA dose scheme, the QRS prolongation potency ratio for racemic bupivacaine:levobupivacaine:ropivacaine was 2.1:1.4:1.[38] In contradistinction, in conscious, chronically instrumented sheep receiving intracoronary LA, QRS width was increased by all 3

drugs, but only the potency and duration of racemic bupivacaine were greater than ropivacaine. Also, these investigators failed to find a significant difference in the frequency of ventricular arrhythmias or premature ventricular contractions between any drug pair. Interestingly, this is in contrast to their other studies in conscious sheep in which intravenous levobupivacaine produced fewer arrhythmias than racemic bupivacaine.[26,27] Racemic bupivacaine doses of 125 to 200 mg administered intravenously over 3 minutes produced fatal ventricular fibrillation in some animals, whereas similar life-threatening arrhythmias were not found with 225 mg levobupivacaine. Two explanations for the discrepancies among these studies performed in sheep may be the dosing regimen and site of LA administration; that is, a single, slow (3 minutes), intracoronary injection of LA has the distinct advantage of reducing CNS-mediated arrhythmogenic effects.[39]

Programmed electrical stimulation (PES) protocols have also recently been used in an attempt to elicit arrhythmias at lower LA plasma concentrations than would be required to produce arrhythmias so as to minimize the effects of LA-induced CNS toxicity on electrophysiologic responses. In anesthetized, open-chest canines receiving incrementally escalating infusions of the long-acting LAs and lidocaine, the incidence of PES-induced extrasystoles or premature ventricular contractions with racemic bupivacaine and levobupivacaine, at the target dose corresponding to a plasma concentration of 8 μg/mL, were significantly greater than in the lidocaine-treated dogs, at the target dose corresponding to a plasma concentration of 32 μg/mL.[40] There was no difference in extrasystoles between ropivacaine and lidocaine, suggesting that ropivacaine may have a lower arrhythmogenic potential than its butyl homologue, levobupivacaine, or racemic bupivacaine (that is if one assumes that extrasystoles or premature ventricular contractions are the harbinger of more malignant arrhythmias). Nonetheless, there were no differences in spontaneous versus PES-induced ventricular tachycardia or fibrillation between groups. As with CNS toxicity, the dose, mode, site of LA administration, and the conscious state of the animal should be considered when interpreting the differing results among the electrophysiologic studies of LA toxicity.

Mechanical Activity

Dose-dependent reductions in contractility occur with systemic LA intoxication. Parameters used to track LA-mediated contractile depression include reductions in systemic blood pressure and elevations in left ventricular end diastolic pressure. More direct measures of reduced inotropy include reductions in dP/dtmax, cardiac output, stroke volume, and ejection fraction (echocardiography). Sonomicrometry has also been used to assess regional changes in myocardial function. Similar to in vitro findings,[41] the extent of cardiac contractile depression is proportional to nerve blocking potency, such that the more potent local anesthetics (i.e., racemic bupivacaine) tend to reduce cardiac contractility at lower doses and concentrations than the less potent local anesthetic agents (i.e., lidocaine).[34,35,42]

Comparisons of the myocardial depressant effects of the new, single S(-) enantiomer LAs with racemic bupivacaine have recently been reported. Subconvulsive doses of levobupivacaine and racemic bupivacaine given to conscious sheep produced a comparable depression in contractility even though the incidence of ventricular arrhythmias was greater with racemic bupivacaine.[26] In the same species, slow (3-minute), intracoronary administration of ropivacaine produced reductions in dP/dtmax and stroke volume that were less potent than those observed with equivalent doses of racemic bupivacaine and levobupivacaine.[39] No significant differences were observed between levobupivacaine and racemic bupivacaine in this sheep study. Similarly, in anesthetized, open-chest canines, ropivacaine exhibited a slightly greater safety margin with regard to contractility as compared with its n-butyl homologue, levobupivacaine, and racemic bupivacaine during incremental LA intoxication via a continuous intravenous infusion.[43] The effective LA concentrations yielding 35% reductions in dP/dt max and percent fractional shortening (echocardiographic index of left ventricular ejection) were 4.03 μg/mL and 2.95 μg/mL, respectively. The plasma concentrations of levobupivacaine that produced these same endpoints of contractile dysfunction were 2.42 μg/mL and 1.28 μg/mL, respectively, and these were comparable to bupivacaine (2.3 μg/mL and 2.12 μg/mL, respectively). Taken together, these data suggest that the differing negative inotropic effects of the long-acting LAs may not be due to chirality alone, because ropivacaine has been shown to be a less potent myocardial depressant than its S(-) butyl homologue, levobupivacaine, and because differences between racemic bupivacaine and levobupivacaine have yet to be reported.

CC/CNS Ratio

In the past, the primary objective of acute toxicity testing was to determine the median lethal dose $(LD)_{50}$ for the purpose of classification and stan-

dardization of drugs. However, due to all the extraneous factors that affect the precision of LD_{50}, including the animal species, strain, sex, route of administration, dosage formulation, etc., the ratio of the LA dosage required for irreversible cardiovascular collapse and the dosage that produces CNS toxicity (convulsions), the cardiovascular collapse (CC)/CNS ratio, has been adopted as a comparative measure of CV toxicity among LAs. Blood level ratios and tissue level ratios have also been used to help determine the mechanisms of lethality from LA overdosage, i.e., differences in tissue uptake among organs. Despite the fact that convulsions are not the desired effect, it is believed that the higher the CC/CNS ratio the better the safety margin. That is, the wider the safety margin between convulsions

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